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EDITED BY

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DIRECTOR OF THE JOHN INNES HORTICULTURAL INSTITUTION

AND

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ARTHUR BALFOUR PROFESSOR OF GENETICS IN THE UNIVERSITY OF CAMBRIDGE

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ERRATA.

Page 92, line 5, *for* "obvate" *read* "ovate."

Page 101, line 4, *for* "+sugar + flavone" *read* "+sugar + flavone).

Page 105, line 9, *for* "with removal of sugars" *read* "without removal of sugars."

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VOLUME IV JUNE, 1914 No. 1

ON THE RELATIONS BETWEEN CHROMOSOMES, SEX-LIMITED TRANSMISSION AND SEX-DETERMINATION IN *ABRAXAS GROSSULARIATA*.

BY L. DONCASTER, SC.D.,
Fellow of King's College, Cambridge.

IN the last of a series of papers on chromosomes and sex in *Abraxas grossulariata* (Currant Moth), I described breeding experiments with a strain which in each generation produced families consisting entirely of females, and showed that in this strain all the ovaries examined, with one exception, had oogonia with 55 chromosomes instead of 56, the normal number in the species[1]. The present paper confirms and amplifies the results previously described, gives an account of the chromosomes in the maturation of the egg in normal females and in the strain which produces unisexual broods, and describes an exception to the normal sex-limited transmission of the *grossulariata* character which can possibly be correlated with an exception in the chromosome number in such a way as to suggest a definite relation between that character and a chromosome. It also gives direct evidence of dimorphism of the eggs in respect of chromosome number, exactly comparable with the dimorphism of spermatozoa which has now been described in so many insects of other orders.

MATERIAL AND METHODS.

The methods adopted were in general the same as described in my previous papers, except that the larvae (usually about half-grown) were dissected in most cases in tap-water instead of Ringer's fluid. I found by accident that this gave clearer division-figures; I discovered that the Ringer's fluid used for the ovaries obtained in the autumn

[1] *Journal of Genetics*, Vol. III. 1913, p. 1.

of 1912 had accidentally been made up by my assistant to only one-tenth of the normal strength, and on making up the correct solution it gave very inferior results. Tap-water gave the best results of all. Sections for both ovarian and maturation divisions were stained with Heidenhain's Iron Haematoxylin; in the latter case the stain must be washed out until the yolk is left almost colourless.

For the maturation of the eggs, I have to thank Miss P. H. Dederer of New York for giving me full information in writing about the methods used by her in studying the maturation divisions of *Philosamia cynthia*. I followed her methods in general, with modifications due to the much smaller eggs and thinner shells of *Abraxas grossulariata*. The eggs were fixed at varying times up to about 3 hours in Carnoy's alcohol sublimate (absolute alcohol, glacial acetic acid, chloroform, in equal parts; sublimate to saturation). They were then washed in 70 % alcohol, and usually treated with iodine in 70 or 90 % alcohol to remove the sublimate, and preserved in 80 % alcohol. For cutting sections it is necessary to remove the shell, always a troublesome and often a difficult operation. In some batches the egg is contracted away from the shell, and the shell can then be removed without much difficulty with needles under a binocular dissecting microscope. In some cases, eggs which had been treated with iodine seemed to have softer shells, but I am not sure that this is general. The removal of the shell seemed usually to be considerably easier in eggs which had been kept for some weeks in spirit. In other batches the protoplasmic layer of the egg sticks to the shell, and to remove it without tearing it from the yolk is a matter of great difficulty. If it is once separated from the yolk, accurate orientation becomes impossible and the polar region often cannot be found in the sections. The polar spindles are at the anterior end of the egg, which in this species can generally be recognised by the less marked sculpturing of the shell. The shell was pricked and torn apart from the posterior end when this could be identified. The shelled eggs were passed through absolute alcohol into cedar oil for some hours or more, placed in xylol for a few minutes, and embedded in paraffin melting at 60°. The sections were cut transversely as a rule, with a thickness of 10 μ.

a. Breeding Experiments.

The main facts established in the previous paper were that in a certain strain families appeared in each generation consisting entirely of females; some of these females again have only female offspring, while others, apparently of about equal number, produce males and

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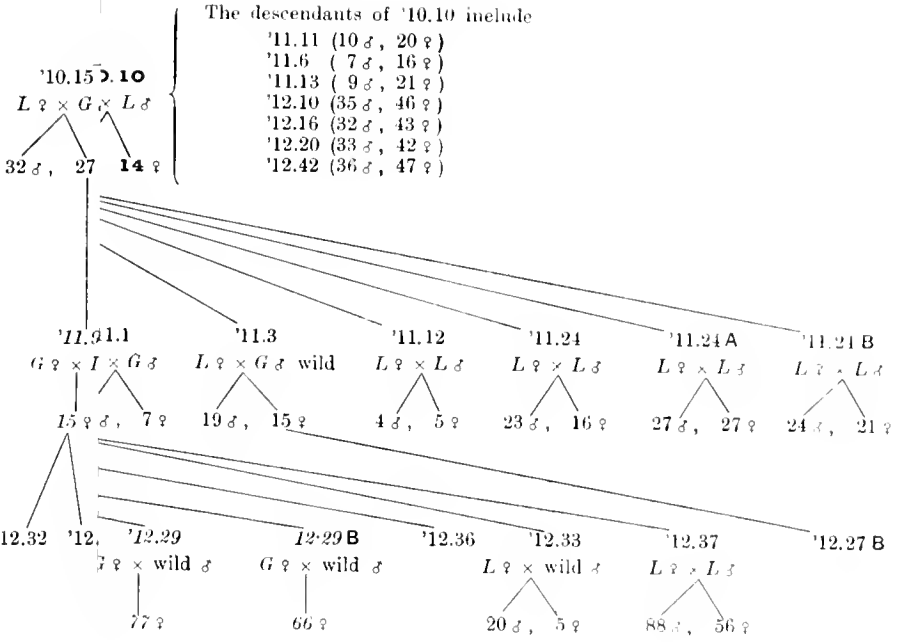


TABLE I.

Pedigree of All female Strain.

(All female broods in *italics*, broods with great excess of females in **thick** type.)

Only the ancestry in the female line is shown; the ancestry of the male parents can be found in the Tables of Matings in this and the preceding paper. The years are those in which the matings were made. *G*—gross, *L* lact. For the sake of clearness, the number of offspring in the normal families of the 1912 matings are not given, they will be found in the Table of Matings.

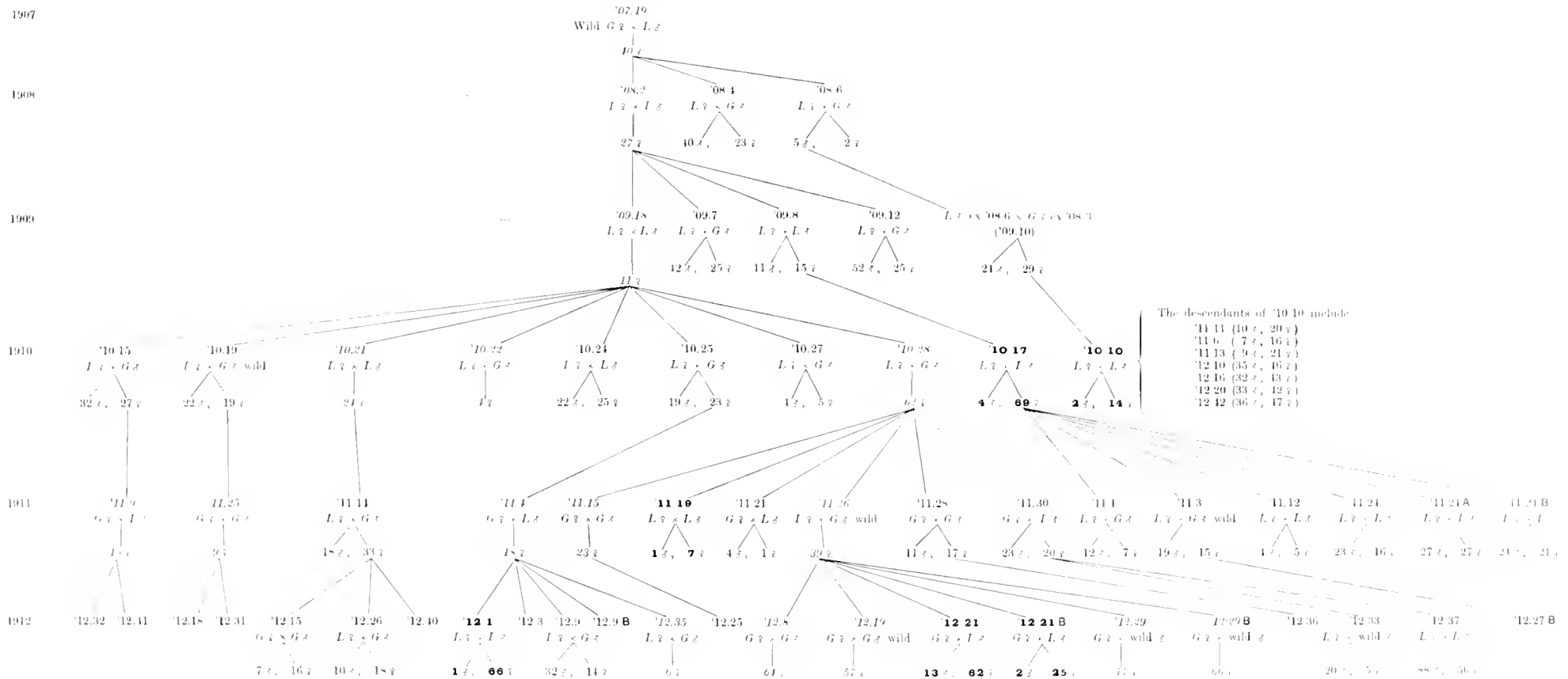


TABLE II. *Matings*, 1912–1913.

This table is a continuation of that given for previous years, *Journ. of Genetics*, Vol. III. 1913, pp. 4, 5. Reference numbers of purely female families are given in italics; those of families with at least twice as many females as males in thick type. Since a number of larvae were dissected for cytological examination, the figures in the 'Total Males' and 'Total Females' columns are often larger than the sum of 'gross. males' + 'lact. males,' etc.

Reference Number	Female Parent	Male Parent	Number of Eggs	Imagos: Gross. Males	Imagos: Lact. Males	Imagos: Gross. Females	Imagos: Lact. Females	Total Males	Total Females
'12.8	gross. ex *'11.26*	gross. ex **'11.11**	65	—	—	7	9	—	36
'12.8 (2)	,, ,,	,, ,,	42	—	—	4	7	—	28
'12.19	,, ,,	wild	98	—	—	38	—	—	57
'12.29	,, ,,	,,	111	—	—	34	—	—	77
'12.29 B	,, ,,	,,	about 83	—	—	52	—	—	66
'12.35	lact. ex *'11.1*	gross. ex '11.30	not counted	—	—	4	2	—	6
'12.1	,, ,,	lact. ex **'11.6**	97	—	—	—	41	1	66
'12·15	gross. ex '11.11	gross. ex '11.30	58	6	—	8	7	7	16
'12.21	gross. ex *'11.26*	lact. ex '11.24 A	73	2	—	—	25	4	37
'12.21 (2)	,, ,,	,, ,,	about 94	7	—	—	37	9	45
'12.21 B	,, ,,	,, ,,	53	—	—	—	21	2	25
'12.3	lact. ex *'11.1*	lact. ex **'11.11**	about 100	—	16	—	28	33	28
'12.5	lact. ex **'11.6**	gross. ex '11.28	,, 80	17	—	18	—	26	26
'12.6	,, ,,	lact. ex '11.12	,, 100	—	35	—	24	40	29
'12.7	,, ,,	gross. ex '11.14	76	21	23	28	13	47	46
'12.9	lact. ex *'11.1*	gross. ex '11.3	38	7	11	5	3	21	9
'12.9 (2)	,, ,,	,, ,,	41	4	6	3	2	11	5
'12.9 B	,, ,,	,, ,,	about 30	7	4	3	5	12	8
'12.16	lact. ex **'11.6**	gross. ex '11.14	,, 50	15	3	12	18	19	30 [1]
'12.16 (2)	,, ,,	,, ,,	,, 36	6	4	4	8	13	13 [1]
'12.18	lact. ex *'11.25*	gross. ex '11.28	,, 85	17	—	13	—	30	14
'12.20	gross. ex **'11.11**	lact. ex '11.14	121	33	—	—	39	33	42
'12.10	,, ,,	,, ,,	about 90	22	—	—	22	26	40
'12.22	gross. ex **'11.13**	lact. ex 11.22 A	39	12	—	—	15	17	17
'12.22 (2)	,, ,,	,, ,,	65	3	—	—	7	13	13
'12.42	,, ,,	lact. ex '11.24	109	33	—	—	41	36	47
'12.25	gross. ex *'11.15*	lact. ex '11.14	69	21	—	2	14	31	23 [2]
'12.26	lact. ex '11.14	gross. ex **'11.13**	36	—	2	—	7	9	12 [3]
'12.26 (2)	,, ,,	,, ,,	33	—	1	1	4	1	6 [3]
'12.27 B	gross. ex '11.3	lact. ex '11.24 B	65	19	—	—	17	20	17
'12.31	lact. ex *'11.25*	wild	68	12	—	7	—	17	13
'12.32	lact. ex *'11.9*	,,	40	11	—	3	—	13	10
'12.32 (2)	,, ,,	,, ,,	45	6	—	3	—	6	6
'12.40	lact. ex '11.14	,, ,,	78	19	1	14	1	25	22 [4]
'12.41	lact. ex *'11.9*	lact. ex **'11.6**	26	—	6	—	3	11	4
'12.33	lact. ex '11.30	wild	about 45	20	—	5	—	20	5
'12.37	,, ,,	lact. ex '11.24 B	not counted	82	—	49	—	88	56
'12.28	wild	lact. ex '11.21 A	83	29	—	—	29	33	31
'12.14	,,	gross. ex '11.21	48	17	—	8	12	23	22 [5]
'12.30	,,	lact. ex '11.14	95	19	—	—	27	20	27 [5]
'12.39	,,	,, ,,	about 75	30	—	—	25	30	27 [5]

[1] The inverted ratio of gross. : lact. in males and females is noteworthy.

[2] Note two exceptional gross. females.

[3] Note great excess of lact. where equality is expected. Some larvae of '12.26 (2) were lost at hatching.

[4] The male and female lact. are unexplained exceptions.

[5] The object of these experiments was to determine whether a male immediately descended from a unisexual family might produce unisexual offspring by a wild female.

females in the normal ratio. The tendency to produce only female offspring has usually descended in the direct female line, but has occasionally missed a generation, so that there were one or two cases of females of bisexual families having only female offspring, and others where such females had families in which males were produced, but only in extremely small numbers.

The complete pedigree of the all-female strain is given in Table I. The details of the 1912 matings are given in Table II; those of previous years have been given in the earlier paper. Table II includes the results of all the matings made, with the exception of some which were nearly or quite sterile, or which have no close bearing on the questions discussed in the present paper. As in the previous paper, the reference numbers of families consisting entirely of females are printed in italics, in both the table and the text; families in which there were at least twice as many females as males are distinguished by thick type.

The moths reared in the year 1912–13 (from pairings made in 1912) confirmed the previous results, but added some new facts.

The chief new point of importance is the fact that broods which are not entirely of one sex, but in which the females greatly outnumber the males, may arise among the offspring of females which belonged to unisexual broods (e.g. **'12.1**, **'12.21**, **'12.21 B**). It appears, in fact, that there is no sharp distinction between unisexual broods and broods in which the sexes are in nearly equal numbers, but that various grades of excess of females occur, leading to the extreme condition in which all the offspring are female. These results therefore suggest that the existence of unisexual broods is not due to the absence of a male-determining factor from the mother, but that there is a tendency, of varying amount, for this factor to be eliminated from the egg. The facts of sex-limited inheritance have already led to the conclusion that the female in *Abraxas* is heterozygous for a sex-factor, and in normal families it must be supposed that this factor is present in half the eggs and absent from the other half. In the strain which produces female families it may be supposed that the factor, whatever it may be, which determines maleness, has a tendency to be eliminated, in some cases from all the eggs, in others from much more than half, instead of its retention or elimination being a matter of chance. It will be seen below that this supposition is to some extent supported by cytological observations on the maturation of the eggs.

There are one or two further points in the 1912–13 families which require notice before passing on to the cytological observations. In

two families ('12.25, '12.40) there were exceptions to the ordinary sex-limited transmission. These will be referred to more fully below. It will be noticed that in several families the same reference number is used, followed either by '(2)' or by 'B.' Such families as *'12.8* and *'12.8(2)* were reared from eggs of the same parents, *'12.8* being from the first eggs laid, *'12.8(2)* from eggs laid later. The object of this was to determine whether the earlier and later eggs gave similar results as regards sex-ratio. The results show that there is never any important difference between the ratios of the sexes from the two lots. When a family is distinguished with 'B' (e.g. **'12.21**, **'12.21B**), it was reared from parents which were brother and sister of those of the family with the same number without 'B.' Thus the male parent of **'12.21B** was brother of the male parent of **'12.21**, the female parent sister of that of **'12.21**. Such pairs of families are in each case closely similar in the 1912–13 families, as they were in previous years, but the fact that two sisters mated to males of closely similar ancestry sometimes give very different progeny (e.g. **'12.1** and '12.3) makes it doubtful whether the resemblance between the offspring of two sisters mated to two brothers will be found in every case.

There were no cases in 1912–13 of females belonging to bisexual broods having only female offspring, such as occurred in three families in the preceding year. Matings of females belonging to broods in which males were very scarce usually gave a smaller preponderance of females, when the sex-ratio differed from equality, than occurred in the mother's family (e.g. '12.16, '12.20); there were no cases of a great increase in the ratio of females to males. It seems, therefore, that when the tendency to produce female offspring is not strong enough to cause all the progeny to be female, it suffers a progressive decrease in intensity in subsequent generations.

Complete or nearly complete sterility occurred in six matings with females of unisexual families, out of a total of 21 such matings, and in four out of 23 other cases. The infertility of females of unisexual families is thus not much greater than that of related moths belonging to bisexual broods.

It may be mentioned incidentally that among the larvae of family '12.27 B, seven were almost totally black. The moths when hatched showed no peculiarity. Two of them paired together gave a brood of 23, which were all nearly or quite black. On the other hand, a male and a female derived from black larvae, paired with moths derived from normal larvae, both gave broods consisting only of normals. The black

character is therefore probably a recessive mutation, but shows no sign of being sex-limited in transmission. The Rev. G. Waddington, S.J., writes to me that he also finds evidence that the black form is recessive to the type. In a brood of black larvae, the completeness of the blackening varies somewhat in different individuals, but even those with least black are conspicuously different from the normal.

b. The Oogonial Chromosomes.

In my former paper on the strain which produces unisexual families, I gave evidence that females belonging to unisexual broods have 55 oogonial chromosomes instead of the 56 which I had previously shown to be the usual number for the species. The evidence consisted chiefly in the observations that of brood '*12.8*, 33 larvae were dissected, all of which were females; nine of these larvae provided altogether 18 oogonial mitotic figures, in which the number 55 was almost or quite certainly correct, and in other larvae a number of figures were found in which 55 was the most probable number. In female larvae of broods '**12.1** and '12.32 also, five figures were found each with 55, and several others in which 55 was most probable but not absolutely certain. Since of these broods '*12.8* was the only one which was known at all certainly to consist only of females (many larvae being still too small to dissect) it was concluded that all females belonging to unisexual broods had 55 instead of 56 chromosomes, but that it was doubtful whether females belonging to bisexual families descended directly from unisexual had always 55, or sometimes 55 and sometimes 56.

The work during the spring of 1913 has definitely shown that not only females of unisexual broods, but also females of bisexual broods which are immediately descended from unisexual broods, always have 55, with one probable exception to be referred to later. In the following account the observations previously recorded on brood '*12.8* will not be described again, but the cases of broods '**12.1**, '12.25, and '12.32, mentioned in the former paper, will be included together with the observations made in 1913.

In all, in addition to the 33 female larvae of '*12.8* recorded in the former paper, the ovaries of about 160 female larvae were dissected out and sectioned. Of these 39 larvae belonging to the unisexual strain, and eight not belonging to this strain, yielded ovaries with figures considered good enough to count. Of the larvae belonging to the unisexual strain, eleven belonged to three broods ('*12.19*, '*12.29*, '*12.29 B*) which consisted entirely of females; fifteen belonged to

broods '**12.1** and '**12.21**, in which females were in very great excess, and the remaining thirteen to broods '12.18, '12.25, '12.31 and '12.32, in which males and females were in approximately equal numbers. In every one of these 39 larvae, with one exception which will be discussed below (brood '12.25), all the really good figures showed 55 chromosomes (Figs. 3—8). The following is an analysis of the counts; all were made by drawing the chromosomes and then counting the chromosomes drawn, and in many cases the figures were drawn again later and the counts compared. I have classified the figures by notes made at the time into five grades of trustworthiness—'excellent,' 'good,' 'fair,' 'probable' and 'doubtful'—the 'doubtful' group including those in which it was not possible to decide with any confidence between 55 and 56. Classes 'excellent' and 'good' include cases where no reasonable doubt is possible.

The counts in the unisexual broods were as follows. Individual larvae are represented with letters (A, B, BB etc.); the number of ovarian mitoses of each class is given after the letter in words.

Excellent	*'12.29*	♀ *JJ*, one.
Total 8	**'12.21**	♀ *B*, one.
	'12.18	♀ *D*, one; ♀ *F*, two; ♀ *G*, one.
	'12.31	♀ *F*, two.
Good	*'12.29*	♀ *CC*, one.
Total 10	**'12.1**	♀ *R*, two; ♀ *S*, one; ♀ *X*, one.
	'12.21	♀ *C*, one; ♀ *I*, two.
	'12.31	♀ *A*, one.
	'12.32	♀ *A*, one.
Fair	*'12.29*	♀ *R*, two, ♀ *U*, one; ♀ *BB*, two; ♀ *CC*, one; ♀ *II*, one.
Total 29	*'12.29 B*	♀ *F*, one.
	'12.21	♀ *B*, two; ♀ *F*, three; ♀ *G*, three; ♀ *K*, one; ♀ *N*, one.
	'12.1	♀ *A*, one; ♀ *D*, two; ♀ *W*, one.
	'12.18	♀ *G*, one.
	'12.31	♀ *A*, one; ♀ *E*, two; ♀ *F*, two.
	'12.32	♀ *B*, one.
Probable	*'12.29*	♀ *O*, one.
Total 14	*'12.19*	♀ *B*, one.
	'12.21	♀ *J*, three; ♀ *K*, one.
	'12.31	♀ *D*, one; ♀ *E*, two; ♀ *F*, one.
	'12.32	♀ *A*, one; ♀ *C*, one; ♀ *N*, two.
Doubtful	*'12.29*	♀ *L*, one; ♀ *GG*, one.
Total 7	**'12.21**	♀ *G*, one; ♀ *N*, one.
	'12.31	♀ *F*, two.
	'12.18	♀ *D*, one.

In addition to the above there is one figure, in an ovary of a larva (♀ *I*) of brood '**12.1**, in which 56 appears the most probable number,

but the chromosomes have begun to divide, so that several appear double, and it is not impossible that two which appear to be distinct are really separating halves of one. Brood '12.25 is not included in the list; the exception which occurs in it is discussed below.

It appears from this list there are eighteen really good figures in which the number 55 is perfectly clear, twenty-nine others in which it is scarcely less certain, fourteen in which it is the most probable, and only seven or eight in which it is doubtful; and it should be noted that of these doubtful figures, five are in ovaries of larvae in which the better figures were counted as 55. I think, therefore, that on this evidence alone it may be taken as certain that females of the unisexual strain, whether they themselves belong to unisexual or bisexual broods, have 55 chromosomes. Confirmatory observations from the maturation-figures of the eggs will be described below.

Comparatively few larvae with no descent in the direct female line from unisexual families were examined, since the number 56 had been determined with confidence as characteristic of the species in previous work[1]. Of those from which ovaries were sectioned, two wild females, three females from brood '12.10, and one from brood '12.22 provided figures which could be counted; all showed 56 (Fig. 1), and were distributed in the various gradês as follows:

Excellent	Wild	♀ *B*, one.
Total 3	,,	♀ *C*, one.
	,,	♀ *D*, one.
Good	'12.10	♀ *A*, one.
Total 4	'12.22	♀ *C*, three.
Fair	'12.10	♀ *K*, one.
Total 2	'12.22	♀ *C*, one.
Probable	'12.10	♀ *O*, two.
Total 2		

These seven larvae, therefore, provided seven good or excellent figures with 56, and four others in which 56 was almost certainly the true number.

One very important point appeared in addition. One wild larva (♀ *A*) not included in the list just given, provided four ovarian mitotic figures, three of them of the 'excellent' and one of the 'probable' class, all with 55 chromosomes (Fig. 2). Two of them had, in addition, one or two minute stained specks on the edge of the chromosome group, in such a position that they might be regarded as chromosomes if they

[1] *Journal of Genetics*, Vol. II. 1912, p. 189.

were not so much smaller than the normal chromosomes of the species. Such specks are not infrequent on the edge of the spindle, and probably have no connexion with chromosomes. In this female, therefore, it must be concluded that only 55 normal chromosomes were present, unless possibly a very much reduced 56th is represented by one of the specks mentioned.

All these facts taken together lead to the conclusion that in *Abraxas grossulariata* the commonest chromosome number in the female is 56, but that in some strains one of these may be very much reduced or absent. There is no necessary connexion between the number 55 and the tendency to produce only female offspring, since all individuals of the female-producing strain have 55, whether they belong to unisexual or to bisexual broods. The nature of the mechanism which results in unisexual families will be dealt with in connexion with the account of the maturation divisions (below, Section *d*).

c. Spermatogenesis of the strain which has 55 oogonial chromosomes in the female.

In my first paper on the spermatogenesis of *Abraxas grossulariata*, I described 28 chromosomes in both first and second spermatocyte divisions; I found no spermatogonial figures which could be counted quite accurately. The testes then examined belonged to individuals of the normal form with 56 chromosomes in both sexes, but it was clearly a point of importance to find out whether the same number occurs in males of the strain in which the female has 55.

I have examined a number of testes of broods '12.3, '12.18, '12.25, '12.26, '12.31, all of which families are either known to have females with 55, or are descended from the same stock. In all of the numerous first spermatocyte figures counted there are clearly 28 chromosomes[1], but this would probably be the case whether the spermatogonial number were 56 or 55. The number in the secondary spermatocyte divisions is somewhat less certain, but there is practically no doubt that it is also 28 in all cases. In testes of the broods mentioned, belonging to the 55-chromosome strain, I have counted 41 good secondary spermatocyte equatorial plates; twenty-nine of these have quite clearly 28, and twelve have 26 distinct chromosomes and a pair of chromosomes in contact. In males of two broods ('08.10, '12.7) not belonging to the 55-chromosome strain, the condition with two chromosomes in contact

[1] The only exception I have seen is in one figure in a testis of a male of '12.7, which does not belong to the 55-chromosome strain. This single figure has 27, but many other figures in the same testis have 28.

seems to be somewhat more frequent; I have counted ten figures with 28 separate chromosomes, and eight in which two are associated. The degree of association varies somewhat, and in a few cases, especially in one male of '12.7, the two are so close together that they might easily be mistaken for one. This is not the case in any of the figures from the 55-chromosome strain, and since such a double chromosome is not found in figures in which 28 separate chromosomes are seen, and since the two members of the pair are not always equal in size, I think it is certain that 28 is the number constantly found in all secondary spermatocytes. In *Biston hirtaria* I have found that in the spermatocytes two chromosomes are constantly associated, and the occasional figures which might be counted as 27 in *Abraxas* are probably due to the less regular occurrence of the same sort of thing.

The spermatogonial divisions are not usually clear enough to give quite reliable counts. I have made very many attempts, and in almost all the best figures I have found 56, whether of the strain with 55 in the female or in males not belonging to this strain. I have altogether twenty spermatogonial figures recorded as 'good' or 'very good' in males belonging to the strain in which the female has 55; of these sixteen show 56, three might be either 56 or 55, and one shows only 55. All the six best figures have clearly 56 (Fig. 9). I have therefore no hesitation in concluding that in all males 56 is the spermatogonial number, and that all spermatozoa contain 28.

d. The Maturation Divisions of the Eggs.

The polar divisions take place in a little patch of protoplasm at the anterior end of the egg, and in searching for them in sections the anterior end can usually be recognised by the somewhat finer granulation of the yolk. During the maturation, the superficial protoplasmic layer of the egg is drawn out, just at the point where the outer polar nucleus will lie, into a fringe which in sections has exactly the appearance of a tuft of cilia (Fig. 14); this usually makes the division-figures easy to find in a longitudinal section, but is of no help in sections transverse to the axis of the spindles. I have not discovered the meaning of the fringe; it is possibly due simply to the superficial protoplasm sticking to the shell at the point where the polar divisions occur. During the maturation divisions, the heads of the spermatozoa, with their radiations, are usually conspicuous objects in the yolk near the anterior pole. One can almost always be found, and not rarely one or two others are present in addition. I cannot give the exact times

at which the various stages of the polar divisions take place, for the usual method in fixing the eggs was to determine as accurately as possible when the moth began to lay, and about two and a half hours later to fix all the eggs laid. In eggs fixed less than an hour after laying, usually the first polar spindle is found; in eggs fixed two and a half hours after laying began, the latest stage found is commonly the anaphase of the second division. The equatorial plate stage of the second division appears to last for some time, since it is the stage most frequently found. This is fortunate, for it is the only stage at which the chromosomes in the inner and outer spindles can be counted with certainty. Even then, on the average not more than one egg in ten has the chromosome groups cut in a plane in which they can be counted, so that of nearly 600 eggs cut and examined, only about 60 gave at all reliable counts. The proportion is considerably higher in batches of eggs which could be shelled easily without damaging them, so that accurate orientation is possible; in such batches, as many as one in three or four may give good figures.

During the first polar division, a mass of granules which stain deeply with iron haematoxylin is left in the equatorial plate as the chromosomes travel to the poles (Fig. 14). This remains in the second division as a flat plate of fine granules between the outer and inner mitotic figures. When, as happens sometimes, one chromosome lags behind the others, it can easily be determined to which spindle it belongs, even when it is in the next section to the remainder of the chromosomes, by its position on the outer or inner side of the granule layer. In most cases, however, all the chromosomes of a group (outer or inner equatorial plate) are found in one section, and except where the contrary is stated, all the figures have been drawn from such cases.

One abnormality which was observed must be mentioned, since it may have considerable theoretical importance. In one egg of brood '13.4, and in two of brood '13.42, there are two separate polar division-figures, near together at the anterior end of the egg (Figs. 15, 16 *a*, *b*). In the egg of '13.4 and in one of those of '13.42 each figure is in the equatorial plate stage of the second division, with an inner and outer spindle; in the second egg of '13.42, each figure shows three vesicular polar nuclei, and two female pronuclei each conjugating with a sperm-nucleus (Fig. 16 *a*, *b*). There must therefore have been two nuclei in these eggs, which are undergoing maturation simultaneously. If in such an egg one nucleus underwent maturation in such a way as to give rise to a male-producing pronucleus, the other to a female-producing, a

bilateral gynandromorph, such as is not uncommon in Lepidoptera, might be produced. Several suggestions have been made by various writers as to the origin of such gynandromorphs, but a binucleate egg of this kind seems as likely a cause of their production as any which has been suggested. In this connexion, reference may be made to the fact recorded in my previous paper on the chromosomes in *Abraxas* (*Journ. of Genet.* III. 1913, p. 9), that in several testes of family '12.25, binucleate spermatocytes were not infrequent. Incidentally, Fig. 16 *b* illustrates a point of considerable cytological interest. Around the conjugating pronuclei there are conspicuous radiations in the yolk, and it is clearly seen that these have a somewhat sinuous course in the protoplasm between the alveoli which contain the yolk-granules. The protoplasm near the pronuclei, and also around the polar nuclei, strongly suggests that their radiations are caused by outgrowths of protoplasmic alveoli, as suggested by Jenkinson[1]. The radiations are not only sinuous, but in several cases are clearly branched. Further, since the two pairs of pronuclei are near together, their radiations are intermingled, and in some cases those belonging to one system quite clearly cross those of the other. Such crossing can be seen in one section, but I have found no case of two radiations crossing when both are in focus; since, however, the section is 10 μ thick, and the crossing radiations are well within the thickness, they cannot be much more than 5 μ apart. As far as can be seen, the two sets of radiations spread from centres quite independently of each other, like the roots of two plants growing near together.

I have examined eggs of only one wild female, which presumably has 56 as the oogonial number, and in the fifty or more eggs which were fixed, there are eight good or fair preparations showing both inner and outer equatorial plates. In all of these there are certainly not less than 28 chromosomes in each plate (Fig. 10 *a*, *b*). In most 28 are clearly shown; in three figures, one plate might be counted as 29. In the case figured, the outer plate has 27 chromosomes which are clearly separate, and, in addition, a pair which might be one dividing, or two at somewhat different levels. I regard 28 in both plates as the most probable number, but the double chromosome may perhaps correspond with the chromosome described by Seiler[2] which becomes double in one of the second polar plates, but remains a single chromosome in the other. I find no constancy of behaviour in the eight

[1] J. W. Jenkinson, *Quart. Journ. Micr. Science*, Vol. XLVIII. 1905, p. 107.

[2] J. Seiler, *Zool. Anzeiger*, Vol. XLI. 1913, p. 246.

eggs which give clear figures, and am inclined to regard the double chromosome merely as one which has begun its normal division somewhat in advance of the rest.

I have good figures in the eggs of ten females of the strain with 55 oogonial chromosomes; in altogether about 37 eggs both inner and outer plates can be counted, and in some 20 more one or other (usually the outer) can be counted.

In all the good figures in which the chromosome number in both inner and outer plates can be determined with certainty, there are 28 in one and 27 in the other (Figs. 11—13). In some families there are about equal numbers of each arrangement, e.g. in brood '13.48 there are two cases with 28, three with 27, in the outer plate. This family is known to include males. In other families there seems to be considerable excess of eggs which have 27 in the inner plate and 28 in the outer; e.g. in brood '13.4 B, excluding five cases in which there is some doubt, there are four clear figures with 28 in the outer and 27 in the inner, nine in which either the outer can be counted as 28 or the inner as 27, although the second plate is not countable, and only two in which the outer spindle has 27, the inner 28. In brood '13.34 there are three with 28 in the outer, 27 in the inner, one with 27 in the inner and the outer is not countable, and one, which is too oblique for quite safe determination, which appears to have 28 in the inner group. In these two families, four females have been reared from '13.4 B, and the surviving six larvae proved to be female by dissection, and in '13.34 ten larvae were dissected, all females. There are thus indications that both broods had a large excess of females, and taken together they show 17 cases with the unpaired chromosome in the outer plate against two or three with it in the inner. Since females of this strain have 55, males 56 chromosomes, it is evident that eggs with 27 in the inner spindle, and therefore in the mature egg-nucleus, must be female-determining. It is thus to be expected that broods which have a great excess of females should show a corresponding excess of eggs with 27 chromosomes in the inner spindle. I have not yet been able to find a batch of eggs in which all have 27 in the inner plate, and family '13.4 appears to contradict the results obtained from '13.4 B and '13.34. Seven females have been hatched and ten dissected in this family, and yet the eggs show a majority of cases with 28 chromosomes in the inner plate (twelve with 28 in the inner plate or 27 in the outer, eight cases of the converse arrangement). In all three families '13.4, '13.4 B and '13.34, there was considerable

mortality among the very young larvae, and it is possible that the excess of females is due not to their being families in which few male zygotes were formed, but that there has been differential mortality in favour of the females. For the present, therefore, the question must be left open, whether all-female families are produced by the unpaired chromosome going into the first polar nucleus in all the eggs, or whether a differential mortality has some share in their production. Since in some cases, e.g. brood '12.29, more than two-thirds of the eggs laid were reared into females (in this case 111 eggs, 77 females, no males) it seems very improbable that the production of all-female families is due to early death of the males, and the cytological results from '13.4 B and '13.34 described above suggest that there are families in which at least in a high proportion of the eggs the odd chromosome goes into the polar nucleus, but the proof that all-female families are due to the extrusion of this chromosome in every egg cannot be regarded as complete until a batch of eggs has been found in which all the inner plates have 27 and the outer plates 28.

That in some families the unpaired chromosome may be extruded in all or nearly all the eggs is made more probable by the observations of Morgan on *Phylloxera*[1]. In this genus, and apparently in other Aphids, the male-producing eggs differ from the female-producing in the fact that in the former one chromosome is always extruded with the polar body, so that males have one chromosome less than females. It seems clear, therefore, that the poles of the maturation spindle are not necessarily alike, since in *Phylloxera* and other Aphids a particular chromosome always goes to one pole. It is not very improbable, therefore, that in *Abraxas* the difference between bisexual and all-female families consists in the amount of unlikeness between the poles of the first maturation spindle. Bisexual families would arise when it is equally likely that the unpaired chromosome should go to either pole; all-female families when it always goes to the outer pole, and families with great excess of females when it goes much more frequently to the outer than to the inner.

Another line of evidence with regard to the part played by the extra chromosome in sex-determination might be provided if gynandromorphs should appear in families which are known to produce binucleate eggs. In the only egg of this kind in which it is possible to count the chromosomes (one of those from '13.42), although the figures are not very good, each appears to have 27 in the outer spindle

[1] T. H. Morgan, *Journ. Exp. Zool.* Vol. XII. 1912, p. 479.

and 28 in the inner, which would indicate that both egg nuclei would be male-determining.

To obtain the complete answer to the questions raised in the preceding paragraphs will require the examination of many more eggs, and it is not unlikely that fresh material will be needed. Meanwhile, one point of great importance appears from the observations made up to the present time—they show that in *Abraxas*, and therefore probably in other Lepidoptera, the presence of two heterochromosomes is characteristic of the male, and one, of the female. This follows of necessity from the fact that some eggs have 27, others 28, and that females of the strain used have 55 and males 56. In strains with 56 in the oogonia and 28 in all the eggs, it must be concluded that the additional chromosome is functionless as regards sex, as in the case of other insects where there is a large and a small heterochromosome in the male. The Lepidoptera thus show a condition which is the exact converse of that found in all cases hitherto described. The connexion of this fact with sex-limited inheritance will be referred to again in the next section.

e. The Exceptional Family '12.25.

In my previous communication on the chromosomes of the unisexual families (*Journ. Genet.* III. p. 9), I mentioned that one female of family '12.25 showed an oogonial figure with 56 chromosomes, while others of the same brood had clearly 55. At that time I had no other clearly countable figures in females which were daughters of unisexual broods, but which themselves belonged to bisexual broods, and I therefore concluded that some females of such broods had 55, others 56. Subsequent work, however, as described above, has shown that with this one exception, all females of the strain with 55 chromosomes, whether they belong to unisexual or to bisexual families, have 55. The single female of brood '12.25 is thus alone in apparently having 56 (cf. Figs. 17, 18). Very careful examination, with both 2 mm. and 3 mm. Zeiss apochromatic lenses, shows that in the single good figure two of the chromosomes counted separately are in contact, and might conceivably be dividing halves of one (Fig. 18). This, however, is made improbable by the fact that one of the halves is itself somewhat divided. Many of the chromosomes show some extent of doubleness, in preparation for division, but none of them are so conspicuously double as the chromosome in question, if it is really one in division, and the distinct doubleness of one of the halves is also inexplicable if the pair are really halves of one chromosome. The evidence therefore seems to me to be

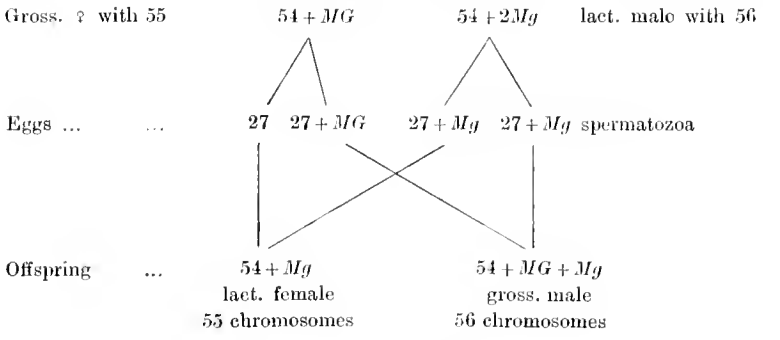
strong that in this figure there are in fact 56 chromosomes in place of the normal 55. I should not lay any great emphasis on a single exceptional case in which only one equatorial plate is available, if it had not appeared, after the record of the exceptional count was published, that family '12.25 was peculiar in another respect. The chromosomes were counted in the winter of 1912–13; when the moths hatched in the following May and June, two females of this family, out of a total of 16 reared, were *grossulariata* instead of *lacticolor*. The parents were *gross.* ♀ × *lact.* ♂; the normal result of such a mating, owing to the sex-limited transmission of the *gross.* character by the female, is that all male offspring should be *gross.*, all females *lact.* The males were all *gross.* (21 were reared), but the presence of two *gross.* females shows that in this case there was an exception to the normal sex-limited transmission.

I have very rarely had such exceptions in previous years, but since the moth is very common, and the possibility of the accidental introduction of a wild larva with the food-plant difficult to guard against with absolute certainty, I have always hitherto regarded such exceptions as at least possibly due to accident rather than to failure of the normal sex-limited transmission. In the present case, however, such an explanation is impossible. The two exceptional females are very closely alike (Figs. B, C), and show peculiarities in their markings which make it certain, not only that they are sisters, but also that they are daughters of the mother of brood '12.25 (Fig. A). Their peculiar features are quite exceptional in wild *grossulariata* (cf. Fig. E), but exist in the mother and other *grossulariata* ancestors of the family. Some of these features, it is true, are found in collaterally related families (Fig D), and it might be argued that two larvae had been accidentally transferred from another brood. Apart from the great improbability of such an accident in carefully controlled experiments, I have no other specimen which combines the peculiarities in the way seen in the females in question, and it seems certain that they are genuine exceptions. Similar exceptions, of course, are well known in other species, e.g. Canaries (Miss Durham) and Pigeons (Staples Browne).

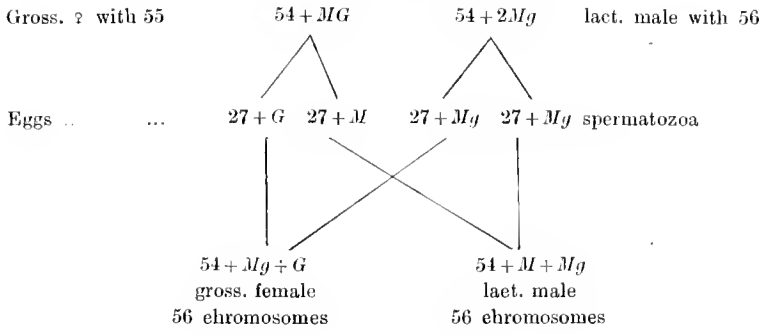
Now it is a very remarkable coincidence, if it be coincidence, that the only exception to the number 55 in the oogonial chromosomes should occur in the same brood as these two very rare exceptions to the normal sex-limited transmission. If, however, it is not coincidence, the facts fall quite simply into line. In the strain with 55 chromosomes,

it has been shown that eggs with 27, fertilized by sperm with 28, give rise to females with 55, while eggs with 28 give rise to males having 56. Since the *grossulariata* factor is normally transmitted only by the male-producing eggs, it must be supposed to be associated with the extra chromosome in the male-determining egg. If, however, the male factor *M* and the *gross.* factor *G* are not borne in one indivisible chromosome, but in a compound chromosome (such as has been described by Seiler in eggs of the moth *Phragmatobia*), and if in these exceptional cases the compound chromosome separated into its components, so that the *G* portion remained in the egg and the *M* portion went into the polar nucleus, an egg would then be produced which was female-determining, bore *G*, and had 28 chromosomes. Such an egg when fertilized by a *lacticolor* male would give rise to a *grossulariata* female with 56 chromosomes, instead of the normal result, namely, a *lacticolor* female with 55. Diagrams will make this clearer.

A. *Normal Case.*



B. *Exceptional Case.*



On this hypothesis, there should of course be as many exceptional *lacticolor* males as *grossulariata* females, unless, as is possible, when *M* and *G* separate, *M* always goes into the polar nucleus. In any case, since only 21 males were reared, the absence of *lacticolor* males from the family is not surprising[1].

Since only one oogonial figure with 56 was found, and this is not entirely unequivocal, it is obviously impossible to found any important theoretical conclusions on the case, but the fact that the abnormal chromosome number was recorded before the existence of exceptional transmission of the *gross.* character was suspected, makes some discussion of the matter justifiable.

It should be mentioned that two other exceptions occurred in the moths hatched in 1913, for which I can offer no explanation. In family '12.40, of which the parents were *lacticolor* ♀ and wild *grossulariata* ♂, among 19 *gross.* males and 14 *gross.* females, there were one male and one female *lacticolor*[2].

Summary.

1. The families reared in the year 1912–13 in general confirm the conclusions given in the former paper on the inheritance of a tendency to produce all-female families in *Abraxas grossulariata*. About half the females belonging to such families usually have only female offspring, but the 1912 matings show that they may sometimes have a few males among a very large excess of females. The tendency to produce only females is thus variable in intensity, and ranges through varying degrees from equality of the sexes to complete absence of males. In families in which males are produced, evidence is given that there is no important difference in the sex-ratio between the offspring of the earlier and later eggs laid by the mother. When there is great excess of females, there appears to be a tendency for this excess to diminish

[1] Since this was written, C. B. Bridges has published a hypothesis to account for exceptions to sex-limited transmission in *Drosophila*. (*Journ. Exp. Zool.* Vol. xv. 1913, p. 587.) He suggests that exceptionally the sex-chromosomes may fail to separate at the maturation division, and both pass into one gamete, so that gametes with two sex-chromosomes, and others with none, would be provided. If a spermatozoon with no *MG* chromosome fertilized an egg with one, a *grossulariata* female would result. In this connexion it may be noted that one primary spermatocyte with only 27 chromosomes was found in a male of '12.7, the father of which was brother to the male parent of '12.25.

[2] A female *lacticolor* could arise from this mating from a spermatozoon lacking the sex-chromosome, according to Bridges' hypothesis mentioned in the footnote above, but it is impossible on this hypothesis to account for a male *lacticolor* in this case, unless 'non-disjunction' occurred simultaneously in the egg.

in subsequent generations. Sterility is not much more frequent in all-female families than in normal families.

2. In counts of mitotic figures in ovaries of more than fifty females descended from unisexual families, 55 chromosomes were found in every case (with one exception in family '12.25; see No. 5 below). About twenty of these figures were so good that doubt about the number is hardly possible, and in several the number 55 is absolutely certain. The number 55 was also counted in oogonia of one wild female, but in three other wild females and in four pedigree individuals not descended from the all-female producing stock there were clearly 56.

3. Males, whether of the stock in which the female has 55 or 56, always have 56 spermatogonial and 28 secondary spermatocyte chromosomes. In some cases, more commonly in the stock in which the female has 56 oogonial chromosomes than in that in which it has 55, two chromosomes in the secondary spermatocytes are in contact, but it is almost certain that there are never really 27. A single figure with 27 in the first spermatocyte was found, but many others in the same testis had 28.

4. Both equatorial plates of the second polar mitosis in eggs of females which have 56 oogonial chromosomes, have 28. Eggs of females with 55 in the oogonia have 28 chromosomes in one polar equatorial plate, 27 in the other. In some families it is equally common to find 27 in the inner or the outer plate, and some of these are known to be bisexual families. In two, however, there is a considerable excess of eggs with 27 in the inner plate, and these are known to be families with excess of females. In one family, in which females are in excess, a majority of the figures counted show 28 in the inner plate. It is suggested that the excess of females in this family is due to mortality of the males. The facts as a whole make it clear that eggs which eliminate the 28th chromosome become females, those which retain it, males; *Abraxas* thus shows a condition which is the converse of that described in most other insects.

5. In the previous paper it was recorded that one female of family '12.25 had 56 chromosomes (only one figure can be counted). Other females of the same brood have clearly 55. In the same brood there was failure of sex-limited transmission of the *grossulariata* character in two cases, in such a way that the *grossulariata* mother transmitted this character to two of her daughters (out of a total of 16), instead of, as normally happens, only to her sons. It is suggested that this may

be correlated with the extra chromosome found in one female of this family, the *grossulariata* bearing chromosome having become separated abnormally from the sex-chromosome.

6. In the section on the maturation of the egg three cases are described of eggs which have two nuclei, both undergoing maturation simultaneously. In each case the two maturation figures are in exactly the same stage; in two they are in the equatorial plate stage of the second polar mitosis, in the third egg both figures show three polar nuclei and conjugation of the egg-nuclei with sperm-nuclei. It is suggested that such an egg, if it had developed, might have given rise to a bilateral gynandromorph, and that such gynandromorphs perhaps arise in this way. In the egg in which there are two pairs of conjugating pronuclei, the sperm-radiations are simmons and branch, and those of one system cross those of the other.

CAMBRIDGE,
January, 1914.

EXPLANATION OF PLATES.

All the microscopic figures, with the exception of No. 18, were drawn with Zeiss 3 mm. apochromat. objective, n. ap. 1·40, and with compens. ocular 12. The drawings are free-hand, and are not all on exactly the same scale.

PLATE I.

Fig. 1. Wild ♀. Oogonial equatorial plate, 56 chromosomes.
Fig. 2. ,, ,, ,, 55 ,,
Fig. 3. ♀ of '12.18. ,, ,, 55 ,,
Fig. 4. Another ♀ of '12.18. Equatorial plate of cell in synapsis zone of ovary, 55 chromosomes.
Fig. 5. ♀ of '12.31. Equatorial plate of cell in synapsis zone of ovary, 55 chromosomes.
Fig. 6. Same ♀ of '12.31. Oogonial equatorial plate, 55 chromosomes.
Fig. 7. ♀ of '12.29. Oogonial equatorial plate, 55 chromosomes, the double chromosome marked × is almost certainly one dividing.
Fig. 8. ♀ of '12.21 Oogonial equatorial plate. The small rings above the chromosome group indicate stained bodies which are certainly not chromosomes.
Fig. 9. ♂ of '12.18. Spermatogonial plate with 56 (cf. Figs. 3, 4, oogonial plates with 55 from females of the same brood).
Fig. 10. *a*, outer; *b*, inner equatorial plate in egg of a wild ♀. 28 chromosomes in each plate.
Fig. 11. Egg of '13.1. *a*, outer polar plate with 28; *b*, inner with 27 chromosomes.
Fig. 12. Egg of '13.34. *a*, outer polar plate, with 27 in one section and one lagging chromosome (separated from the rest by a line) in the next section; *b*, inner polar plate with 27.
Fig. 13. Egg of '13.48. *a*, outer polar plate with 27; *b*, inner with 28.

PLATE 2.

Fig. 14. Egg of '13.3. Second polar mitoses, side view, to show the appearance resembling cilia. Combined from two successive sections.

Fig. 15. Egg of '13.4. Showing two maturation figures in one egg. The two parts of the figure are drawn from sections five sections (50μ) apart.

Figs. 16*a*, 16*b*. Egg of '13.42, with two maturation figures in one egg.

Fig. 16*a*. Combined from two successive sections, showing three polar nuclei, the two inner in contact, in each maturation figure. The outer nuclei of both figures are in one section, the two inner pairs in the next.

Fig. 16*b*. Conjugation of the two female pronuclei with two sperm-nuclei. Combined from the two sections next following the sections from which 16*a* was drawn. Note that the sperm-radiations are sinuous, bifureate, and those of one system cross those of the other. The clear space around the conjugating nuclei is due to contraction at fixation.

Fig. 17. ♀ of '12.25. Oogonial equatorial plate with 55 chromosomes. (Only 54 are visible in the figure; one has been accidentally omitted.)

Fig. 18. Another ♀ of '12.25. Oogonial equatorial plate with 56 (exceptional). The only doubt about the number 56 arises from the fact that the two chromosomes marked × might possibly be one in division. Since the outer is itself double, this is very improbable. Drawn with Zeiss 2 mm. apochrom. n. ap. 1·40, compens. oc. 12.

PLATE 3.

Fig. A. Female parent of brood '12.25.

Figs. B, C. The two *grossulariata* females which appeared as exceptions to the normal sex-limited transmission of the *gross.* character, in brood '12.25. Note the close resemblance of the pattern in the two specimens, and their general resemblance to their mother. The chief peculiarities are

(*a*) the union of the costal and discal spots to form a definite hook-shaped or ?-shaped mark across the middle of the fore-wing;

(*b*) the reduction, but not complete absence, of the spots at the tip of the fore-wing on the outer side of the yellow band;

(*c*) the union of the basal line of spots on the hind wing into a continuous band, from which the discoidal spot projects outwards, but is continuous with the band;

(*d*) the almost complete union of the distal row of spots on the hind wing, and the absence of the most anterior marginal spot, which is usually present at the costal angle.

These points are indicated by letters in Fig. C.

Fig. D. A *grossulariata* female of brood '12.15, closely related to '12.25. This is the specimen most nearly resembling the *gross.* females of '12.25 among the moths bred in 1912.

Fig. E. A typical *grossulariata* female; showing the difference between the females of '12.25 and ordinary *grossulariata*.

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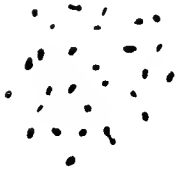

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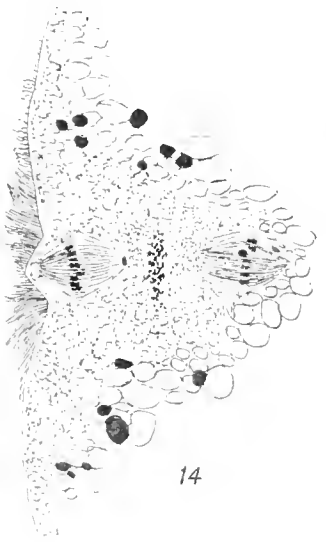

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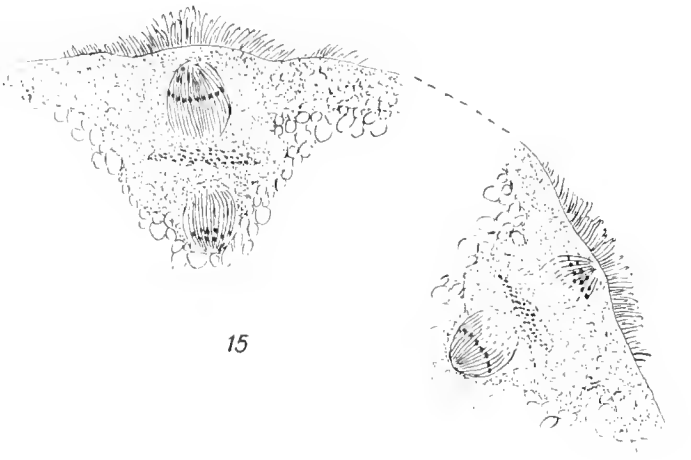

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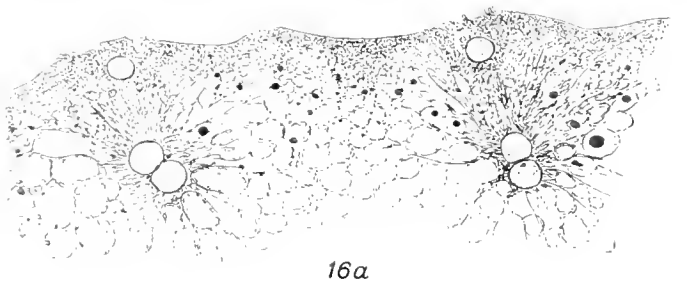

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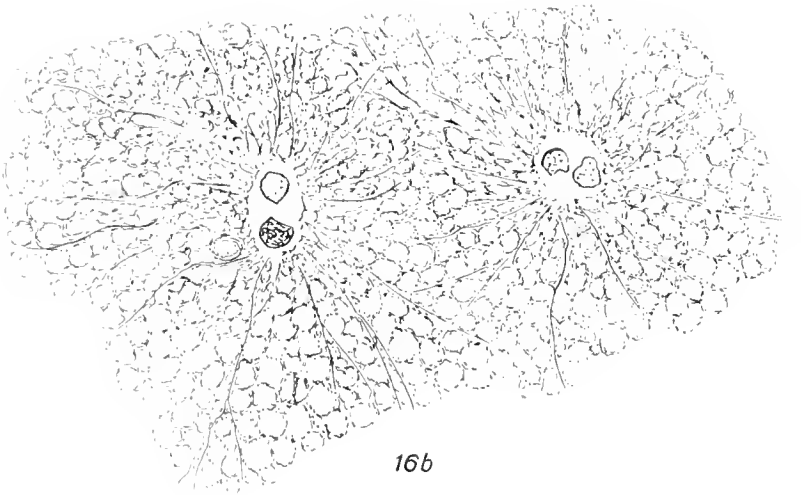

16b

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A

B

C

D

E

ON INHERITANCE OF WEIGHT IN POULTRY.

By R. C. PUNNETT, M.A., F.R.S.,
and P. G. BAILEY, M.A.

CONTENTS.

Introduction.

Few experiments have been undertaken with the object of investigating the inheritance of size or weight in animals. Beyond the observations of Goldschmidt[1] and of Phillips[2] on ducks, and of Castle[3] on rabbits practically nothing definite is known. Nor are all of these observations sufficiently extensive to advance our knowledge greatly. Goldschmidt only reared an F_2 generation from one cross and that consisted of but 8 individuals. Moreover as his weight records cease at the age of 10 weeks little is to be learned from them that bears upon our present enquiry. Castle's work on the weights of rabbits is too scanty to give much definite information. Phillips' experiments however are more extensive and suggestive. From the cross Mallard × Rouen he reared 13 F_1 and 33 F_2 birds. The F_1 birds were intermediate in size as compared with the parents. The mean of the F_2 birds was also near that of the F_1, but their range of variation was considerably greater. None however were as heavy as the Rouen, nor were any as light as the Mallard. The numbers however are too small to be able to state definitely that these classes do not appear in

[1] *Zeit. f. ind. Abst. Vererb.* 1913. [2] *Journ. Exp. Zool.* 1912; *ibid.* 1914.
[3] *Publ. Carneg. Inst.*, Wash., No. 114, 1909.

F_2. Nevertheless the great range of variation in F_2 suggests an interpretation by means of several segregating factors, though this cannot be decided definitely without raising an F_3 generation. Whether size in animals can be expressed in terms of Mendelian factors is therefore still an open question.

The present paper gives an account of some experiments which we have been carrying out during the past few years with the object of learning something about the transmission of weight in poultry. For this purpose two breeds were selected which differed considerably in size, but not sufficiently to prevent natural crossing. At the same time it was considered advisable for reasons of economy that the larger breed used should not be too large; otherwise it would not have been possible to rear so many offspring to maturity. The two breeds eventually selected were the Gold-pencilled Hamburgh and the Silver Sebright Bantam. The choice was determined with the idea of following up the inheritance of other characters in addition to size, and in this set of experiments we have been investigating also the inheritance of gold and silver ground colour, and the peculiar assumption of the hen's plumage by the cock which is characteristic of the Sebright. An account of these characters and their inheritance will be given later. For the present we shall confine ourselves to what we have been able to learn from this cross with regard to the transmission of size. More correctly perhaps we ought to say weight, since our estimation of size depends entirely on the weight of the bird. Generally speaking the bird that looks heavier will be found actually heavier when weighed, but it is not always possible to be certain from appearance alone. A taller but more slender and "leggy" bird will sometimes be found to weigh less than what appears to be a smaller though better furnished one. Weight is doubtless a character which depends in some degree on structural characters capable of analysis. We have not found it practicable to attempt such analysis for the present. Our criterion throughout has been the weighing pan alone. Observations start with the egg weight. The newly-hatched chicken is also weighed, and a record is then taken weekly or fortnightly up to the end of the 35th week. It would of course be desirable to have a complete record of every bird from the day of hatching to the day of natural death. In practice this is not possible owing to the difficulty of keeping so many birds. A bird, especially a cock, may go on growing more than a year after it is hatched. But we have found that such growth, when it occurs, is intermittent. Birds hatched from February to May receive a check in their growth at the end of the year,

if not before, and the weight reached by them at the age of 35 weeks is not exceeded for several months. Later on the bird may show a further period of growth, but after it has reached the age of 8—9 months, or in many cases even less, there is a distinct interval of several months during which the weight remains nearly constant. This is certainly true for the breeds with which we have been working, and we have consequently kept and recorded the weights of the birds bred up to 35 weeks, after which the majority have been killed. When we speak of the weight of a bird we mean this weight of the first year's growth unless the contrary is stated. We may add that all our birds were kept together on the same land and were fed and treated, so far as possible, precisely in the same way.

The Hamburgh-Sebright Cross.

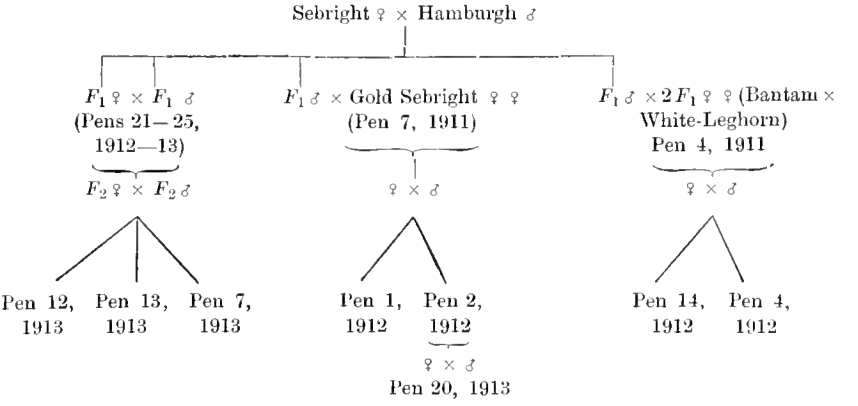
The original stock with which the experiments were started in 1910 consisted of a trio of Gold-pencilled Hamburghs and 2 Silver Sebright hens. The Hamburgh ♂ when 3 years old weighed 1540 grams; when 35 weeks old it is likely that he would have been some 150—200 grams lighter. The Sebright ♀♀ weighed 570 and 620 grams respectively. We have no record of the weight of a Silver Sebright cock of this strain but he would probably have weighed about 750 grams. During the course of our experiments we also used some Gold Sebrights. These were procured from a different source and were markedly smaller than the Silvers, a cock weighing but 570 grams and 3 hens 455, 455, and 480 grams respectively. We raised a few birds from our original Hamburgh ♂ with one of the Hamburgh hens, viz. 1 ♂ and 4 ♀♀. In both sexes these birds were rather larger than the F_1 ex Sebright × Hamburgh (cf. Table I, p. 26). We subsequently mated up a brother and sister from these pure Hamburghs. The offspring were distinctly smaller and throughout their growth were sickly looking birds. Whether due to close inbreeding or to some other cause the phenomenon was very marked, and we have not made use of observations on these birds in our account. With healthy birds the weight of the Hamburgh is nearly double that of the Sebright.

The original cross was made between the Silver Sebright hen and the Hamburgh cock (cf. Pl. IV, fig. 1). From it two cockerels were reared in 1910, and in the following year 8 ♂♂ and 7 ♀♀ were raised. With regard to size both sexes were fairly uniform. At 35 weeks old the ♂♂ ranged from 1140 to 1360 grams, while at the same age the ♀♀ were between 940 and 1110 grams (cf. Table I).

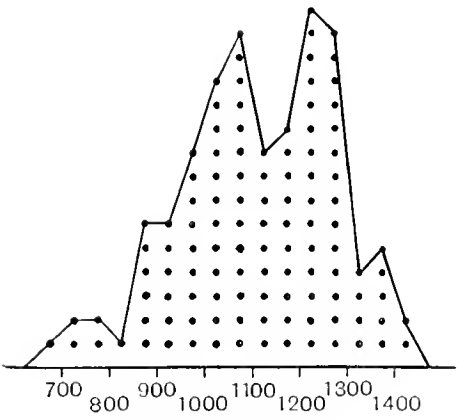
TABLE 1.

Parents	Reference	Weights of Male offspring in grams.														Weights of Female offspring in grams.									
		500—600	600—700	700—800	800—900	900—1000	1000—1100	1100—1200	1200—1300	1300—1400	1400—1500	1500—1600	1600—1700	1700—1800	1800—1900	400—500	500—600	600—700	700—800	800—900	900—1000	1000—1100	1100—1200	1200—1300	1300—1400
Pure Sebright	— —	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—
Pure Hamburgh	Pen 9, 1912	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	3	1	—	—
Silver Sebright ♀ × Hamburgh ♂ ...	Pen 9, 1911—12 ...	—	—	—	—	—	—	5	2	1	—	—	—	—	—	—	—	—	—	—	6	1	—	—	—
F_1 ♀ · F_1 ♂	Pens 21—25, 1912—13	—	1	1	7	15	26	19	29	9	2	—	—	—	—	—	3	17	25	27	36	8	4	1	—
F_2 ♀ × F_2 ♂	Pen 12, 1913	—	—	—	—	—	1	2	4	2	1	2	1	—	—	—	—	—	—	—	1	3	5	2	2
F_2 ♀ × F_2 ♂	Pen 13, 1913	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—
F_2 ♀ × F_2 ♂	Pen 7, 1913	—	—	3	3	—	—	—	—	—	—	—	—	—	—	—	2	2	3	—	—	—	—	—	—
Gold Sebright ♀ ♀ · F_1 ♂ ..	Pen 7, 1911	—	2	—	1	2	1	1	—	—	—	—	—	—	—	—	—	6	1	1	3	—	—	—	—
Bants. ex Pen 7, 1911	Pen 1, 1912	1	—	7	7	2	—	—	—	—	—	—	—	—	—	2	6	4	2	1	—	—	—	—	—
Bants. ex Pen 7, 1911 . .	Pen 2, 1912 . . .	—	2	1	1	—	—	—	—	—	—	—	—	—	—	5	3	—	—	—	—	—	—	—	—
Bants. ex Pen 2, 1912 ..	Pen 20, 1913 . . .	2	3	4	—	—	—	—	—	—	—	—	—	—	—	2	1	—	—	—	—	—	—	—	—
♀ (ex Bant. × Large) × F_1 ♂	Pen 4, 1911	—	—	—	—	1	2	2	3	1	3	—	—	1	—		—	—	1	3	6	1	2	—	—
♀ (large) × ♂ (large) ex Pen 4, 1911	Pen 14, 1912	—	—	—	—	—	2	2	3	2	1	1	—	1	—	—	—	—	—	—	1	1	2	1	2
♀ (small) × ♂ (small) ex Pen 4, 1911	Pen 4, 1912	—	—	2	1	3	1	—	—	—	—	—	—	—	—	—	—	3	2	—	—	—	—	—	—
♀ Brown Leghorn × F_1 ♂	Pen 21, &c.	—	—	—	—	—	1	1	3	2	3	1	1	—		—	—	—	—	—	—	—	—	—	—
♀ Brown Leghorn × Large F_2 ♂ ...	Pens 10 and 16, 1913	—	—	—	—	—	—	2	7	2	8	11	1	2	1	—	—	—	—	—	—	—	—	—	—
F_1 ♀ × Brown Leghorn ♂	Pen 19, 1913	—			—	—	1	—	—	1	2	1	1		—	—	—	—	—	—	—	—	—	—	—
Total of above 3 crosses	— —	—	—	—	—	—	2	3	10	5	13	14	3	2	1	—	—	—	—	—	—	—	—	—	—
Small F_2 ♀ · Brown Leghorn ♂ ...	Pens 14 and 26, 1913	—	—	—	—	—	1	3	12	5	1	1	—	—	—	—	—	—	—	—	—	—	—	—	—
Pure Brown Leghorn	— —	—	—	—	—	—	—	—	—	—	1	2	1	—	—	—	—	—	—	—	—	—	—	—	—

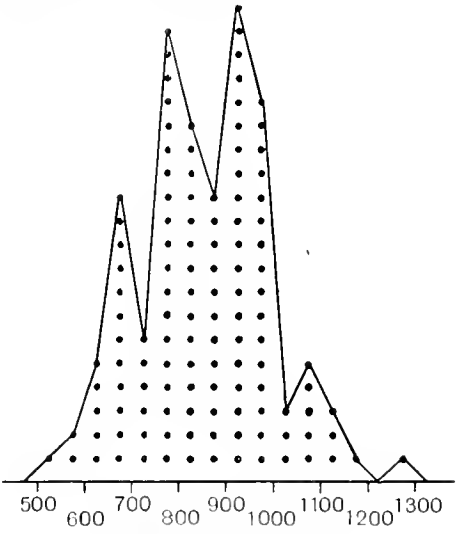
In general build and habit the F_1 ♀♀ were all remarkably alike (cf. Pl. IV, fig. 3). Of the F_1 cocks there were two classes, viz. those with henny plumage (Pl. IV, fig. 2), and those with normal plumage. Within each class however the individual members were very similar to one another. The difference in plumage was not found to be correlated with any size difference either in F_1 or in subsequent generations. The F_1 birds then from this cross were rather smaller than the pure Hamburghs, though of course very much larger than the Sebrights. Of these F_1 birds 13 were bred from, viz. 7 ♂♂ and 6 ♀♀. Most of them were mated with one another but 2 ♂♂ were run respectively with Gold Sebright ♀♀ (p. 30) and with 2 F_1 ♀♀ from a bantam × White Leghorn cross (p. 31). The figure below provides a concise scheme of the experiments.



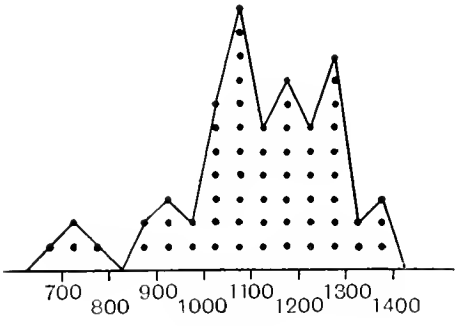
The F_2 generation. During 1912 and 1913 five pens of F_1 birds were bred from. Each of these contained a single pair, except that in 1912 one of the F_1 cocks was run with two of his sisters. We have analysed the results given by each pen separately, but they are very uniform, and as we can detect no significant difference we shall consider them together. In all we have raised 233 F_2 birds to maturity, viz. 112 ♂♂ and 121 ♀♀. In both sexes the range of size is very considerable. The ♂♂ vary from 680—1420 grams while the pullets weigh from 540—1290 grams. Allowing for sexual difference the range of variation is evidently similar in the two cases, and in each of them the weight of the largest bird is distinctly more than double the weight of the smallest. For each sex also the range of variation is such that the smallest birds are smaller than the Silver Sebright bantam, while the largest are larger than the Gold Hamburgh.



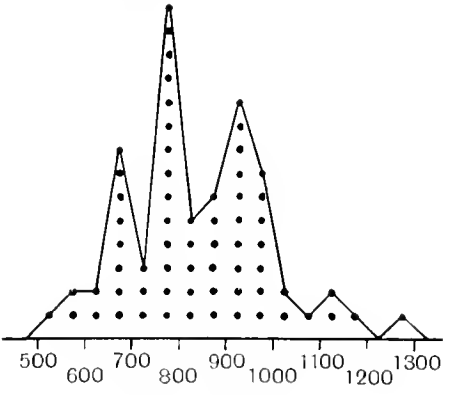
F_2 males, 1912 and 1913.



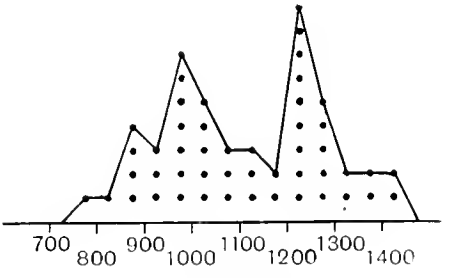
F_2 females, 1912 and 1913.



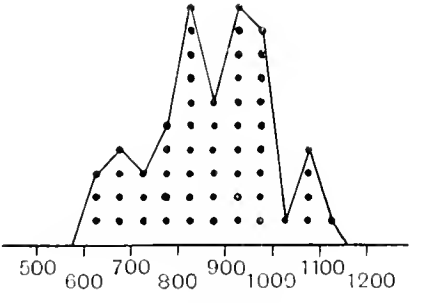
F_2 males, 1912.



F_2 females, 1912.



F_2 males, 1913.



F_2 females, 1913.

Fig. 1. Weight curves of F_2 birds at 35 weeks.

In Fig. 1 the F_2 weights at intervals of 50 grams have been arranged to give the curves of variation[1]. For each sex these curves are irregular. Probably they cannot be regarded as simple curves of error, but appear rather to suggest that we are dealing in either instance with compound curves made up of several simple ones. Further analysis however must come from the breeding pen, and in this direction we have already taken the first step with some experiments in raising a further generation from certain of the F_2 birds.

The F_3 generation. It was clear that the first step towards analysing the F_2 birds was to test whether the largest and the smallest bred true. With this purpose three pens of F_2 birds were mated up in 1913, viz. a pair of the largest birds and two pairs from among the smallest.

(A) For the pen of larger birds we selected an F_2 hen (Pl. IV, fig. 6) which was more than 100 grams heavier than any other F_2 ♀ yet bred. Her weight at 35 weeks was 1290 grams. The F_2 ♂ chosen (Pl. IV, fig. 5) weighed 1390 grams at the same age, and was among the heaviest of the 1912 birds. (In 1913, however, we bred two heavier F_2 cockerels.) From this mating 13 ♂♂ and 13 ♀♀ were reared during the past season. The ♂♂ ranged between 990 and 1630 grams, averaging 1360 grams, while the ♀♀ were between 930 and 1350, averaging 1150 grams. The offspring of the large F_2 birds must all be reckoned on large side, though they showed a good deal of variation. Nothing approaching a bantam was produced by these two birds.

(B) Of the two pens of small F_2 birds put up in 1913 one (Pen 13) consisted of a pair of the smallest F_2 birds reared by us. The ♂ (Pl. IV, fig. 5) weighed 680 grams and the ♀ (Pl. IV, fig. 6) but 540 grams. These figures are rather below the average weights of the original Sebrights. Unfortunately the hen died before she had laid many eggs and we only reared five birds from her. Of these 2 were ♂♂ and 3 were ♀♀, and all of them were below 600 grams.

(C) The other pair of small F_2 birds was rather larger than that just discussed. Here (Pen 7, 1913) the ♂ weighed 740 and the ♀ 710 grams. She must probably be regarded as belonging to the smaller of the intermediates, rather than as pure extracted bantam.

[1] The curves for the two years 1912 and 1913 have been given separately. The average weight of the birds in the latter year is somewhat lower than in the earlier one. This is due to an outbreak of diphtheritic roup which affected about half of the F_2 birds in 1913. Many of these birds had probably not recovered their normal weight when killed at the age of 35 weeks.

From this mating were reared 6 ♂♂ and 7 ♀♀. The 6 ♂♂ ranged from 720 to 860 grams, averaging 800, half being about the same size as the father and half rather larger. Of the 7 ♀♀ four were smaller than their mother while the other three were of about the same size or a little larger. In other words there were nearly equal numbers in both sexes of regular bantams and of birds rather larger. The results are in accord with the view that the father was a true bantam, and that the mother was heterozygous for a factor which had the effect of increasing the weight of a bantam by about 25 %.

These F_2 results taken together suggest strongly that size in poultry depends upon definite factors, and that these factors segregate in gametogenesis. More experimental data are required before we can hope to determine the nature and number of these factors, and we have already designed some further experiments with this end in view. Meanwhile we may give an account of some other data we have got together in connection with the inheritance of size.

F_1 ♂ × Bantam. We mentioned above that two F_1 ♂♂ ex Silver Sebright × Gold Hamburgh were reared in 1910, but that owing to the lack of F_1 ♀♀ we were unable to breed an F_2 generation in the following year. One of these F_1 ♂♂ was put to two Gold Sebright hens in 1911 (Pen 7, 1911) and from this mating 7 ♂♂ and 11 ♀♀ were reared. Of the former 2 were bantams under 700 grams, while the remaining 5 ranged from 800—900 up to 1100—1200. Of the pullets 6 were under 700 grams, 3 were of the usual F_1 size and the remaining 2 were intermediates. From these birds two pens, 1 and 2, were mated up in 1912.

Pen 2, 1912. ♂ 316 (weight 620 gr.) × ♀ 283 (weight 620 gr.). This pen gave 7 ♂♂ ranging between 680 and 880, and 8 pullets all under 600. These birds were evidently breeding nearly true to small size. In the following year the strain was further tested by mating together two of these 1912 birds from Pen 2. They were ♂ 232 (weight 690 gr.) and ♀ 678 (weight 470 gr.). They produced in 1913 10 ♂♂ between 540 and 750 grams, and 3 ♀♀ all under 600.

The experiment shows that the original F_1 ♂ ex Sebright × Hamburgh must have been producing some "bantam" gametes. Such gametes, meeting with the gametes carried by pure bantams, gave rise to birds which belonged to and bred true to the small bantam size.

Pen 1, 1912. The other pen mated up with birds from the F_1 × Sebright cross consisted of a cock and 2 hens. The former was rather

above bantam size, weighing 825 grams, while the hens were respectively 620 and 650 grams. The progeny showed a good deal of variation (e.g. Table I). A few were of the smallest bantam size, others were near the parents, while a few were distinctly larger. Two of the cockerels for instance were between 900 and 1000 grams while one of the pullets was over 800. The result shows that birds which are not quite of bantam size may give both birds distinctly larger and others as distinctly smaller than themselves.

The Hamburgh Sebright × Brown Leghorn Cross.

These experiments were made primarily with the object of following up the transmission of the female form of plumage that may occur in cocks. They offer also some interesting data in connection with the inheritance of size which may be considered here. The ♂♂ alone were reared, and as they generally show their type of feathering at 6 months old they were for the most part killed at that age. Had we been able to keep them for a further 3 months it is probable that the larger birds would have grown relatively more than the smaller ones. With Brown Leghorn hens were crossed F_1 ♂♂ and two large F_2 ♂♂ (Nos. 28 and 139 of 1912) whose weights, 1200 and 1250 grams respectively, were those of F_1 birds. A few birds were also raised from an F_1 ♀ mated with a Brown Leghorn ♂. Similar results were obtained in all of the above cases (cf. Table I) and they may conveniently be considered together. The figures have been put into the form of a curve in Fig. 2 (on p. 32). It is evidently bimodal. What is of peculiar interest is that one of the modes, the higher, practically coincides with that of the pure Brown Leghorn, while the lower one is the same as the single mode on the curve got from the crossing of Brown Leghorn ♂ with two small F_2 ♀♀. The bearing of this will be considered later.

Hamburgh-Sebright × Bantam-Leghorn.

We have already mentioned that in 1910 we failed to breed any F_1 ♀♀ from the Hamburgh-Sebright cross. Of the two F_1 ♂♂ bred that year one was put to Gold Sebright ♀♀ as stated above, and the other was run with two hens which had resulted from an artificial cross between a White Leghorn and a bantam[1]. They were nearly of the same weight, one being 1020 and the other 1040 grams. 29 birds were produced from the pen (Pen 4, 1911), viz. 16 ♂♂ ranging from

[1] These two hens were given to Professor Bateson in 1908 by Dr Hagedoorn, who stated that they were made by artificially inseminating a bantam hen with the sperm of a White Leghorn cock. The nature of the bantam used is unknown.

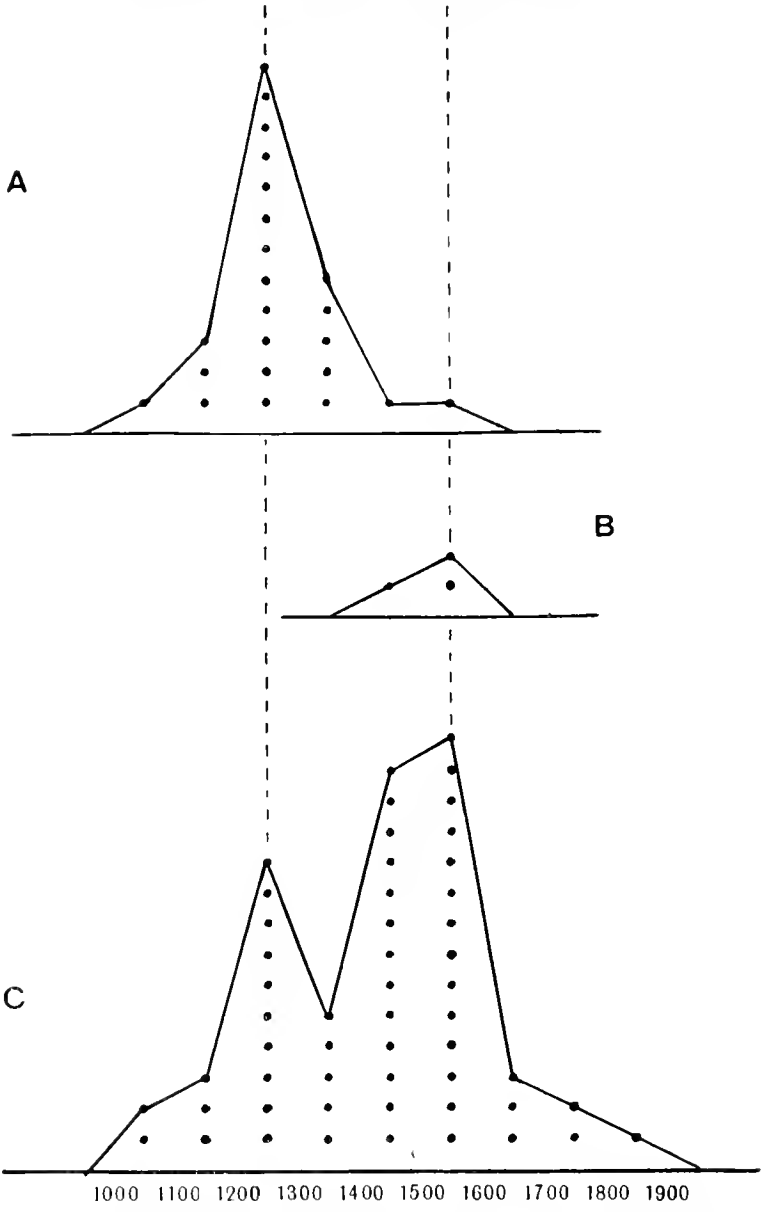


Fig. 2. Weights of Hamburgh-Sebright × Brown Leghorn Crosses at 24 weeks.

A. Male offspring from small F_2 ♀ ♀ × Brown Leghorn ♂ (Pens 14 and 26, 1913).
B. Pure Brown Leghorn ♂ ♂.
C. Male offspring from F_1 × Brown Leghorn and from two large F_2 birds × Brown Leghorn.

940 up to 1710 grams, and 13 ♀♀ between 765 and 1190 grams. The wide range of variation accorded with the nature of these two hens sent by Dr Hagedoorn, and this was confirmed by the following year's work. The largest and the smallest pair were mated up in 1912. From the largest pair (Pen 14, 1912) were produced 12 ♂♂ and 7 ♀♀. The average weight of the ♂♂ was not very different from that of the 1911 family, but the 7 ♀♀ were a distinctly heavier lot (cf. Table 1). The smallest pair of birds (Pen 4, 1912) produced 7 ♂♂ and 5 ♀♀. As the table (p. 26) shows all of these birds were small, the majority being smaller than the parents. In the case of the pullets the range of variation of those from the small parents is entirely outside the range of those from the large parents; in the case of the cockerels the two ranges just overlap. These experiments were discontinued after 1912 in favour of the Hamburgh-Sebright cross. The general results however, the great range of variation in F_2, and the fact that the extremes tend to breed true to size, offer an interesting corroboration of the Hamburgh-Sebright story.

We may now proceed to suggest a scheme by which the phenomena we have described may be interpreted in terms of genetic factors.

Hypothetical Explanation.

In discussing such a tentative scheme several distinctive features about the results must be borne closely in mind. Among them the most important are:

(1) The fact that from the Sebright-Hamburgh cross the range of variation in F_2 is beyond that of either of the original parent forms. The smallest birds are definitely smaller than the Silver Sebright and the largest are well above the weight of the Gold Hamburgh. This phenomenon was more marked in the F_3 generation when the smallest and largest F_2 birds were bred from. It must therefore be supposed, in devising an explanation in terms of genetic factors, that the Gold Hamburgh does not contain all the factors concerned, and that the Silver Sebright does not lack them all.

(2) The F_1 birds are not quite so large as the Gold Hamburghs. They must however contain a single dose of all the factors concerned. Hence we must suppose that the factors with which we are dealing produce a less marked result when the bird is heterozygous for them than when it is homozygous.

(3) Extreme variants in F_2 are relatively scarce. Indeed the smallest and largest extremes as shown by the F_3 generation did not

occur in our F_2 generation at all. We regard this as due to the fact that an insufficient number of birds was bred in F_2 to give them. Where F_1 birds are heterozygous for four factors, the two extremes, i.e. that homozygous for all four factors and that lacking all four, will only occur once in 256 birds. The extremes were however readily obtained in F_3 and we have little doubt that we should have obtained them in F_2 had we been able to breed a sufficiently large number of birds.

Bearing these points in mind we have constructed a scheme involving the following assumptions.

(*a*) That we are dealing with a case in which we are involved with the presence or absence of four genetic factors each of which affects the weight of the bird.

(*b*) That when none of these factors are present the birds are of the minimum size.

(*c*) That two of these factors, **A** and **B**, produce an increase of 66 % respectively on the minimum size when the birds are homozygous and 38 % when the birds are heterozygous for either of them.

(*d*) That the remaining two factors, **C** and **D**, produce an increase of 30 % respectively when the birds are homozygous, and 25 % when the birds are heterozygous.

(*e*) That the constitution of the Gold Hamburgh is **AABBCCdd** and of the Silver Sebright **aabbccDD**.

If we take the minimum weight as 100 for birds of the constitution **aabbccdd**, the maximum weight for birds of the constitution **AABBCCDD** becomes $100+66+66+30+30=292$. Hence F_1 birds of the constitution **AaBbCcDd**, themselves of grade 226, should when bred together give a generation ranging from grade 100 up to grade 292. Since four factors are concerned the extreme members of the series will only make their appearance in this generation once, on an average, among 256 birds. Table II shows the proportion and constitution of these 256 birds which go to form the F_2 generation. Opposite the constitution in each case the weight grade is given, and in the other two columns these theoretical weight grades have been translated into expected actual weight on the basis of the range of variation as found by experiment in the F_2 and F_3 generations. For example the smallest ♂ obtained in Pen 13, 1913, weighed about 550 grams and the largest bred in Pen 12, 1913, reached a weight of rather over 1600 grams (cf. Table I, p. 26). In order to obtain the actual weights of the females as they should be on our scheme we have multiplied the male

TABLE II.

Constitution	Weight grade	Weights of Males	Weights of Females	Constitution	Weight grade	Weights of Males	Weights of Females
1*AABBCCDD*	292	1620	1300	8*AaBbCcdd*	201	1110	890
2*AABBCCDd*	287	1580	1270	4*AaBbccDD*	206	1130	905
1*AABBCCdd*	262	1440	1150	8*AaBbccDd*	201	1110	890
2*AABBCcDD*	287	1580	1270	4*AaBbccdd*	176	970	775
4*AABBCcDd*	282	1550	1240	2*AabbCCDD*	198	1090	870
2*AABBCcdd*	257	1415	1130	4*AabbCCDd*	193	1060	850
1*AABBccDD*	262	1440	1150	2*AabbCCdd*	168	925	740
2*AABBccDd*	257	1415	1130	4*AabbCcDD*	193	1060	850
1*AABBccdd*	232	1275	1020	8*AabbCcDd*	188	1030	825
2*AABbCCDD*	264	1450	1160	4*AabbCcdd*	163	900	720
4*AABbCCDd*	259	1420	1130	2*AabbccDD*	168	925	740
2*AABbCCdd*	234	1290	1030	4*AabbccDd*	163	900	720
4*AABbCcDD*	259	1420	1130	2*Aabbccdd*	138	760	610
8*AABbCcDd*	254	1390	1110	1*aaBBCCDD*	226	1240	995
4*AABbCcdd*	229	1260	1010	2*aaBBCCDd*	221	1215	970
2*AABbccDD*	234	1290	1030	1*aaBBCCdd*	196	1080	870
4*AABbccDd*	229	1260	1010	2*aaBBCcDD*	221	1215	970
2*AABbccdd*	204	1120	900	4*aaBBCcDd*	216	1190	950
1*AAbbCCDD*	226	1240	995	2*aaBBCcdd*	191	1050	840
2*AAbbCCDd*	221	1215	970	1*aaBBccDD*	196	1080	870
1*AAbbCCdd*	196	1080	870	2*aaBBccDd*	191	1050	840
2*AAbbCcDD*	221	1215	970	1*aaBBccdd*	166	916	735
4*AAbbCcDd*	216	1190	950	2*aaBbCCDD*	198	1090	870
2*AAbbCcdd*	191	1050	840	4*aaBbCCDd*	193	1060	850
1*AAbbccDD*	196	1080	870	2*aaBbCCdd*	168	925	740
2*AAbbccDd*	191	1050	840	4*aaBbCcDD*	193	1060	850
1*AAbbccdd*	166	916	735	8*aaBbCcDd*	188	1030	825
2*AaBBCCDD*	264	1450	1160	4*aaBbCcdd*	163	900	720
4*AaBBCCDd*	259	1420	1130	2*aaBbccDD*	168	925	740
2*AaBBCCdd*	234	1290	1030	4*aaBbccDd*	163	900	720
4*AaBBCcDD*	259	1420	1130	2*aaBbccdd*	138	760	610
8*AaBBCcDd*	254	1390	1110	1*aabbCCDD*	160	880	705
4*AaBBCcdd*	229	1260	1010	2*aabbCCDd*	155	855	685
2*AaBBccDD*	234	1290	1030	1*aabbCCdd*	130	715	570
4*AaBBccDd*	229	1260	1010	2*aabbCcDD*	155	855	685
2*AaBBccdd*	204	1120	900	4*aabbCcDd*	150	825	660
4*AaBbCCDD*	236	1290	1030	2*aabbCcdd*	125	690	550
8*AaBbCCDd*	231	1270	1020	1*aabbccDD*	130	715	570
4*AaBbCCdd*	206	1130	905	2*aabbccDd*	125	690	550
8*AaBbCcDD*	231	1270	1020	1*aabbccdd*	100	550	440
16*AaBbCcDd*	226	1240	995				

weights by $\frac{4}{5}$, a proportion which experience has shown us to express very nearly the relation between average weights of the sexes in the material we have worked with.

In Table III we have interpreted the experimental results in terms of the scheme just outlined. It is clear from this table that the scheme covers the facts fairly closely. The chief discrepance is in the figures for the F_2 generation where the proportion of heavy birds among the pullets is somewhat below expectation. Apart from this the actual results from the various matings, both with regard to mean weight and range of variation, tally closely with the figures deduced theoretically on our hypothesis. It is not impossible that an even closer agreement might be arrived at if the values given to the various factors were altered[1]. At this stage however we are more concerned in demonstrating that an explanation of these phenomena in terms of definite genetic factors is possible. To determine the action of each factor with precision would be a long and laborious undertaking, nor do we propose to pursue the matter so far. But if our hypothesis is adequate we ought to find birds of different intermediate sizes breeding true. We should for example be able to establish a breed of the constitution **AAbbccDD** and of weight grade 196 (Table II). Such a grade is exactly intermediate between **AABBCCdd** and **aabbccDD**, i.e. between the Gold Hamburgh and the Silver Sebright. We ought also to be able to establish another intermediate race of the constitution **aabbCCDD** which would breed true to a grade rather above that of the Sebright. Experiments on these lines are already in progress. Moreover, until we have succeeded in establishing such strains we cannot know for certain what amount of variation is due to non-inheritable fluctuations apart from the operation of definite genetic factors which affect weight. In the present paper we have not taken such possible fluctuations into account as there seems no reason why they should not affect all of the birds similarly.

We alluded above to some experiments in which F_1 and F_2 birds from the Sebright-Hamburgh cross were mated with pure Brown Leghorns (p. 31), and we pointed out that in the case of the F_2 and

[1] For the sake of simplicity we have conceived of our factors as acting in each case directly on a minimum and constant basal weight. But it is of course possible to regard them as acting also upon the weight resulting from the other factors present. For example we have supposed that a single dose of **D** gives an increase of 25 % on the minimum weight of 100, whether **A**, **B**, or **C** are present or not. But it may be that **D** produces a 25 % increase on whatever is present. And of course it is possible that weight factors may act upon one another.

TABLE III.

Parents	Zygotic Constitution of Parents	Reference to Experimental Results	Weight of Male Progeny in grams.														Weight of Female Offspring in grams.									
			400—500	500—600	600—700	700—800	800—900	900—1000	1000—1100	1100—1200	1200—1300	1300—1400	1400—1500	1500—1600	1600—1700	400—500	500—600	600—700	700—800	800—900	900—1000	1000—1100	1100—1200	1200—1300	1300—1400	1400—1500
Silver Sebright ...	*aabbccDD* ...	Calculated	—	—	—	*	—	—	—	—	—	—	—	—	—	—	*	—	—	—	—	—	—	—	—	—
		Observed	—	—	—	—	—	—	—	—	—	—	—	—	—	—	*1*	*1*	—	—	—	—	—	—	—	—
Gold Hamburgh ...	*AABBCCdd* ...	Calculated	—	—	—	—	—	—	—	—	—	—	*	—	—	—	—	—	—	—	—	*	—	—	—	—
		Observed	—	—	—	—	—	—	—	—	—	*1*	—	—	—	—	—	—	—	—	—	*3*	*1*	—	—	—
Sil. Sebr. × G. Hamb.	♂ = *AABBCCdd*	Calculated	—	—	—	—	—	—	—	—	*	—	—	—	—	—	—	—	—	—	*	—	—	—	—	—
	♀ = *aabbccDD* ...	*Pen 9, 1911—12* ...	—	—	—	—	—	—	—	*5*	*?*	*1*	—	—	—	—	—	—	—	—	*6*	*1*	—	—	—	—
F_1 ♀ × F_1 ♂ ...	♂ = *AaBbCcDd*	Calculated	—	1	4	6	9	30	48	36	71	16	26	8	1	1	6	12	31	64	46	45	42	8	1	—
	♀ = *AaBbCcDd*	*Pens 21—25, 1912—13*	—	—	*1*	*4*	*3*	*9*	*24*	*17*	*27*	*9*	*2*	—	—	—	*3*	*15*	*23*	*20*	*28*	*7*	*4*	*1*	—	—
		(see note below) ...	—	—	*(1*	*4*	*7*	*15*	*26*	*19*	*29*	*9*	*2)*	—	—	—	*(3*	*17*	*25*	*27*	*36*	*8*	*4*	*1)*	—	—
F_2 ♀ × F_2 ♂ ...	♂ = *AABbCeDd*	Calculated	—	—	—	—	—	—	—	—	2	2	6	5	1	—	—	—	—	—	—	2	8	5	1	—
	♀ = *AABBCCDd*	*Pen 12, 1913*	—	—	—	—	—	—	—	—	*1*	*1*	*1*	*1*	*1*	—	—	—	—	—	—	*1*	*1*	*2*	*1*	—
		(see note below) ...	—	—	—	—	—	—	*(1*	*?*	*4*	*?*	*1*	*2*	*1)*	—	—	—	—	—	*(1*	*3*	*5*	*2*	*2)*	—
F_2 ♀ × F_2 ♂ ...	♂ = *aabbccDD* ..	Calculated	—	—	1	1	2	—	—	—	—	—	—	—	—	—	2	1	1	—	—	—	—	—	—	—
	♀ = *aabbCcDd* ...	*Pen 7, 1913*	—	—	—	*3*	*3*	—	—	—	—	—	—	—	—	—	*2*	*2*	*3*	—	—	—	—	—	—	—
F_2 ♀ × F_2 ♂ ...	♂ = *aabbccDd* ...	Calculated	—	—	1	1	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—
	♀ = *aabbccDd* ...	*Pen 13, 1913*	—	*?*	—	—	—	—	—	—	—	—	—	—	—	—	*3*	—	—	—	—	—	—	—	—	—
Gold Sebright × F_1 ♂	♂ = *AaBbCcDd*	Calculated	—	1	3	1	1	6	2	2	1	—	—	—	—	1	2	3	5	4	—	1	1	—	—	—
	♀ = *aabbccdd* ...	*Pen 7, 1911*	—	—	*?*	—	*1*	*?*	*1*	*1*	—	—	—	—	—	—	—	*6*	*1*	*1*	*3*	—	—	—	—	—
Bantams ... ... (ex Pen 7, 1911) ..	♂ = *aabbCeDd* ...	Calculated	—	3	8	4	11	4	2	—	—	—	—	—	—	3	10	12	5	2	—	—	—	—	—	—
	♀ = *aabbCcDd* ♀ = *Aabbccdd* ♀	*Pen 1, 1912*	—	*1*	—	*7*	*7*	*?*	—	—	—	—	—	—	—	*2*	*6*	*4*	*?*	*1*	—	—	—	—	—	—
Bantams ... ... (ex Pen 7, 1911)...	♂ = *aabbccdd* ...	Calculated	—	1	2	—	1	—	—	—	—	—	—	—	—	1	2	1	—	—	—	—	—	—	—	—
	♀ = *aabbCcDd* ...	*Pen 2, 1912*	—	—	*?*	*4*	*1*	—	—	—	—	—	—	—	—	*5*	*3*	—	—	—	—	—	—	—	—	—
Bantams ... ... (ex Pen 2, 1912)...	♂ = *aabbCcdd* ...	Calculated	—	1	1	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	—	—	—	—	—
	♀ = *aabbccdd* .	*Pen 20, 1913*	—	*?*	*3*	*4*	—	—	—	—	—	—	—	—	—	*?*	*1*	—	—	—	—	—	—	—	—	—

Note. The numbers given for Pens 21—25, 1912-13, and for Pen 12, 1913, are those for the healthy birds which were not attacked by roup in 1913 (cf. note on p. 29). The larger series in brackets given below in each case gives all birds including those affected.

larger F_2 birds the results could be expressed in the form of a bimodal curve whereas a unimodal curve was given by the smaller F_2 birds. These results are readily explained on the hypothesis we have just

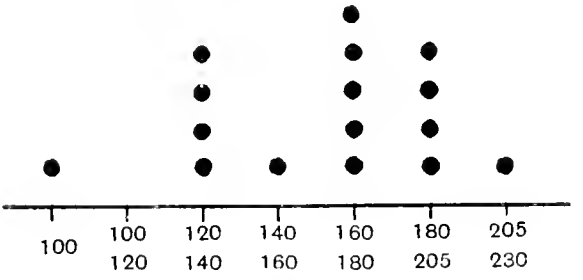
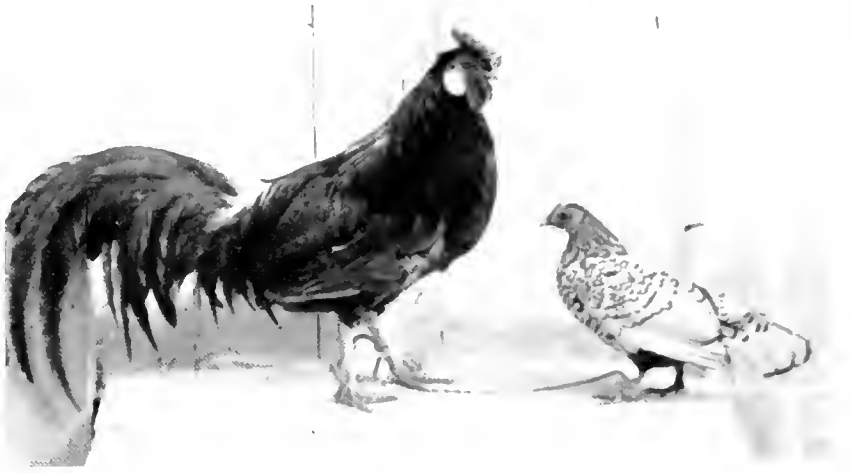


Fig. 3.

outlined. The gametes produced by an F_1 bird are of 16 sorts, viz. ABCD, ABCd, ABcD, ABcd, AbCD, AbCd, AbcD, Abcd, aBCD, aBCd, aBcD, aBcd, abCD, abCd, abcD, abcd. With abcd gametes such a series would give rise to the grades 226, 201 (2), 176, 188 (2), 163 (4), 138 (2), 150, 125 (2), 100. These grades as shown graphically in Fig. 3 give rise to a bimodal curve in which the greater mass is collected on the upper mode. It is the form of curve which we should expect when F_1 or similarly constituted birds are crossed with some uniform strain. It is the form of curve which was actually obtained when F_1 (and similar F_2) birds were crossed with the pure Brown Leghorn (cf. Fig. 2, p. 32). The results offer an interesting corroboration of our views as to the nature of the F_1 birds from the Sebright-Hamburgh cross.

In connection with our interpretation we may call attention to the following point of theoretical, and possibly also of practical interest. On our hypothesis two strains of intermediate and similar weight are possible, viz. AAbbCCdd and aaBBccDD. A cross between two such strains would result in F_1 birds rather larger than either, while the F_2 generation would be like that shown in Table II. In other words a cross between two medium sized strains of the same average size may lead in F_2 to the production of strains considerably larger and also considerably smaller than either of the parent strains, both of which can be readily fixed. It is possible that the great size of many floral varieties, e.g. in daffodils, has been effected on these lines from crosses between medium sized forms differing in constitution for the factors affecting size. It is not impossible that the increase sometimes observed on

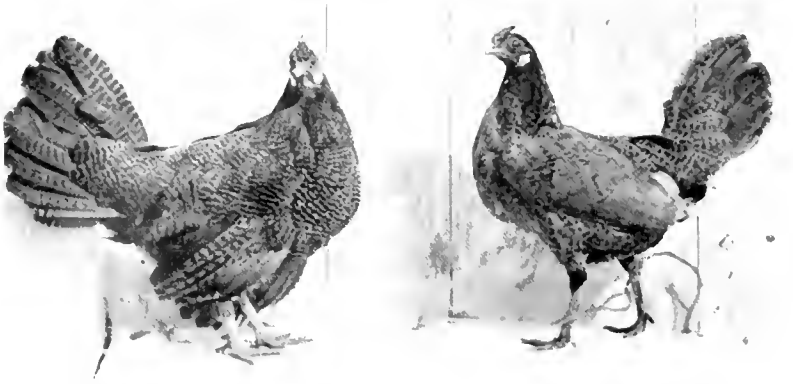


Gold-pencilled Hamburgh ♂. Silver Sebright ♀.

Fig. 1.

Gold-pencilled Hamburgh ♂. F_1 ♂ (ex Sebright × Hamburgh).

Fig. 2.

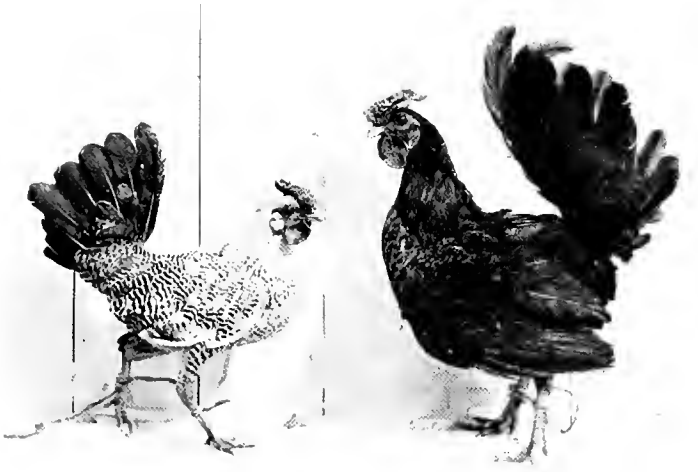


Gold-pencilled Hamburgh ♀. F_1 ♀ (ex Sebright × Hamburgh).

Fig. 3.

Silver Sebright ♀. F_1 ♀ (ex Sebright × Hamburgh).

Fig. 4.



Small F_2 ♂. (In Pen 13, 1913.) Large F_2 ♂. (In Pen 12, 1913.)

Fig. 5.

Small F_2 ♀. (In Pen 13, 1913.) Large F_2 ♀. (In Pen 12, 1913.)

Fig. 6.

crossing strains of animals or plants of similar size is not due to increased vigour resulting from the cross, but to the bringing together of independent growth factors each capable of producing some effect. Were this the case it would be apparent in the F_2 generation where fixable strains both larger and smaller than the parent forms should make their appearance.

In conclusion we may once more state that the scheme which we have put forward is a tentative one and may have to be recast in any or all of its details in the light of future knowledge. Nevertheless there can be little doubt that it expresses an essential truth in connection with the inheritance of the complex character designated weight. The facts of breeding offer a clear indication that weight may depend upon the presence or absence of definite genetic factors segregating from one another in gametogenesis on lines with which students of these matters are already familiar.

The experiments of which an account is given above form part of a series of investigations on heredity in poultry for which the means have been provided out of the Fund controlled by the Development Commission.

EXPLANATION OF PLATE IV.

In all six figures the birds are taken to the same scale.

Fig. 1. Gold Pencilled Hamburgh ♂ and Silver Sebright ♀.

Fig. 2. ,, ,, ♂ and F_1 ♂ ex Silver Sebright × Gold Hamburgh.

Fig. 3. ,, ,, ♀ and F_1 ♀ ,, ,, ,,

Fig. 4. Silver Sebright ♀ and F_1 ♀ ex Silver Sebright × Gold Hamburgh.

Fig. 5. Small F_2 ♂ (Pen 13, 1913) and Large F_2 ♂ (Pen 12, 1913).

Fig. 6. ,, F_2 ♀ (,,) ,, F_2 ♀ (,,).

A PRELIMINARY NOTE ON THE FACTORS CONTROLLING THE GINNING PERCENT OF INDIAN COTTONS.

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The cotton crop, as it is harvested, consists of the lint in which the seed is embedded. This seed-cotton is then ginned, by which process the seed and lint are separated. The ginning percent of a cotton, as the term is here used, may be defined as the number of pounds of lint obtained from 100 lbs. of seed-cotton.

Now the area under a given crop is largely controlled by the price the actual cultivator receives for his produce. In the case of cotton, the cultivator parts with the seed-cotton for which the price paid by the purchaser is almost directly proportional to the ginning percent as determined by sampling[1]. The character, which forms the subject of the present note, is, consequently, one of great economic importance, and for this reason has formed the basis of a considerable series of experiments. From the scientific aspect the problem is an attempt to resolve a complex character into its simpler component factors.

The range of variation found in the ginning percent of the different types of cotton at present cultivated may be usefully indicated. In the crop at present in general cultivation in the United Provinces—consisting of a mixture of various types of *G. neglectum* Tod.—the ginning percent is 30—33. Improved forms, isolated by selection, are now being introduced for which this figure approaches 41. Races of *G. cernuum* Tod. have been cultivated in which the ginning percent is as high as 44 or 45. At the other extreme lie *G. indicum* Lamk. and *G. arboreum* Linn. *sp.* Pl. with a ginning percent of 25—26, the former giving the Bani cotton of Central India, perhaps the best indigenous cotton of India, and

[1] See Leake and Parr, *Agri. Journ. of India*, Vol. VIII. 1.

the latter being the sacred perennial cotton only found now in the vicinity of Hindu temples. Lastly *G. intermedium* Gammie, a form cultivated as a mixed crop round Allahabad and the West of Bengal, has a ginning percent as low as 15.

A brief consideration is sufficient to indicate that the ginning percent is not a simple character. It is directly dependent on the weight of seed and weight of lint. In practice the fact becomes still more evident: thus, among the offspring of numerous crosses which have been made between two parent types, each having a ginning percent of 25—26, forms have been isolated for which the figures diverge as much as 36 and 18.

The direction of the present investigation will be most profitably indicated by a short discussion of the *a priori* considerations on which it was based. From the definition it is clear that the exact figure for the ginning percent is dependent on the weight of two distinct bodies—the lint and the seed. This weight is, in each case, dependent on the values of several characters which, in their turn, may vary. Thus, in the case of the seed, the weight depends on the volume, on the specific gravity, and on the number of seeds. In the case of the fibre the characters affecting the weight are not so obvious. They may, however, be considered as the weight of the individual fibres—clearly not a unit character as this in its turn depends on such conditions as length, thickness (mean diameter), size of lumen and specific gravity of fibre wall—and the number of fibres. The relation between the number of seeds and number of fibres in a given sample is again determined by the number of fibres arising from a single seed. The total number of fibres may also be derived from the number of fibres arising from a unit area of seed coat surface—a number to which the term "density" will be applied. Calculated in terms of "density" the number of fibres will be seen to be governed by the area of seed coat surface which, in its turn, is expressible in terms of volume. This consideration is of advantage inasmuch as it indicates that the weights of both the seed and fibre are directly affected by the same character—the volume of the seed. While, however, the weight of seed will vary as the cube of the seed radius, the weight of lint will, if the "density" remains constant, vary as the surface area, which varies as the square of the seed radius. Hence, other factors being constant, the ginning percent will increase as the volume of the seed diminishes. The calculations in terms of "density" are, however, open to objection inasmuch as the number of fibres is probably determined early in the course of development of the ovule while the volume

of the seed, and hence the surface area and "density," will, in part at any rate, be determined later by the nutrition supplied to the ovule throughout development. It seems, therefore, advisable to use the number of fibres arising from a single seed in preference to the figure obtained for the density for the fibres.

From such *a priori* considerations, there is reason to suppose that the ginning percent actually recorded will be the resultant of at least the four characters:

(1) Volume of seed,

(2) Specific gravity of seed,

(3) No. of fibres arising from a single seed

which may be simple characters, and

(4) Weight of the individual fibres, obviously a complex character.

A further consideration will show that the problem is probably even more complex. The seed and lint are developed in a closed cell of the fruit, each cell of which contains a number of seeds, frequently eight. According to the conditions of environment, the nature of the tissue, and, consequently, the space in which the seed must develop, will vary. It is probable, therefore, that, during the course of development an unknown, but appreciable, effect is produced by the mutual pressure thus brought into play. Conclusive evidence of the exact extent of such mutual pressure is not so far available. It is clear, however, that the effect will not directly influence the ginning percent but that such effect as it may produce will be indirect and through one or other of the four characters already noted above. The importance of a recognition of this mutual pressure lies in the fact that the actual values obtained for these characters may not represent the true potential capacity of the plant. This has already been noticed by Balls[1]. The present discussion, which concerns the factors directly controlling the ginning percent, is not affected thereby, and the problem as it now stands may be defined as the determination of the degree to which each severally of the four characters given above influences the ginning percent and of the extent to which they, in combination, account for the range of variation recorded in that character.

Methods. In a preliminary note of this nature it is not possible to give in detail the precautions it has been found necessary to adopt. The main outlines of the method only can be indicated. The unit used as a sample is the seed-cotton derived from a whole and not diseased

[1] Balls, W. L., *The cotton plant in Egypt*, p. 170.

boll. The sample is gathered after the capsule has expanded thoroughly and when the lint and seed are thoroughly dry. From this sample fibres to the number of about 2000 are accurately counted and weighed. The remaining lint is removed from the seed by a small hand gin and the weight of lint and seed recorded as is also the number of seeds. The volume and specific gravity of the seed are then determined by displacement in water. The data thus obtained give

(1) weight of a known number of fibres,
(2) weight of total fibre,
(3) weight of seed,
(4) number of seeds,
(5) volume of seed,

from which the figures required can be derived by direct calculation.

The method is not ideal as, apart from the precautions necessary in the process of sampling, a single complete determination, with subsequent calculation, occupies at least two hours—a point of considerable importance where the value of the results is to a large extent determined by the number of the observations.

During the course of the investigation it soon became apparent that the specific gravity of the seed, even within the limits of a single sample, was subject to marked fluctuation. Evidence, as far as it was obtainable, indicated, however, that such fluctuation depends on the conditions which prevail during the process of ripening. Any inherent difference in specific gravity due to the nature of the plant is small in comparison with such observed fluctuation. It has been considered advisable, therefore, to eliminate this character by reducing the specific gravity in all cases to an uniform figure of 1·10, such a correction involves a corresponding correction of the observed ginning percent. There thus remain three characters, volume of seed, number of fibres arising from a single seed and weight of the individual fibres, which, from the given *a priori* considerations, there is reason to suppose might influence the ginning percent. The object of the present work is to determine how far fluctuations in the ginning percent can be accounted for by these characters only and whether a search for other characters must be undertaken.

The units used for the expression of these values are

volume of seed	...	cubic millimetres,
No. of fibres per seed	...	in thousands,
fibre weight	...	weight of 1000 fibres in milligrams.

The material on which the preliminary determinations, here dealt with, have been made consists of 232 samples derived from a wide series of plants of the Asiatic series cottons, and covers, as far as possible, the full range of variation found. Full details cannot be given here, but a few typical instances illustrating the range may be recorded.

Seed volume	Number of fibres per seed	Fibre weight	Gin percent	Remarks
67	1·2	8·1	11	A form from China
66	2·9	7·4	23	An F_7 plant
45	3·3	6·6	30	*G. arboreum*
67	3·3	10·5	31	A form from China
36	5·2	3·7	34	*G. neglectum*, yellow flowered
62	7·6	5·9	40	*G. neglectum*, white flowered
42	6·3	5·9	44	,, ,,

These few illustrations are sufficient to indicate the wide degree of variation found in the characters under consideration, and it is noteworthy that there appears to be no direct relation between the ginning percent and any of the remaining characters, even in the selected series here given. That this must be so is again illustrated by abstract consideration. I am indebted to Mr Udny Yule for the following formula which indicates the relation between the four characters concerned:

$$\text{gin } \% = \frac{100\,k/1{\cdot}1}{1 + k/1{\cdot}1}$$

$$\text{where } k = \frac{\text{number of fibres per seed} \times \text{wt. of 1000 fibres}}{\text{volume of one seed}}.$$

From this it is clear that the ginning percent cannot be directly measured from any single one of the characters here dealt with. It is necessary, therefore, to proceed further and calculate the coefficients of correlation.

For this purpose the characters may be numbered as follows:

(1) Ginning percent,

(2) Number of fibres per seed,

(3) Wt. of 1000 fibres,

(4) Volume of seed.

The correlations between these characters are found to be

$r_{12} + {\cdot}7933$	$E \pm {\cdot}0164$
$r_{13} + {\cdot}0530$	$E \pm {\cdot}0442$
$r_{14} - {\cdot}2208$	$E \pm {\cdot}0420$
$r_{23} - {\cdot}2285$	$E \pm {\cdot}0421$
$r_{24} + {\cdot}0966$	$E \pm {\cdot}0439$
$r_{34} + {\cdot}5147$	$E \pm {\cdot}0326$

From these figures the partial correlation coefficients may be calculated. These have been determined as follows[1]:

$r_{12\cdot 4} = +\cdot 8392$
$r_{13\cdot 4} = +\cdot 1992$
$r_{23\cdot 4} = -\cdot 3260$
$r_{12\cdot 3} = +\cdot 8285$
$r_{14\cdot 3} = -\cdot 2898$
$r_{24\cdot 3} = +\cdot 2566$
$r_{13\cdot 2} = +\cdot 3952$
$r_{14\cdot 2} = -\cdot 4909$
$r_{34\cdot 2} = +\cdot 5540$
$r_{23\cdot 1} = -\cdot 4449$
$r_{24\cdot 1} = +\cdot 4577$
$r_{34\cdot 1} = +\cdot 5406$

$r_{12\cdot 34} = +\cdot 9760$
$r_{13\cdot 24} = +\cdot 9198$
$r_{14\cdot 23} = -\cdot 9282$
$r_{23\cdot 14} = -\cdot 9255$
$r_{24\cdot 13} = +\cdot 9266$
$r_{34\cdot 12} = +\cdot 9346$

From a consideration of these coefficients it is clear that the four characters concerned form a closely interrelated group in which variation in any one character is very fully accounted for by variation in one or other of the other three. Further, of the three characters by which the ginning percent may be affected, one only, namely the number of fibres per seed, has any marked effect on the value of the ginning percent.

Certain other conclusions may be drawn from the above figures of which one or two may be mentioned here. In the first place the high negative correlation ($r = -\cdot 9282$) between the ginning percent and the volume of the seed indicates the validity of the conclusion, based above (p. 42) on *a priori* considerations, that the ginning percent, other factors being constant, will increase as the volume diminishes. Secondly, the high negative correlation between the number of fibres per seed and the weight of 1000 fibres ($r = -\cdot 9255$) seems to indicate that the area in which the fibre can develop is limited as a result of which any increase in the number must be accompanied by a diminution in the space occupied by each individual fibre. This conclusion has already been foreshadowed above.

Lastly, while variation in the number of fibres per seed will produce a marked direct effect on the value of the ginning percent, variation in either the fibre weight and the volume of the seed can produce but a small effect in that the correlation between these and the number of fibres per seed is low ($r_{23} = -\cdot 2285$ and $r_{24} = -\cdot 0966$).

The main and all-important conclusion, however, to be drawn from these results lies in the fact that there is here definite proof that the ginning percent is a complex character, the variation found in which can be almost completely accounted for by the variation found in the three

[1] Cf. Yule, *An introduction to the Theory of Statistics*, 2nd edition, p. 241 et seq.

characters, number of fibres per seed, weight of the individual fibres and volume of seed. It follows, therefore, that the determination of the ultimate causes of variation in the ginning percent cannot be made directly. Rather, these must be sought indirectly through their effect on the three characters under consideration and especially on the number of fibres per seed.

The conclusion just reached opens up a wide field of investigation. Before practical use can be made of the above conclusions in plant breeding it is necessary to determine the true value which can be assigned to any given plant for these characters. In all observations involving multiple characters a considerable fluctuating variability is found in different samples from the same individual, and it is necessary to determine the extent of such fluctuations. The somewhat tedious nature of the determinations militates against any rapid conclusion being arrived at and, though considerable progress has been already made, a full exposition of the results must await further experiment. Sufficient information has, however, been obtained to show that such individual variations are of a magnitude readily distinguishable from the differences found between different races. A single instance, based on determination of the number of fibres per seed, must here suffice.

	Average number of fibres per seed	Maximum range of error
A single plant culture of Type 9[1]	4561	762
A field crop of Type 9	6279	2761

There is thus good reason for believing that further investigation will make it possible to elucidate such cases as that given above where two plants, both having a ginning percent of 25—26, when crossed gave rise to offspring of which the ginning percent varied from 36—18. There is also hope that the ultimate physiological causes of fluctuation in the ginning percent may be traced.

In conclusion the writer desires to express his great indebtedness to Mr G. Udny Yule and to Dr G. T. Walker, F.R.S., of the Indian Meteorological Dept., for their kindly help, also to the Authorities of the Imperial College of Science and Technology and especially to Prof. J. B. Farmer, F.R.S., for the facilities given him at that College for carrying out these investigations during a period of leave from India, and lastly to his sisters, too numerous to mention individually, for their kindly help in checking the somewhat lengthy calculations.

[1] For an account of the types see Leake, *Journal of Genetics*, Vol. I. No. 3 (1911), pp. 209—211.

IMMUNITY TO FUNGOUS DISEASES AS A PHYSIOLOGICAL TEST IN GENETICS AND SYSTEMATICS, EXEMPLIFIED IN CEREALS.

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CONTENTS.

One of the fundamental phenomena in the parasitism of fungi on plants, playing a decisive *rôle* in the selection of immune sorts, is the specialization of parasites. The majority of parasitic fungi are, by their nature, sharply limited in the choice of their hosts, and are attached to definite genera and species of plants. In many cases they are limited to one plant genus. In others, especially in the group of rusts and mildews, there are cases in which the fungus is limited to a few, or even to one host-species only.

In rusts, mildews and a few other families of fungi, the differentiation went so far that the same morphological species are not seldom divided into many independent physiological races, so-called "biologic forms," which are attached to different host-plants. In some instances the various stages of fructification of the same fungus may be differently specialized[1].

The study of the causes of the immunity and susceptibility of plants hitherto has not made much headway; the phenomena of parasitism being too complex, and the drawing up of general statements a matter for the future. But still it has been shown, by the works of Eriksson, as well as by Marshall and his school (17), that immunity depends, not on the anatomical peculiarities of plants, but on the properties of their protoplasmic cell-contents. Salmon and Miss Gibson have also established that positive chemotactic attraction of the germ tubes of fungi by the juice of the host-cells, is not sufficient to produce the normal growth of fungi on the plant. In fact it is now clear to us that immunity depends on very complicated physiological inter-relations between the protoplasm of host-cells and fungus, and that the external differences in regard to immunity to fungal diseases, which are perceptible in various races of plants, are an indication of internal hereditary differences in the constitution of their plasma.

Starting from the nature of immunity, as we understand it at the present day, and from the fact of the specialization of parasites—the connection between the phenomena of immunity and genetics becomes evident. It is obvious that fungi, and, in particular, narrowly specialized ones, may be used in some cases as a physiological test (or,

[1] A very interesting case of this was recently reported by J. Norton for the autoecious rust of asparagus, *Puccinia asparagii* D.C. In regard to the "uredo" or summer stage, some immune sorts of asparagus have been found, but these resistant plants are all susceptible to the same fungus in the "aeeidio" stage. (*Methods used in breeding Asparagus for Rust Resistance*, by J. B. Norton. Bureau of Plant Industry, Washington. Bul. No. 263, 1913, pp. 23—24.)

strictly speaking, reagent) for the recognition of the species and races in systematic and genetic studies of plants.

The general idea of a connection between immunity and genetics is certainly very conspicuous, and there are many data to prove it. It is enough to mention the well-known fact that nearly allied species of animals are very often susceptible to the same diseases; nearly related genera of plants very often suffer from the same insects. Several genera of parasitic fungi are exclusively connected with definite families of plants, as for example *Phragmidium* with the family *Rosaceae*.

The general indications of the possibility of applying fungal reactions of plants in ascertaining their affinity, may be found in mycological literature. But, notwithstanding the certainty of a relation between immunity and genetics, very few cases are known of the actual use of fungi or other parasites as tests in genetic and systematic work. There are probably two reasons which explain the neglecting of this method: firstly, because in the majority of cases, fungi are not sufficiently specialized to be employed for the delicate differentiation of nearly related plants, and secondly, on account of the usual divergence of the work of pathologists, genetists and systematists.

As to examples in literature of the use of parasites as physiological tests, I can cite very few. First, Prof. Klebahn in his monograph on rusts (7), pp. 140—141, in the paragraph entitled: "Verwendung der Spezialisierung des Schmarotzers zur Unterscheidung der Arten und Wirte," mentions a case in which by the aid of narrowly specialized rust *Melampsora ribesii purpureae* he found a mistake in the denomination of a willow plant in his garden. A second example occurs in the work of Eriksson entitled: *Ein parasitischer Pilz als Index der inneren Natur eines Pflanzenbastards* (4). In this work he has shown that the hybrid of wheat and rye is immune to brown rye-rust *Puccinia dispersa* and susceptible to brown wheat-rust *P. triticina*, which proves that this plant is nearer to wheat than to rye (16, p. 104). A third case is furnished by E. M. Vasiliev's observations on the injurious insects of maize in which he makes an attempt to connect the number of species of these insects with the origin of this plant (15)[1].

[1] In Darwin's *Variations of Animals and Plants under Domestication*, second edition, Vol. II, Chap. XVIII, we find the following footnote about the affinity of *Aperea* and guinea-pigs. "I sent to Mr H. Denny, of Leeds, the lice which I collected from the wild *Aperea* in La Plata, and he informs me that they belong to a genus distinct from those found on the guinea-pig. This is important evidence that the *Aperea* is not the parent of the guinea-pig; and is worth giving, as some authors erroneously suppose that the guinea-pig since being domesticated has become sterile when crossed with the *Aperea*."

Working both on the immunity and systematics of cereals, I often had an opportunity of proving the connection between the relationship and fungal reactions of these plants. My observations led me to the conclusion that fungi, as a physiological test, may be very useful in the genetic and systematic study of this plant group.

Some of the results obtained have been published (16). Here I propose to cite several general conclusions from work done, and then add new data.

To begin with, a few words about the application of fungal tests for the purposes of the systematics of cereals. At the present time attention, in the systematic study of these plants, is being directed towards numerous small constant botanical units, the so-called "races." The principal justification of this work is a very real need for the drawing up of a detailed catalogue of cereal races, useful alike to the worker in genetics and to the practical agriculturist. In the description of these races not only morphological characters are taken into account, as is usual in purely systematic studies, but also physiological ones.

The extremely narrow specialization of many fungi found on cereals, and the well-known fact of the existence of differences in the degree of susceptibility to diseases amongst various sorts of them, warranted first of all the attempt to use fungal tests for the recognition of the races.

In fact, by the aid of this method, I was easily able to divide many varieties[1] of wheat and oats into races. The important *rôle* in the systematic study of such a character as the degree of immunity is increased, as practice in its application has shown, by the circumstance that the physiological individualisation of race is very often accompanied by morphological characters which, however, are externally not very conspicuous. In these cases the peculiarity in behaviour towards fungi obliges the observer to pay more attention to this or that previously unsuspected race, and in the end he usually succeeds in finding in it some other confirmatory differences[2].

[1] We use here the word "variety" in a purely botanical sense. (See F. Koernicke, *Handbuch der Getreide*, 1885.) In the group of cereals "varieties" usually are collective notions, and some of them include many independent morphological and physiological forms—"races"—the smallest systematic units.

[2] Parenthetically we must remark that the definition of degree of susceptibility to a certain disease is not quite simple. In fact, the exactitude of many old works in this respect is so small that in our days we are obliged to obtain these data anew. More details about the definition of degree of immunity are given in our paper (16), Chapter I.

With respect to wheat the application of fungal reactions is a specially grateful method, because in this instance we have at our disposal many narrowly specialized parasites, in other words many sensitive physiological reagents, as brown and yellow rusts, mildew, etc. When one test is not sensitive enough to distinguish our races, we may have recourse to a second, a third, and so on.

In the above-mentioned paper (16) many examples of such a division of varieties of oats and wheat into races are given. As a, perhaps, interesting result, I may mention that many races were found to exist in some of the less common varieties, as *Avena diffusa* As. and Gr. var. *brunnea* Keke., var. *cinerea* Keke., var. *montana* Al., and in several rare varieties of *Triticum vulgare* Vill.

In the species *Triticum dicoccum*, which is represented by many varieties and races, it was ascertained that there exist two groups of races: one immune to brown rust, the other susceptible to it. The genetic significance of this will be dealt with later on. Here we must observe only, that the resistant forms of *Tr. dicoccum*, which were in our collection, are morphologically, in the structure of ears and leaves, very like several varieties of *Tr. durum*. The degree of immunity of these latter to brown rust is also nearly the same as in the resistant races of *Tr. dicoccum*.

The most interesting example of the application of this method to the systematic study of wheat I met with, is the following:

In the investigation of 580 sorts belonging to the species *Tr. vulgare*, which in general is very susceptible to mildew (*Erysiphe graminis* D.C.) and to different rusts, we found to our surprise a spring race which was perfectly immune to mildew. Notwithstanding many attempts at artificial infection in the field, in the greenhouse or under bell-jars, this race remained quite immune. Not one pustule of mildew was found on this wheat, whereas other races of *Tr. vulgare* in these conditions were severely attacked by the above fungus. This wheat proved also to be relatively very immune to brown rust (*P. triticina*). The seeds of this wheat were obtained from a German seed merchant under the name of "Persian Wheat."

Such an extraordinary immunity as distinguished this race from other races of *Tr. vulgare* and from the majority of races belonging to other species made me pay exceptional attention to this form, and the preliminary investigations so far made have revealed many further peculiarities in it. Although a number of morphological characters show that it belongs to common wheat, namely to *Tr. vulgare* var.

fuliginosum Al.[1]—a variety with black bearded hairy ears and red grains—yet this form is distinguished by many other characters from all our races of *Tr. vulgare*, which are not included in the classification of Koernicke.

The straw of this wheat is relatively full of pith. Usually, the varieties of *Tr. vulgare* have a hollow straw. The stem-knots of this wheat are visibly hairy: generally they are smooth in the species *Tr. vulgare*. The rachis of "Persian Wheat" is only half as broad as in common wheats. Ordinarily in wheat, only flowering glumes possess an awn. In this "Persian Wheat," the empty glumes are also awned[2]. Besides these, there are other small morphological differences in the structure of ear and leaf.

By crossing this wheat with other varieties of *Tr. vulgare*, it was observed that the percentage of successful results was very small. The same fact was repeated the next year. Usually in the same conditions the percentage of success in our crossings of different varieties of *Tr. vulgare* was very high. In the F_1 hybrids (♀ "Persian Wheat" × ♂ *Tr. vulgare* var. *lutescens* Keke.) about 70 % of the spikelets were sterile.

Finally in some of our crossings of susceptible races of wheat with immune ones, the susceptibility to mildew was clearly dominant, and F_1 hybrids were severely attacked by this fungus, as was seen in the experiments of Prof. Biffen with barley. But in the case of crossing "Persian Wheat" with a very susceptible race (*Tr. vulgare* var. *lutescens* Keke.), only after many efforts did we succeed in infecting slightly the F_1 hybrids, when in the same conditions the susceptible parent was severely attacked. In other words immunity in this instance is not "recessive."

Without dwelling further on this case, we must remark only that all that has just been said about this wheat allows a systematist to separate it from other varieties of *Tr. vulgare*, and makes it of special interest for the genetist.

An instance I came across in the investigation of barley is analogous to that described by Prof. Klebahn with willows. In our collection we had some samples of naked two-rowed barley from different parts of Russia. In the classification of Koernicke (1885), which we made use

[1] The name *Tr. vulgare* var. *fuliginosum* Al. is a collective name. We know quite ordinary susceptible wheats of the species *Tr. vulgare* which have black, hairy, bearded ears with red grains, like "Persian Wheat," and which must be placed in Koernicke's classification under the name "var. *fuliginosum*."

[2] This character is observable also in a few races of *Tr. vulgare*, belonging to different varieties, which are cultivated in Asiatic Russia and Persia.

of for the identification of barleys, all naked two-rowed barleys are represented by one variety—*Hordeum distichum* var. *nudum* L. To this variety we had referred, after identifying, all our naked two-rowed sorts of barley. But from observation of these barleys during two years, it was noticed that one of these parts (a pure line) was noticeably less susceptible to *Puccinia simplex* Eriks. than others, although growing side by side. This circumstance obliged me to pay more attention to the form in question, and in the result it was found that we had a very rare variety, which was wanting in the old classification of Koernicke. It is distinguished from var. *nudum* L. by weak development of the lateral spikelets (as in var. *deficiens* Steud.), and it was described only in the posthumous article of Koernicke (8), published in 1908, under the name of *Hordeum distichum* var. *nudideficiens* Keke. We received this variety from Caucasus (Daghestan).

I now append some examples of the connection of the fungal reaction of cereals with their genetics.

Characteristics of the Eight Species of Wheat in relation to Rust and Mildew.

After the work of Prof. Biffen and Nilsson-Ehle, which proved that immunity and susceptibility to fungal diseases is subject to the Mendelian rules of inheritance, it would seem very natural to suppose that the distribution of these characters amongst hundreds of varieties and races of cereals is quite accidental and without any definite order, as immunity and susceptibility may be combined by the aid of crossing with any group of morphological characters. Especially it would be natural to suppose it to be so in such a group as wheats, seven species of which (*T. vulgare, T. compactum, T. durum, T. polonicum, T. turgidum, T. Spelta* and *T. dicoccum*) have been proved to be fertile by crossing[1].

In reality, it is far from being so.

After investigation of about 800 sorts (represented by pure lines) of spring and winter wheat, collected from different parts of Europe and Asia, with regard to fungi prevalent in European Russia (*Puccinia triticina* Eriks. and *Erysiphe graminis* D.C.), and after classifying the sorts and tabulating these data, we came to the conclusion that in general each of the eight species of wheat, including dozens of varieties and races, has a definite characteristic behaviour in relation to fungi (16, pp. 29—54, 94—102).

[1] See works of Vilmorin, Beijerinck, Rimpau, Tschermak, Biffen and others.

For example, all the cultivated varieties of *T. durum* and *T. turgidum* are relatively immune to brown rust. All known wild and cultivated varieties of *T. monococcum* are perfectly immune to brown rust.

Many scattered data, which were found in old and recent literature, referring to the same or other varieties, prove the correctness of this conclusion (the literature is given in the above-mentioned paper 16, pp. 94—99, 5—6) and allow us even to apply, in some degree, this generalisation to the relation of the species of wheat to other fungi, as yellow rust *P. glumarum*. For instance, all varieties of *T. monococcum*, in accordance with published data, are perfectly immune to yellow rust. The different varieties of *T. durum* and *turgidum* are relatively immune to the same rust.

In general, the characteristics of the eight species of wheat in relation to the fungi by which they are attacked in Russia are as follows:

In relation to *Puccinia triticina* Eriks.[1]

susceptible	*resistant*
Tr. vulgare Vill. (there are a few immune races)	*Tr. durum* Desf.
Tr. compactum Host.	*Tr. polonicum* L.
Tr. Spelta L.	*Tr. turgidum* L.

perfectly immune

Tr. monococcum L.

Tr. dicoccum Schr. has both susceptible and immune races.

In relation to *Erysiphe graminis* D.C.

susceptible	*resistant*
Tr. vulgare Vill. (with the exception of a few races)	*Tr. durum* Desf.
Tr. compactum Host.[2]	*Tr. polonicum* L.
Tr. Spelta (a little less than the preceding ones).	*Tr. turgidum* L.
	Tr. monococcum L.

Tr. dicoccum Schr. has both susceptible and immune races[3].

[1] In the paper (16) are given the coloured plates, illustrating the differences in susceptibility of wheats and oats to brown, black and crown rusts.

[2] Only one race, belonging to the variety *Tr. compactum* var. *creticum* Mazz. proved to be relatively resistant to mildew. (The same was observed in America by Prof. Reed.)

[3] These characteristics of species are based on observations in fields under different conditions of manure, soil and climate. In greenhouses brown and yellow rusts do not develop to any considerable extent even by artificial infection; on the contrary, the

Only in *Tr. vulgare* and partly in *Tr. compactum* there are a few relatively immune races—exceptions to the general characteristic of these species, as susceptible to brown rust and mildew. One of the extreme exceptions is the above-mentioned "Persian Wheat"; several of the other more or less immune races, without any doubt, represent products of artificial crossing in recent times (16, p. 96).

Also, briefly speaking, in the group of wheats we meet with a case of specific peculiarities of whole species in their fungal reactions, notwithstanding the great polymorphism of these species.

The genetic significance of these data I shall shortly touch upon. The practical importance of this generalization for the selection of immune sorts is evident, for it simplifies considerably the work of the plant-breeder.

Characteristics of the Species of Oats in relation to Rusts.

As in Russia, so in England and other countries, oats are attacked very severely by two species of rust: crown or leaf rust *P. coronifera* Kleb., and black or stem rust *P. graminis* Pers.

Observations in Moscow showed that the majority of cultivated and wild oats are very susceptible to crown rust.

Of 323 sorts of *Avena sativa* L. (*A. diffusa* Aschr. and Gr., and *A. orientalis* Schreb.) examined, 297 belonging to the majority of known botanical varieties of cultivated oats (8) proved to be very susceptible; 21 less susceptible, and 5 races (belonging to the varieties var. *cinerea* Kcke., var. *brunnea* Kcke., and var. *grisea* Kcke.) proved to be relatively very immune to crown rust. The great majority of these

conditions of greenhouses are very favourable to mildew of cereals; the fungus in the conidial stage lives, for example, in greenhouses much longer than in the open air, and in general the plants are always more attacked by mildew in greenhouses than in fields. And even more or less resistant races of wheat, for instance different representatives of *Tr. durum, polonicum*, may be severely attacked in the greenhouse, as also under the bell-jar, by *Erysiphe graminis*. Immune races do not "lose" their immunity in greenhouses. The difference in susceptibility may be observed during the first days of infection, but the fungus develops better under these conditions. The most important fact is that even under these conditions such races as "Persian Wheat" or several races of *Tr. dicoccum* remain uninfected.

By what is said above is removed the controversy relating to the characteristics of *Tr. durum* and *Tr. polonicum* in our work and that of Prof. Reed in America (*Phytopathology*, Vol. II, No. 2, 1912), who defined the degree of susceptibility of 78 sorts of wheat by the aid of artificial infection of seedlings under bell-jars. The data of the characteristics of other species of wheat, in Russia and America, in general coincide.

last 26 races belong to varieties with black and grey grains (flowering glumes), and in general they are morphologically different from susceptible races (16, pp. 15—18, 94—95).

The various examined races of wild oats, belonging to the species *A. fatua* L., *A. Ludoviciana* Dur., and *A. sterilis* L., all proved to be very susceptible to crown rust.

Avena brevis Roth., *A. strigosa* Schreb., and *A. nuda* L. var. *biaristata* Aschr. and Gr. are relatively immune to this rust.

A different result was obtained with *black rust*. Of 350 examined sorts of cultivated and wild oats, belonging to nearly all known botanical varieties, *only two races* of the species *A. sativa* L. proved to be relatively immune. One of them (more resistant) belongs to the var. *brunnea* Keke., the other (less resistant) to the var. *montana* Al., two varieties with dark flowering glumes, and both these races are morphologically very different from the other susceptible races of the same varieties; for instance, they are very low plants and are characterized by very thin straw; practically both are of small value. ***All other cultivated and wild varieties belonging to six species are badly attacked by P. graminis.***

In other words, as a result of these observations, we come to a simple statistical conclusion as to the very slight probability of plant-breeders' finding oats resistant to black rust.

This conclusion will be quite logical if we remember that black rust of oats is a very weakly specialized fungus, which lives freely not only on the genus *Avena*, but also on *Alopecurus*, *Millium*, *Bromus*, *Lamarckiana*, *Phalaris*, *Koeleria*, *Festuca* and other genera of *Gramineae*. For genetists it is quite natural to conclude that if the fungus does not distinguish generic differences, there is very little probability that it will sharply distinguish racial differences in the species *A. sativa* L.[1]

[1] A similar argument may be applied to the ergot of cereals—*Claviceps purpurea* Tul. The same biologic race of this fungus, according to Stäger's experiments, lives on rye, barley, wheat, *Anthoxanthum*, *Hierochloa*, *Arrhenatherum*, *Dactylis*, *Poa*, *Briza* and other genera of *Gramineae*. Theoretically, therefore, there is very slight probability for plant-breeders to find a great difference among races of rye, barley and wheat in their susceptibility to this fungus. The great difference between rye, barley and wheat in the degree of infection by ergot (the two latter are very rarely attacked by ergot), is evidently connected with the different modes of flowering of these cereals. Rye usually flowers with opened glumes, wheat and barley with closed glumes; and the closed mode of flowering prevents the two latter from being infected by ergot. Eventually all the

Fungal Reactions of Species of Wheat and their Genetic Relationships.

Now turning again to the general characteristics of the species of wheat and oats in relation to narrowly specialized fungi, we shall see that they have not only significance for plant-breeders, but deserve serious attention on the part of students of genetics. As is known, the genetic relations of cereals are far from being solved. Every new criterion for the understanding of this problem is useful and valuable. It is especially so because the usual criterion of degree of affinity—sterility or *vice versa*—fertility of hybrids cannot always be used in the group of cereals. For instance, the seven species of wheat are so nearly allied that they give fertile hybrids. To understand the genealogy of this group, botanically restricted but nevertheless represented by an immense number of independent forms, we must employ finer methods.

On looking into the characteristics of eight species of wheat in relation to rusts and mildew, we cannot help being struck by their complete agreement with several genetic conceptions which are more or less established concerning their relationship.

eight species of wheat, according to our observations in Russia, may be slightly attacked by ergot, especially when wheats are cultivated side by side with rye.

In his second paper on "Studies in the Inheritance of Disease-resistance," *Journ. of Agr. Sc.*, Vol. IV, Part 4, 1912, Prof. Biffen communicates a curious fact of the occurrence in the F_2 hybrids of Rivet (*Tr. turgidum* L.) with several varieties of *Tr. vulgare* of some plants which were attacked by ergot, although the parent forms had never been seen to be attacked by this fungus. Prof. Biffen explains this fact, as a result of combination of two Mendelian factors of susceptibility to ergot, which are separated in their parents, and in separate form cannot produce the susceptibility of wheat to ergot.

The apparent contradiction of this case of distinct difference in susceptibility to ergot of wheat plants to the above-mentioned general statement, however, is easily removed by a simpler and more probable interpretation of this fact, than that given by Prof. Biffen.

Already in 1891 Prof. Rimpau, in his *Kreuzungsprodukte landwirtschaftlicher Kulturpflanzen*, pp. 11—12, had noticed the fact that in the same crossing of Rivet and *Tr. vulgare* there appear in F_2 hybrids *some sterile plants*. The sterile plants of cereals as is known flower usually with open glumes, remain many days in this state, and commonly are badly attacked by ergot. (See E. Tschermak, "Die Blüh- und Fruchtbarkeitsverhältnisse bei Roggen und Gerste und das Auftreten von Mutterkorn," *Fühlings landwirtschaft. Zeitung*, LX. 1906.) The sterile plants of F_1 of the hybrid of wheat and rye, for example, are severely ergotized.

Evidently the same fact of appearance of sterile plants was observed in the experiments of Prof. Biffen at Cambridge, and, as might be supposed, *these sterile plants* were attacked by ergot.

(*a*) *Tr. monococcum* L. is unanimously separated by genetists as an independent species from all the other seven, principally on account of the sterility of its hybrids with the other seven species of wheat, which has been proved by many investigators [Vilmorin, Beijerinck, Koernicke, Tschermak] (18), whereas all these latter (*Tr. vulgare*, *compactum*, *dicoccum*, *turgidum*, *durum*, *polonicum*, and *Spelta*) in crossing give more or less fertile hybrids[1]. The wild progenitors of our cultivated varieties of *Tr. monococcum* have been known for a very long time, whereas the wild prototypes of other species were found only a few years ago.

The genetic individualisation of *Tr. monococcum* is confirmed too by its fungal reactions. All its known wild and cultivated varieties are perfectly immune to brown and yellow rusts, and in this respect occupy a separate place among other species of wheat. In literature we find also indications that they are equally immune to stinking smut, *Tilletia tritici* (6).

(*b*) *Tr. compactum* Host.—dwarf wheats, according to modern views are so nearly allied to common wheats, *Tr. vulgare* Vill., that many authors unite them into one species. As we see in the table given above, their fungal reactions on mildew and brown rust are the same.

(*c*) *Tr. polonicum* L. and *Tr. turgidum* L. by systematists and genetists (Koernicke, Schulz, Beijerinck and others) are considered as species nearly allied to *Tr. durum* Desf. "Bei einzelnen Formen" (of *Tr. turgidum*)—says Schulz—"kann man zweifeln, ob man sie zu *Tr. turgidum* oder *Tr. durum* zurechnen soll" (10, p. 150). "*Tr. polonicum*"—says Beijerinck—"ist ohne Zweifel nur eine halbmonströse Abart von *Tr. durum*" (3). "Es giebt unter *Tr. durum*"—says F. Koernicke—"Sorten, deren Körner in der Länge, Gläsigkeit und der hellen Farbe genau denen von *Tr. polonicum* gleichen" (8, p. 397). These three species are alike not only in the structure of their ears, but also in their vegetative organs.

The characteristics of these species in their fungal reactions to *P. triticina*, *P. glumarum* and *Erysiphe graminis* are identical.

(*d*) *Tr. dicoccum* Schr.—Emmer—according to the current view, is a polymorphic progenitor species from which the susceptible and

[1] Only quite recently M. Blaringhem reported in *C. R. de l'Acad. des Scienc.*, 1914, T. 158, No. 5, that he succeeded in obtaining a few fertile hybrids ♀ *Tr. monococcum* L. var. *vulgare* Keke. × ♂ *Tr. durum* var. *Macaroni*, as a result of many crossings of these species. Many of his other crossings of *Tr. monococcum* with different species, proved to be unsuccessful or sterile.

immune species as *Tr. vulgare*, *Tr. durum* and others (except *Tr. monococcum*) are descended. This view, as is known, has been confirmed in our days by the finding of numerous forms of wild *Tr. dicoccoides* in Palestine and Syria by A. Aaronsohn, and in Persia by Strauss. In accordance with this view we find in the species *Tr. dicoccum*, races both immune and susceptible to mildew and brown rust.

The susceptibility to brown wheat rust and mildew of some races of wild *Tr. dicoccoides*, which were kindly sent to me by Mr A. Aaronsohn and were examined in Moscow in their relation to fungi, once more confirmed their near relationship to cultivated wheats. Prof. Tschermak and A. Aaronsohn proved also that *Tr. dicoccoides* gives fertile hybrids with cultivated wheats (14).

Fungal Reactions of Species of Oats and their Genetic Relationships.

The same parallelism of fungal reactions and the genetic relations of plants is observable in oats. According to the present views which are based on systematic study and experiments in the crossing of wild with cultivated forms, oats have a *polyphyletic* origin. According to recent work by Dr Trabut in Algeria (13), Dr Thellung in Switzerland (12) and A. F. Malzev in Russia, we regard *A. fatua* L., *A. sterilis* L. and *A. Ludoviciana* Dur. as the ancestors of our cultivated forms *A. sativa* L. (*A. diffusa* Asch. and Gr., and *A. orientalis* Schreb.)[1].

As was said before, the representatives of all these species examined in Moscow proved to be equally susceptible to the narrowly specialized crown rust *P. coronifera*, like the majority of cultivated oats.

A. strigosa Schreb. and *A. brevis* Roth., two rarely cultivated species, which are morphologically much alike, and which by our crossing in Moscow proved to give fertile hybrids[2], are relatively immune to crown rust. In this respect, they are distinguished from the above-mentioned wild and cultivated oats.

Many attempts at crossing of *A. strigosa* (two varieties) with cultivated *A. sativa* (*A. diffusa* Asch. and Gr.) repeated during two years in the Moscow Agricultural Institute proved to be unsuccessful, whereas under the same conditions the crossing of *A. fatua* and *A. sativa* was

[1] The origin of naked cultivated oats, represented by very different morphological forms hitherto, is far from being clear.

[2] This crossing was done after their similar reaction on fungi was known to us, and in this case the similarity in fungal reaction suggested the possibility of crossing these two species.

successful and the hybrids were fertile. This fact confirms the peculiar genetic place of *A. strigosa* amongst other cultivated oats, which was suggested by its fungal reaction.

Finally, the isolated genetic position of *A. strigosa* is proved by the fact, which we find recorded (11), that it is also immune to smut *Ustilago avenae*, whereas the cultivated forms of *A. sativa* are usually severely attacked by this fungus[1].

Some other examples might be given illustrating how, by the aid of genetic knowledge, the differences of various cereals in their behaviour to fungi become clearer; and *vice versa* how fungal reaction helps us to understand the genetic relation of plant forms. But the complete enumeration of all these examples would be out of place in this paper.

One objection may be raised against the broad application of fungal reaction for genetic purposes.

This is the phenomenon of so-called "bridging species"—cases in which the biologic form of a fungus, after living on certain of its host-species ("bridges"), becomes capable of infecting a species, which it cannot infect after living on its other host-species. Pole Evans also showed with black rust of wheat *P. graminis*, that very susceptible F_1 hybrids of immune and susceptible varieties may serve as a "bridge" between susceptible and immune sorts.

But against this, in the first place, there are only very few cases known of existence of "bridging species[2]." In the case of fungi of cereals, they are found only in *Puccinia graminis forma* sp. *tritici* and not in *P. glumarum*, *P. triticina*, *P. simplex* (2, 5). Furthermore, we must not forget that the biologic forms of *P. graminis* are relatively weakly specialized fungi; for example, *forma* sp. *tritici*, as has been shown in different countries, can infect not only wheat but, more or less, barley also[3].

[1] Morphologically *A. strigosa* is very like *A. barbata* Pott.; and Dr Trabut and Thellung account the latter as the progenitor form of the former species. It would be very interesting, therefore, to know the fungal reaction of *A. barbata*.

[2] "Bridging species" are found in *P. Symphyti Bromorum* F. Mull. (M. Ward and Freeman), *Erysiphe graminis* D.C., living on *Bromus* (E. Salmon), in *P. graminis forma* sp. *tritici* (Freeman and Johnson), and *Sphaerotheca Humuli* on *Alchemilla* (Steiner).

[3] One of the conclusions to which Freeman and Johnson came after their numerous experiments with biologic forms of *P. graminis* is that "two biologic forms may inhabit the same cereal without being identical" (5, p. 75). This statement, as also the fact, well-known to mycologists, of the existence in Australia of a different race of *P. graminis f. tritici*, which cannot infect *Berberis*, and all that is known about the differenee in

Secondly, as has already been remarked by Prof. Biffen (2, pp. 428—429), two facts speak against the great *rôle* of "bridging species": first, the fact that immune varieties may be grown dozens of years in close proximity to susceptible ones, severely attacked by fungi, and still remain quite resistant; the second fact is the possibility of obtaining immune races by crossing.

Finally, it may well be conceded that the exactitude of fungal reactions must be studied before using them for genetic purposes, as with reactives in chemistry. With fungi of cereals, this preliminary work is happily already more advanced than it is with any other group of fungi[1].

In conclusion, we need only remark that the degree of sensitiveness of fungal reaction, for instance, with cereals up to the present time is not exceeded by that of the so-called "serum" methods, applied to plants. It is hardly necessary to add that fungal reaction technically is much simpler than the "serum" method in its application for the recognition of individuals.

March, 1914.

Note. While this paper was being printed there appeared in the *Zeitschrift für Pflanzenzuchtung*, April 1914, Bd. II, Heft 2, a very interesting paper by Dr Zade entitled: "Serologische Studien an Leguminosen and Gramineen." In his investigation with cereals, using the "serum" reactions as a chemical test for the genetics of these plants, Dr Zade, as we understand his tables, comes to quite the same conclusions concerning the genetic relationship of oats and wheats, to which we came using fungal tests for the same purpose.

So, the tables of experiments on different species of *Avena* show that the *A. fatua* gave almost the same reaction as *A. sativa*.

A. strigosa, which according to our investigation is genetically more distant from *A. sativa* than *A. fatua*, gave in the experiments of Dr Zade, when he used the weak "serum" solution of *A. sativa*, a much weaker reaction than *A. fatua*.

specialization of this fungus in different countries, suggest the suspicion that it is possible, in the same biologic form, more than one race of fungus may exist, and these races differ more or less in their specialization. And, perhaps, in some instances the same phenomenon of "bridging species" is the result of unconscious selection of different races of fungi by the aid of different hosts. Certainly this question can be solved only by means of pure cultures of fungi.

[1] For the purpose of greater exactness, the conditions of the use of fungal reactions must be borne in mind too, because, like chemical reactions, they change under differing conditions; although in general the *rôle* of external conditions (climate, soil, manure, etc.) in changing immunity is very often too exaggerated in mycological literature (16, pp. 99—102).

The similarity in the case of wheats is even more striking. So, *Tr. monococcum*, according to Dr Zade's experiments, occupies a separate place. Three species *Tr. durum*, *Tr. polonicum* and *Tr. turgidum*, which have the same fungal reactions, had the same "serum" reaction. The *Tr. vulgare*, *Tr. compactum* and *Tr. Spelta*, both in relation to fungi and in their "serum" reaction are very similar.

The similarity of "serum" reaction of *Tr. dicoccum* with that of *Tr. durum*, *polonicum* and *turgidum* again does not contradict our results; for probably Dr Zade used for his experiments a variety of *Tr. dicoccum* which is immune to brown rust. It is to be regretted that Dr Zade does not give the names of the varieties with which he worked. This makes a complete comparison of results difficult.

On account of the great polymorphism of the species *Tr. dicoccum* (and *Tr. dicoccoides*) which is very important for the construction of the genealogy of wheats and which was not taken into consideration by Dr Zade, we cannot agree completely with the concluding genealogical table of wheats, given on p. 144. We believe that the difference in varieties which occurs in species of cereals cannot be neglected in genetic work.

The striking parallelism of fungal and "serum" reactions once more confirms the possibility of using both these methods for genetic and systematic purposes.

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HEREDITARY LEFTHANDEDNESS, WITH A NOTE ON TWINNING.

(STUDY III.)

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This study is based chiefly on data collected by Dr Albert Ernest Jenks, Professor of Anthropology, University of Minnesota, in the form of questionnaire blanks and filed at the Eugenics Record Office, Cold Spring Harbor Long Island. My best thanks are due to Professor Jenks for his generous contribution, and to Dr Charles B. Davenport for kindly placing the blanks at my disposal for examination and record in the form of charts. The material includes also six pedigrees from various other sources. Respecting this body of data the primary objects here sought are to make it more widely accessible by putting it into more available form, and to analyze it with a view to testing my earlier conclusions, namely, that lefthandedness is hereditary and closely follows the behaviour of a Mendelian recessive character. The questions of the cause and anatomical basis of this trait will not be touched upon[1]; these are similar to those involved in physiologic "unit characters" generally. Scarcity of data respecting ambidexterity precludes effective discussion of this character from a hereditary viewpoint. The pedigrees include a considerable number of twins; this circumstance invites a consideration also of hereditary twinning; and explains the inclusion of pedigree chart Fig. 80.

[1] This matter is discussed in my earlier papers.

The main body of data is somewhat unsatisfactory in respect of the absence of collateral histories; these are indispensable for complete studies seeking evidence of Mendelian inheritance; however, the abbreviated histories are still of the greatest value, and warrant careful analysis and permanent record.

Three remarkable five-generation discontinuous histories of hereditary lefthandedness are included, charted in Figs. 1 to 3. Here the absence of collateral histories and record of the total number of individuals in the several childships is especially regrettable, and precludes attempts at further analysis.

Four histories of direct transmission are charted in Figs. 4 to 7. Chart 6 is noteworthy in that all the affected individuals are males. Chart 5 suggests recedence of the sinistrality factor, since the lefthanded individual of the last generation is the result of a double lefthanded mating. However, the three lefthanded individuals of the preceding generation contradict this general deduction, since the female parent was presumably righthanded. Moreover, the fact that the mother had also 3 nieces, 1 nephew, and 3 great-nieces who were lefthanded suggests almost equally cogently that in this family lefthandedness was dominant. This particular history will be further discussed below in an attempt to interpret apparent cases of dominant lefthandedness.

The blanks include also eleven, in six of which only an uncle or an aunt of the lefthanded individual is known to be similarly affected, and in five only a great-uncle or great-aunt. Several of these are charted and discussed below (Figs. 69—74).

Forty-eight blanks record lefthandedness in both parent and child. The histories with more than one affected member in the fraternity are charted in Figs. 8 to 16. This set includes only one instance of double lefthanded parentage (Fig. 16). The expected total frequency on Mendelian assumptions is vitiated by the presence of one "normal." In the other histories of this set, one of the parents is normal as respects use of hand. On the assumption that the "normals" are heterozygotes the majority of the charts fulfil Mendelian expectancy for $RR \times DR$ crosses (Figs. 10, 11, 13, 9, and 15). Chart Fig. 8 suggests dominance of the lefthanded trait, as also to a lesser extent, in view of the limited childships, charts Figs. 12 and 14. A strict Mendelian interpretation of this group of charts (Figs. 8 to 16) involves the further assumptions of degrees of bias to sinisterity and variation in relative hereditary prepotency. An attempt will be made to support the legitimacy or plausibility of these assumptions below.

The remaining 39 histories of parent-child "transmission" may be summarized thus:

father to son	9
father to daughter ...	10
mother to son	13
mother to daughter ...	7

The numerical variation is small, and of no significance as indicating prepotency of one or the other parent to transmit the character differentially to either sex.

Adding to these numbers those from charts 8 to 16 the same conclusions become still more obvious, thus:

father to son	9 + 8 = 17
father to daughter ...	10 + 5 = 15
mother to son	13 + 6 = 19
mother to daughter ...	7 + 5 = 12

The following table summarizes the data respecting fraternities of four or more individuals:

Number of lefthanded and righthanded in fraternities of four or more individuals. Parent-child histories.

Left	Right
5	4
3	7
1	4
1	3
3	3
1	3
3	3
2	2
1	5
1	7
1	5
1	6
2	4
1	4
2	7
1	3
1	5
1	4
Totals 31	79
or 1 :	2·55

Four fraternities give the expected proportion for a $DR \times RR$ cross; several others may be said to approximate expectancy. But only by assuming that a certain number of the normal consorts were duplex for dextrality (DD), and that the character is occasionally imperfectly dominant, can the ratio of the totals (1 : 2·55) be made to suggest Mendelian principles.

Six blanks record lefthandedness appearing in great-grandparent, parent and child (charts, Figs. 17 to 22). Pedigree chart, Fig. 22, is of especial interest in that it gives a final generation of two pairs of twins, all lefthanded. Since both pairs are severally of the same sex, they are presumably identical: the fact that identical twins are rarely unlike with respect of use of hand, suggests very strongly the hereditary character of this trait. The facts shown in this chart as also in chart Fig. 17, again suggest dominance of the lefthandedness factor in certain strains; or "imperfection of dominance" of the righthandedness factor.

Fourteen histories show transmission from great-grandparent to child (Figs. 23 to 36). The absence of information concerning collateral lines, and of complete childships in every instance, forbids deduction beyond the general statement that lefthandedness in the ancestry presages a certain amount of lefthandedness among offspring. The inference is suggested that the normal parents are heterozygous, in which event the Mendelian proportion of 1 lefthanded to 3 righthanded would be expected. This expectation is practically met in charts 24 and 33. With the exception of charts 35 and 36, the remaining charts of this group also are in close accord with Mendelian formulae.

Four blanks record a four-generation history of lefthandedness with the direct ancestors of the penultimate generation normal (Figs. 37 to 40). All show a lefthanded offspring from a double "normal" mating. The ancestry of the normals concerned, however, suggests their heterozygous nature; one in every four would therefore be expected to be lefthanded. The histories as given are in close accord with this ratio.

Sixteen blanks record lefthandedness in three consecutive generations (Figs. 41 to 56). Chart, Fig. 41 is interesting in that the direct ancestors in the two earlier generations were both ambidextrous, suggesting a specificity of this condition as compared with dexterity and sinisterity. In charts, Figs. 44, 50 and 52, the lefthanded condition is distributed in the fraternities approximately as would be expected in a $DR \times RR$ cross, most probably the actual fact.

Hereditary transmission from grandparent to child appears in 44 blanks. The distribution may be summarized thus:

Grandfather	to	son's	son ...	...	9	19
„	„	„	daughter	...	9	
„	„	daughter's	son ...	...	9	29
„	„	„	daughter	...	2	12
Grandmother	to	son's	son ...	...	10	11
„	„	„	daughter	...	3	24
„	„	daughter's	son ...	...	2	
„	„	„	daughter	...	9	11

The summary seems to show that a grand-daughter rarely inherits lefthandedness from her mother's father, and a grandson from his mother's mother; but the disproportion is due more likely to the relatively small number of histories as the grosser summaries show (second and third columns). In this group again nothing appears sufficiently striking to indicate prepotency on the part of one or the other parent (grandparent).

The following table summarizes the data for this group where the fraternities are larger than three individuals:

Grandparent—child histories.

Left		Right
1		5
1		7
2		3
1		6
1		4
2		3
1		6
1		4
1		3
1		3
1		6
1		3
1		4
1		4
1		4
1		4
Totals 18		69
or 1	:	3·83

On the most probable assumption, namely, that the parents involved are heterozygous, the expected proportion of 1 to 3 is strikingly and suggestively met by 1 to 3·83. Moreover, three fraternities contain lefthanded and righthanded individuals in the exact proportion of 1 to 3; and six in the proportion of 1 to 4.

One hundred and sixty-eight (168) blanks record absence of lefthandedness in the direct ancestry; including 51 (26 ♀ + 25 ♂) individuals to and including all the great-grandparents, 92 (40 ♀ + 52 ♂) to and including all the grandparents, and 36 blanks (43 lefthanded individuals) with statements, "unable to get information," or "so far as is known." This set includes a fraternity of three lefthanded, including a pair of twins (Fig. 57). Of another lefthanded individual the statement is given that the lefthanded character is considered a "birth-mark," the mother having been obliged by reason of injury to right arm to use the left during pregnancy. Another history, in which no trace of lefthandedness is said to appear back to and including the great-grandparents, includes two lefthanded males among 9 other males and 3 females, including two pairs of ordinary twins (Fig. 58). Still another records an individual "lefthanded from birth," an extraordinary fact.

This latter group of data admits of three interpretations: (1) absence of hereditary influence; (2) spontaneous origin of lefthandedness, which may be thereafter transmitted by heredity; and (3) the "normals" may all be heterozygotes. Moreover, mild degrees of lefthandedness may have escaped notice or may have been masked or lost in later life; or the information may in a certain number of instances be inaccurate. That it does not indicate absence of hereditary influence appears from such pedigrees as the one shown in Fig. 59 where the direct ancestors for two generations on both sides are normal. This could be carried backward in time to any previous generation without violence to Mendelian principles. This chart is Fig. 10 of my paper, "Studies in Human Heredity," 1912. These three histories, Figs. 57, 58 and 59, as also Figs. 60 and 61, suggest forcibly that lefthandedness cannot be due to the absence of a factor. As stated in an earlier paper the antithetic condition due to factor absence is probably ambidexterity. This will be further discussed below.

The data in charts Figs. 60 to 62 were supplied by Professor F. A. Hodge of Winthrop College, South Carolina. Concerning the penultimate fraternity of six of chart 62 the statement is recorded that these children were "made to wear gloves on the left hand when infants

to keep them from becoming lefthanded. Charts 60 and 62 both contain a fraternity fulfilling expectancy from a $DR \times RR$ cross.

The following table summarizes the data for this group:

Histories of fraternities with righthanded ancestry.

Left	Right	Left	Right
2	5	1	4
1	4	1	3
1	3	1	3
1	4	1	6
2	6	2	12
1	8	1	3
1	7	1	4
1	4	2	4
1	4	1	6
1	4	3	3
1	3	1	5
1	3	1	4
1	6	1	3
2	4	1	7
1	3	1	5
1	4	2	4
1	4	1	8
1	3	1	4
1	5	1	10
1	6	1	3
1	6	1	5
1	3	1	3
2	5	2	6
1	8	2	3
3	5	2	4
1	3	1	6
1	6	2	4
2	4	2	5
1	5	1	4
2	5	1	3
2	4	1	3
1	4	1	4
1	7	1	3
2	3	2	3
1	6	—	—
	Totals ...	90	321
	or	1 :	3·56

The most probable assumption is that the parents involved are heterozygotes. The sum ratio, 1 to 3·56, is very close to expectancy. Sixteen fraternities actually show the 1 to 3 ratio; and sixteen others a 1 to 4 ratio. Still other ratios are significantly close. This body of data seems all but conclusive in showing the general recessive nature

of the lefthandedness "determiner," and the general agreement with Mendelian laws of inheritance.

Charts Figs. 63 to 67 present striking histories from the foregoing group. In the first four the ancestors to, and including, the grandparents (Fig. 64; also great-grandparents) are said to have been normal. The immediate parents may be presumed to be in the simplex condition (heterozygous dominants). These four pedigrees show unmistakeably that lefthandedness is due to a positive factor (presence of a "determiner"). The reported incidence of lefthandedness in the several fraternities defies reconciliation with strict Mendelian principles[1]. Similarly, pedigree Fig. 67. Here the entire childship of a double lefthanded mating is reported normal. This history, as also many other facts, show that righthandedness also depends upon a positive factor. Charts 63 and 67 are directly contradictory, not only with reference to each other, but also as concerns numerous other pedigrees and the principles of lefthanded inheritance (Mendelian) deduced from them. These will be further discussed among exceptions considered below.

Chart Fig. 68 is from the parent-child group. It is especially interesting in fulfilling the Mendelian expectation of a $DR \times RR$, the probable cross.

Charts Figs. 69 to 74 are the most important and most extensive among the "collateral" group noted above. Concerning the lefthanded male parent of 71 it is recorded that he "bats lefthanded in base-ball." The fraternities of Figs. 69 and 74 give the Mendelian ratio for the most probable crosses involved, namely $DR \times DR$, and the remainder are not seriously at variance with expectancy in view of the probable ancestry.

Chart, Fig. 75, was contributed by Professor F. A. Hodge, Winthrop College, S.C.; and Fig. 76 by Mr Frank J. Sconce, of Fairview, Illinois (filed at the Eugenics Record Office, Cold Spring Harbor, Long Island). Both histories demonstrate the recessive nature of decided lefthanded bias, and leave no doubt regarding the general valency of Mendelian laws in hereditary lefthandedness. Chart Fig. 77 (Prof. Hodge) appears to countervail this deduction; but the reported lefthanded parents have but a mild bias, as I have learned from subsequent inquiry, since "they write with the right hand, and do only certain things with the left hand." Complete recession, under the circumstances, would hardly be expected. This pedigree was charted as Fig. 32 of my "Studies in Human Heredity."

[1] The possibility is suggested that lefthandedness (and ambidexterity) may be degrees of the same condition due to an "inhibitor" to righthandedness.

Chart Fig. 78 was given to me by Professor J. P. Campbell, of the University of Georgia. It shows peculiarly well the inadequacy of only the direct ancestry in a study of hereditary lefthandedness and the hereditary influence and significance of collateral ancestry, and suggests the rarity of "pure" strains with respect of dexterity. Also, it is of especial interest in giving a pair of duplicate twins, only one of which is lefthanded (and both "cross-eyed"). This condition is the exception of what usually obtains in the case of such twins. It suggests more forcibly perhaps than any other fact the verity of "degrees of bias" to use of hand.

Chart Fig. 79 is supplied by Mr J. H. Green, of Clifton Forge, Virginia. It is a fairly complete five-generation history of a large family in which appear both lefthandedness and a tendency to twinning. Nine pairs of twins appear; one member of pair 5 is lefthanded; but these are not identical twins. The other members of this fraternity are another pair of twins; and the lefthanded condition appears in the expected ratio for the $DR \times DR$ cross. Coincidence of lefthandedness and twinning in the same fraternity is frequent; but no very likely explanation suggests itself regarding causative relationship. The explanation of lefthanded duplicate twins however lies most probably in the ancestral presence of both conditions; hereditary twinning would then produce a lefthanded *pair* when the determiner for lefthandedness was coincidently present in the case of a duplicate set (cf. Figs. 22 and 57). The complete absence of lefthandedness in the product of mating $\varphi X \times \sigma Y$ is interesting as suggesting a "pure" extraction from a "tainted" stock.

A summary of the 79 histories charted gives a proportion of 173 lefthanded males to 143 females. This result is the opposite of the one usually recorded, namely, that females show a heavier incidence of lefthandedness. My earlier studies gave an approximate equality; and the discrepancy here noted is not sufficiently great to have significance as contradicting the general conclusion that males and females are equally "susceptible" to lefthandedness.

Discussion.

No attempt has been made in the foregoing to explain contradictions and exceptions. In view of the present study, and of two earlier ones, no one, I believe, will seriously dispute the conclusion that lefthandedness is hereditary. That it follows in inheritance Mendelian principles, a

number of apparently serious contradictions may perhaps still give some ground for question. However, the main mass of data points to strict Mendelian hereditary conduct. The proportion of 1 lefthanded to $1\frac{1}{2}$ righthanded obtained in both of my earlier studies when all histories were included cogently suggests the operation of Mendelian laws, when account is taken of the fact that the majority of the crosses were either $DR \times DR$ (1 : 3) or $DR \times RR$ (1 : 1). Moreover, a considerable number of histories gave approximately the expected proportions for such crosses, where the most probable condition of the parents was indicated by the ancestry.

Major C. C. Hurst, on the basis of a study of a number of pedigrees, confirms my conclusion that lefthandedness is a Mendelian recessive[1]. Dr E. Stier's pedigrees of double lefthanded matings (6 cases) force the same conclusion, when allowance is made for occasional imperfection of dominance or slight degrees of bias. In no instance are *all* of the offspring lefthanded; but with one exception the majority are lefthanded.

My present study gives additional, and more complete, confirmation of my earlier conclusion. A general survey of these charts permits no doubt that lefthandedness is hereditary. This conclusion is further suggested by cases of duplicate twins, where both members of the pair are lefthanded (Figs. 22 and 57—exception Fig. 78). When therefore lefthandedness appears in a fraternity, at least one of the parents is simplex for the lefthandedness factor, frequently perhaps both. Summarizing the 69 pedigrees in the group where the ancestry is said to be normal, and where the fraternities contain four or more individuals, the ratio of lefthanded to righthanded is as 1 : 3·56 (page 73). This is so close to Mendelian expectancy for $DR \times DR$ crosses as to furnish almost a demonstration of the actual fact. The slight preponderance of righthandedness is undoubtedly to be accounted for by the occasional $DD \times DR$ crosses with possible imperfections of dominance. Moreover, the inclusion of sixteen 1 : 3 ratios, and the same number of 1 : 4 ratios still more firmly supports the conclusion. Likewise, the ratios of the other two tables above discussed and the average of all, 1 : 3·31. A number of the pedigrees here charted give also the exact ratio expected on Mendelian assumptions.

Again, double lefthanded matings give complete lefthanded fraternities (Figs. 5, 16—almost, 75, and 76), as expected from pure recessive parents. The apparent contradiction, Fig. 77, is to be explained on

[1] Likewise more recently Professor Francis Ramaley (*Am. Nat.*, Dec. 1913).

the basis of faulty record, since later inquiry elicited the information that the "lefthanded" parents were only slightly so, and that they wrote with the right hand. The very fact of the ability to write well with the right hand indicates slight bias, which would be expected to be only imperfectly conformable with strict Mendelian formulae. Chart 67 may perhaps legitimately be similarly interpreted, in the absence of more definite information. The contradictions, Figs. 5, 39, 63, 64, 65 and 66, still remain. Here presumably "normal" parents have all lefthanded children.

Already in my first study I was led to the idea of "degrees of bias." This phenomenon has only become more clear in my second and the present studies. How shall we account for the origin of degrees, and what is their significance in inheritance? This discussion further involves a consideration of the apparent dominance of lefthandedness in certain strains (Figs. 5, 8, 12, 14, 22, 46).

Both phylogenetic and ontogenetic facts show that the ancestral condition with respect of use of hand was ambidexterity. Anthropological data point in the same direction. Both lefthandedness and righthandedness represent variations from the ambidextral condition. The antithetic condition to both (i.e. the one characterized by nulliplicity or absence of determiner) is natural ambidexterity.

The spontaneous origin of lefthandedness and righthandedness in ontogeny suggests, granting full validity to the biogenetic law, that racially these conditions also arose spontaneously, i.e. discontinuously; not as the result of a slow accumulation of slight variations or fluctuations—a mutation, not an acquired character now become hereditary. The fundamental anatomic variation presumably inheres in a foetal asymmetry of the cerebral blood supply producing probably an unequal development (microscopic) of the hemispheres. This phase of the question is discussed in my former papers.

The usual variation both phylogenetic and ontogenetic is toward a righthanded bias. Ancestrally those individuals that varied toward a lefthanded bias came, perhaps, to some extent under the influence of natural selection. Present lefthanded individuals would thus trace their ancestry back to those earliest ancestors who escaped elimination.

The dominance of righthandedness over lefthandedness is also in accord with the principle of progressive evolution through discontinuity. There can be little doubt that under present conditions of community life, dexterity gives considerable advantage over sinisterity or even perhaps ambidexterity. Indeed righthandedness may easily be conceived

of as having contributed to a considerable degree to the attainment of the present level of civilization. The teleological significance of the dominance of righthandedness is obvious. However, the cause of prepotency is here as obscure as elsewhere. The evidence on this point is conflicting; now ancestral conditions (racially older) seem to be dominant, in other instances the new, and again the more intense, are prepotent. Progress by discontinuous variation would seem to be possible only through the prepotency of determiners signifying improved adjustment.

Genetic studies of bias toward use of one or the other hand promise to throw some light on the question of the stability of "determiners" or "genes." Degrees of prepotency of characters, so strikingly evident in the case of the heredity of use of hand, suggests considerable lability of genes.

Elucidation of the whole matter proceeds with much plausibility from the recent suggestion by Miss Elderton, that dominance may not attach to a character but to the individual with the character, "i.e. that the same character can in certain individuals be dominant and in others be recessive," p. 34. Whether by reason of a numerically preponderant variation towards dexterity relative to sinisterity, or a numerically equal but more decided bias in that direction, righthandedness early came to be the numerically predominant condition, now present approximately in the ratio of 4 to 1. Under later civilization lefthandedness became an increasingly greater handicap. Thus individuals in whom this handicapping character of great intensity was dominant may have been eliminated through differential selection. In this wise only recessive lefthandedness of high degree has come to remain almost exclusively, and the ideas of dominance and recession have come to be attached to the character itself. Less extreme degree of lefthandedness due to a greater power of adaptability and training may thus have to some extent remained of a dominant nature.

Thus the parents in charts 63 to 66 may possibly have been left-handed individuals of so slight degree as to have escaped notice as such in childhood or forgotten at maturity, but of dominant nature; then, whether duplex or simplex, all the children would be expected to be lefthanded. Likewise in pedigrees like Figs. 67 and 77 the parents may be simplex dominants (for lefthandedness), thus of mild degree, and their naturally lefthanded offspring of slight bias may by slight or even unpremeditated training have been made to acquire right-handedness.

In conclusion; the data with reference to hereditary lefthandedness are overwhelmingly in accord with Mendelian theory. Certain real exceptions appear; these, however, are no more forbidding than those relating to certain other human characteristics, and readily permit of complete reconciliation by aid of the suggested hypotheses of degrees of intensity of bias, and mild dominant and intense recessive strains of lefthandedness. Whether this interpretation of "exceptions" be thought admissible or not, the mass of strictly conformable evidence and data is still so considerable as to compel the conclusion of the general validity of Mendelian law in the inheritance of lefthandedness.

A Note on Twinning.

Davenport has called attention to the inheritance of this tendency, and illustrates the phenomenon by two suggestive pedigrees recorded by Stocks and by Wakley. Dr James Oliver has more recently published abbreviated pedigree charts of 28 sets of twins, showing an hereditary tendency to twinning. The data are perhaps not yet of sufficient quantity to warrant deductions as to laws of twin heredity. Charts 79 and 80 show perhaps simply that twins in one's ancestry gives a fair prospect of twins in one's progeny. The fact that "normal" parents may have twins suggests that the factor (or factors) for twinning is a Mendelian recessive. This is further suggested by the circumstance that a normal (A) × twin (B) cross produced a fraternity of 10, all normal (Fig. 79). However, the pedigrees cited by Davenport seem to leave little doubt that the twinning tendency is in some instances "dominant"; probably a complex of factors is involved. Moreover, contrary to Dr Oliver's data, which shows a preponderance of female to male twins to the extent of 2 to 1, chart 79 shows a preponderance of males in almost the reverse ratio, 7 females to 11 males. The same deduction is indicated in a pedigree (Fig. 80)[1] supplied by Mr R. G. Reaves, of Greenville, Tennessee, in which there are 14 pairs (1 with sex unknown) including 18 males and 8 females (6 male pairs, 1 female pair, and 6 bi-sexual pairs).

[1] This is of course only a partial pedigree in the earlier generations; it includes, however, all known pairs of twins; the total number of descendants of the first mating charted is approximately 1100. Childship (M) fulfils Mendelian expectancy for the probable cross, $DR \times RR$.

Twin birth is presumably simply a lesser degree of multiple-birth, characteristic of lower mammals. In general, the higher the degree of development (specialization) the fewer the individuals at a birth. In horses twins are rare; in certain sheep they are the rule. Fewer individuals at a single birth implies evolutionary advance. From this viewpoint, the suggested recession of human twin birth to the more normal birth becomes intelligible in terms of the dominance of a derived or advanced, over a racially older, condition.

The pedigrees given by both Oliver and Davenport show transmission through males only. In both of my own histories, the transmission is more frequently through the female line.

On the basis of the experimental evidence from invertebrates, Amphioxus, and amphibia identical (monochorial; duplicate) twins are commonly explained in terms of a relatively independent development of accidentally separated blastomeres, at the two-cell stage of development. The stimulus or causative agent to separation (or "budding" of embryonic disc as in armadillo—Patterson; or "fission,"—Assheton) may conceivably be either mechanical or chemical (nutritive—Patterson). If the former, an inheritance of the tendency would hardly be expected. The latter circumstance seems the more probable. The chemical factor for disjunction might inhere either in the male or the female gamete. On this basis heredity of duplicate twins becomes intelligible.

But occasionally bi-sexual (heterosexual or ordinary) pairs of twins occur in such pedigrees (*vide* Oliver, Davenport, and Figs. 79 and 80). Ordinary twins are commonly supposed to be due to the practically simultaneous ovulation of both ovaries, or a double ovulation on the part of the functioning ovary. Such tendency may well be hereditary, but it could obviously show itself only in females. It is inconceivable how the spermatozoon could exert influence on the ovary such as to stimulate to double ovulation. Inheritance on the above explanation would have to follow an alternation of generations where males intervened; i.e. only females of such a pedigree could determine twins of the ordinary type. In view of the several pedigrees here involved, it must remain doubtful whether such is the case, though pedigrees 79 and 80[1] are suggestive of this condition. Both ordinary and identical twins appear in the same pedigree, and both conditions seem hereditary according to similar principles, the tendency for both being apparently transmitted either by male or female. It seems in the light of known

[1] Twin pairs of the same sex are known to be "identical" only in the instances so specified; regarding the remainder the information was uncertain.

pedigrees of human twinning that the explanation of ordinary and duplicate twins is probably not as simple a matter as has been supposed. It would appear that the sperm is as potent as the egg, in determining twins, either ordinary or identical.

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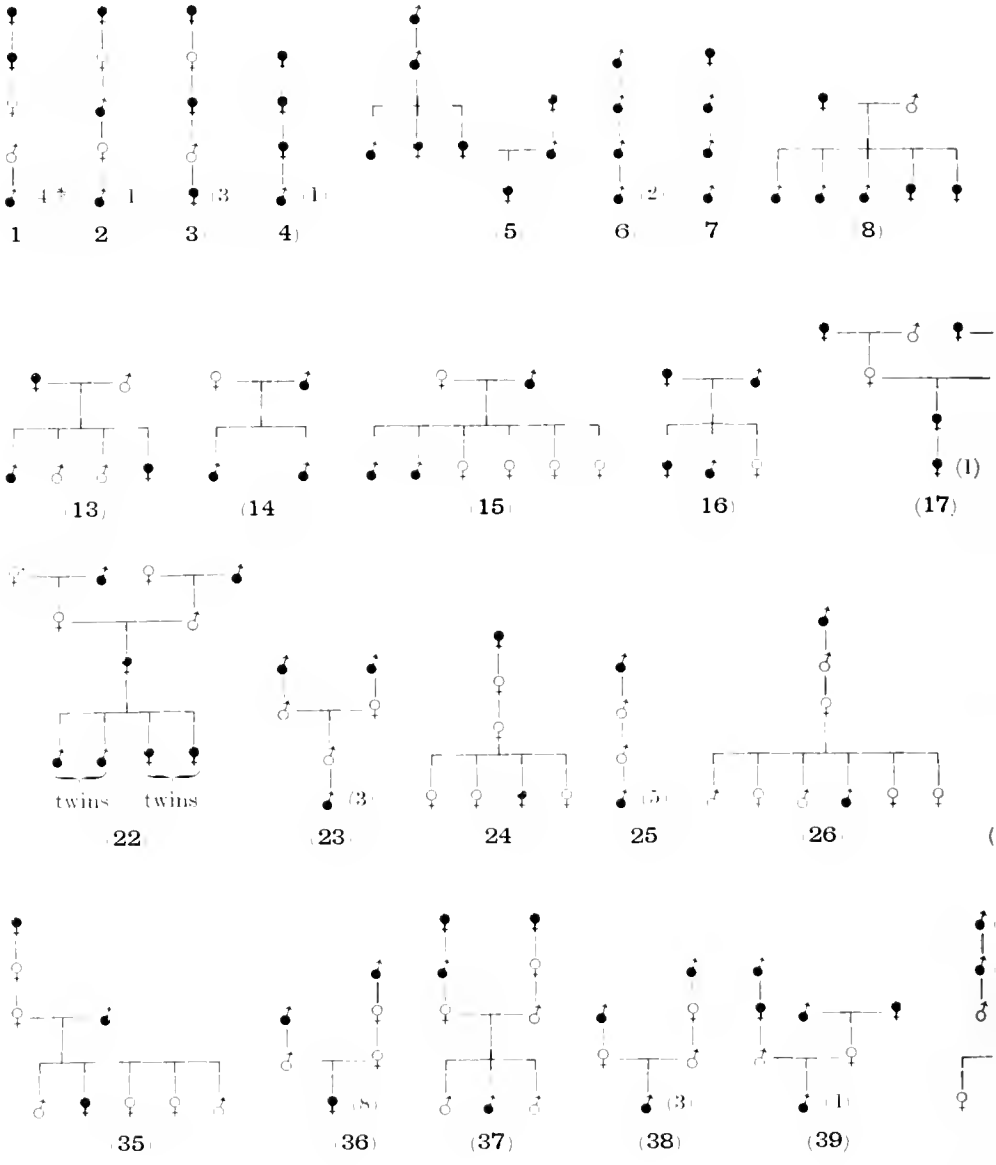
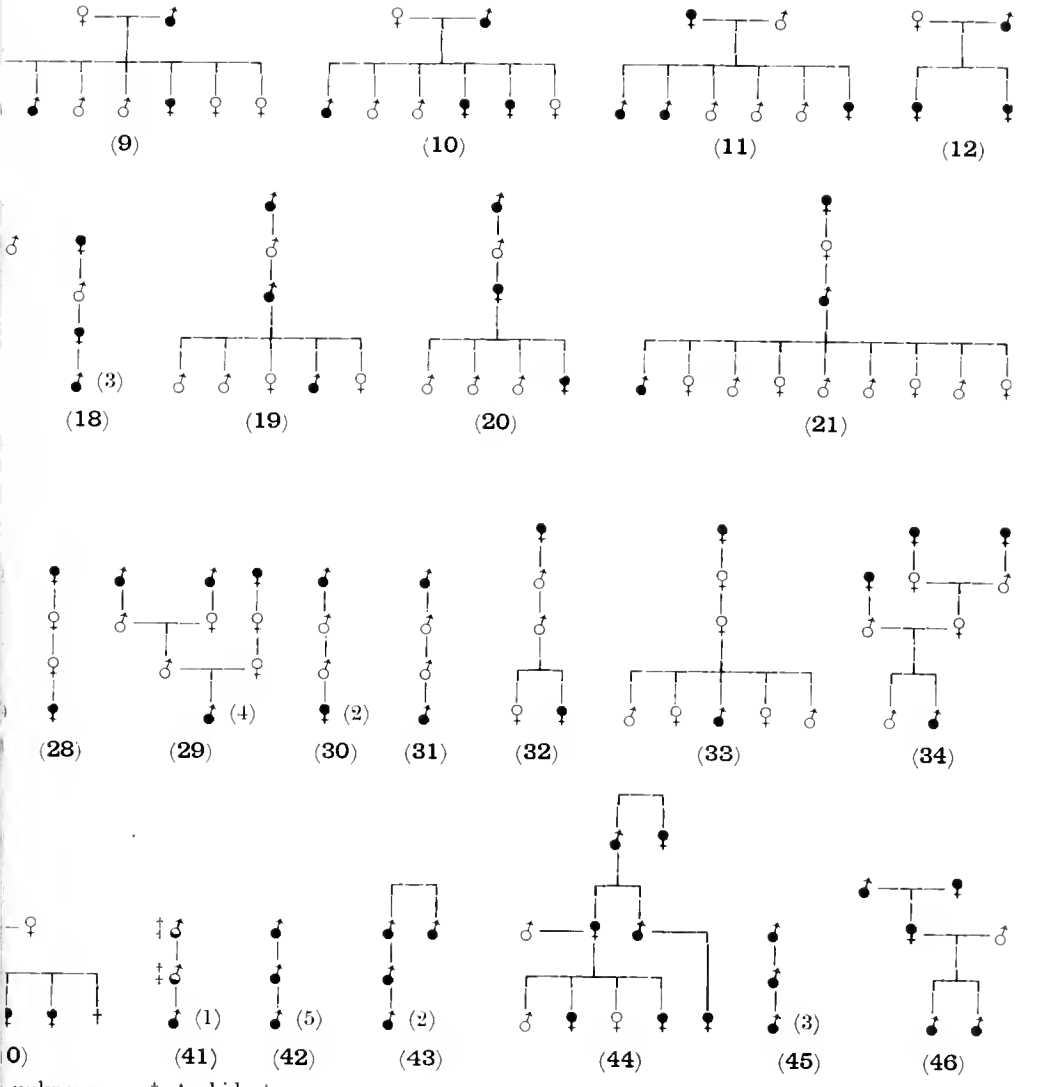


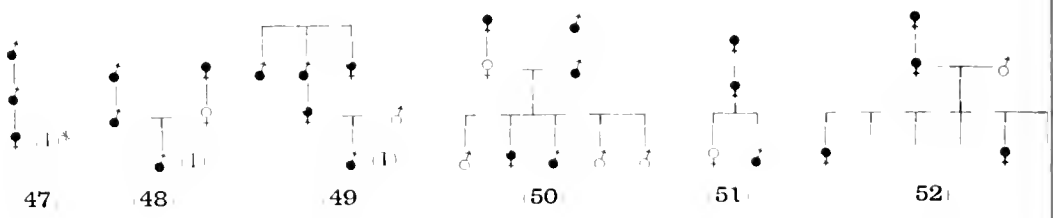
1—3 Lefthandedness traceable five generations in the direct line. (Three histories.)
8—16 Lefthandedness appearing in parent and child. (Forty-eight histories.)
23—36 Lefthandedness appearing in great-grandparent and child. (Fourteen histories.)
41—56 Lefthandedness appearing in grandparent, parent and child. (Sixteen histories.)

* The numeral gives the order in the fraternity; the total number of indiv



4—7 Lefthandedness appearing in four consecutive generations. (Four histories.)
7—22 Lefthandedness appearing in great-grandparent, parent and child. (Six histories.)
7—40 Lefthandedness appearing in great-grandparent, grandparent, and child. (Four histories.)

s is not recorded. Normal consorts are generally omitted in the charts.



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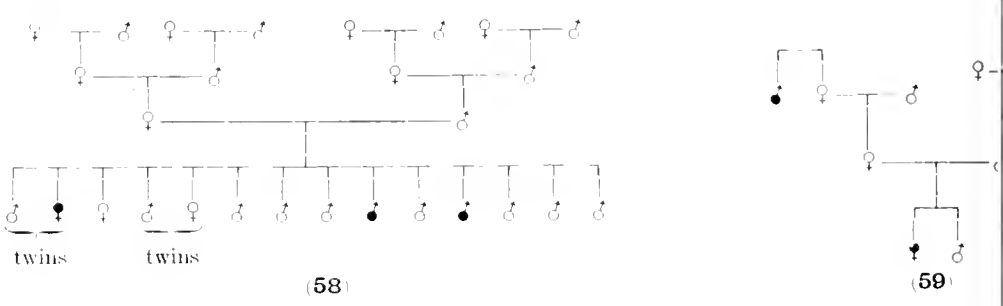
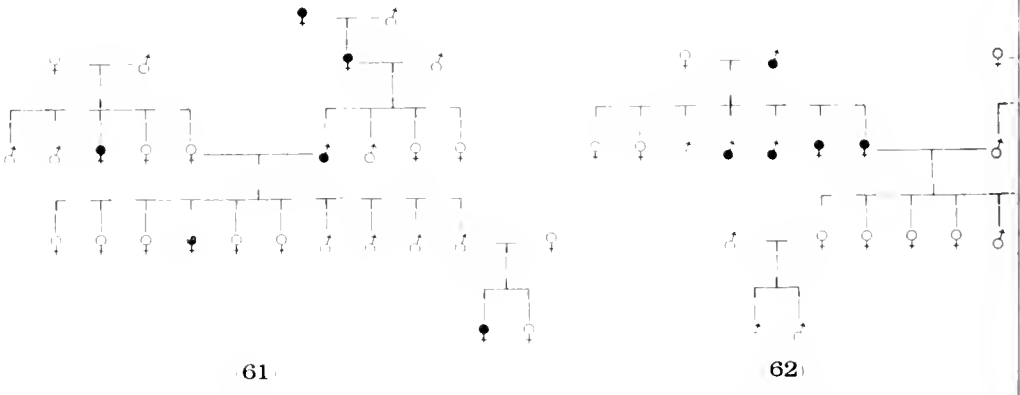


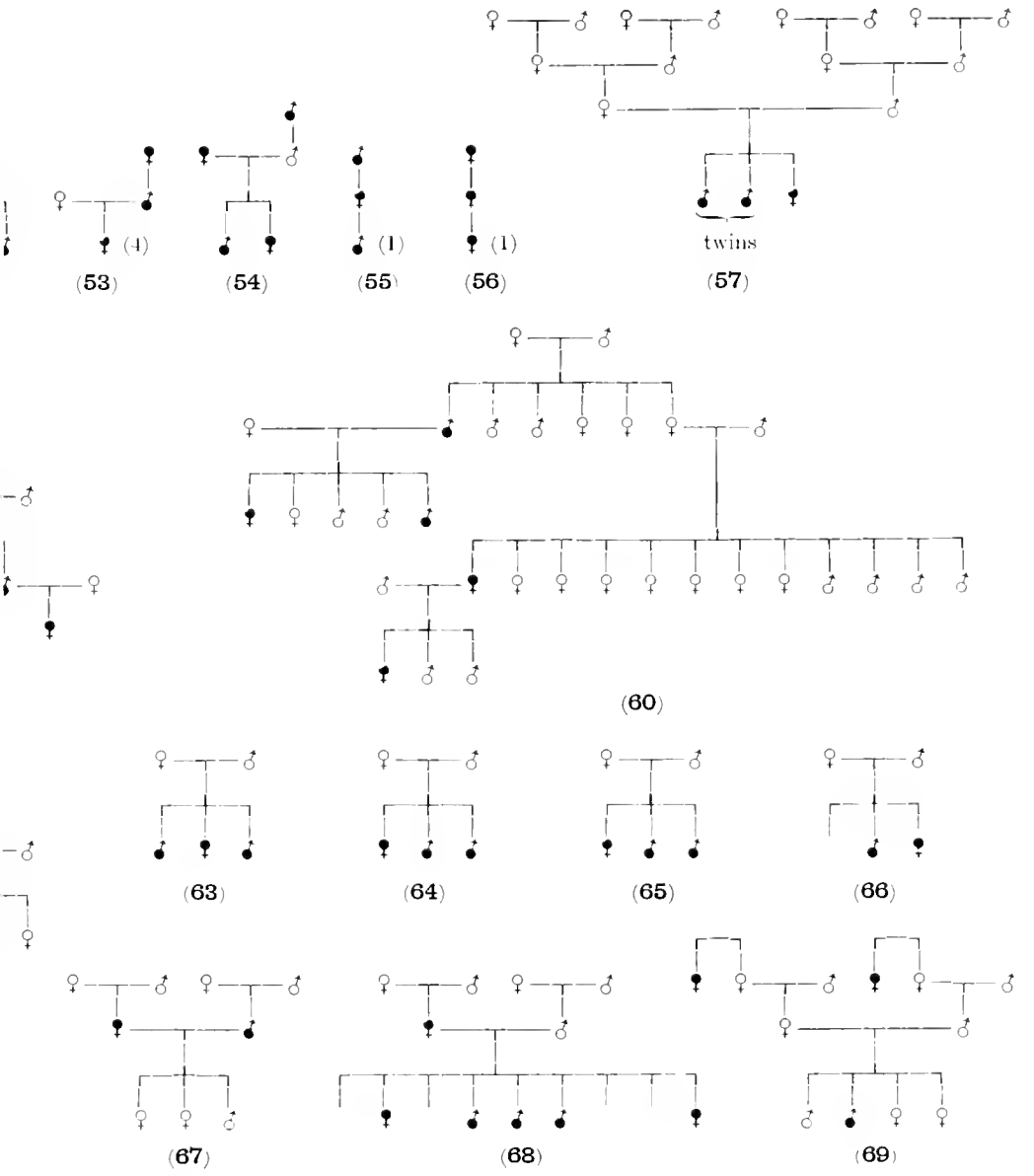
58 59



61 62

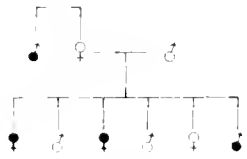
41–56 Lefthandedness appearing in gran

* The numeral gives the order in the fraternity; the total number of indi



ent, parent and child. (Sixteen histories.)

ils is not recorded. Normal consorts are generally omitted in the charts.



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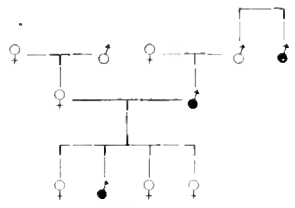

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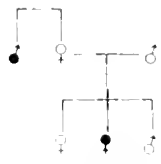

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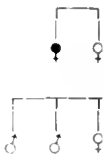

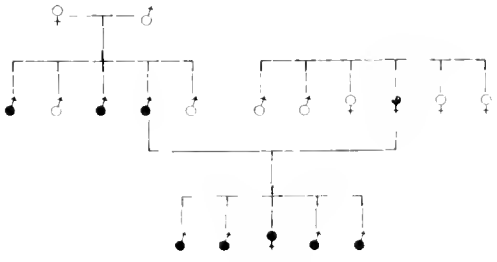

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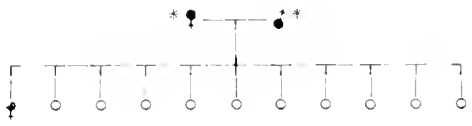

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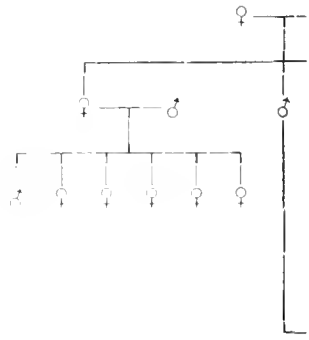
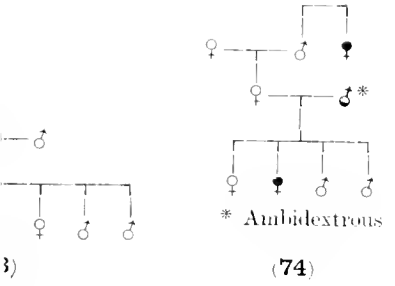
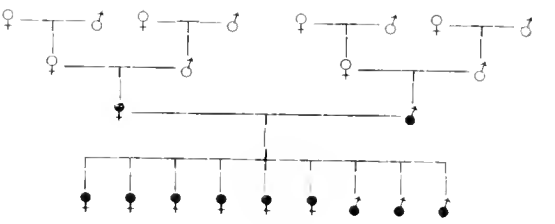


* Cf. text, pp. 74 a


* Ambidextrous

(74)



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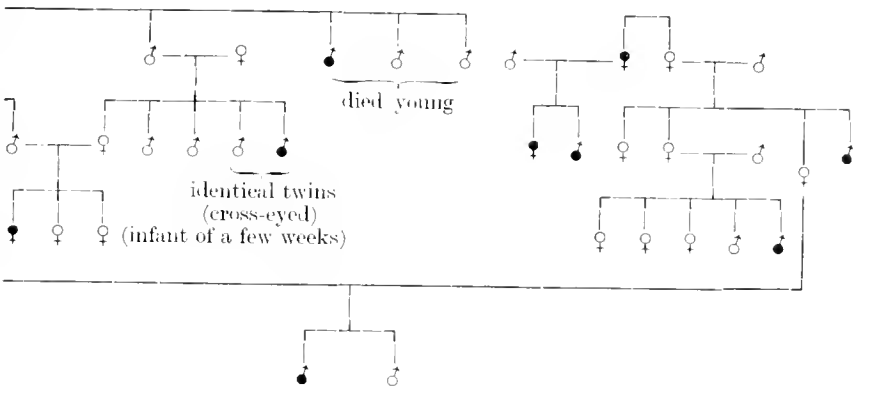
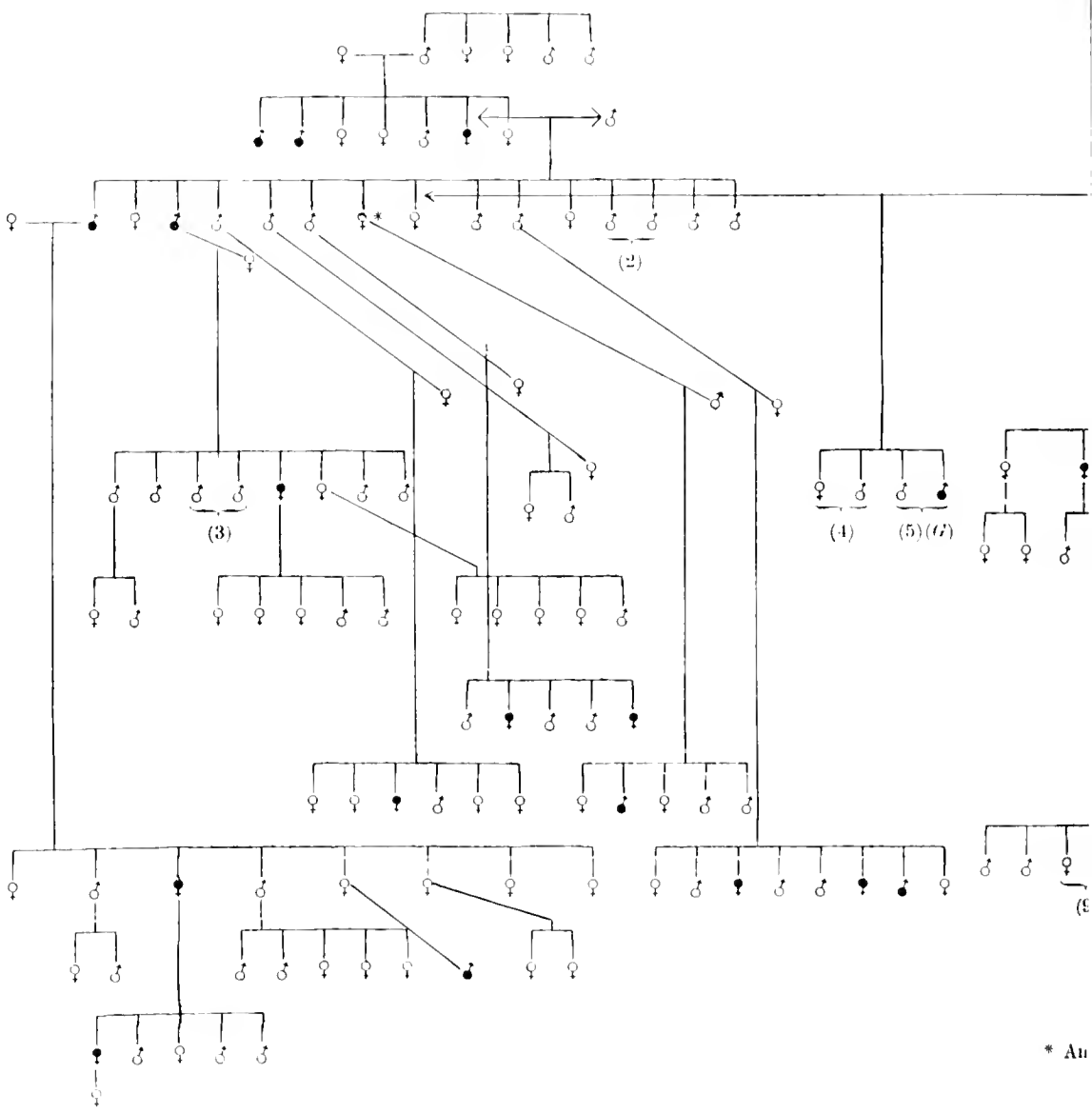
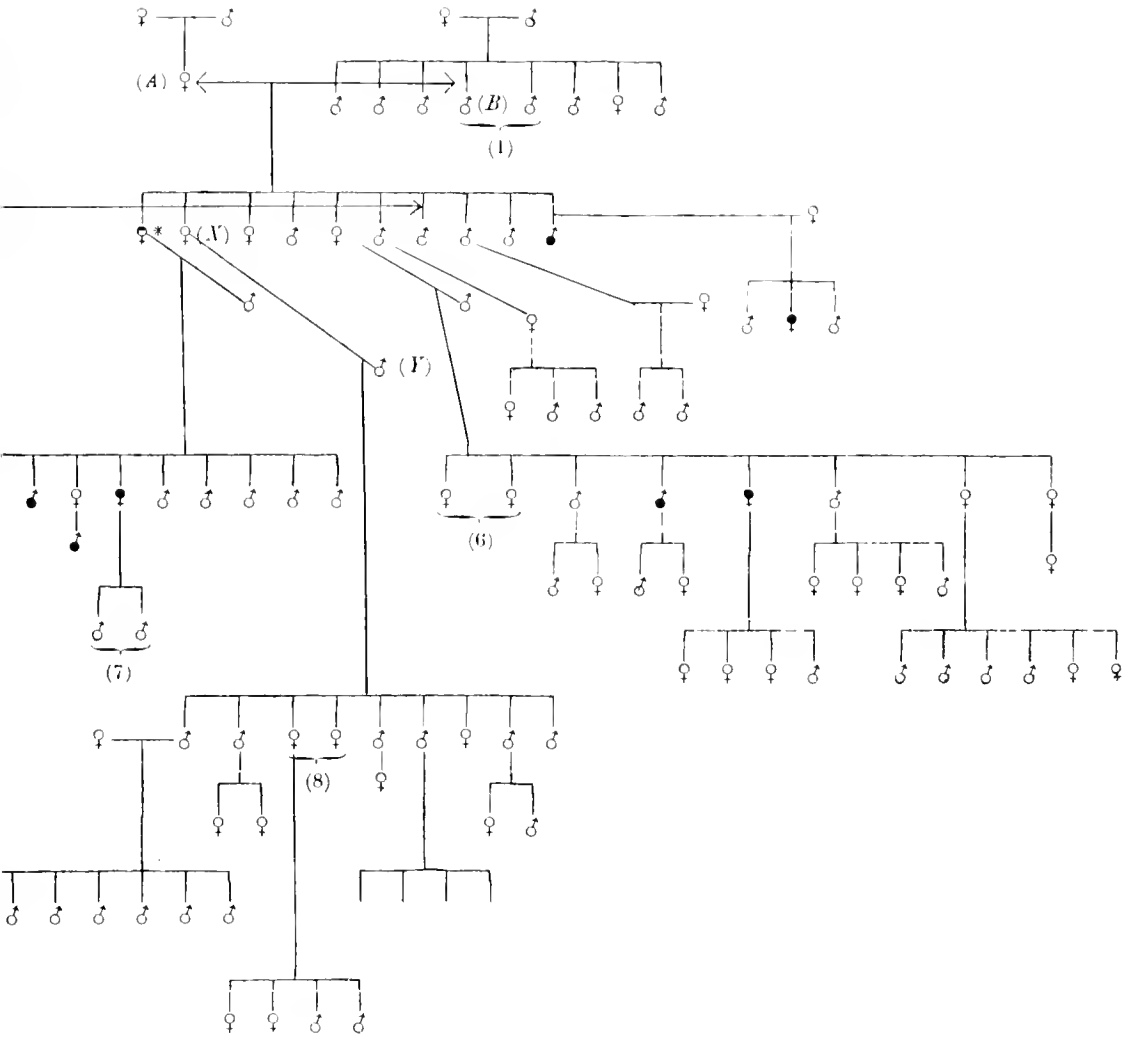

died young
identical twins
(cross-eyed)
(infant of a few weeks)

(78)



* Au

FIGURE 79



xtrous.

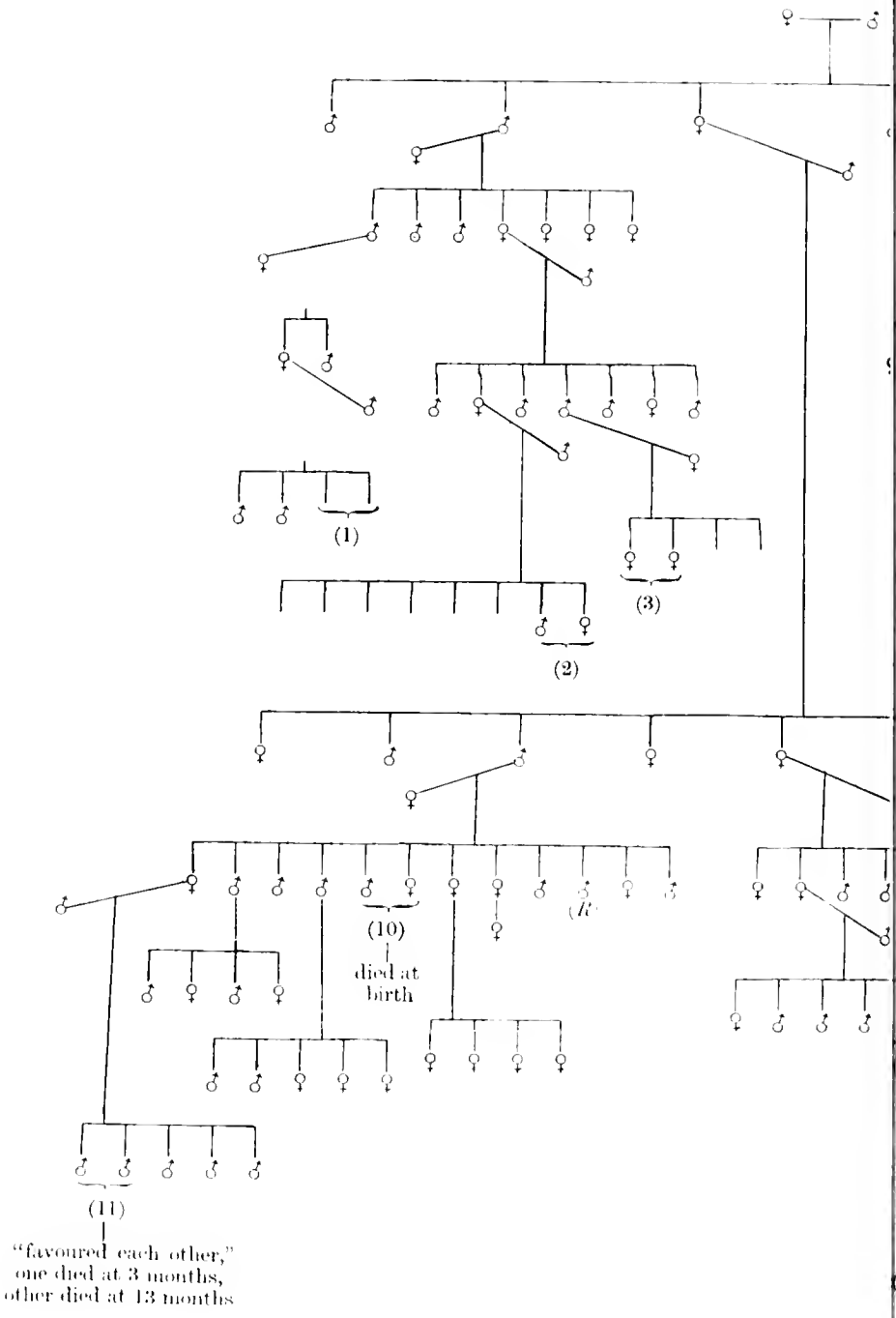
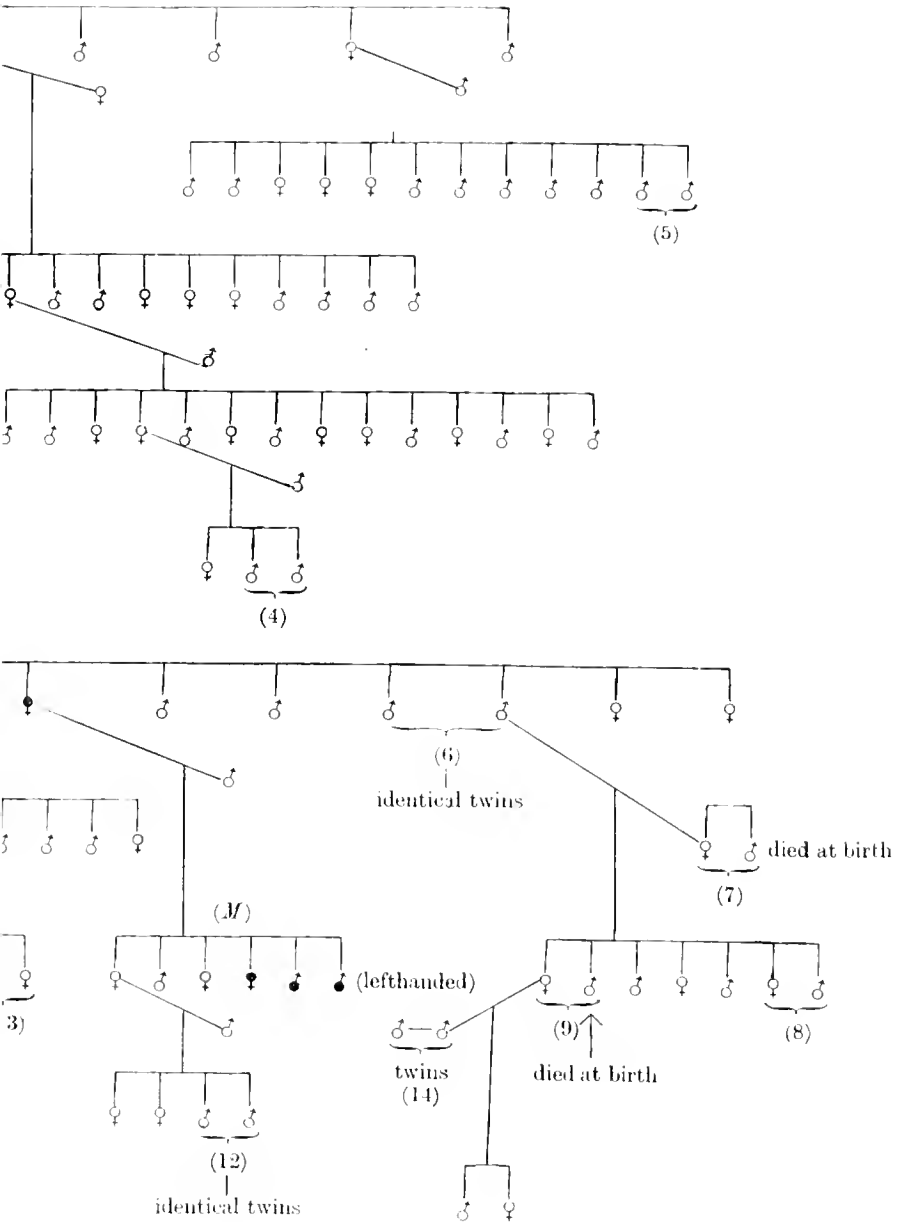

(1)
(2)
(3)
(10)
died at birth
(11)
"favoured each other," one died at 3 months, other died at 13 months

FIGURE 80


(5)
(4)
(6)
identical twins
died at birth
(7)
(M)
(lefthanded)
3)
(9)
(8)
twins
(14)
died at birth
(12)
identical twins

A PECULIAR NEGATIVE CORRELATION IN OENOTHERA HYBRIDS.

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With all the careful attention which has been given to the Oenotheras by students of genetic phenomena, there is no other group of organisms in which these phenomena seem so far from a satisfactory interpretation. Fundamental difficulties are encountered whenever attempts are made to apply to the Oenotheras rules of genetic behavior which are readily demonstrated in other groups of organisms. Equal confusion has arisen by the application of genetic experiences with the Oenotheras to species in which typical Mendelian phenomena appear. A hereditary mechanism must exist in Oenothera fundamentally different from that which distributes the Mendelian unit-characters. It becomes increasingly clear that the data on record are inadequate for the discovery of the essential features of this mechanism. It is conceivable that different mechanisms for the distribution of the hereditary characters may exist in different sections of the genus and that this may partly account for the slowness with which a comprehensive view of the genetic phenomena in this field has been made possible. Under these circumstances there is demanded a large amount of purely inductive work by the strictest pedigree methods and individual analysis. Before we can hope to explain the phenomena we must know what phenomena are presented for explanation. As a modest contribution to our knowledge of the genetic facts in Oenothera which need interpretation the following data are presented.

Early in June 1912 I received from Dr. A. F. Blakeslee, Storrs, Connecticut, three rosettes grown from unguarded seeds of *Oenothera rubricalyx* Gates, the seeds having been received by him from Dr. R. R. Gates, the discoverer of this particularly beautiful form.

The rosettes had the general morphological features of my *O. rubrinervis* forms, but the leaves were darker green and of more shining surface. The buds were more slender and more nearly terete, but the capsules were long and decorated, when young, with the 2–4 deep-red longitudinal bands usually seen on immature *rubrinervis*-capsules. The buds were brilliantly red-pigmented throughout, except on the ovary and the distal portion of the free tips, which were green. The stems were also brilliantly red-pigmented, as described by Gates. All three of the plants were of the same type, and it seems probable that they were true *O. rubricalyx* Gates, despite the fact that they came from unguarded seeds under circumstances doubtless favorable to crossing with many other Oenotheras[1]. As my results in breeding these plants differ strikingly from those reported by Gates, the possibility of the hybrid nature of my original plants of this form must be kept in mind; but it must also be kept in mind that even if these three plants were hybrids, they must each have been produced by the union of only two germ-cells, and as I have used in the self-fertilization and in all the crosses described below only one of the three original plants, any complication from their possibly hybrid nature is reduced to the lowest possible minimum. Such a possible hybrid origin will not detract in the least from the interesting correlations with which this paper deals. It should be added that in making reciprocal crosses generally, I have used in both crosses *the same pair of individuals*, so that no unsuspected complications can have been introduced by genotypic differences in individuals wrongly assumed to be identical because they were phenotypically alike. This is a detail of technique of the utmost importance; it will be found that the genetic problems in Oenothera are sufficiently complex when the elements which enter into the related crosses are reduced to the lowest possible terms.

Oenothera rubricalyx appeared in 1907 in a mixed progeny consisting of 112 offspring of four self-fertilized *rubrinervis*-plants grown at Woods Hole the preceding year, from unguarded[2] seeds received from de Vries

[1] Several features of my plants suggested a relationship to *O. grandiflora*, particularly the rather lax rosettes, strong red spotting of the young rosette-leaves, and the development of buds more slender and rounder than in my *O. rubrinervis* cultures, but as none of the characters presented by the offspring showed accentuated resemblance to *O. grandiflora*, I doubt the reality of this suggested relationship.

[2] Gates does not state that the seeds received from de Vries were from open pollinations, but the experience of *Oenothera*-students with *rubrinervis*-cultures, makes probable no other interpretation of the occurrence of 2 *O. Lamarckiana* and 1 *O. oblonga* among the 45 plants which included the *rubrinervis*-parent of the original *O. rubricalyx*.

(Gates, 1913 b). The original *rubricalyx*-plant, self-fertilized, yielded 9 *O. rubricalyx*, 1 *rubrinervis*, 2 undetermined[1]. Three self-fertilizations of these *rubricalyx*-specimens produced in the next generation 57 *rubricalyx* and 22 *rubrinervis*, showing in one family of 44 plants a ratio 3 : 1, and another family grown from seeds of an open-pollinated *rubricalyx*-plant in the same generation, yielded 71 *rubricalyx*, 38 *rubrinervis* and 4 doubtful[2]. Owing to various misfortunes the progenies from several other self-fertilizations could not be classified, but both *rubricalyx*- and *rubrinervis*-plants were apparently present. From these results Gates concluded that *rubricalyx* originated as a heterozygote which differed from the parent in a single, dominant, purely quantitative, Mendelian character, and strangely enough at the same time concluded that it would always split into *rubricalyx* and *rubrinervis* (Gates, 1911).

The small number of plants which reached maturity in Gates's pedigrees aroused the desire to test more thoroughly the apparently unique behavior of the *rubricalyx*-character. Since my experiments with this form were undertaken, Gates (1912) has announced the discovery of a "homozygous" individual of *O. rubricalyx*, which breeds true to this character, thus proving the incorrectness of his conclusion regarding the continued splitting of the *rubricalyx*-progenies, but on the other hand strengthening his assumption that the *rubricalyx*-character is Mendelian in inheritance. His oft repeated emphasis of the view that *O. rubricalyx* differs from *O. rubrinervis* in a purely quantitative character which acts as a monohybrid Mendelian dominant over the *rubrinervis*-type of pigmentation will have a special interest in relation to the results described below. In a paper published since this was written Gates (1914) wavers between the treatment of the *rubricalyx* type of pigmentation as a Mendelian and as a non-Mendelian character. His most positive declarations on the subject are that it is non-Mendelian; but if he sincerely holds to this conviction it is strange that he should continue to treat the genetic behavior of this character as if it threw valuable sidelights on Mendelian phenomena.

During the past season (1913) I have had five pedigrees derived from one of the three individuals of *O. rubricalyx* grown by me in 1912. These were *O. rubricalyx* self-fertilized, *O. rubricalyx* × *rubrinervis*,

[1] These two undetermined plants are listed in one place as *O. rubricalyx*. See Gates, 1911, Table II, p. 365.

[2] In the same place (Gates 1911, Table II) this family is erroneously indicated as the product of a self-fertilization and the four doubtful plants are included under *O. rubrinervis*.

O. rubrinervis × *rubricalyx*, *O. rubricalyx* × *Lamarckiana* and *O. Lamarckiana* × *rubricalyx*. All of these were sown on the same day, grown under the same conditions, potted, described and photographed at approximately the same ages, and set into the field in adjacent rows on the same day. Each of these families will be considered under a separate heading.

Oenothera rubricalyx Gates.

Pedigree No. 11410(1) × Self = 1231.

Seeds sown January 30th, 1913, germinated in 16—20 days and yielded 119 plants, which at 10 weeks of age were clearly divisible into two groups in respect to morphological characters, and into two independent groups in regard to the pigmentation of the leaves. These four groups may be briefly characterized as (*a*) the *rubrinervis*-like group, (*b*) the *nanella*-like group, (*c*) the spotted group, and (*d*) the unspotted group. Two plants died unclassified. The remaining 117 were distributed as follows: (*ac*) 59, (*ad*) 48, (*bc*) 8, (*bd*) 2. These categories may be briefly characterized as follows:

Plants of the (*a*) group containing 107 individuals, or 91·45 per cent. of the entire family, had rather lax, ascending rosettes of relatively narrow leaves, resembling those of *O. rubrinervis*, but in general a little darker green and with more reddening of petioles and midribs in the older leaves, the spotted sub-group (*c*) usually being a little more strongly crinkled and a little more prominently dentate toward the base of the blades than the unspotted sub-group (*d*), as seen in the upper series of plants in Plate V. These may be compared with pure-bred *O. rubrinervis*-rosettes of similar age, which are shown in the lower series on the same Plate. The adult plants all had the tall, wide-branching form usually seen in vigorous strains of *O. rubrinervis*, with the characteristic long capsules of that species, but the buds were as in the parent, relatively a little more slender than in my long-controlled strains of *O. rubrinervis*.

In the following table, bud-measurements of these *rubricalyx*-plants both red-stemmed (*ac*), and pale-stemmed (*ad*), may be compared with corresponding measurements from four pure *O. rubrinervis*-pedigrees taken at about the same time (Aug. 1) and under similar conditions. Each measurement entered in the table is an average from five buds taken at random from five different plants in the indicated pedigree. All

measurements are in centimeters and were made with a micrometer-caliper by my scientific assistant, W. F. Friedman, to whose faithful work and painstaking care it is a pleasure to give this grateful recognition.

Character	Ped. No.	*O. rubrinervis* 1213	1214	1215	1216	Av.	*O. rubricalyx* 1231 (*ac*)	1231 (*ad*)
Length of ovary ...	..	1·11	1·13	1·07	1·17	1·13	1·06	1·00
Thickness of ovary[1]	...	0·33	0·32	0·32	0·31	0·32	0·30	0·28
Length of hypanthium ...		3·63	3·93	3·55	3·03	3·53	4·13	3·77
Thickness of hypanthium[2]		0·31	0·28	0·25	0·28	0·26	0·24	0·24
Length of cone ...	...	4·21	4·43	3·95	4·06	4·16	4·61	4·07
Thickness of cone[3]	...	0·81	0·84	0·75	0·74	0·78	0·74	0·72
Length of free tip	...	0·59	0·63	0·66	0·66	0·63	0·74	0·60
Length of anther	...	1·54	1·58	—	1·66	1·59	1·49	1·45

Plants of the (*b*) group were distinguishable by their closer rosettes, caused by the relatively short, ascending petioles and nearly horizontal blades. They were recognized at once as *nanella*-like rosettes, though their longer, narrower, less crinkled leaves distinguished them strikingly from the *nanella*-form derived from *O. Lamarckiana*. The adult plants proved to be of little more than *nanella*-stature (25—40 cm.) as shown in fig. 1, but of very unique aspect, due to the broad-lanceolate, acuminate, dark green, nearly uncrinkled leaves. The buds were long, slender and the cones nearly terete. Gates (1914) reports the occurrence of dwarfs in his cultures of both *O. rubricalyx* and *O. grandiflora*, and their recurrence in some of the F_2 families from crosses between these two species.

The group (*c*) differed from the (*d*) group in having conspicuous red spots on the dorsal surface of the leaf-blades, as shown in the accompanying Plates. As Gates had found self-fertilized *rubricalyx* yielding progenies containing both *rubricalyx* and *rubrinervis*, I at once inferred that I was getting the same result, and that the spotted rosettes belonged to the *rubricalyx*- and the unspotted rosettes to the *rubrinervis*-type. This did not prove to be true, however, as the entire (*a*) group, both spotted and unspotted, had the intensely pigmented hypanthia and bud-cones characteristic of *O. rubricalyx*. In the adult stage the spotted and unspotted groups, (*c*) and (*d*), were definitely differentiated from one another in only one feature, namely, in the pigmentation of the stems. The group grown from spotted rosettes, (*ac*), had intensely red-pigmented stems, the pigment being particularly conspicuous about the base of the

[1] Greatest diameter.

[2] Least diameter.

[3] Greatest diameter parallel with sides.

central spike and on the upper lateral branches. They were in this respect like their parent. The unspotted group, on the other hand, had the stems only slightly reddened, with the upper part of the main-stem and upper laterals not conspicuously pigmented with anthocyan. In the dwarf group, (*b*), only six plants bloomed, all belonging to the sub-group with spotted rosettes. Five of them had no anthocyan in the buds, and one had the pigmentation of *O. rubricalyx*. All had strongly reddened stems.

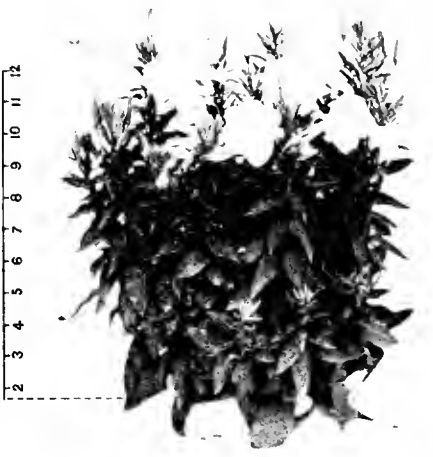

Fig. 1. A dwarf plant with dark red stems and green buds from self-fertilized *O. rubricalyx*. (The included scale of inches was drawn from a rule photographed on the same negative; 1 inch = 2·5 cm.)

Gates has not mentioned red-spotting of the young rosette-leaves as a characteristic of *O. rubricalyx*, but on the other hand he now includes this character among the features which differentiate *O. grandiflora* from *O. rubricalyx* (Gates, 1914). The fact that half of my *rubricalyx*-plants had strongly spotted leaves may perhaps mean, therefore, that my original *rubricalyx*-plants were hybrids. Another possibility may be suggested. Gates (1914) characterizes the rosette of *O. grandiflora*

as having "*pale red blotches*"; in my cultures that species had *deep* red blotches. There can be no doubt that anthocyan production is strongly influenced by sunlight and temperature, and possibly other environmental factors, which differ in different localities. The deeper red spots on *O. grandiflora* in my cultures might be due to genotypic differences between Gates's material of that species and mine, but it seems rather more likely that they indicate a condition in my cultures favorable to the intensification of the anthocyan colors. It is conceivable that a form which does not possess red spots at all under one condition may be strongly spotted under some other condition. This is directly illustrated by the spotted plants themselves, for the spotting is an evanescent character, which completely disappears as the rosettes grow older. The occurrence of spotting in the young rosette-leaves in my cultures of *O. rubricalyx*, does not necessarily prove, therefore, that my plants are hybrid derivatives of *O. rubricalyx* instead of that species itself, although on certain grounds the assumption that they are hybrids may seem to be the more tenable hypothesis.

As the *rubricalyx*-plants in this pedigree were of two types with respect to the pigmentation of the stems, it was thought possible that such splitting might also occur in the homozygous strain discovered by Gates, but a letter from A. W. Sutton, Esq., Reading, England, informs me that all of the *O. rubricalyx*-plants from Gates's pure-breeding stock have the brilliant red stems[1].

Oenothera rubricalyx × *rubrinervis* F_1.

Pedigree Nos. 11410(1) × 1123(8) = 1232.

The male parent was from a pure cross-bred strain of *Oenothera rubrinervis* which had been fully controlled in my cultures in seven consecutive generations, each of these ancestral generations having resulted from a cross between two typical *rubrinervis*-specimens of as widely separate relationship as my several cultures provided. All the preceding generations had been uniformly typical *O. rubrinervis* with the exception of an extremely small percentage of aberrant individuals which have appeared from time to time as characteristic mutants(?).

Seeds from this cross yielded 205 individuals of which 8 died unclassified. These were, like the progeny from the selfed *O. rubricalyx*

[1] Seeds of this form are now being offered for sale by Sutton and Sons under the name "Oenothera Afterglow.'

(No. 1231), divisible into a spotted and an unspotted group, in the ratio 103:94. No *nanella*-plants were seen, and all were similar in morphological features to *O. rubrinervis*, as shown in the second row of rosettes from the top of Plate V, though in the 10-weeks-old rosettes about two-thirds of the unspotted group had somewhat broader, darker green leaves than the remaining one-third. Later, however, this distinction could no longer be seen; the broader- and narrower-leafed groups were kept separate throughout their development, but no distinction was visible between them in the mature plants. With the exception of three plants which were slightly divergent in characters of foliage and branching, all were of the same vegetative form, and indistinguishable in this respect from the (*a*) group of selfed *O. rubricalyx*. In pigmentation, however, a striking situation was presented. The unspotted rosettes developed into plants of the same type as the corresponding group in the selfed *O. rubricalyx* family, having greenish stems and brilliantly pigmented buds of the *rubricalyx*-type. The spotted rosettes, on the other hand, produced brilliantly red-pigmented stems, with buds of the *Lamarckiana*-type of pigmentation, the cones being merely pink in longitudinal bands of greater or lesser width, and the hypanthia green. No plant of the latter group had buds as strongly pigmented as in *O. rubrinervis*, the male parent, and the minus variations ranged to cones nearly, though not quite, completely free from anthocyan.

Oenothera rubrinervis × *rubricalyx* F_1.

Pedigree Nos. 1123(8) × 1410(1) = 1233.

Seeds from this cross produced 152 plants which were likewise clearly separable into a spotted and an unspotted group, but with no other apparent distinction. Two-months-old rosettes are shown in the second row from the bottom of Plate V. The two groups consisted of 62 spotted and 89 unspotted rosettes, 1 having died unclassified. Plants of both groups had red on the under side of petioles and leaf-blades, especially when the latter were going into decline. Several plants which had one or two obscure red spots or fine red specks, but proved on their subsequent development to belong to the unspotted group, are included with that group in the ratio given above. One plant which was marked on May 2 as spotted, was found two weeks later without spots, and was transferred to the unspotted group, where its identity was lost. Among the adult plants one individual from the unspotted

group had the bud- and stem-characters of the spotted plants, from which I infer that the original determination of the character of this plant was the correct one, and it is therefore included with the spotted plants in the above reckoning. Only 25 of each group were set into the field to grow to maturity. All the adult plants had the branching habit and long capsules characteristic of *O. rubricalyx* and *O. rubrinervis.* Eight in the spotted group had slightly broader, darker green, more strongly crinkled leaves than their sibs, in these particulars more closely approaching the characters of *O. Lamarckiana,* but their stems and buds did not differ in pigmentation from those of the other plants in the spotted group. One plant in the spotted group had the buds colored as in *O. rubrinervis,* but was so heavy in all its parts as to suggest the likelihood that it was a triploid form. Its capsules were notably thicker and shorter than those of its sibs. I have no special record as to the color of the stem of this plant, but as it stood in the red-stemmed group, it would probably have been noticed if different in this respect from the rest of the group. All the rest of the spotted group had brilliant red stems and *Lamarckiana*-like buds with pink cones and green hypanthia. The unspotted group, with the one exception already mentioned, had greenish stems and the typical *rubricalyx*-coloration of the buds, i.e., with hypanthia and cones uniformly and intensely red-pigmented, except on the distal portion of the free tips of the sepals, which were free from anthocyan.

Oenothera rubricalyx × *Lamarckiana* F_1.

Pedigree Nos. 11410(1) × 118(10) = 1234.

Of this pedigree I secured 123 plants which were again divisible into spotted and unspotted groups, but the spotting was not so pronounced as in the *rubricalyx-rubrinervis*-hybrids, so that it was not quite certain whether the unspotted group was a natural group or simply the minus-end of a single fluctuating series. The grouping of the young rosettes gave 97 spotted and 26 unspotted plants. None of these rosettes closely resembled *O. Lamarckiana,* being more lax, the leaves having longer petioles and more tapering bases; and they were also more ascending than in the rosettes of *O. Lamarckiana.* They may be compared with *O. Lamarckiana* in Plate VI, in which *O. rubricalyx*-rosettes are at the top, *O. Lamarckiana* at the bottom, and the present series of hybrids in the second row from the top. The foliage

in the spotted group was perceptibly darker green and the leaves slightly broader and more crinkled than in the unspotted group. As the rosettes grew older, a small number were noted, which were differentiated from the rest in being rather coarsely crinkled, with the obvate to oblong-obvate blades abruptly contracted, with a slight undulation, to a triangular-winged petiole. The leaves of these had more numerous and more conspicuous red spots than their spotted sibs. That this group grades into the more common type of spotted plants, is shown by the fact that I separated only 6 out of the 9 plants which subsequent development proved to belong to the same natural group. Fifty plants were set in the field, including 30 spotted and 20 unspotted plants, the members of each of these groups being taken at random, with the exception of one of the crinkled-leafed, strongly spotted plants just described, which was discovered before the reservations were made for the field cultures. Aside from this one individual, therefore, the distribution of the different types in the field should accurately represent, within the limits of the probable error, the composition of the entire progeny. The adult plants were all of one type with respect to general habit of branching and the long capsules (2·5—2·7 cm.), being in these regards like my selfed *O. rubricalyx*. Among them were three classes of individuals sharply distinct from one another, namely,

(*a*) 9 [27][1] with very dark, dull-red stems, and buds wholly free from red pigment;

(*b*) 5 [17] with brilliant red stems, and *Lamarckiana*-like buds, i.e., with pink cones and green hypanthia; and

(*c*) 36 [79] with greenish stems and *rubricalyx*-buds, i.e., brilliant red hypanthia and cones.

It would have been desirable to make an actual determination of the relative amounts of anthocyan in the stems of groups (*a*) and (*b*), but for lack of time this determination was not made. It was my impression, however, that the dull blackish-red stems of the former contained more anthocyan than the brilliant stems of the latter. If this impression is correct, the three grades of pigmentation of the buds are completely associated with three grades of pigmentation in the stems, but the bud-series and the stem-series run in opposite directions,

[1] In brackets are given the number of each type which should have been present if the entire progeny had been grown to maturity. These are the significant numbers, as the proportionality among the *observed* numbers is distorted by the fact that a relatively larger number of unspotted rosettes were grown to maturity, than of spotted ones.

thus: green with dark red, pink with bright red, red with green. The fine red specks often seen on the ovaries show a partially independent series, being absent in (*a*), numerous in (*b*) and scarce in (*c*).

The distribution of the adult plants shows that the classification of the rosettes into spotted and unspotted categories was not in this case a natural grouping, as it was in the reciprocal *rubricalyx-rubrinervis*-crosses, and this difference is doubtless due to the fact that my *O. Lamarckiana* has moderately spotted rosettes while *O. rubrinervis* is unspotted, as shown by Plates V and VI (bottom series in each). All plants of groups (*a*) and (*b*) had spotted rosettes, and two-thirds of the former were noticeably more strongly spotted than the rest of the spotted plants. All the unspotted rosettes and most of the moderately spotted ones developed into plants of type (*c*). It is thus seen that although the pigmentation of the rosette is not in this hybrid combination, as sharply diagnostic of the natural groups, the nature of the association between the pigmentation of the rosette and that of the adult organs is the same, in direction, in the *rubricalyx-Lamarckiana*-cross as in the *rubricalyx-rubrinervis*-cross. Stated more generally, there is a positive correlation between the red-pigmentation of the rosette-leaves and that of the adult stems, and a corresponding negative correlation between the red-pigmentation of the rosette and the red-pigmentation of the buds.

Oenothera Lamarckiana × *rubricalyx* F_1.

Pedigree Nos. 118(10) × 11410(1) = 1235.

Seeds of this cross produced 117 plants which were at one time grouped provisionally into 36 *Lamarckiana*-like, 18 *rubricalyx*-like and 37 *rubrinervis*-like, besides a considerable number of individual aberrants. As these names proved later to be inappropriate it is desirable to translate them for our present use into terms of their pigment-characters. The "*Lamarckiana*-like" group and the "*rubricalyx*-like" group had red-spotted rosettes, and the "*rubrinervis*-like" group had unspotted rosettes. Unfortunately, several of the divergent rosettes which received individual description, were not noted with reference to the presence or absence of red spots, and only 113 are now classifiable on the basis of the red spotting of the rosette-leaves; of these, 64 were spotted and 49 unspotted. Most of the slightly aberrant rosettes developed into adult plants not perceptibly divergent from other plants which had not been noticeably aberrant in the rosette-

stage. Two had heavier buds than the rest, nearly like those of pure *rubrinervis* in size and form, but each of these had greenish stems and *rubricalyx*-colored buds; they are included among the number referred to group (*c*) below. Aside from these two plants and several specimens which failed to bloom, the 78 plants which had been set into the field were referable to three phenotypes, indistinguishable from those presented by the reciprocal family. The several types of pigmentation were distributed in the following proportions:

(*a*) 7 [9][1] with dull, dark-red stems and buds devoid of anthocyan.

(*b*) 17 [22] with brilliant red stems, pink cones and green hypanthia.

(*c*) 51 [82] with greenish stems and intensely red hypanthia and cones.

The relation of these groups to the rosette-characters of the same family are the same as in the reciprocal family; both (*a*) and (*b*) were completely included among the spotted rosettes, while (*c*) included all the unspotted rosettes and 26 (i.e. essentially 50 per cent) of the spotted ones.

Similar phenomena in other Oenothera-crosses.

The negative correlation between the redness of the stems and that of the buds, which has been so strikingly manifested in these *rubricalyx*-crosses, is by no means limited to the combinations of *O. rubricalyx*. In an extensive series of crosses between *O. Lamarckiana* and several biotypes of *O. cruciata*, glimpses of the same inverse relation in the distribution of the red pigmentation, have been frequently seen. The results of the latter crosses will be published in detail in another place, and only several examples of many that might be presented will be introduced here to illustrate the criss-cross distribution of the red pigment on the stems and buds. The forms of *O. cruciata* have green buds and usually more or less strongly reddened stems. *O. Lamarckiana* has pink cones and green hypanthia associated with only moderate reddening of the stems, the stems having a degree of red-pigmentation similar to that of the above-described "greenish"-stemmed plants among the *rubricalyx*-hybrids. A cross between a certain elementary form of *O. cruciata* ♀ and *O. Lamarckiana* ♂ produced in the F_1 (Ped. No. 1140) four types characterized as follows with respect to pigmentation:

(*a*) 2 [2][1] with red stems and buds entirely green.

[1] See footnote on p. 92.

(*b*) 10 [18] with bright red stems, very slightly reddened bud-cones and green hypanthia.

(*c*) 2 [3] with pink stems, and with cones and hypanthia red throughout.

(*d*) 5 [5] with stems nearly green and buds entirely green.

A plant of the type (*b*), which type made up the bulk of the F_1 family, pollinated by a plant of the parental biotype of *O. cruciata* (i.e., a sesqui-reciprocal hybridization of the form $(A \times B) \times A$), produced 43 [97] offspring with red hypanthia and red cones on pink stems, and 5 [6] with faint reddening on the cones, green hypanthia and intensely red stems (Ped. No. 12107). The parental form of *O. cruciata* had only moderate reddening of the stems, so that the red stems of the hybrid types (*a*) and (*b*) represent a marked intensification of the pigmentation as compared with both parents, just as the red hypanthia and strongly red-pigmented bud-cones of hybrid type (*c*) show a remarkable advance over the pigmentation of the buds in *O. Lamarckiana*, the only one of the parents which had any red coloration of the buds.

In crosses between *O. Lamarckiana* and two other biotypes of *O. cruciata*, the F_1 hybrids, when *O. Lamarckiana* is the mother, are in each cross of uniform type, having the bud-cones reddened to about the same extent as in *O. rubrinervis* (i.e., much more strongly reddened than in *O. Lamarckiana*) while all the vegetative parts are pale green and absolutely devoid of red-pigmentation. The reciprocal crosses produced in the one case two types, in the other case four types, some of which had pink cones and green hypanthia, others entirely green buds, but all had strongly reddened stems, the green-budded plants having a stronger pigmentation of the stems than the pink-coned plants. In the latter pedigrees the green-budded forms can be partially sorted out from the pink-coned forms in the rosette-stage, because of the more prominent red spots on the dorsal surface of the young rosette-leaves in the former.

The pigmentation of *O. rubrinervis* as compared with its parent *O. Lamarckiana*, may be related to the same phenomenon, for the bud-cones of the mutant-form are much more strongly pigmented than those of *O. Lamarckiana*, while the rosettes are entirely free from red spots and the stems are nearly green, while *O. Lamarckiana*, on the other hand, has the rosettes sparsely spotted and the stems moderately reddened. Thus, *O. rubrinervis* shows a progressive variation in the amount of red pigment in the buds, while the leaves and stems present a retrogressive variation in respect to red pigmentation.

Discussion.

The behavior of the red pigments in these *O. rubricalyx*-hybrids, is in striking contrast with that reported by Gates (1909 to 1913 b), whose reciprocal crosses between *O. rubricalyx* and *O. Lamarckiana* appeared to consist predominantly of *rubricalyx* and *Lamarckiana*. To what extent the differences between his results and mine are due to the very slender basis for the conclusions regarding his crosses of these species, cannot be determined, and consequently it is impossible to say how much of these differences is to be referred to putative genotypic differences between the particular individuals which entered into his crosses and those which were used in mine. Whatever may be the fact in regard to the lack of genotypic identity between his parent plants and mine, the results described in this paper have an important bearing on the several propositions set forth by Gates in regard to the origin and genetic behavior of the *rubricalyx*-character, namely (*a*) that the difference between *O. rubricalyx* and *O. rubrinervis* is a purely quantitative one; (*b*) that the *rubricalyx*-character is a typical monohybrid Mendelian character; and (*c*) that the method of inheritance of a character is determined by the nature of that character itself.

Many Mendelian color-patterns have been discovered in various plants and animals, which are inherited quite independently of the actual quantities of pigment present in the organism as a whole, the same pattern being associated sometimes with weak pigmentation at other times with intense pigmentation; dissimilar and independently inheritable patterns may also affect different parts of the same individual, and in every such case it is demonstrable that a "qualitative" and not alone a "quantitative" difference is present. Such experiences incline the geneticist to the interpretation of any striking change in a color-pattern as something more than a quantitative change in the amount of pigmentation present. Still, in a case as simple as that which Gates's material seemed to him at first to present, in which the new color-pattern completely includes the original one, the interpretation of the change as a purely quantitative one is possible, though perhaps not in any case particularly probable. Gates (1914) has now shown that in the case of the *rubricalyx*-character, also, the pattern is measurably independent of the actual quantity of pigment, since he secured pale red buds with the characteristic *rubricalyx*-distribution of the pigment. The results from my crosses show even more strikingly

that the mere quantity of anthocyan which a plant produces does not make the difference between the color-pattern of *O. rubricalyx* and that of *O. rubrinervis*, for certainly those plants with red-spotted leaves and dark-red or brilliant-red stems, produced many times as much anthocyan as the greenish-stemmed plants, though only the latter had the *rubricalyx*-type of buds.

It is interesting to see that even Gates (1914) occasionally catches a glimpse of the fact that the difference between *O. rubrinervis* and *O. rubricalyx* is something more than a mere quantitative difference in the amount of red pigment produced by each. He says (1914, p. 246) "It is then perfectly clear that, although *extent* of pigment on the buds behaves, with very few exceptions, as a definite unit-character, showing the phenomena of dominance and absence; yet the *amount* of pigment is very probably reduced to a half in the F_1 hybrids, and it is certainly diluted very much (probably one-half) again on crossing back with *grandiflora*....The cause of the definiteness of distribution of the pigment in the buds is a problem in morphogenesis." After this statement one may be surprised to read (p. 270) that "the most careful observation and study shows that the red pigmentation-character *R* is in the last analysis, inherited in quantitative fashion."

The Mendelian behavior of the *rubricalyx*-character was inferred by Gates from very insufficient evidence. When an attempt is made to interpret my results on a Mendelian basis, difficulties are encountered which seem at present nearly insurmountable. If we suppose that my original *rubricalyx*-plant (No. 11410(1)) was a heterozygote in respect to two independent determiners for the *rubricalyx*-type of buds, the progeny secured by self-fertilizing this plant was in as close agreement with expectation, as the small number of plants would require, the ratio being 10·7 : 1 instead of 15 : 1; but if there were no further complications, such a constitution for the *rubricalyx* parent would lead to the expectation of *rubricalyx* and non-*rubricalyx* in the ratio 3 : 1 in every cross between this plant and other plants which lacked both of the supposed determiners for the *rubricalyx*-character. Instead of this, the ratios in the four hybrid families here reported, were 0·91 : 1, 1·44 : 1, 1·80 : 1 and 2·65 : 1, or if we combine the two *rubricalyx-rubrinervis* families, the result is 165 : 183 or nearly 1 : 1, while the two *Lamarckiana* crosses combined give 161 : 75, or about 2 : 1. Both of these ratios actually occur in typical Mendelian inheritance, and could be accounted for here by such additional assumptions as these; namely, (*a*) that my *rubrinervis* plant, No. 1123(8), not only lacked the two independent

rubricalyx-determiners, but that it was also heterozygous for a third gene which inhibits the action of one of the genes for the *rubricalyx*-type of pigmentation, but not the other; and (*b*) that my *Lamarckiana*-plant, No. 118(10), contained a factor whose union with one of the *rubricalyx*-determiners produces, in the absence of the other, a non-viable zygote. While such assumptions are perfectly proper as working hypotheses, they have no other value. Assumptions of a still more radical kind would have to be made to account for the peculiar alternative relation between the pigmentation of leaves and stems on the one hand and that of the buds on the other hand.

In the enormous mass of genetic data already recorded for the Oenotheras, there is but here and there a situation which bears more than a remote resemblance to a Mendelian behavior, and in these cases the observed phenomena usually present only a more or less misshapen caricature of the beautiful regularity of procedure which has such far-reaching applicability among many other groups of organisms. It appears to me undesirable therefore to speak of the *rubricalyx*-character as a Mendelian unit-character because it happened to constitute 75 per cent of one family of 44 plants. I believe that the only other character in Oenotheras which has been accepted as Mendelian in inheritance, namely, the *brevistylis*-character, may well be put to the test of a fuller genetic analysis.

In view of these facts, one can only view with astonishment the performance of Heribert-Nilsson (1912) in maintaining that the remarkable series of genetic puzzles presented by the Oenotheras can find an explanation through the recombinations of plural Mendelian determiners. His entire thesis falls to the ground the instant we begin to figure out some of the very simplest and most obvious consequences of such an explanation. His abandon in the application of this hypothesis was made possible only by his belief that students of the Oenotheras have generally failed to use strictly individual analysis in their investigations. Having myself never mixed the seeds from two different mothers or from two different crosses, I am unwilling to believe that Heribert-Nilsson has not greatly overestimated this source of difficulty in interpreting the genetic phenomena in Oenothera, although it is a valuable service to have pointed out so strongly as he has done the importance of the strictest possible adherence to the "isolation principle." The importance of this emphasis may be seen, when so careful a worker as Dr. Gates grows a culture from the mixed seed of four different self-fertilized parents, belonging to a group in which there was obvious

genotypic impurity, on the ground that "this made no difference in the experiments [he] then had in view" (Gates, 1913 b, p. 143). It is a little difficult at the present time to imagine the nature of those genetic experiments which would not be injuriously affected by such an origin of the foundation stock. It can only be a matter for regret that Gates (1914) has also used for his so-called "reciprocal" crosses between *O. rubricalyx* and *O. grandiflora*, a strain of the latter species from Alabama, for the one cross, and one from Birkenhead, England, for the "reciprocal" cross, especially in view of the fact that he has found much evidence of complex hybridizations in the latter locality (Gates, 1913 a). Finally, his "back-crosses" of the F_1 plants from this second hybrid family have been made not upon plants of the parental strain, but upon those of the Alabama strain. It is hardly to be hoped that the elements of genetic behavior in Oenothera will be discovered by these methods.

The conclusion reached by Gates (1910) that the method of inheritance of a character is determined by the nature of the character itself, is also brought sharply into question by the peculiar situation in my *O. rubricalyx*-crosses, for I am clearly dealing with the same character which Gates has repeatedly called a monohybrid Mendelian dominant; but in my cultures it is either not Mendelian at all, or, if Mendelian, is affected in a complex way by several different determiners. The remarkable diversity in the nature of the characters which have been proved to be typically Mendelian in inheritance in various plants and animals, should have made Gates's conclusion impossible. It is not the externally visible, physical or chemical nature of a character which determines the method of its inheritance, but the nature of the inheriting-"mechanism" to which it is related, and the manner in which its determiner or determiners are related to that mechanism. Baur (1910) has discovered a case which clearly illustrates this point, and I also have been able to confirm his results (Shull, 1914). We have shown that "chloralbinism" in plants may result from several different causes. In some cases the absence of chlorophyll is due to the absence of a definite Mendelian gene, inherited equally well through both the male and female gametes; in other cases it appears to be a purely cytoplasmic defect, inherited in characteristically non-Mendelian ways, sometimes only through the mother, sometimes through the father and the mother, but with such irregularity that its inheritance cannot be related to Mendelian phenomena. Whether Mendelian or non-Mendelian, the character itself appears to be the same, namely, the

absence of the chloroplasts. Numerous investigators have shown that the anthocyan pigments of many plants are determined in quantity, quality and distribution by normal Mendelian genes, but the studies described in this paper, as well as those of Gates, strongly indicate that in Oenothera the inheritance of the red-pigmentation is determined by some other hereditary system, and the same inference may be drawn from most of the other genetic phenomena thus far recorded for Oenothera. Further experimentation must discover a mechanism adequate for the interpretation of these genetic phenomena. Until that mechanism is found it will be impossible to decide what constitutes a unit-character in Oenothera, or to decide whether any particular genetic differentiation represents a case of segregation, "fractionation," or some other method of distribution of characters.

Summary.

An investigation of the genetic phenomena presented by *Oenothera rubricalyx* Gates and its hybrids has shown that the bright red hypanthia and cones of that species are separable in inheritance from the brilliant red stems with which, according to Gates's description, it was always associated in his cultures.

In the F_1 hybrids from reciprocal crosses between this species and *O. rubrinervis* and *O. Lamarckiana* a remarkable series of negative correlations appear in the distribution of the red pigment, the brilliantly pigmented buds characteristic of *O. rubricalyx* being invariably associated with a low degree of red-pigmentation in the stems and rosettes: pink-coned buds with green hypanthia, characteristic of *O. Lamarckiana*, being on the other hand, invariably associated with brilliant red stems, while buds entirely free from anthocyan are associated with dull dark-red stems.

A self-fertilized plant of *O. rubricalyx* produced offspring having *rubricalyx*-buds and green buds in the ratio 10·7 : 1, all the green-budded plants having *nanella*-stature and characteristic dark-red stems. One plant having *rubricalyx*-pigmentation was likewise of the dwarf type.

The ratio of *rubricalyx*-budded plants to non-*rubricalyx* in the crosses with *rubrinervis* was approximately 1 : 1, and in crosses with *Lamarckiana* 2 : 1.

By complicated auxiliary hypotheses these ratios could be explained in accord with Mendelian inheritance, but the fact that Oenothera apparently has a unique mechanism for the distribution of hereditary

characters makes such subsidiary hypotheses of no value except as a basis for further investigations.

Other Oenothera crosses are cited, which indicate that the inverse relation between the red-pigmentation of the buds and that of the stems is not limited to crosses of *O. rubricalyx*. *Oenothera rubrinervis* represents a progressive variation in the pigmentation of the bud-cones and a retrogressive variation in the pigmentation of the stems.

It is held that three conclusions arrived at by Gates regarding the origin and genetic nature of the *rubricalyx*-character are erroneous, namely, (*a*) that the character represents a purely quantitative difference from *O. rubrinervis*, (*b*) that it differs from the latter species in a single monohybrid Mendelian unit, and (*c*) that the nature of a character itself, instead of the nature of the inheriting-mechanism to which it is related, determines the manner of inheritance of that character.

LEGENDS FOR PLATES.

PLATE V.

Rosettes of *O. rubricalyx* (upper series), *O. rubrinervis* (lower series) and of their reciprocal F_1 hybrids. Each set of 3 hybrid rosettes stands next to those of the seed-parent type. Inside diameter of pots about 7·5 cm. Photographed April 19, 1913, about 11 weeks after the seeds were sown.

PLATE VI.

Rosettes of *O. rubricalyx* (upper series), *O. Lamarckiana* (lower series) and of their reciprocal F_1 hybrids. Arrangement the same as in Plate V. Photographed April 9, 1913, about 10 weeks after the seeds were sown.

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ON A SUPPOSED SYNTHESIS OF ANTHOCYANIN.

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In a recent paper dealing with the anthocyanin pigments of plants Everest[1] makes suggestions which are quite in contradiction to the views generally held on the subject.

His main contention is that anthocyanins are reduced products of flavones, whereas most investigators have considered them to be oxidised products of some aromatic chromogen. He also raises the question of the mechanism of their production and the first part of his paper is devoted to a criticism of an hypothesis which was brought forward by one of us some years ago.

The said hypothesis was based upon various observations connected with the distribution, appearance and disappearance of anthocyanin pigments in plants, whereby it appeared that the formation of the latter is in some way intimately connected with photosynthesis and the products of this process, i.e. sugars. Hence it was suggested tentatively[2], as there was no absolute evidence either for or against it, that if anthocyanins are formed from flavones (which occur in the plant as

[1] Everest, A. E., "The Production of Anthocyanins and Anthocyanidins," *Roy. Soc. Proc.* 1914, B, Vol. LXXXVII. p. 444.

[2] Wheldale, M., "On the Formation of Anthocyanin," *Journ. of Genetics*, 1911, Vol. I. p. 133. And later, Wheldale, M., "The Flower Pigments of *Antirrhinum majus*. I. Method of Preparation," *Biochemical Journ.* 1913, Vol. VII. p. 87.

glucosides), the reactions controlling the formation of anthocyanin might be as follows:

glucoside (of flavone) + water $\rightleftharpoons$ chromogen (flavone) + sugar

[illegible] X (flavone) + oxygen = anthocyanin[1].

It was suggested that one or more hydroxyls, *though not necessarily all*, of the flavones were combined with sugar and that after hydrolysis of certain, *though again not all*, of the hydroxyls, further changes, such as oxidation or condensation, or both, might take place at these points thus opened to attack, with the formation of anthocyanin. Hence the anthocyanins themselves would be glucosides, and at no time would the products taking part in the reaction be completely in the nonglucosidal state.

The first criticism offered by Everest is that there are no known glucosides of the flavones having more than one or two hydroxyl groups substituted by sugar molecules. The hypothesis was brought forward in full knowledge of this fact, but it must be borne in mind that no accurate work has been done with a view to actually ascertaining the number of sugar molecules attached to flavones *in the plant*. Certain stable glucosides of various flavones, with only one or two sugar molecules attached, have, from time to time, been extracted and identified, and probably the fact that they could be identified is due to their stability. It is quite conceivable that, greater precautions being taken, other and less stable compounds might be isolated. No special precautions have in fact been taken, certainly in the majority of cases, to avoid decomposition of possible unstable glucosides. Until further investigations have been carried out on this point, no definite statement can be made as to the number of sugar molecules attached to flavones while in the plant.

The second criticism offered by Everest is based upon a misconception of the hypothesis. According to the evidence of Willstätter and Everest[2], anthocyanins (as glucosides), can be distinguished from anthocyanidins (non-glucosides), by their solubility in amyl alcohol, the anthocyanidins being soluble.

Hence if the flavones are completely hydrolysed and then give rise to non-glucosidal anthocyanin (which is an incorrect interpretation of Wheldale's hypothesis) it should be possible to isolate anthocyanidins

[1] The second equation is incorrectly quoted in Everest's paper, no allowance being made for condensation.

[2] Everest, *loc. cit.*

from the plant by means of amyl alcohol and this, as Everest notes, is contrary to all observation.

But, as stated above, it is not suggested in Wheldale's hypothesis that anthocyanins or flavones are at any time entirely free from sugar so that the hypothesis is not, on this point, in contradiction to observed facts.

Further, still on the incorrect interpretation of the hypothesis, Everest states, "one would expect that by taking the yellow glucoside, hydrolysing, then reducing with removal of sugars, the anthocyanidin produced would combine with the sugar present to form an anthocyanin. This is not the case." Under any circumstances this criticism would be of no value as it is well known that glucosides are not formed *in vitro*, except in a few cases under very special conditions such as are certainly not realised in the above experiment.

There is however yet another point, namely that Everest maintains that *no hydrolysis at all* of flavone glucosides is necessary as a preliminary to the formation of anthocyanin (glucosidal). For he obtains anthocyanin from extracts of many plants containing flavones as glucosides by simple reduction in the cold (though this result was not obtained with artificial quercitrin, a glucoside of quercetin). The value of this criticism depends on whether the products formed by reduction are really anthocyanins.

This question must now be considered, and forms the second main part of Everest's paper. The reactions described are as follows. When the flavones, quercetin, morin and luteolin are treated, in alcoholic solution acidified by hydrochloric acid, with nascent hydrogen formed, for instance, by the action of sodium amalgam, a purple-red or red colour is produced. In the case of quercetin (we do not know whether this is equally true for morin and luteolin) the red product gives with alkalies a green reaction similar to that given by anthocyanin.

Everest has prepared these red substances from quercetin and from various plant extracts. When quercetin was used the red product was soluble in amyl alcohol—hence it was regarded as an anthocyanidin. When plant extracts were employed, the red product was insoluble in amyl alcohol—hence it was considered to be an anthocyanin.

Everest apparently assumes the red products to be anthocyanins or anthocyanidins, as the case may be, solely on the basis of the green reaction with alkalies, since the mere fact of the solubility or insolubility of the substances in amyl alcohol cannot be taken as evidence of their anthocyanin nature.

Moreover the fact that both natural anthocyanin and the artificial red products can be reduced to a colourless form and then reoxidised to the coloured state, cannot be taken as proving that the artificial substance is anthocyanin, since innumerable coloured aromatic substances give leucocompounds on reduction which regain their colour on oxidation.

The grounds for thinking that these substances are anthocyanins seem to us slight, and our observations lead us, in the absence of further evidence, to feel considerable doubt on the point for the following reasons:

1. The red product from quercetin is soluble in ether whereas all natural anthocyanins are quite insoluble in this solvent.

2. The only case in which anthocyanins and their antecedent chromogen have been analysed is that of *Antirrhinum*, and here the anthocyanins are oxidised, and not reduced products, of the flavone concerned, i.e. apigenin[1].

3. When apigenin is treated in alcoholic solution with sodium amalgam and acid, as was done with quercetin, we obtained a red product and the reaction clearly followed a similar course in the two cases. Analyses showed the product from apigenin to be a reduced substance and its reactions with acids and alkalies do not in any way resemble those of anthocyanin. With alkalies the red colour is deepened and with acids the red colour becomes yellowish.

Since, in the case of apigenin, the product is certainly not an anthocyanin, it obviously throws doubt on Everest's assumption that the similar products from quercetin and other flavones are anthocyanins. Thus, in the only case where the flavone and the naturally derived anthocyanin have been isolated, the product formed by reduction of the same flavone is quite a different substance from the natural anthocyanin.

The evidence of a considerable amount of work by Keeble, Armstrong and Jones[2] shows that in the vegetative organs as well as in the flowers of various plants the distribution of anthocyanin coincides with the

[1] Wheldale, M. and Basset, H. Ll., "The Flower Pigments of *Antirrhinum majus*. 3. The Red and Magenta Pigments," *Biochemical Journ.*, 1913, Vol. VII. p. 87.

[2] Keeble, F. and Armstrong, E. F., "The Distribution of Oxydases in Plants and their Rôle in the Formation of Pigments," *Roy. Soc. Proc.*, 1912, B, Vol. LXXXV. p. 214: "The Rôle of Oxodases in the Formation of the Anthocyan Pigments of Plants," *Journal of Genetics*, 1912, Vol. II. p. 277: Keeble, F., Armstrong, E. F., and Jones, W. N., "The Formation of the Anthocyan Pigments of Plants. Part I. The Chromogens," *Roy. Soc. Proc.*, 1913, B, Vol. LXXXVI. p. 308: "The Formation of Anthocyan Pigments of Plants," Part 6, *Roy. Soc. Proc.*, 1913, B, Vol. LXXXVII. p. 113.

presence of oxidising enzymes. Anthocyanin is therefore formed in tissues in which oxidation most readily takes place. The above authors have latterly inclined to the view that reduction precedes oxidation, but this view rests on the supposition that the products of reduction of the flavones are anthocyanins.

It is difficult to reconcile Everest's assumption that anthocyanins are reduced flavones, with the fact that they are shown to occur only in those parts of plants where oxidases, and therefore oxidations, occur.

There remains then the question of the derivative from quercetin which gives a green alkali reaction. This substance was prepared by us from an alcoholic solution of quercetin; the residue, after evaporation, was extracted with ether containing a little alcohol and several combustions were made of the product. The results showed the product to be a reduced derivative of quercetin.

A determination of the molecular weight by the raising of the boiling point of alcohol gave a molecular weight approximately equal to that of quercetin. Hence the substance is a simple reduction product of quercetin, whereas the natural anthocyanins derived from apigenin are both oxidised and condensed products. Nothing can be gained at present by comparing the composition of the reduction product from quercetin with other analyses of natural anthocyanins, since the antecedent chromogens of the latter are not known.

Further discussion on the matter is useless in the absence of exact experimental evidence. In the case of quercetin, in order to secure any convincing evidence, it would be necessary to isolate a natural anthocyanin known to be derived from quercetin and to compare the results of its analysis with those obtained for the reduction product.

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OUR PRESENT KNOWLEDGE OF THE CHEMISTRY OF THE MENDELIAN FACTORS FOR FLOWER-COLOUR.

BY M. WHELDALE,

Fellow of Newnham College, Cambridge, and formerly Research Student of the John Innes Horticultural Institution, Merton, Surrey.

SINCE the inheritance of flower-colour involves, in the majority of cases, the inheritance of some kind of anthocyanin pigment, any knowledge of the chemistry of the factors for colour must of necessity be concerned with the chemistry of anthocyanin. During the last few years a considerable amount of work has appeared, i.e. that published by Combes, Grafe, Tswett and Willstätter, on the chemistry of anthocyanin apart from its connection with Mendelian problems. It seems advisable at every stage to bring the results of such work to bear, when possible, upon the evidence obtained by those who have approached the subject more directly from the Mendelian point of view.

In the present paper an attempt has been made to state, as far as possible, from all the evidence available, exactly how much we know of the chemical mechanism underlying the Mendelian factors for flower-colour.

The colour varieties of *Antirrhinum majus* having provided useful material for the chemical interpretation of some Mendelian factors, an account will be first given of the pigments of this species.

The Varieties of Antirrhinum majus.

The original wild type of *Antirrhinum majus* has magenta flowers, the colour being due to at least seven pairs of factors (Wheldale, 25, 26, 29; Baur, 5, 6), but of these, only four need be mentioned in the present paper, and the type homozygous in all factors may be represented as:

YYIIRRBB.....magenta.

Of these factors:

Y represents the power to produce a very pale yellow (ivory) pigment in the tube of the corolla and a deep yellow pigment in the lips of the corolla with a still deeper yellow patch[1] on the palate (Pl. VII, fig. 2). The composition of a plant bearing yellow flowers may be represented as:

$$YY(y)iirrB(b)B(b)\ldots\ldots\ldots\text{yellow.}$$

I represents the power to form ivory pigment in the tube and lips, and to inhibit the formation of yellow pigment in the lips except on the palate. The composition of a plant bearing ivory flowers (Pl. VII, fig. 3) is:

$$YY(y)II(i)rrB(b)B(b)\ldots\ldots\ldots\text{ivory.}$$

R represents the power to produce red anthocyanin pigment in the corolla[2]. If the ivory factor is absent, the red is mixed with yellow and the result is bronze (Pl. VII, fig. 4):

$$YY(y)iiRR(r)bb\ldots\ldots\ldots\text{bronze.}$$

If the ivory factor is present, the result is rose doré (Pl. VII, fig. 5):

$$YY(y)II(i)RR(r)bb\ldots\ldots\ldots\text{rose doré.}$$

B represents the power to convert the red anthocyanin into bluish-red or magenta anthocyanin. If the *I* factor is absent, the result is a mixture of magenta and yellow, i.e. crimson (Pl. VII, fig. 6):

$$YY(y)iiRR(r)BB(b)\ldots\ldots\ldots\text{crimson,}$$

but if the ivory factor is present, the result is magenta (Pl. VII, fig. 7):

$$YY(y)II(i)RR(r)BB(b)\ldots\ldots\ldots\text{magenta.}$$

If the *Y* factor is absent, the plant produces dead white flowers (Pl. VII, fig. 1), though it may be carrying any or all of the remaining factors:

$$yyI(i)I(i)R(r)R(r)B(b)B(b)\ldots\ldots\ldots\text{white.}$$

[1] The patch on the palate is common to all varieties except white.

[2] In the original publications on *Antirrhinum*, the factor *R* was used to denote a tingeing only of anthocyanin, an additional factor *D* being present in the fully coloured varieties, but this distinction is not really necessary in the present paper.

1. White

2. Yellow

3. Ivory

4 Bronze

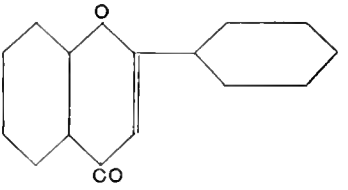
5. Rose doré

6. Crimson

7. Magenta

The Yellow Pigments of Antirrhinum.

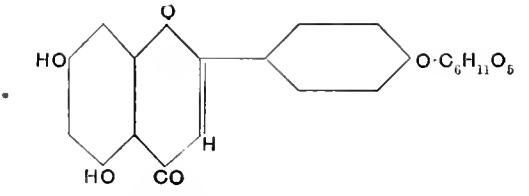
Examination was first made of the yellow pigments of *Antirrhinum* and they were found to be members of a group of natural yellow colouring matters known as flavones. These substances are derivatives of β-phenylbenzo-γ-pyrone:



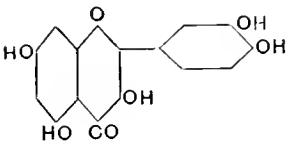
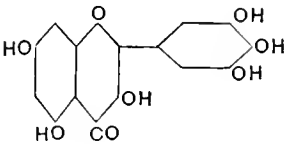
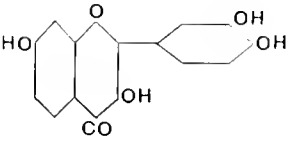
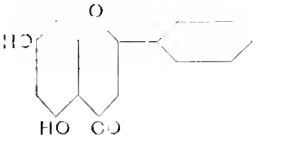
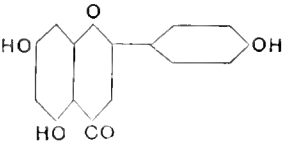
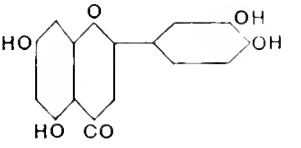
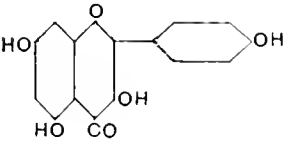
and they differ from each other, as regards constitution, in the number and position of their hydroxyl groups. The accompanying table (on page 112) sets forth the reactions and distribution of the more common flavones. The actual extent of their distribution, however, is by no means represented by the genera mentioned, for our knowledge of the occurrence of the flavones in plants is practically limited to the researches of Perkin (21), and a few other workers who have investigated these particular species solely from the point of view of the dyeing properties of the plant extracts. The results of these investigations have shown that the capacity for dyeing in the above cases is due to the presence of particular flavones.

There is little doubt that the flavones are universally distributed among plants and form a class comparable to sugars, proteins, tannins, etc., but no work has yet been done with a view to ascertaining the distribution of the various members of the class.

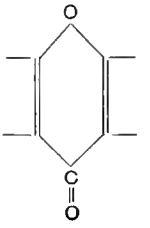
The flavones occur as a rule in the plant in the form of glucosides, one or more of the hydroxyl groups being replaced by sugar, as for instance:



In this condition the flavones are very little coloured (see below) and readily soluble and as such they occur in the cell-sap throughout the plant. A characteristic reaction of the flavones is to give an

Flavone	Constitutional Formula	Melting Point	Products of Decomposition from Alkali Melt	Distribution
Quercetin		Sublimes above 250	Phloroglucin and protocatechuic acid	Free and as various glucosides in *Quercus* (bark), *Rhamnus* (berries), flowers of *Cheiranthus*, *Crataegus*, *Viola*, *Prunus*, *Hibiscus*, leaves of *Ailanthus*, *Rhus*, *Arctostaphylos*, *Calluna*, *Eucalyptus* and many others
Myricetin		357	Phloroglucin and gallic acid	*Myrica* (bark), leaves of *Rhus*, *Haematoxylon*, *Arctostaphylos*
Fisetin		Above 360	Resorcinol and protocatechnic acid	*Rhus* (wood)
Chrysin		275°	Phloroglucin and benzoic acid	*Populus* (buds)
Apigenin		347	Phloroglucin and *p*-oxybenzoic acid	Leaves of *Apium*, *Reseda*
Luteolin		327	Phloroglucin and protocatechuic acid	Leaves of *Reseda*, *Genista*, *Digitalis*
Kampherol		276	Phloroglucin and *p*-oxybenzoic acid	In flowers of *Prunus*, *Delphinium*, leaves of *Polygonum*, *Indigofera*, *Robinia*

intense yellow colour with alkalies; this reaction is readily seen when parts of plants, which are free from chlorophyll, are immersed in ammonia vapour. White flowers under these conditions turn canary-yellow. When water extracts of glucosides of the flavones are hydrolysed by boiling with an acid, the flavone separates out from the solution owing to the fact that it is less soluble than it was when in the form of a glucoside. The flavones themselves are yellow crystalline substances, as a rule readily soluble in alcohol, sparingly soluble in ether, and almost insoluble in water. Their solutions give yellow or orange precipitates of lead salts with lead acetate and generally a green or brown coloration with ferric salts. The colour of the flavones is due to the chromophore group:



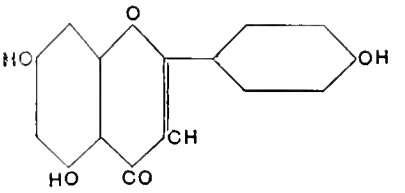
in conjunction with hydroxyl groups, the auxochromes, the intensity of colour depending on the position of the latter (Smiles, 22). When the hydroxyl groups are replaced by sugar or by acetyl or benzoyl radicals a colourless, or practically colourless, compound is produced, since the effect of the auxochromes is eliminated.

The fact that the flowers of the ivory variety of *Antirrhinum* become yellow in ammonia vapour suggested that the pale yellow or ivory pigment might be a flavone.

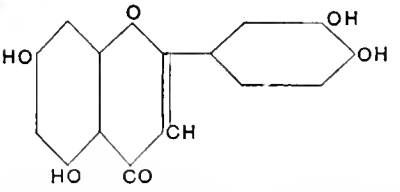
Ivory pigment was prepared from the upper lips of ivory flowers in the following way (Wheldale, 32). Large quantities of material were boiled with water, filtered, and poured into lead acetate solution which precipitates the pigment as a canary-yellow lead salt. This was filtered off and decomposed by dilute sulphuric acid which precipitates the lead as lead sulphate, the pigment being set free again in solution. After filtering off the lead sulphate, the pigment in the dilute acid solution was boiled, by which means the glucoside is hydrolysed, and on cooling, the free pigment is deposited as a brownish-yellow precipitate which is filtered off and dried.

The crude pigment was then purified by extracting in a Soxhlet thimble over boiling ether in which the ivory pigment is soluble and

was thereby purified from oxidised products formed in hydrolysis. The ether extract was crystallised from alcohol, the crystals being in the form of pale yellow plates. The pigment was next converted into its acetyl derivative by boiling with acetic anhydride. The acetyl derivative is pure white (owing to replacement of the hydroxyl groups by the acetyl residue) and crystallises in long needles. The pigment may be again obtained in a completely pure state by hydrolysing the acetyl derivative with alcoholic soda solution. From the analyses and melting points of the acetyl derivative and of the pure pigment (Wheldale and Bassett, 33), the latter was identified with apigenin, a flavone of known constitution occurring in Parsley (*Apium Petroselinum*), and in small quantities in *Reseda luteola*:



The yellow pigment of the yellow variety was found to be present in the epidermis only of the lips of the corolla, the inner tissues containing apigenin. Hence the crude pigment, prepared in the way described above from the upper lips of the yellow variety, always consisted of a mixture of apigenin and yellow pigment. From preliminary experiments it seemed likely that the yellow pigment was the flavone, luteolin. On this assumption, a separation of the two pigments was brought about by means of hydrobromic acid which forms a compound with luteolin but not with apigenin. The yellow pigment was identified with luteolin by means of its melting point and other properties and the melting point of its benzol sulphonyl derivative (Wheldale and Bassett, 34). Luteolin has been isolated from *Reseda luteola* and it is also found in *Genista tinctoria* and in leaves of *Digitalis*. It is represented as:



and owes its intense colour to the ortho-position of the two hydroxyl groups in the side ring, which structure does not occur in apigenin.

Hence we may regard the dominant ivory factor in *Antirrhinum* as the power to inhibit the formation of luteolin.

The difference between the ivory and yellow varieties can however be expressed in a more fundamental way. When the flavones are fused at a high temperature with caustic alkali, a splitting of the molecule takes place into a phenol, usually phloroglucin, and a hydroxybenzoic acid. It is most probable that the flavones, conversely, are synthesised in the plant from the same phenols and acids, or their derivatives. Hence the particular flavone synthesised depends on the hydroxybenzoic acid formed in the plant. In the ivory variety the constitution of the living molecule is such that only a monohydroxy acid can be formed, whereas in the yellow a dihydroxy acid is formed in addition. More than this cannot at present be said and no particular benefit is gained at present by postulating an enzyme connected with the process.

From the white variety no flavone could be extracted, nor do the flowers give the canary-yellow colour when subjected to ammonia vapour.

The Anthocyanin Pigments of Antirrhinum *and* Centaurea.

By crossing yellow and ivory varieties with whites carrying suitable factors, the anthocyanin-containing varieties can be produced in F_1:

YYiirrbb (yellow) × *yyiiRRbb* (white) ⟶ *YyiiRrbb* (orange)

„ × *yyiiRRBB* (white) ⟶ *YyiiRrBb* (crimson)

YYIIrrbb (ivory) × *yyiiRRbb* (white) ⟶ *YyIiRrbb* (rose doré)

„ × *yyiiRRBB* (white) ⟶ *YyIiRrBb* (magenta)

Thus it is obvious that in each of the above cases the constituents of anthocyanin are separated in the two parents but when they come together again in F_1, the pigment can be formed.

The universal distribution of flavones, the similarity in properties between flavones and anthocyanins, and their intimate connection in the above crosses led to the suggestion (Wheldale, 27, 28, 30), that anthocyanins may be derivatives from flavones and that the white variety contains some factor which modifies the flavone with formation of anthocyanin.

In the plant, the anthocyanins are present as glucosides, in solution in the cell-sap of the epidermis of the corolla. From water or alcohol solutions, they are precipitated as green lead salts, by addition of lead acetate.

Pure anthocyanin was prepared from five varieties of *Antirrhinum* in the following way (Wheldale and Bassett, 35). Crude pigment was obtained by the method described for yellow and ivory varieties. Since anthocyanin only occurs in the epidermis, while apigenin is found in the inner tissues of the corolla, all crude pigment consisted of mixtures of anthocyanin and apigenin, and, in some cases, of luteolin in addition. Anthocyanin was purified from flavones by extracting the mixture for several months with boiling ether. The residue of crude anthocyanin was then dissolved in the minimum amount of alcohol and precipitated by ether in which it is insoluble. The dried precipitate was again extracted with ether to remove all traces of flavone. In this way anthocyanin was obtained in a purer condition than by crystallisation, since from a mixture flavone and anthocyanin readily crystallise out together.

Combustions of pure anthocyanin prepared from rose doré and from bronze showed the anthocyanin pigments to be identical in both cases, the difference of colour in the flowers being merely due to the presence of luteolin in the latter case.

In the same way combustions of pure magenta anthocyanin from magenta and from crimson showed that crimson in the flower is a mixture of magenta anthocyanin and luteolin. The same magenta anthocyanin was also obtained from a variety—ivory tinged with magenta—which is formed from magenta by loss of a deepening factor. Hence the anthocyanin pigments in the tinged and full-coloured varieties are identical.

From analyses of anthocyanin from both red and magenta varieties, both pigments were found to contain much higher percentages of oxygen than the flavone:

	C	H	O (by difference)
Red anthocyanin ...	51·81 %	5·01 %	43·18 %
Magenta anthocyanin	50·50	5·11	44·39
Apigenin	66·66	3·70	29·64

A determination of the molecular weights of the two anthocyanins showed them to be of the order of magnitude of 700 for magenta and 570 for the red. Hence, if derived from flavones, of which the molecular weight is about 270, changes other than oxidation must be involved. It is most probable that there is also condensation of two or more molecules of flavone or condensation of a flavone molecule with other aromatic substances. If the latter be the case, condensation with one

substance might produce red anthocyanin and a further condensation with a second substance magenta anthocyanin, and the power to form these substances would be represented by the red and blue factors.

Hypotheses in this connection are, however, of little value until we have more knowledge of the constitution of the pigments themselves. Nothing, moreover, can be said at present of the nature of the factors causing the deepening or dilution of anthocyanin characteristic of deep and pale varieties.

At this point some account must be given of the work of Willstätter (36), on the anthocyanin of the Cornflower (*Centaurea*). The author states that in the flower three pigments are present, a red, a violet, and a blue. Willstätter is of the opinion that the anthocyanin of *Centaurea* contains a nucleus of the type of Fig. 1, colour being due to the presence of the quinonoid structure:

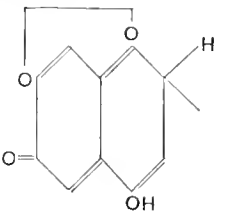


Fig. 1.

The above would represent the nucleus of the violet pigment, which is also in itself an acid. Under certain conditions a change readily takes place to a colourless isomer, the oxonium oxygen becoming divalent:

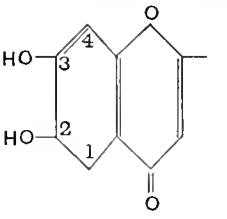


Fig. 2.

The isomer is really a flavone with an extra and unusual hydroxyl group in position 2.

The blue pigment in the flower, according to Willstätter, is the potassium salt of the compound in Fig. 1, the position of the potassium being uncertain. If the cell-sap is alkaline, the blue salt predominates or is alone present.

If there is excess of acid in the cell-sap an oxonium salt with an organic plant acid is formed:

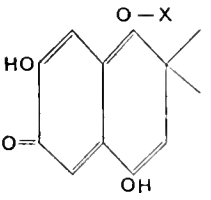


Fig. 3.

and crystalline salts, which the pigment readily forms with hydrochloric acid, are regarded as artificial compounds of a similar kind:

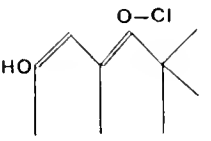


Fig. 4.

In the presence of excess of water, both the red and the blue forms will also pass to the colourless isomer, but when a neutral salt, such as sodium nitrate or chloride, is present the isomeric change is prevented by the formation of additive compounds:

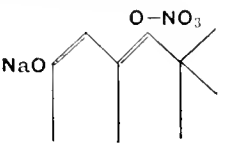


Fig. 5.

For preparing the pigment, the methods are long and elaborate, but consist mainly in extracting the pigment from dried flowers by means of water, sodium nitrate being added to protect the pigment, and finally precipitating the pigment with alcohol.

The blue pigment—cyanin—is a glucoside. On hydrolysis it is decomposed into glucose and a pigment, cyanidine. Both cyanin and cyanidine readily form crystalline compounds with hydrochloric acid. Analyses of cyanidine chloride gave as simplest formula $C_{16}H_{12}O_7$. HCl but no account is given of any determination of the molecular weight.

The hypothesis advanced by Willstätter as regards the constitution of anthocyanin is not at present based on any experimental evidence beyond the existence of the salts with acids. The chief argument

against the hypothesis is that flavones, of the constitution indicated in Fig. 2, are unknown.

No reference is made by Willstätter to the pigments in the actual red and purple *varieties* of the Corn-flower. If the pigments of the varieties are identical with the red and purple pigments found in the blue type, we must suppose the factorial differences merely to affect the acidity and alkalinity of the sap, the sap of the red variety being acid, of the purple, neutral, and of the blue type, alkaline, which does not appear to be a very reasonable suggestion.

Grafe (13) also, working with flowers of scarlet *Pelargonium*, has obtained an anthocyanin which forms very fine crystals, in combination with acetic acid, of the composition, $C_{18}H_{26}O_{13} . 2CH_3COOH$. The inheritance of colour in *Pelargonium* has not yet been investigated, so that these results cannot be brought into use at present.

Factors involved in the Formation of the Anthocyanin pigments.

The next question to be considered is the nature of the factor, *R*, connected with the formation of anthocyanin.

It was originally suggested (Wheldale, 30), that the *R* factor might be an oxidase and that the formation of anthocyanin might be represented as:

$$\text{glucoside} + \text{water} \rightleftharpoons \text{flavone} + \text{sugar}$$

$$x\,(\text{flavone}) + \text{oxygen} \longrightarrow \text{anthocyanin}$$

the first reaction being controlled by a glucoside-splitting enzyme, the second by an oxidase.

Since the views in connection with oxidases and oxidase reactions are very confusing, a short statement, as regards the action of oxidising enzymes, may not be out of place at this point.

It has long been known that solutions of certain compounds, such as guaiacum, α-naphthol, and benzidine are oxidised to coloured products in the presence of plant extracts. The water extracts, for instance, of some plants when added to guaiacum tincture at once produce a blue colour (direct oxidase reaction). Extracts from other plants do not produce this blue colour, unless hydrogen peroxide is also added (indirect oxidase reaction). If a comprehensive examination be made with these tests (Clark, 7, 8; Wheldale, 31), it will be found that the members of many orders and genera (*Umbelliferae, Labiatae, Boraginaceae* and others) commonly give a direct action whereas the members

of other orders (*Cruciferae, Caryophyllaceae, Crassulaceae,* and others) only as a rule give an indirect action. It will be noted at the same time that the plants whose juices give the direct action, also turn brown on injury, or when exposed to chloroform vapour. Also the extracts themselves, on exposure to air, rapidly turn brown or reddish-brown.

It is to Bach and Chodat (4) that we owe the first suggestion that an oxidase consists of two elements, an oxygenase which oxidises some substance to the state of a peroxide and a peroxidase which then transfers the oxygen of the peroxide to oxidisable substances. The hypothesis has been modified both by the authors themselves and by other workers: the oxygenase has been eliminated and the more simple hypothesis of a peroxide-peroxidase system has been retained.

It is now the opinion of various authors (Kastle and Loevenhart, 15; Moore and Whitley, 20; Wheldale, 31) that those plants which give the direct reaction form in their metabolism some substance which is able to autoxidise and form an organic peroxide so that the system peroxide-peroxidase is realised.

When, in the metabolism of the plant, no substance capable of rapid autoxidation is formed, the system is not able to convey the oxygen of the air to the artificial acceptor, i.e. guaiacum, but can only oxidise the latter when hydrogen peroxide is added.

Hence the division of plant oxidising enzymes into direct and indirect oxidases is purely artificial. The factor of importance is the peroxidase which is practically universally distributed and is the only factor which can be concerned in the formation of anthocyanin, for anthocyanin is formed equally in plants giving the direct or indirect oxidase reaction, and moreover in a large number of cases of plants giving the direct action the albinos possess this power as well as the pigmented types.

The view that anthocyanin may be formed from a chromogen by the action of an oxidising enzyme has been supported by Keeble, Armstrong and Jones (14, 16, 17, 18, 19). These authors, by means of an excellent micro-chemical test for oxidases, have shown that there is, in many cases, an intimate connection between distribution of oxidases in tissues and the distribution of anthocyanin.

The above authors employed dilute solutions of α-naphthol and of benzidine, in presence of hydrogen peroxide, as tests for oxidases, the material used being chiefly colour varieties of flowers and stems of *Primula sinensis.*

The results obtained were as follows. Broadly speaking, in all coloured varieties, peroxidases were found in the epidermis and bundle sheathes, the tissues in which anthocyanin is distributed. Flowers of the dominant white varieties were found to give oxidase reactions only after certain inhibitors were artificially removed from the flower. Albino varieties gave the oxidase reaction as fully as coloured varieties. Hence, if the hypothesis that colour in *Primula* is due to the action of an oxidase on a chromogen be true, we must assume that albinism is, in this case, due to the absence of chromogen. Since the chemical nature of the chromogen is unknown in *Primula*, this assumption can, for the present, be made, though all *Primula* albinos certainly contain a flavone.

In the same way all albinos of the Sweet Pea and the Stock contain both peroxidase and flavone, and here again, if the Keeble and Armstrong hypothesis be correct, we must assume the absence of chromogen to be the cause of albinism.

But in the case of *Antirrhinum*, peroxidase is present in both the ivory parent and the white parent which on crossing produce magenta. Since the evidence is strongly in favour of apigenin being the chromogen, some third substance or reaction must be postulated and the high molecular weights of the anthocyanins lend support to the hypothesis that there is some further process involving condensation.

From the work of Keeble, Armstrong and Jones we can only at present make the deduction that anthocyanin is formed in tissues where oxidation can take place, and there is no evidence among the well-known Mendelian cases of colour inheritance of a factor being represented by a peroxidase.

Quite similar results to those of Keeble and Armstrong have been obtained by Atkins (2, 3), working with flowers of many species and varieties of *Iris*. The author shows that the distribution of peroxidases and inhibitors is correlated with the natural colouring of the flowers.

The hypothesis that anthocyanin is formed by oxidation and condensation or by oxidation alone of the flavones is entirely refuted by Combes (9, 10, 11, 12), who maintains that anthocyanin, on the contrary, is formed by reduction from flavones. His evidence may be summed up as follows:

(1) There are two pigments in *Ampelopsis hederacea*, a yellow (flavone) and a red (anthocyanin).

(2) The flavone can be converted into a red pigment, identical with natural anthocyanin, by treating an alcoholic solution of the yellow

pigment with sodium amalgam in presence of hydrochloric acid. Hence the anthocyanin is a reduction product of the flavone.

(3) The anthocyanin can be converted into a yellow pigment, identical with the natural flavone, by means of hydrogen peroxide. Hence the flavone is an oxidation product of the anthocyanin.

As regards the formation of anthocyanin from the flavone in *Ampelopsis*, the method of procedure was as follows (Combes, 9):

"La solution dans l'alcool à 90° du composé jaune brun, acidifiée par l'acide chlorhydrique et soumise à l'action de l'hydrogène naissant fourni par l'amalgame de sodium, prend progressivement une coloration rose violacé qui devient de plus en plus foncée. Filtrée et neutralisée, la liqueur obtenue fournit par évaporation une substance pourpre.

Ce corps, comme l'anthocyane naturelle extraite des feuilles rouges, cristallise en aiguilles pourpres groupées en rosettes. Après deux cristallisations dans l'alcool et trois cristallisations dans l'eau, le pigment artificiel obtenu en partant du composé jaune naturel, aussi bien que le pigment anthocyanique naturel, commencent à se décomposer au bloc Maquenne à 165°; la fusion instantanée a lieu à 212°—215°, tandis que le composé jaune commence à se décomposer à 182°, et fond instantanément à 226°—229°."

The artificial anthocyanin had, moreover, the same qualitative reactions as the natural anthocyanin, i.e. it turned red on addition of acid and green, becoming brown-yellow, with alkali. It gave a green precipitate with lead acetate and a green-black coloration with ferric chloride. On addition of sodium bisulphite, the colour of the artificial anthocyanin disappears, but reappears on acidification with sulphuric acid, a reaction which is also characteristic of the natural pigment.

By the sodium amalgam method, Combes (11) was also able to obtain, in several cases, artificial anthocyanin identical as far as qualitative reactions are concerned with the natural product. The materials used were:

(*a*) A crystalline yellow-brown pigment isolated from Privet (*Ligustrum*) leaves. The resulting anthocyanin crystallised in needles.

(*b*) A yellow-brown crystalline pigment isolated from leaves of a variety of Vine which does not redden naturally. Crystalline anthocyanin resulted.

(*c*) A crystalline pigment from *Narcissus incomparabilis*; the resulting pigment had properties of anthocyanin.

(*d*) From artificially synthesised flavones (names not given). The resulting pigment had the properties of anthocyanin.

As regards the conversion of anthocyanin into flavone by oxidation the following method was adopted by Combes (10): "Le pigment anthocyanique extrait des feuilles rouges Vigne-vierge (*Ampelopsis hederacea*), purifié par deux cristallisations dans l'alcool et trois cristallisations dans l'eau, et dissous dans l'alcool à 90°, donne une solution de couleur pourpre, qui, additionnée de son volume d'eau oxygénée, passe peu à peu au rouge-brun, puis au jaune. De la solution jaune, ainsi obtenue, j'ai extrait un pigment jaune-brun cristallisé en aiguilles groupées en rosettes."

The artificial yellow pigment was found to have the same properties and melting point as the natural yellow pigment and was considered to be a flavone.

In the absence of further evidence, it is difficult to avoid making the following criticism of the experiment just quoted. Since no mention is made of any methods employed in the preparation of crystalline anthocyanin from *Ampelopsis*, it is highly probable that this product contained flavone as impurity, since most careful purification is necessary to remove all traces of flavone. Thus, from a mixture of flavone and anthocyanin extracted from *Antirrhinum*, both pigments will crystallise out together in plates which have, in spite of the presence of flavone, a deep red colour. If the mixture from *Ampelopsis* were of such a nature, on treating with hydrogen peroxide, the anthocyanin would be decomposed though not necessarily into flavone, and the flavone present as impurity could then be extracted from the resulting solution.

A similar observation, however, to the above is made by Willstätter (36), with regard to Cornflower anthocyanin. "Eine interessante Umwandlung erleidet das Cyanidin, wenn wir seine alkoholische Lösung mit verdünntem Wasserstoffsuperoxyd anstatt mit Wasser erwärmen. Nach der Entfärbung setzen wir einige Tropfen verdünnter Salzsäure zu und erhitzen im Wasserbad weiter. Die Flüssigkeit wird gelb, durch Extrahieren mit Äther lässt sich ein Produkt isolieren, das schöne, hellgelbe Krystalle bildet und mit Alkalien tief gelbe Lösungen liefert."

From the above one would make the deduction that, if the anthocyanin from Cornflower were pure from flavone, a flavone might be formed by destruction, though not necessarily oxidation, of the anthocyanin.

This method was also tested on pure anthocyanin from *Antirrhinum*. The pure pigment, prepared by methods previously described and entirely free from flavone, was treated in a similar way with hydrogen peroxide. The colour disappeared, giving a yellowish-brown solution and precipitate, but from neither solution nor precipitate could any flavone be extracted. From this it would appear that no flavone is formed by the action of hydrogen peroxide on the anthocyanin of *Antirrhinum*.

Observations of a similar kind to those made by Combes on the effect of sodium amalgam on acid alcohol solutions of flavones have also been made by Keeble, Armstrong and Jones (19). By treating the alcoholic extracts of various flowers with zinc dust and hydrochloric acid they obtained results which may be summarised as follows:

	Zinc and hydrochloric acid	Alkali
Pale yellow ("primrose") Wallflower	red solution	green
Yellow Daffodil	„ „	?
Yellow Crocus	„ „	?
Cream Polyanthus	„ „	?
Chinese Primrose	„ „	?
Primula sinensis (dominant white)	„ „	?
„ „ (recessive white)	extremely slight	?

Tswett (23, 24) also has obtained a red pigment by treating colourless extracts of apples with strong mineral acids in the presence of formaldehyde or acetic aldehyde. A careful examination of this artificial anthocyanin showed it to have very similar properties to natural anthocyanin. The chief point of difference appears to be its solubility in ether, for natural anthocyanin is insoluble in this medium, unless, according to Tswett, it has stood with hydrochloric acid for 3—5 days. In addition Tswett obtained similar red pigments, though he did not study them in detail, from pears (aldehyde not necessary), white grapes, bananas, flesh of red grapes, white petals of roses and Cyclamen. Negative results, however, were obtained with leaves of white cabbage, mesophyll of red cabbage, Pelargonium leaves, orange peel, petals of white pinks, white petals from buds of red pinks, flowers of lily of the valley, carrots, potatoes, Kohlrabi, and barley seedlings.

The reaction which underlies some, if not all, of the above phenomena is the following: when the alcoholic solution of some flavones is warmed and acidified with strong hydrochloric acid and sodium amalgam or zinc

dust is added, an intense red or purplish-red coloration is produced (Abderhalden, 1).

Hence the coloration given by many plant extracts in alcoholic solution when treated with hydrochloric acid and sodium amalgam or zinc dust is obviously due to the presence of flavone in the extracts, and it is useless to postulate the nature of the reaction in the absence of analyses of the red product.

The following points must then be considered:

(*a*) The question as to the identity of the artificial and natural anthocyanins. The only characteristic reactions in common between the two substances are the red colour with acids, the green colour with alkalies and the decolorisation with sodium bisulphite, and it is possible that these reactions might be given by two derivatives which were similar, though not necessarily identical. The artificial anthocyanin, after removal of alcohol and hydrochloric acid by evaporation, is soluble in ether and in water, whereas natural anthocyanins are always insoluble in ether and also almost insoluble in water except when in the condition of a glucoside.

(*b*) If the natural and artificial anthocyanins are not in any cases identical, the reaction is still interesting as showing that coloured products, very similar in properties to anthocyanin, can be obtained by reduction from the flavones.

(*c*) If the natural and artificial anthocyanins are in all cases identical, which is unlikely, then the reactions, by which the latter are formed, must be very different from those taking place in its formation in the plant, since, for instance, artificial anthocyanin can be produced, according to Combes, from members of the genus *Narcissus*, which do not form natural anthocyanin. Artificial anthocyanin, on the other hand, cannot be obtained from many species which do produce natural anthocyanin.

(*d*) There is a possibility, however, that in some cases natural anthocyanin may be formed by the treatment with nascent hydrogen, as for instance in the case of plants which produce the anthocyanin but in which the formation of pigment is inhibited in the flowers. It is conceivable that the materials necessary for the formation of anthocyanin are present but there is inhibition for some unknown reason. The treatment with sodium amalgam may remove the cause of inhibition and natural anthocyanin may be formed. Only isolation and accurate

analyses of both artificial and natural anthocyanin from the same plant can provide evidence which is of value.

Conclusions.

1. There are three varieties of *Antirrhinum majus*, ivory, yellow and white which do not form anthocyanin. Ivory is dominant to yellow and contains a factor "*I*" which is absent from yellow.

2. It has been shown that the pigments in the ivory and yellow varieties are flavones. Ivory contains a pale yellow flavone, apigenin, and yellow, in addition to apigenin, a deeper yellow flavone, luteolin. Hence the "*I*" factor may be represented as the power to inhibit the formation of luteolin.

3. The white variety contains no flavone.

4. When either the yellow or ivory is crossed with a white of suitable composition, an F_1 containing anthocyanin is produced. Therefore it appears likely that the anthocyanin is formed from a flavone by the action of some factor contained in the white.

5. It has been suggested that anthocyanin is either an oxidation or a condensation product of a flavone or both.

6. Two anthocyanins have been isolated from *Antirrhinum*, red and magenta, the latter containing a "*B*" factor which is absent from the red.

7. Analyses of the red and magenta pigments have shown that they both contain a higher percentage of oxygen than the flavones. Also, magenta has a higher percentage of oxygen than red.

8. Determinations of the molecular weights of the red and magenta pigments indicate that the anthocyanin molecules are at least twice or three times as large as the flavones. Hence, in addition to oxidation, condensation must have taken place, either between flavone molecules, or between the flavone and some other aromatic substance. In the latter case, one substance, the "*R*" factor, may give red anthocyanin and a second substance, the "*B*" factor, magenta.

9. The view that anthocyanin is in part, at any rate, an oxidation product is confirmed by the researches of Keeble, Armstrong and Jones, who have shown that anthocyanin is formed in tissues most rich in oxidising enzymes, though there is no good evidence, from the well-known Mendelian cases, of albinism being due to loss of an oxidising enzyme.

10. From recent researches on the pigments of the Cornflower, Willstätter states that the flower contains three pigments; a purple pigment which is an acid and owes its colour to the presence of a quinone nucleus and which readily passes to a colourless isomer which is a flavone derivative; a blue pigment which is the potassium salt of the purple; and a red pigment which is an oxonium salt of the purple with an organic acid.

11. As a result of recent work, Combes has brought forward the hypothesis that anthocyanin is not an oxidation product but, on the contrary, a reduction product of the flavones. As evidence, he quotes experiments which bring about the formation of anthocyanin from flavones by means of sodium amalgam in acid solution and the formation of flavones from anthocyanin by treatment of the latter with hydrogen peroxide. The evidence, however, cannot be considered conclusive in the absence of analyses of the products.

12. A similar formation of artificial anthocyanin has been obtained by Tswett, and by Keeble and Armstrong from plant extracts by treatment with aldehyde and strong acid or nascent hydrogen in presence of alcohol and strong acid. The artificial product has some of the properties of natural anthocyanin but differs in its solubilities. Further analyses are required for the determination of its identity.

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A COMMENTARY ON THE GENETICS OF THE CILIATE PROTOZOA.

By CLIFFORD DOBELL.

PREFACE.

For many years the ciliate Protozoa have been favourite objects of study, and consequently they have furnished much curious information concerning many problems of genetics. This information is, however, to a large extent still unincorporated into current biological conceptions. It has therefore seemed to me that it would not be an altogether thankless task to attempt to extract from the immense and often hardly accessible literature dealing with this group of organisms, such facts as are likely to interest workers in the field of genetics; and to present these facts in a summary fashion easily comprehensible to the reader unversed in protozoology. This is my aim in the following pages.

From its very nature, therefore, it will be apparent that I do not aim at setting forth in this article anything more than a selected body of facts extracted from the works cited in the appended bibliography—a consideration which I would ask the writers of these works to bear in mind, if they should find much that they have contributed to our knowledge passed over in silence, or treated in cavalier fashion. Without rigid and critical selection it would have been impossible to compress all the accumulated facts of many years into anything less than several bulky volumes.

In the presentation of the facts I have adopted the following plan. I have begun with a very brief general account of the Ciliata—in order to make clear some essential peculiarities of these organisms: I have then given a short account of the classical researches of Maupas[1]—since current conceptions concerning the Ciliata are largely based (explicitly

[1] I may remind the reader that the work on the Ciliata before the time of Maupas has been exhaustively dealt with by Bütschli (1887–89).

or implicitly) on these: I have then reviewed and commented upon the work which has been done since his day: and finally I have drawn a few of the more obvious conclusions—conclusions which will appear to many, I am afraid, the least satisfactory feature of this article. Since the works of many of the older investigators—Bütschli, Balbiani, Engelmann, and the rest—are in many ways no less admirable and important than the more modern researches of R. Hertwig, Enriques, Jennings, Popoff, Woodruff, and others, I have more than once referred to these; but such references have been relegated to footnotes.

To facilitate the numerous cross-references in the body of the paper I have adopted the plan of numbering the paragraphs. A parenthetic reference thus (§ *x*) signifies that on referring to the paragraph numbered *x*, the reader will find additional information relating to the matter under discussion.

I am fully conscious of having made many omissions, but I trust that misstatements concerning the facts are few. Such criticisms as I have ventured to make are based upon my personal knowledge of the ciliates, and I should be the first to welcome corrections or counter-criticisms.

A general analysis of the subject-matter of this article will be found on page 183. I have added it to facilitate reference to the subjects dealt with: and I have placed it at the end because I should prefer the article to be read as a whole before special sections of it are consulted. My commentary aims at being a consistent whole, and its several parts—considered singly—may therefore appear obscure or incomprehensible. If the reader's curiosity has been sufficiently excited, he will accordingly proceed now to the first chapter.

CHAPTER I.

The Organization of a Ciliate, and the Chief Events in its Life.

1. A typical ciliate possesses a peculiar organization, which is often misunderstood. Its body is built upon the plan shown in Fig. 1 (A). It is clothed, more or less, with locomotory ciliary appendages (*c*) often greatly differentiated. The animal possesses a mouth (*mo.*) defining the ventral surface; and as one end is specialized as a "head" end, we can further distinguish anterior and posterior, dorsal, and right and left lateral regions of the body—which is generally asymmetric. In the protoplasm there are usually vacuoles (*f.v.*) in which food is digested,

and also one or more rhythmically contractile vesicles (*c.v.*) serving probably for excretion or for regulation of the water-content of the protoplasm. Many other organs may be present.

2. The characteristic nuclear apparatus consists of two nuclei or systems of nuclei—a meganucleus (*M*), and a micronucleus (*m*). The latter may be represented by one, two, or many nuclei; whilst the meganucleus is frequently segmented, or otherwise modified.

3. Reproduction is usually effected by transverse fission of the body into two. The meganucleus is divided by a simple constriction, but the micronucleus (or micronuclei, as the case may be) divides mitotically. (See Fig. 1, B.) Unequal bipartition or budding occurs in some forms.

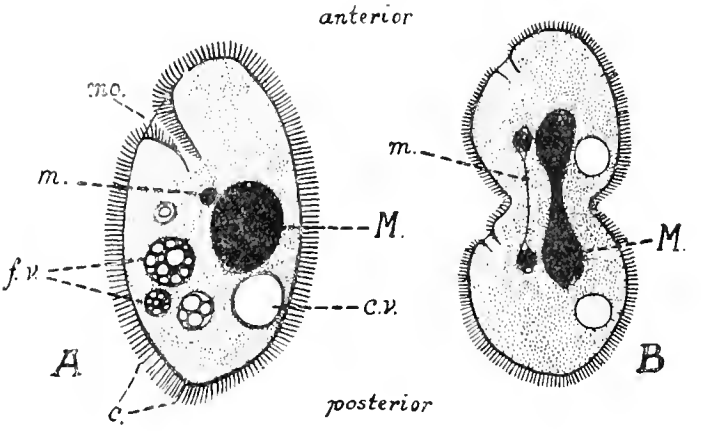

Fig. 1.

4. Most ciliates are known to be able to encyst, and so withstand the drying of the liquid in which they live, or other adverse conditions. In some forms temporary cysts are formed during fission.

5. Most ciliates perform from time to time a peculiar act called conjugation. The details of this process vary considerably in different forms, but the following is an approximately true general account of the events occurring during a typical conjugation[1]. (See Fig. 2, A—H.) The organisms unite in pairs, the protoplasm subsequently fusing and becoming continuous at the point of contact (Fig. 2, A, B). Each

[1] This account is based on the cases of *Colpidium colpoda* (Maupas, 1889) and *Paramecium caudatum* (Calkins and Cull, 1907), which are perhaps as "typical" as any other cases, and are fairly simple because there is only a single micronucleus and meganucleus in these forms.

individual's micronucleus (or one of them in a multimicronucleate form) now undergoes two[1] successive mitotic divisions, forming thus four micronuclei (Fig. 2, B). Three of these four nuclei degenerate (Fig. 2, B, C) and the remaining one again divides into two—a "stationary nucleus" (*s.n.*) and a "migratory nucleus" (*m.n.*). Each

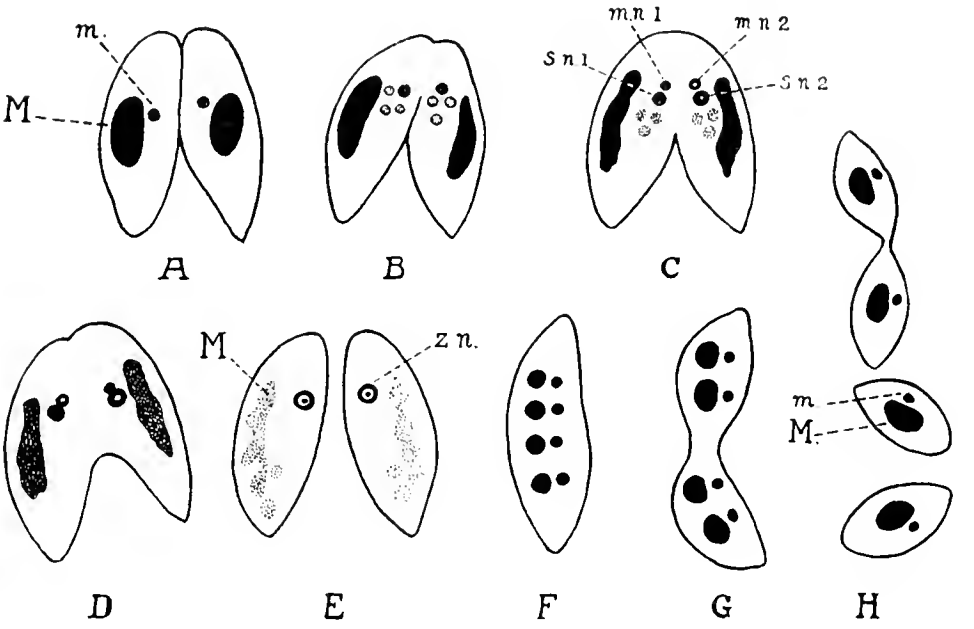

Fig. 2.

migratory nucleus now passes into the opposite individual of the pair, and fuses with its stationary nucleus (Fig. 2, C, D, E). Each individual thus comes to have a single micronucleus (*z.n.*) which is the product of its own stationary nucleus and the migratory nucleus of its partner (Fig. 2, E). The two organisms now separate, and their meganuclei degenerate and disappear (Fig. 2, E). At the same time, the micronucleus (fusion nucleus, *z.n.*) divides thrice in succession—producing eight daughter nuclei (Fig. 2, E, F) in each individual. Of these eight nuclei four increase in size, becoming new meganuclei, four remain small as new micronuclei (Fig. 2, F, G). By two successive transverse fissions of the whole organism, four small individuals are subsequently formed (Fig. 2, G, H), each with the nuclear arrangement characteristic

[1] In some forms three or more such divisions occur.

of ordinary individuals[1]. They grow and divide subsequently in the ordinary fashion (§ 3).

6. In *Vorticella* and its allies, conjugation of a different type occurs[2]. The two conjugating individuals are of different size—a large and a small, respectively sessile and motile. As in the previous case, they come in contact and fuse—their meganuclei sooner or later degenerating. The micronucleus in the larger individual divides twice successively by mitosis, giving rise to four nuclei, of which three degenerate and disappear. In the smaller individual the micronucleus undergoes three successive divisions, thus producing eight nuclei, of which seven degenerate. The two conjugating individuals now fuse completely[3]. Their two remaining nuclei divide once more, one of the two daughter nuclei so formed in each degenerating, the other persisting. The latter then come together and fuse. The single uninucleate individual resulting from conjugation then reorganizes its nuclear system in a manner comparable with that described above (§ 5)—the details of the process probably being different in different forms[4].

7. A "reduction" or halving of the chromosomes (meiosis) has been shown to occur during the micronuclear divisions which take place at the beginning of conjugation[5]. The halving appears to occur usually

[1] The details of the process whereby the new nuclear apparatus is constructed after conjugation may be very different from those outlined above. In all cases, however, the reorganization is of essentially the same nature—the formation of a new meganucleus and micronucleus from the single fusion nucleus.

[2] This account is based upon *V. monilata* (Maupas, 1889).

[3] According to Maupas (1889) the membrane clothing the body of the smaller individual is cast off and degenerates—it is not absorbed into the body of the larger.

[4] The accounts given above are conformable with the views of nearly all those workers who have studied the conjugation of the ciliates since the time of Maupas. It should be stated, however, that this accepted version of the sequence of nuclear events has been questioned by some workers. Hoyer (1899) believed that in *Colpidium* no nuclear fusion occurs during conjugation—the migratory nuclei are simply exchanged, and the stationary nuclei then degenerate. The new nuclear apparatus is therefore formed, in each individual, entirely from the nucleus which it receives from its partner. More recently, Dehorne (1911, 1911 *a*) has stated that the same thing happens in *Paramecium*, and that he has confirmed Hoyer's account of *Colpidium*. Dangeard (1911, 1911 *a*) has contradicted this in the case of the latter form (wrongly called *Colpoda* in his first paper), and maintains that the accepted version is correct. I believe that Hoyer and Dehorne are wrong in their contention. Maupas, R. Hertwig, Calkins and Cull, and all other recent workers who have studied conjugation, are unanimously agreed that a nuclear fusion does occur.

[5] Chromosome reduction has not been shown to occur in *Paramecium*. Calkins and Cull (1907) believe that the chromosomes are halved at the first micronuclear division, but they are "far too numerous to count." They judge them to be approximately 165 in number at this stage, whilst at other divisions the number is "greater than this." In *Stentor* (2 species) the chromosomes appear to be reduced to a quarter—not a half—of the usual number during the "reduction" divisions (Mulsow, 1913).

at the second division. (See Prandtl (1906), Enriques (1907), Popoff (1908 *a*), etc.)

8. It is possible that a certain amount of cytoplasm is always exchanged during conjugation[1]. This has been described in at least one form—*Stentor*. (See Mulsow, 1913.) In the parasitic form *Anoplophrya*, according to Collin (1909), each conjugating individual receives half the meganucleus of its partner[2]. After the exchange, however, it degenerates and disappears. The meganucleus is not known to behave in this manner in other forms.

9. After this brief account of division and conjugation[3], we may consider the interpretation to be put upon these events and the comparisons which may be drawn between them and the conditions which obtain in other animals. Failure to make a correct comparison, confusion of ideas, and misuse of words, have unfortunately introduced a great many unnecessary complications into this relatively simple matter. A real analysis of the vital phenomena of the Ciliata is possible only when we discard the erroneous *a priori* deductions of the modern cell doctrine and certain evolutionary speculations—with all their misconceptions and slipshod phraseology—and consider these organisms objectively. I have pointed out elsewhere how this may be done, so that it will be unnecessary to repeat what I have already written. (See Dobell, 1911.) I shall now merely state a few obvious homologies between a ciliate and a metazoon which are fundamentally important for a comprehension of what follows. These homologies are as correct as I believe it possible to make them with the words at my command. But I would emphasize here that the very peculiar organization and behaviour of the ciliates—which are in many ways radically different from all other organisms—render all descriptions of them, in terms borrowed from those other organisms of a different order, only approximately true.

10. First, a ciliate is not unicellular—it is not the homologue of one cell in the body of a metazoon, but is homologous with an entire

[1] For those who regard the chondriosomes, and not the chromosomes, as the "bearers of hereditary characters," it may be of interest to add that chondriosomes have been described in many different ciliates, but their behaviour during conjugation has not yet been studied.

[2] First described in *Anoplophrya* by Schneider, in 1886.

[3] It should be stated that the conjugation here described is that characteristic of most free-living ciliates. In some parasitic forms quite different processes occur. I may mention *Opalina*, which forms small ciliated gametes (Neresheimer, 1907; Metcalf, 1909), and *Ichthyophthirius*, which exhibits a peculiar form of self-fertilization (Neresheimer, 1908; Buschkiel, 1911).

metazoon. The ciliate is a non-cellular but complete organism: it has a body formed on the same fundamental plan as that of a metazoon, but it possesses no cellular differentiation. Cytoplasm and nuclei are specialized for special functions, but they are not sub-divided into separate units or "cells."

11. Secondly, it is clear that the meganuclear system of a ciliate is the equivalent of the nuclei of the somatic cells of a metazoon; and the micronuclear system of a ciliate is the equivalent of the germ-cell nuclei of a metazoon. These homologies—first made clear by Maupas (1889)—rest upon the observed behaviour of the nuclei during conjugation, and at other times in the life-history. It seems to me unnecessary to adduce here all the evidence which can be used to establish this proposition.

12. Thirdly, conjugation is a process of reciprocal fertilization (in the cytological sense): but it is coupled with a process of complete reorganization[1] unlike anything known to occur in other organisms. The conjugating individuals may be suitably called *conjugants*, and those which have just conjugated and separated, *exconjugants*. It is obvious that the conjugant is not a gamete, but a sexual individual: and the exconjugant is a zygote—but of a very remarkable kind; for it is the remains of the "parent" organism reorganized after the addition of a foreign nucleus.

13. Fourthly, the migratory nucleus is a microgamete nucleus and the stationary nucleus a macrogamete nucleus. This is evinced by their behaviour before and during conjugation: for the micronuclear divisions which precede conjugation (§ 5) are homologous with the maturation divisions of metazoan germ-cells (§ 7), and the active migratory nucleus is in some (? all) cases of smaller size than the stationary nucleus. (*Didinium* (Prandtl, 1906), *Paramecium* (Calkins and Cull, 1907), etc.)

14. Fifthly, organisms which—like *Paramecium*—conjugate in the manner described in § 5, must be regarded as hermaphrodite[2] individuals at the time of conjugation: for they each produce the equivalents of a "male" gamete and a "female" gamete. Organisms which conjugate like *Vorticella* (§ 6), however, are of a different sexual nature. The large conjugant is a female individual, producing the equivalent of a "female" gamete. The small conjugant is a male individual, producing the equivalent of a "male" gamete. There is an additional complication

[1] First definitely stated by Engelmann (1875).

[2] A conclusion drawn by Engelmann (1875) and earlier workers, but from incorrect premisses.

here, moreover, because the conjugants as well as their gamete nuclei fuse during conjugation, so that only one complex zygote results. It seems to me necessary to lay special emphasis on the preceding statements, because the terms "gamete," "sex," "male" and "female" are almost always misused in the case of the ciliates, and a very great deal of confusion has arisen in consequence[1]. It is manifest that "male sex" or "female sex" is properly predicated of individuals, which are ultimately known by the gametes which they produce. To suppose that a "male gamete" is itself a male is absurd. This misleading term really signifies "a gamete produced by a male individual." The gamete itself has no sex—within the legitimate meaning of the word.

15. Lastly, it should be clearly realized that among the ciliates the sole method of reproduction is by division. Sexual "reproduction" (in the sense of "multiplication") does not occur[2]. The zygote is a conjugant reorganized—a new individual raised upon the ruins of an old, without any increase in the number of individuals. After conjugation, the exconjugant reproduces by fission (the earliest divisions being sometimes modified, cf. § 5) in essentially the same manner as any other individual. All "generations" are produced *asexually*—by fission or budding. The only "sexually produced generation" is the exconjugant, which is merely the conjugant reorganized—an individual thrown into the melting pot, with a bit of another individual, and recast. In the case of the Vorticellids (§ 6), two whole individuals, as well as their gametes, fuse to form one zygote; so that sexual "reproduction" in these forms results in a reduction—not increase—in the number of individuals.

To convince himself of this, the reader need only refer to almost any of the recent works dealing with genetic experiments on ciliates. He will find that the same author assumes a conjugant to be a cell, a gamete, or a sexual organism, at pleasure—according to the requirements of the argument. Examples of such inconsistencies could be given by the dozen.

[2] "Ce n'est pas, en effet, un des résultats les moins surprenants des recherches sur la fécondation des Ciliés, de voir que son évolution et ses manifestations si complexes n'aboutissent à la multiplication ou à la reproduction d'aucun être nouveau" (Maupas, 1889, p. 435).

CHAPTER II.

The Life-cycle of a Ciliate according to Maupas.

16. I propose in this chapter to state very briefly the chief general conclusions drawn by Maupas from the extensive investigations which culminated in his great works of 1888 and 1889. His clear and precise conceptions will form a convenient fixed point from which to contemplate the work which has since been done.

17. Maupas claimed to have proved that the lives of ciliates run in cycles[1]. There is first a period of asexual multiplication (agamic period, or period of immaturity), during which the progeny of an exconjugant continue to multiply, without conjugating or being able to conjugate. At the end of this period—of definite length for each species—the organisms attain the age of puberty, after which conjugation is possible and normally occurs in nature. Unless they conjugate, the organisms continue to multiply for a further definite number of divisions. This second period—during the whole of which the animals are sexually mature—is called by Maupas the eugamic period. If, for any reason, conjugation does not occur during this period, the animals now enter a third period of multiplication—the period of senescence—which is also of definite duration. Throughout this period the animals suffer more and more from old age, until they finally die after completing a given number of fissions. Those organisms which are able to conjugate successfully during the eugamic period become "karyogamically rejuvenated," or reorganized, and start another cycle of development like that just described.

[1] This view was first definitely expressed—so far as I am aware—by Claparède and Lachmann (1860) and by Balbiani (1860). It has been stated by Woodruff (1909 *a*) that Dujardin "maintained that the life-history of infusoria comprises a cyclical change in vitality which terminates in death." I know of no passage in his writings which can be construed in this fashion. He certainly did not believe in a "cycle" such as that described by Maupas, for he held that a sexual act had not been proved to occur in any ciliate. Dujardin (1841, p. 87), suggested that there is possibly a limit to multiplication by fission, but he did not maintain this proposition or take sides on the issue. Balbiani's statements, however, are very definite. He says that he has established the fact that there is a limit to asexual reproduction in the ciliates. The period of asexual multiplication terminates with natural death of all individuals belonging to the same cycle; or "by a return of sexual reproduction, indicating the completion of one of these cycles and the beginning of a new cycle"; or by encystation. (See Balbiani, 1860.) His views were therefore at this time—he frequently changed them—very similar to those of Maupas.

18. A concrete case may be given in illustration. The life-cycle of *Stylonychia pustulata* may be tabulated thus[1]:

Divisions.

1—Exconjugant.
⋮ } Period of immaturity (agamic period).
130—Age of puberty.
⋮ } Eugamic period.
170
⋮ } Period of senescence.
316—Death.

The length of one complete cycle for this species is thus 316 divisions, but Maupas found that the cycle is of different length in different species. For example, *Stylonychia mytilus* died of old age after 319 successive bipartitions, *Oxytricha* and *Onychodromus grandis* after 320—330, *Leucophrys patula* after 660.

19. The period of time occupied by a developmental cycle depends, of course, upon the rate at which division takes place. This, according to Maupas, depends upon four factors: (1) the individual temperament of the species; (2) the biological adaptation of the species to its form of nutrition; (3) the quality and quantity of the nutrition[2]; (4) the temperature. Light and other external factors he found to have no appreciable effects.

20. Three conditions are, according to Maupas, necessary for successful conjugation. These are: (1) hunger; (2) sexual maturity; (3) diversity of ancestry of the conjugants ("fécondation croisée")—that is, the conjugants must not be related[3]. "External physical conditions play no determining part in the appearance of conjugation[4]." Hunger—

[1] The division at which the eugamic period ends and the period of senescence begins is variously stated by Maupas to be the 160th and the 170th—180th.

[2] Earlier experiments had been made by Balbiani (1860) in this connexion. He states that he placed three individuals of *Paramecium aurelia* separately in three different watch-glasses. In one of these he placed 6 drops of pure water, in another 6 drops of a pepper infusion containing many bacteria and amoebae, in the third 3 c.c. of the same infusion. After 16 days, the first animal had divided once, and both the daughter individuals died. After a similar length of time he found the second animal had produced 17 offspring: whilst the third had produced in 17 days so numerous a progeny that he estimated them at approximately 2100.

[3] If they are, their union is sterile, or produces weakly progeny which ultimately die.

[4] Bütschli (1887) after analysing all the evidence available at the time, had concluded that "external conditions which call forth conjugation have not yet been determined."

caused by transferring animals from a culture containing plenty of food into one containing none or little—induces conjugation, provided that the animals are "karyogamically mature," and unrelated. In cultures containing closely related animals it brings about encystation or abortive attempts to conjugate. If the organisms are too richly nourished during the eugamic period, conjugation does not occur.

21. Senescence, which occurs in the last period of the life-cycle in organisms which have failed to conjugate, is manifested by the following phenomena: decrease in the size of the organisms, degeneration of the nuclear apparatus (in various ways), reduction of the mouth-parts and appendages, physiological degeneration (loss or inhibition of various functions), and sometimes by "morbid sexual hyperaesthesia," leading to abnormal and sterile conjugations between closely related individuals. The final result is always death.

22. Maupas did not share the view—which had then recently been revived by Weismann[1]—that the Ciliata are "immortal." He believed that his researches had shown that these animals are not capable of multiplying by fission for an endless period. Unless conjugation takes place at the proper season, the doom of the organism or its offspring is sealed, and old age and death are as inevitable for *Paramecium* as for man.

CHAPTER III.

The Results of Later Investigators.

23. Having briefly outlined the conclusions reached by Maupas, I shall now review the chief results which have accrued from the investigations of subsequent workers. For the sake of convenience, I shall sub-divide these results into two main groups—according as they concern (A) the asexual or (B) the sexual period in the life-history: and I shall consider the experimental results, in each subject studied, as far as possible in chronological order.

[1] Weismann's conception of the "immortality" of the Protozoa was familiar to earlier investigators—e.g., Ehrenberg and Dujardin. The first expression of this idea with which I am acquainted is in the work of the former (Ehrenberg, 1838). Moreover, he clearly realized the allegorical nature of this "immortality"—"welche poëtisch genug an Unsterblichkeit und ewige Jugend grenzt." "Man theile sich in zahllose neue Theile, um zahllose Jahre zu leben und jung zu seyn." Woodruff (1909*a*), however, is wrong in stating that Ehrenberg held that the "Protozoa are so simply organized that they are not subject to natural death." Calkins (1902*a*) is also wrong in stating that "Ehrenberg's view was vigorously contested" by Dujardin. (Cf. also footnote to § 17, *supra*.)

A. The Asexual Period.

24. We may first consider the duration of the period of multiplication by fission. It is important to inquire how far Maupas was justified in concluding that this period is of a limited duration, and that—without conjugation—old age and death form the natural termination of the ciliate life-cycle.

25 The first experiments to be considered are those of Joukowsky (1898), who kept four cultures—each started from an exconjugant—of *Pleurotricha lanceolata*. One of these continued for 458 generations[1], without conjugation or degeneration. This line was still developing normally when it was abandoned. Another line persisted for 250 generations, a third for 442—both finally dying. The fourth was followed to the 220th generation. It developed further, but abnormally. Two similar cultures of *Paramecium caudatum* reached 150 and 170 generations, and then died. Conjugation was observed twice, and degenerate forms were found at the end of the time. The first culture (*Pleurotricha*) is interesting, on account of its long duration; but from the others no definite conclusions can be drawn.

26. Kulagin (1899) studied *Paramecium*, and suggested that "senescence" in old cultures is really due to the accumulation of harmful substances in the bodies of the organisms or the surrounding medium. Satisfactory evidence for this conclusion he does not give.

27. Simpson (1901) found that in his cultures of *Paramecium* the rate of division of the organisms gradually decreased, until they finally died. He did not attempt to ascertain the determining factors.

28. Calkins (1902 (with Lieb, 1902), 1902 *a*, 1904), also investigated *Paramecium*, and made a similar observation. He succeeded in keeping one line for 742 generations, without conjugation occurring; others for shorter periods. In all cases he found that the rate of fission gradually became slower, and the animals entered a "period of depression." From this they were able to recover if stimulated with various chemicals and extractives. "The well-marked cycles, with periods of depression which demanded stimulation of a decided character, were approximately of six months duration, while intermediate cycles of less importance were about three months long" (Calkins, 1904). "One natural way in

[1] It should be remembered that all "generations" are asexually produced among the ciliates. The term does not connote any of those secondary meanings associated with the conception of "generation" in sexually reproducing organisms.

which the weakened descendant may be restored to new vigor is by conjugation with another individual" (Calkins and Lieb, 1902). "A couple of hundred generations, more or less, uses up the potential of vitality, whereupon, unless the potential is renewed [by conjugation or stimulation], the race dies out with some indications of protoplasmic old age" (Calkins and Lieb, 1902).

29. Calkins (1904) denied that the meganucleus increases in size during depression periods, or that the micronucleus degenerates (*contra* Maupas). "Temperature changes have little or nothing to do with the decline of vigor" (Calkins, 1902). Depression is not morphological, but physiological, and is shown by pathological divisions and the formation of monsters, decrease in the rate of division, and death. The recovery from depression—brought about by chemical stimuli—he regarded as a case of "artificial parthenogenesis." This is based, apparently, upon the erroneous supposition (§§ 12, 14) that the conjugant—or would-be conjugant—is a gamete.

30. R. Hertwig has formulated a hypothesis to account for the "depression" periods observed by himself, Calkins, and many others, in cultures of ciliates. It is involved in his "theory of the karyoplasmic ratio" (Kernplasmatheorie) which may be stated as follows: the mass-relation of nucleus to cytoplasm expressed as the quotient $\frac{K}{P}$—that is, the mass of nuclear substance divided by the mass of cytoplasm—is a ratio whose magnitude is of fundamental importance for all vital processes influenced by the nucleus, for assimilation and organizing activity, for growth and division (R. Hertwig, 1908, p. 5, *et alibi*—cf. 1899, 1902, 1903, 1903*a*, 1905).

31. Hertwig (1899, 1903, 1903*a*), from studies of *Paramecium*, *Dileptus*, and other Protozoa, believes that depression is due to the development of an abnormal karyoplasmic ratio. During a number of successive generations, the nucleus gradually increases in size at the expense of the cytoplasm—in other words, $\frac{K}{P}$ becomes larger. The resulting disproportion causes depression, or inhibition of vital activities. It may be compensated by elimination or absorption of nuclear substance, or by conjugation—both of which are considered to restore the karyoplasmic ratio to normal: or it may be so great a disproportion that the animal is unable to recover, and consequently dies. Hertwig found that the meganucleus actually is disproportionately large in animals in a state of depression.

32. Popoff (1907) has obtained results which he interprets in a similar manner. He cultivated a line of *Stylonychia mytilus* for 3½ months. During this time he observed "depression periods"—like those of Calkins (§ 28)—which became deeper and deeper until the culture finally died out. In depressed individuals he found the meganucleus abnormally large (§§ 29, 31), and often fragmented or of irregular shape: the micronuclei frequently multiplied, and the whole organism was smaller (§ 21). No conjugations were observed[1], though depressed animals were "inclined to conjugate." They recovered from depression by resorption of the excess of chromatin in the meganucleus (?)—thus restoring the karyoplasmic ratio to normal (§ 31). Similar depression periods were caused in *Paramecium* by overfeeding. Recovery occurred as a result of conjugation. The morphological changes observed were similar to those seen in *Stylonychia*; and Popoff sees "a complete parallel" in the nuclear behaviour during depression with that seen during conjugation. "Everything goes to show that the cause of depression is not to be sought in accidental changes in environment (such as food, water, etc.), but that it lies in the organism itself."

33. In a series of papers, Enriques (1903–1910) has maintained that the "depression" or "senescence" seen in cultures of ciliates is not due to "old age" or any internal cause, but is merely the result of adverse environmental conditions—the most important being overgrowth of bacteria. "Depression" is due to prolonged poisoning with bacterial toxins. If these are not allowed to accumulate, and the culture medium is frequently renewed, no depression occurs, and the animals are capable of multiplying asexually *ad infinitum*. The chief evidence advanced by Enriques in support of the last proposition is the statement that he has kept *Glaucoma* in cultures for 683 generations, without it conjugating or showing any signs of "depression" or "senile decay" (1905). Other organisms kept for shorter periods gave similar results—e.g. *Oxytricha*, *Stylonychia*, *Vorticella* (1903, 1905*a*, 1910, etc.). "Agamic reproduction can be continued as long as one likes, if the technique is good and bacteria are not too numerous. No change of food is necessary" (1909*a*). Enriques strongly criticizes the work of Calkins (§ 28) and Popoff (§ 32).

34. In his later papers Popoff (1909*a*, 1909*b*) has shown that "depression" such as he observed in his cultures (§ 32) may be brought about by treating the animals with certain chemicals. He cultivated

[1] Possibly because the animals were all descended from the same ancestor? (§ 20).

Stylonychia in water containing CO_2 and *Paramecium* in water containing ammonia. In both he observed increase in the size of the meganucleus, followed by its fragmentation, multiplication of the micronucleus, and loss of power to divide or assimilate food. The nuclear changes are compared with those occurring during conjugation, but the animals treated in this manner never conjugated. Similar effects were not produced by other chemicals (NaCl, $MgCl_2$, $MgSO_4$, glucose). Popoff interprets these results as indicating that katabolic products accumulate during a depression period and cause a disorganized condition of the organism which can be corrected by conjugation and its concomitant reorganization (§ 31).

35. Results similar to those of Popoff (§ 34) have been obtained with *Paramecium* by Sun (1912), by adding uric acid or calcium phosphates to the culture liquids.

36. Gregory (1909) has kept a line of *Tillina magna* for 548 generations[1]. "Depression periods" were observed, but chemical stimulation had little effect—"rejuvenation took place, but only to a slight degree." The culture finally died, without conjugation occurring. Gregory interprets her results in the same terms as Calkins (§ 28). She denies that the relative size of the meganucleus increases during depression: but the conclusion appears to rest upon the use of a fallacious "coefficient," and it appears from the few measurements given that her results do not really contradict Popoff's (§ 32). Moody (1912) has obtained closely similar results with *Spathidium*, which she cultivated for 218 generations. Death, however, was probably due to "abnormal conditions of the environment." She also denies that the size of the nucleus increases during depression, but her measurements do not bear this out. She employs the same peculiar "coefficient" as Gregory[2]. Calkins, it may be added, has succeeded in keeping *Blepharisma* for 224 generations, and *Actinobolus* for 446 generations. Both lines then died. (See Calkins and Gregory, 1913.)

37. The most extensive work on the duration of the asexual period has been done by Woodruff. Woodruff's earliest work (1905) was on the hypotrichous forms *Oxytricha*, *Pleurotricha*, and *Gastrostyla*—the

[1] This organism divides into 2 or 4 daughter-individuals inside a temporary cyst. In one place (p. 399) the number of generations is given as 546.

[2] The statistics given for *Actinobolus* (Table 5) are to me absolutely unintelligible. The volume of the cytoplasm is given in every case as less than that of the nucleus (which is inconceivable); yet the average ratio of nucleus to cytoplasm is found to be 1 : 20.

longest lines of which he succeeded in keeping for 860, 448 and 288 generations respectively. He observed Calkins's "cycles" in division rate (§ 28), with corresponding "depression periods" resulting finally in death. "Rejuvenescence" was brought about by beef extract (*Oxytricha*). "The number of generations which constitute a cycle is not at all constant," and the "cycles" themselves show secondary cycles or "rhythms"—i.e. minor fluctuations in division rate. No conjugations occurred.

38. Quite recently Woodruff (1913 *b*) has published some measurements of individuals from his long line of *Oxytricha*. He finds that the mean size of both the organism and the nucleus is "smallest at periods of high reproductive activity, and becomes progressively larger as the division rate falls." During depression periods there is an *increase* in the relative amount of cytoplasm as compared with nucleus (§§ 31, 32). As Woodruff appears to have selected his individuals at random, at unknown inter-division moments, it is not surprising that he finds the karyoplasmic ratio very variable[1]. This consideration seems to me to render his measurements of very questionable significance.

39. In a long series of papers, Woodruff (1908 *a*–1914)[2] has reported upon his investigations of *Paramecium aurelia*[3]. "This race of *Paramecium* has attained so far (May 1, 1912) 3029 generations during the five years it has been under daily observation. The number of generations attained during each of the first five years of its existence is as follows: first year 452, second year 690, third year 613, fourth year 612, and fifth year 662. The mean rate of division for the entire period is over three divisions in forty-eight hours. Periods of marked physiological depression have not occurred—such variations in the rate of reproduction as have appeared being either normal rhythms or the effects of environmental changes of temperature and culture medium. The organisms of the present generation are in as normal morphological and physiological condition as the original 'wild' individual isolated to initiate the culture" (Woodruff, 1912). Later (Woodruff, 1913) we learn that 3340 generations have been reached: still later (Woodruff, 1913 *a*) "more than 3800 generations" in "over 6 years"; and finally it is announced (Woodruff, 1914) that the 4102nd generation has been

[1] See § 47 *infra*.

[2] Summaries of the work of Woodruff and his collaborators will be found in Woodruff (1912 *a*) and Middleton (1913).

[3] *P. caudatum* has given comparable results on a smaller scale. Concerning the specific distinctions of these two forms see Woodruff (1911 *b*). Compare also § 78 *infra*.

attained[1]. Conjugation has never occurred in this line[2]. It is concluded that the "cycle" of Calkins (§ 28) "is probably an artificial one which is brought about by the subjection of the race to an environment which is not suitable for its prolonged existence" (Woodruff, 1911 *d*).

40. Woodruff and Baitsell (1911, 1911 *a*) have found that "when *P. aurelia* is bred on a varied culture medium, or on a constant culture medium of beef extract, cycles do not occur, but rhythms (§ 37) persist. It is not possible by constant environmental conditions to eliminate the rhythms." The "constancy" of the environment in these experiments is, however, not above suspicion.

41. Woodruff (1911, 1911 *a*) has proved that the excretion products of *Paramecium* have "a decidedly depressing effect on the rate of reproduction," and this no doubt accounts to some extent for depression periods in cultures. "These excretion products are essentially specific in their action" (1913 *a*). This result should be compared with those of Enriques (§ 33), Popoff (§ 34) and Sun (§ 35). The last named investigator has found—like Woodruff—that culture fluid in which *Paramecia* have been growing is toxic to other *Paramecia*: but she has made the curious observation that the toxicity is lost if the fluid be boiled.

42. Baitsell (1912) has succeeded in cultivating several lines of *Stylonychia pustulata* for a large number of generations. The longest line ended at the 572nd generation (cf. Maupas, § 18). "Conjugation never occurred and abnormal or degenerating animals did not appear, but after a gradual decline in the fission rate the culture finally died out." This result was probably not due to "the ending of any definite life-cycle," but to unsuitable cultural conditions. More recently, Baitsell (1914) has come to the same conclusion from studies of *Oxytricha* and *Pleurotricha*.

43. If we consider the results obtained by all the investigators mentioned in the preceding paragraphs, it seems highly probable that "depression" or "senescence" in ciliates is due to unhealthy surroundings —unsuitable food, toxins produced by the organisms themselves or their cultural companions, etc.—and is not due to any inherent inability to live or frustrated necessity to conjugate. Woodruff's conclusion concerning *Paramecium* seems equally applicable to all other ciliates: "It is probable that most, if not all, normal individuals have, under suitable

[1] Dr Woodruff informs me that the number of generations now reached (March 20, 1914) is 4310.

[2] But see § 101 *infra*.

environmental conditions, unlimited power of reproduction without conjugation or artificial stimulation" (Woodruff, 1911 *d*).

44. Concerning the morphological manifestations of "depression" there is not complete unanimity among investigators. For whereas some believe that depressed organisms are of smaller size (Maupas, Calkins, Popoff), others maintain that they are larger (Woodruff, Gregory): and similarly some observers find an increase in the relative size of the meganucleus (Hertwig, Popoff, Sun), others a decrease (Calkins, Moody, Woodruff). Some of these conclusions cannot be accepted unreservedly. It seems on the whole probable that hypertrophy of the meganucleus often accompanies depression: and I have little doubt that the discordant conclusions result in part from the loose application of the terms "depression" or "senescence" to a variety of different conditions.

45. We may now consider some remarkable work by Popoff (1908) on what may be called the mechanics of division. The aim of his investigations was to test the truth of one of R. Hertwig's deductions from his "Kernplasmatheorie" (§ 30). According to Hertwig, the normal karyoplasmic ratio of an organism (or cell) becomes gradually altered owing to the growth of nucleus and cytoplasm at unequal rates, until a condition of "karyoplasmic tension" is finally set up. This tension, or unbalanced condition, is the factor which determines division of the organism (or cell) as a whole—the division giving rise to new individuals in which the karyoplasmic ratio is once more normal. Division is thus regarded as a regulatory reaction to an abnormal mass-relation between nucleus and cytoplasm.

46. Popoff's earliest results (1908)[1] were obtained with *Frontonia*, a large holotrichous ciliate of symmetrical and regular shape suitable for measuring. It possesses a single meganucleus—upon which the measurements were made—and several micronuclei. Cultures were kept at various temperatures, and the volume of nucleus and cytoplasm computed from measurements made on animals at known intervals of time between one division and the next. The following results may be selected as illustrating Popoff's general conclusions. (See Fig. 3.)

47. At a temperature of 25° C. *Frontonia* divides once every 17 hours, during which time the cytoplasm grows at a uniform rate (Fig. 3). The nucleus, however, begins immediately after division to decrease slightly in volume—probably owing to loss of liquid during

[1] These experiments were begun originally by Wierzbizki, another of Hertwig's pupils (Hertwig, 1905).

its recovery from the preceding division. At the second hour, however, the nucleus begins to grow, but it does so more slowly than the cytoplasm. Accordingly, the karyoplasmic ratio $\left(\frac{P}{K}\right)^1$ gradually increases. In the just divided organism, the "karyoplasmic norm" $\frac{P}{K} = 67$; but after 15 hours' growth this has increased to 100. The period from the 2nd to the 15th hour is called by Popoff the "period of functional growth of the nucleus," and at the end of it the karyoplasmic ratio reaches its maximum—the "moment of karyoplasmic tension." During

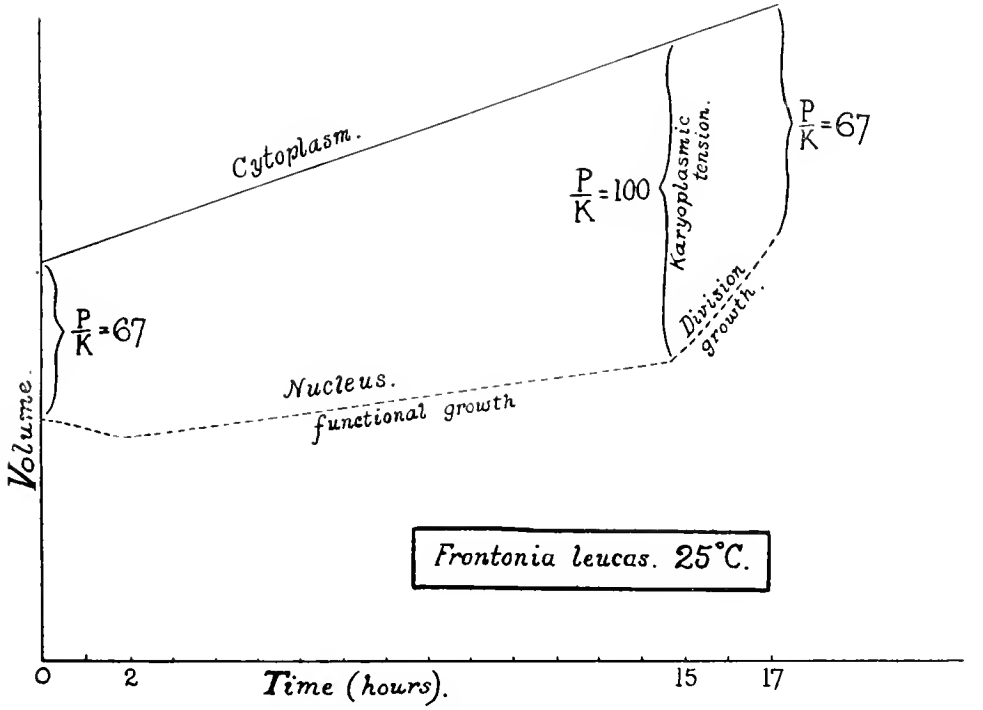

Fig. 3. (From Popoff, 1908, modified.)

the next two hours, the nucleus grows ("division growth") more rapidly than the cytoplasm, so that at the 17th hour the karyoplasmic ratio is once more normal $\left(\frac{P}{K} = 67\right)$. Division now takes place—each daughter-

[1] Popoff reckons the karyoplasmic ratio as $\frac{P}{K} = \frac{\text{volume of cytoplasm}}{\text{volume of nucleus}}$, which is Hertwig's karyoplasmic ratio inverted (§ 30).

individual possessing at its formation a normal karyoplasmic ratio. And so the cycle of events continues.

48. Popoff (1909) obtained confirmatory results with *Paramecium*. The value of $\frac{P}{K}$ and the rate of division vary with the temperature in both cases; but the general conclusions given above appear valid for all experiments, *mutatis mutandis*.

49. The "moment of karyoplasmic tension" is the moment at which division is determined. If this were not so, indeed, it would be difficult to understand how any division could occur[1]. Popoff (1908) established this by ingenious vivisection experiments. The karyoplasmic ratio of a *Frontonia* was changed by cutting off a piece of its cytoplasm. If this was done during the period of *functional growth*, the cytoplasm continued to grow until the normal karyoplasmic ratio was again established. Division was consequently delayed, but finally took place in the usual way. If, however, a piece of cytoplasm was removed during the period of *division growth*, the animal did not regulate its karyoplasmic ratio by compensatory growth of cytoplasm; but it divided at the proper time (17th hour) with an abnormal karyoplasmic ratio. This seems to indicate that the moment of karyoplasmic tension determines division. And, moreover, Popoff elicited the curious fact that the *plane* of division is also determined at this moment: for an animal from which cytoplasm had been removed during the period of division growth divided, into two unequal-sized individuals, at the plane which would have been median had the animal been whole.

50. Moody (1912) has made some measurements of *Spathidium* which—she believes—tally with Popoff's results for *Frontonia* and *Paramecium* (§§ 47, 48). But unfortunately no real confirmation of his laborious and important researches has yet appeared. It is easy to criticize or disbelieve his conclusions, but to test them by further experiments performed in the same manner is an arduous task. And without further experimental evidence, criticism of this work becomes merely an academic discussion of probabilities—of no real value.

51. Inquiries have been instituted into the effects of chemical and physical agents upon the rate of fission in ciliates, and some results of this work may suitably be considered here. We have already seen

[1] In the case cited above, for example, it will be seen that the karyoplasmic ratio is normal (67) just before division. As it is the same in the newly divided organism, one naturally asks why division should occur at all—assuming it to be determined by the karyoplasmic ratio. Some such explanation as that given above therefore becomes necessary.

(§§ 28, 36, 37) that certain chemical (and physical?) stimuli are able to increase the rate of division in "depressed" cultures[1], but similar effects have also been produced in normal organisms.

52. Peters (1904) has laid emphasis on the fact that the salt content of the medium in which ciliates are cultivated is a most important factor. He found as a result of numerous experiments that an excess of KCl in an otherwise normal medium accelerates the division of *Stentor*. Small quantities of chloroform act similarly upon *Paramecium*.

53. Calkins and Lieb (1902) who studied the effects of alcohol on "depressed" *Paramecium* found that this substance acts, in a certain concentration, as "a continued stimulus which sustains the high rate of division even during periods of depression of the control series." But Woodruff (1908) has shown that minute doses of alcohol sometimes increase and sometimes decrease the rate of fission. In the former case, "the effect is not continuous, but gradually diminishes and finally the rate of division falls below that of the control." A larger dose of alcohol will again increase the rate of fission, but again for a limited period only[2]. These results may be compared with those of Daniel (1909) who finds that *Stentor* and *Spirostomum* can acquire a specific resistance to alcohol—becoming "acclimatized" to small but gradually increasing doses. Both Woodruff and Daniel found that alcohol-resistant ciliates are more susceptible to certain other chemicals.

54. The toxicity of certain salts to *Paramecium* has been studied by Woodruff and Bunzel (1909). Estabrook (1910) has studied the effects of various chemicals on the growth of the same form. All the substances tried[3] behaved in a similar manner: "When very weak they have no effect whatever. In greater concentrations, all retard the later stages of growth" and cause "other injury to the organism." It is surprising to find, however, that complete growth can occur in a medium consisting of nothing but distilled water and a little NaCl. It may be noted here that Pütter (1905) has found that *Paramecium* and other ciliates can live for many days in distilled water containing no free oxygen.

55. Many of the earlier workers found that temperature has a marked influence on the rate of fission (§ 19). All later workers have

[1] And the decrease in rate of fission during "depression" may also be due to chemical causes.

[2] Cf. also Matheny (1910).

[3] These were NaCl, nicotine, strychnine, and alcohol, in different concentrations.

reached the same conclusion. [Cf. Jonkowsky (1898), Popoff (1908), Rautmann (1909), Woodruff and Baitsell (1911 *b*), Sun (1912), etc.] The higher the temperature, the more rapid the rate of fission. For example, Popoff (1908) found that *Stylonychia mytilus* divides about once every 8 hours at 25° C., every 16 hours at 18° C., every 30 hours at 14° C., every 48 hours at 10° C. Woodruff and Baitsell (1911 *b*), Sun (1912) and Jollos (1913) point out that the increase in the rate of division with increase of temperature follows van't Hoff's rule for the velocity of a chemical reaction.

56. There is, of course, a maximal and a minimal temperature beyond which a ciliate cannot live and divide. These limits were determined by Rautmann (1909) for *Paramecium* as 35° C.[1] and 5° C. respectively. More recently Hutchison (1913), who has studied several forms, concludes that each species "has a resistance peculiarly its own," but "the amount of variation within the species may be considerable." Different strains of *Paramecium caudatum*, for instance, showed very different powers of thermal resistance.

57. Rate of fission may also depend to some extent upon the form of nutrition (§ 19). An illustration of this is given by Jonkowsky (1898) who found that *Pleurotricha* reproduced more rapidly when fed carnivorously on *Uronema* than when kept in infusions of hay, flour, or albumin.

58. It was found by McClendon (1909) that the rate of fission in *Paramecium* is accelerated if the animal is subjected to centrifugal force for a certain time. His controls, however, seem to have been abnormally slow in dividing, and the mortality excessively high.

59. According to Peters (1904) KCl not only accelerates division in *Stentor* (§ 52) but also modifies its character. The organism undergoes an abnormal process of "budding" often forming very minute "dwarfs." Further information about this curious phenomenon is to be desired. The dwarfs seem to have originated by what may be called "chemical vivisection" or mutilation of the individual. It would be interesting to know whether they are viable and able to reproduce—whether they reorganize to normal size and form—whether they produce dwarf or normal offspring.

60. According to Jonkowsky (1898) the size of a ciliate may depend upon its food. *Pleurotricha* was found to vary from 200 μ to 15 μ in

[1] It was found that a temperature as high as 45° C. might be withstood if the animals were exposed to it for only a very short time. Compare also Jollos (1913).

length—giant individuals of this species and *Onychodromus* resulting from cannibalism.

61. We may now pass to a consideration of variations and their hereditary transmission—a matter to which the foregoing facts inevitably lead us. The nature of "heredity" in the asexual reproductive process of a ciliate should be clearly realized, and I would therefore remind the reader of a few important facts before introducing this subject. It is often assumed that "inheritance" in a ciliate is a very simple matter—one organism merely dividing into two exactly similar organisms. A moment's thought, however, will make it clear that when a complex creature with morphologically and physiologically differentiated ends divides transversely across the middle, the acquisition of the parent's form by the two daughter-individuals is by no means so simple a matter as at first sight it might appear. Indeed, it is probably true that all the complex buccal and appendicular structures —in fact the entire ciliary coat and all its derivatives—of the parent are resorbed or undifferentiated during division and a set of corresponding organs formed by new growth and differentiation in each daughter organism. Details of this process in certain complex forms have been worked out especially by Wallengren (1901) and Griffin (1910). In no known case is the differentiated protoplasm of the parent passively parcelled out or "handed down" to the offspring in the fashion contemplated by *a priori* speculators. Only certain inclusions[1] (food, symbiotic algae, etc.), behave thus. The nuclei, of course, divide during division of the organism as a whole, but in the meganucleus undifferentiation and subsequent re-differentiation often occur during fission.

62. Simpson (1902) has shown that when a *Paramecium* divides into two, the products—measured at given later moments—may differ from one another in size. But Jennings (1909) has shown that "these 'variations' are mere temporary fluctuations, without effect in heredity" (§ 63).

63. Jennings (1908*a*, 1909, 1911) has now demonstrated, by long series of careful measurements and observations, the important fact that the species of *Paramecium* called *caudatum* and *aurelia* are not homogeneous—that each consists of an assemblage of distinct races (Jordan's "little species," Johannsen's "pure lines") which differ *inter se*, but which *in se* are constant. These races differ from one another not only in size and form but also in physiological respects. The

[1] But see § 81 *et seq.*, *infra*.

distinctive characters of 6 such "pure lines" of *caudatum* and 5 of *aurelia* are fully set forth by Jennings and Hargitt (1910).

64. The nature of these pure lines will be instantly envisaged in the accompanying diagram (Fig. 4). We here see (upper line) outlines

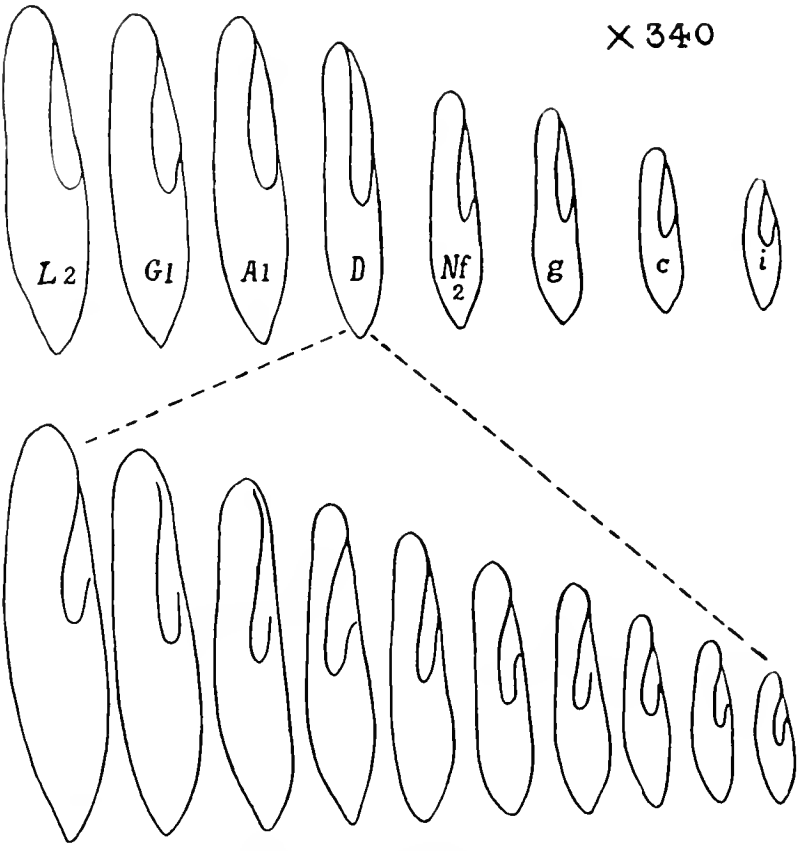

Fig. 4. (From Jennings, 1909.)

of individuals of mean size belonging to eight different races of *Paramecium*, drawn to the same scale. The differences in size are remarkable —the actual mean length of the largest race (L_2, *caudatum*) reaching 230 μ, of the smallest (i, *aurelia*) only 90 μ.

65. Within each race the individuals may differ considerably from one another in size—as a result of growth, nutrition, and external conditions. This is shown diagrammatically in Fig. 4 (lower line), which depicts the relative dimensions of 10 individuals belonging to

the race *D* (upper line). The largest individual has a length of 256 μ, the smallest 80 μ. But the races all breed true to their mean dimensions. "Breeding from the extreme specimens—the largest and smallest—of a single race, we get several hundred individuals from each. *Both produce progeny of the same mean size.* Each produces a whole series of varying individuals, just like the original racial series; the series produced by the largest individual is exactly like that produced by the smallest, or by any other. *Selection within the pure race is of no effect on the size*" (Jennings, 1909). All the races appear to be "singularly resistant, remaining quite constant in most respects, so far as has been determined" (Jennings, 1911). The importance of these conclusions for every worker engaged in the study of genetics in the Ciliata is obvious. There can be little doubt that Jennings's conclusions will be found to hold good for ciliates other than *Paramecium*. He himself (cf. Jennings and Hargitt, 1910) has shown that there are many indications pointing to this conclusion: and he has also made it clear that non-recognition of the existence of pure lines in general populations of ciliates is a source of error which can lead—and probably has led—to many wrong conclusions concerning variation in these organisms.

66. Many interesting observations have been made upon the production and asexual transmission of variations, and the more important of these may now be considered. We may begin with certain normal "modifications" which are due to differences of temperature. R. Hertwig found that the value of the karyoplasmic ratio (§ 30) in a given species of ciliate varies according to the temperature at which the organisms are cultivated[1]. As a general rule, the size of the meganucleus is relatively larger in animals kept at a low temperature, smaller in those at a high temperature. Further, this difference is both relative and absolute, for the organisms themselves are larger at a low temperature than at a high temperature.

67. Confirmation of Hertwig's conclusions (§ 66) has been given by Popoff (1908, 1909) and Rautmann (1909), for *Frontonia*, *Stylonychia*, and *Paramecium*. An illustration may be taken from the case of *Stylonychia* (Popoff, 1908), summarized in the following table[2]:

[1] The value of the karyoplasmic ratio also appears to be different in different races of *Paramecium*. See note by Popoff and Rautmann (Popoff, 1909, p. 180).

[2] *L*, *B* and *W* stand for length, breadth and width. The length of the nucleus is the combined length of its two components, the width the mean of the two widths. The units of measurement are not microns but divisions of the ocular micrometer used by Popoff. I have given the measurements to the first decimal place only.

	Mean Dimensions of					
	Body			Meganucleus		
Temperature C°.	L.	B.	W.	L.	B.	W.
25	9·7	6·6	4·5	4·6	0·9	0·8
17—19	11·4	8·7	5·3	5·7	1·0	1·1
10	12·8	9·0	6·3	5·8	1·3	1·2

The karyoplasmic ratios $\left(\frac{P}{K}\right)$ calculated for these three temperatures are respectively 80·7, 77·4, 74. The dimensions of the micronuclei in *Frontonia* appear to vary like those of the meganucleus (Popoff, 1909), but in *Stylonychia* they were not studied.

68. These size-differences are not permanent; apparently they are manifested as a direct response to temperature—like differences in rate of fission (§ 55). Rautmann (1909) has found, however, that rise of temperature, rise of fission-rate, and rise of karyoplasmic ratio do not all run parallel. For increase in rate of fission (in *Paramecium*) accompanies increase in temperature—the temperatures tried being 5° to 35° C.; whereas the karyoplasmic ratio $\left(\frac{P}{K}\right)$ increases with the temperature up to 25°, but then begins to sink. When an organism is subjected to a change of temperature, it can regulate its karyoplasmic ratio to that characteristic for the new temperature in the space of time which elapses between one division and the next—for a temperature interval of 5° C.

69. Jollos (1913) experimenting with pure lines of *Paramecium caudatum*, has found that although differences in size are produced by changes of temperature—as described by Hertwig, Popoff and Rautmann—nevertheless these differences are transitory. At a new temperature, a change of size is at first observable, but later it disappears. The animals appear to become adapted to the new temperature and then to readjust themselves to their original proportions. Jollos also states that resistance to extremes of temperature can be induced in *Paramecium* to a slight degree. But here again the modification is impermanent—being lost if the organisms are returned to a normal temperature.

70. In one case Jollos (1913) claims to have produced a permanent change ("mutation") in *Paramecium*. From a race subjected to a high temperature he obtained a small race which is *permanently* small—at high, normal, and lower temperatures. This race has conjugated without reverting to the original size. It differs also in being less susceptible

than the original race to sudden changes of temperature. The original culture was derived from a single individual: and after conjugation an exconjugant was similarly isolated and cultivated, so that Jollos's belief that he has assisted at the production of a "mutation" appears[1] to be justified.

71. Jollos (1913) has further recorded experiments with arsenic compounds. He finds that *Paramecium* may acquire an increased resistance to arsenic if treated with it for some time[2]. An arsenic-resistant race so produced remains resistant for a considerable time if bred further in an arsenic-free medium. But the increased resistance gradually diminishes in the course of time, until it is finally lost. The loss is more rapid if the animals are subjected to sudden changes in temperature and nutrition. For such a partially permanent change Jollos proposes the name of "enduring modification" (Dauermodifikation). (See also *infra*, § 113.)

72. Estabrook (1910) has found no evidence that a race of *Paramecium* of a given mean size can be transformed by chemicals (§ 54) into a larger or a smaller race. Temporary changes in size were observed, but regarded as due to "variations in the nutritive and other conditions of the normal environment." Concerning another ciliate, however, Prowazek (1909) has published a peculiar observation. *Leucophrys patula* was said by Maupas to be dimorphic—some organisms being large, others small[3]. Prowazek has confirmed this: and he adds that he has been able, by treating cultures of the smaller form with minute doses of quinine, to extract from them races of the larger form. These, however subsequently reverted to the smaller form.

73. Among the records of experimentally produced variations the researches of Popoff occupy a prominent place. From studies relating to the karyoplasmic ratio (§§ 45—49) he surmised that variations in size might originate through alteration of the relative proportions of nuclear and cytoplasmic matter. Thus if an organism with a given karyoplasmic ratio x chanced to divide into two new individuals of unequal size, but each with a normal ratio (x); then it might be supposed that the new individuals would give rise to new races of

[1] His paper is only a preliminary note, without adequate evidence for his statements.

[2] Compare the similar but more definite results previously obtained by others with trypanosomes—reviewed in an earlier paper in this Journal (Vol. II. p. 201, 1912).

[3] Maupas thought the small forms were probably sexual individuals. Prowazek never saw them conjugate, and appears not to share this view: but what his own interpretation of the dimorphism may be (? sexual) I cannot comprehend. It is certain, at all events, that the large and small individuals are not females and males (§ 14).

larger or smaller individuals. Popoff believes that he has actually observed this in *Stentor* and *Frontonia* (1909) and *Stylonychia* (1908). As a result of unequal division, large and small races arose. They both had a normal karyoplasmic ratio, but differed in size. (See Fig. 5, which shows the relative sizes of a large (A) and small (B) race of *Stentor* —the animals being outlined at the moment of division. A large (C) and small (D) race of *Frontonia* is similarly compared—the animals outlined immediately after division.) Unfortunately, it is not clear from Popoff's statements that he actually saw the unequal divisions which

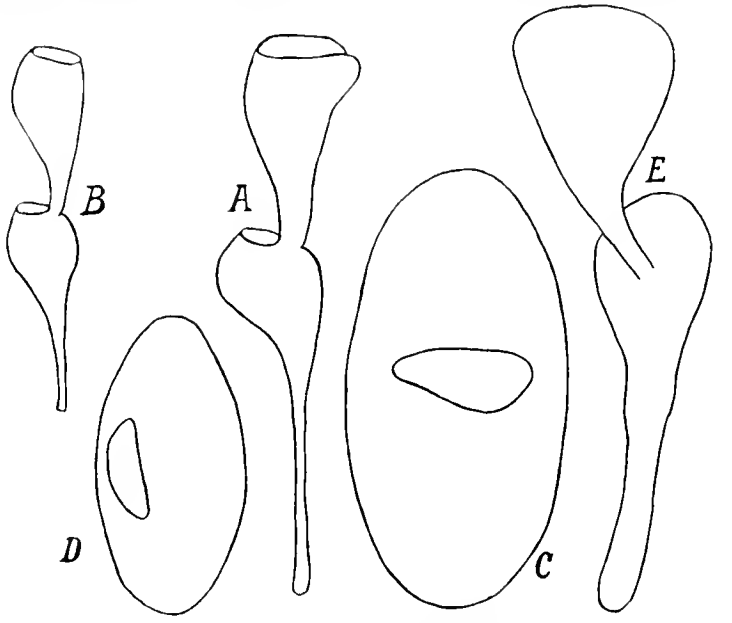

Fig. 5. (From Popoff, 1909.)

are supposed to have originated these new races. And the criticism of Jennings[1] that Popoff may merely have isolated pure lines (§ 63) from a mixed population is one which seems both plausible and cogent.

74. More convincing are Popoff's other experiments (1909) on *Stentor*. This ciliate possesses a meganucleus of a form comparable with a string of beads. By centrifuging an animal which was about to divide, Popoff caused the nucleus to be unequally distributed to the two daughter-individuals—one receiving 16 "beads," the other only 3, whilst the latter animal was similarly only a quarter of the size of the

[1] See Jennings and Hargitt (1910).

former. Both individuals reorganized themselves successfully after fission, and continued to multiply normally for about a week[1]. They produced giant and dwarf races respectively, according to expectation. It was also ascertained that the individuals of the smaller race had reorganized their meganuclei so that they consisted of the normal number of "beads."

75. In another experiment Popoff (1909) suddenly cooled a *Stentor* which had begun to divide. The division was thus made to regress. Placed at a normal temperature, the animal then reorganized itself in a peculiar way into a single individual. It then grew to a very large size, and subsequently divided. A race of giant *Stentors* was obtained in this way which continued to divide and breed true for as long as the culture was kept (about $1\frac{1}{2}$ months). [The size of this race can be gauged from Fig. 5, E, which shows a dividing individual drawn to the same scale as the other *Stentors*, A and B.] The karyoplasmic ratio of the giant individuals appeared to be the same as that of the normal race from which they were derived. One of the giant *Stentors* from the race just described is said to have divided unequally into a large and a small individual. From the former, an even larger race was bred. All attempts to obtain still larger races failed.

76. Popoff (1909) tried to produce new races of *Stentor* by cutting off pieces of protoplasm from an individual and so changing the karyoplasmic ratio. The experiments were inconclusive, though it is stated that a small piece of a *Stentor* containing a small piece of nucleus can reorganize itself into a small individual capable of dividing several times—always forming small individuals. Death always followed, however, and no small race was produced in this fashion[2]. It is to be hoped that all Popoff's experiments will soon be repeated by other workers.

77. Somewhat similar experiments have been made on *Paramecium* by Calkins (1911) and Peebles (1912), and the latter concludes: "The removal of a portion of the cytoplasm does not result in the production of smaller individuals. After several generations have been produced the normal size is regained."

[1] The cultures were then lost.

[2] Regeneration has, of course, been studied in some detail in *Stentor*—e.g. by Balbiani (1888, 1892, 1893), Gruber, etc. But I know of no record of the production of a *race* of different size as a result of mutilation. It seems probable, moreover, that vivisected *Stentors* sooner or later reorganize their meganuclei so that they consist of the normal number of "beads." (Cf. Balbiani (1888, 1893), Prowazek (1904), etc.)

78. The two species of *Paramecium* called *aurelia* and *caudatum* differ from one another, *inter alia*, in that the former has two micronuclei, the latter one. [See Maupas (1888, 1889), Hertwig (1889).] Calkins (1906), however, advanced the view that both forms belong to the same species. Of a pair of exconjugants of *caudatum* (1 micronucleus) he found that one reorganized after conjugation as a *caudatum* form, the other as an *aurelia*—with 2 micronuclei. The *aurelia* form persisted for a number of generations, but finally reverted to the *caudatum* type. Jennings and Hargitt (1910) and Woodruff (1911 *b*) nevertheless regard *aurelia* and *caudatum* as "good" species. In this I agree, and I think the conclusions of the former explain Calkins's results—"in rare cases specimens of the *caudatum* races have two micronuclei, those of *aurelia* races but one[1]."

79. It may be noted here that Sun (1912) has observed divisions in *Paramecium* in which the nuclei are abnormally distributed—the daughter-nuclei all remaining in one individual whilst the other receives none. Enucleate and supernucleate forms may thus arise. The formation of similar enucleate *Stentors* has been observed by Prowazek (1904). Individual *Paramecia* containing only a micronucleus have been found by Kasanzeff (1901) and Sun (1912). The former found them in starved cultures, and believed that they arose by an irregular division or through "hunger degeneration" of the meganucleus. No evidence of the production of new races in this fashion has yet been brought forward.

80. Abnormally nucleate races of *Paramecium* have, however, been experimentally produced by Lewin (1910). By cutting an individual transversely through the meganucleus, he obtained two organisms, one of which retained the micronucleus whilst the other had none. The latter continued to divide, thus producing an "amicronucleate" race. In another case, Lewin cut an abnormally dividing organism so that one-half contained a meganucleus only, the other a meganucleus and both daughter-micronuclei. The former gave rise to an "amicronucleate" race—multiplying slowly, but normally: the latter produced a race with two micronuclei. Both bred true for a considerable time[2].

[1] It is perhaps worth noting here that Powers and Mitchell (1910) have described what appears to be another species of *Paramecium* (called *P. multimicronucleatum*) which resembles *caudatum* but possesses from 2 to 7 micronuclei.

[2] Le Dantec (1897) had previously stated that ciliates deprived of their micronuclei by cutting in this manner are able to regenerate this organ. A full account of his experiments has never been published, nor has any confirmation of his statements yet appeared.

81. Teratological variations[1] are not very uncommon in ciliates, especially in old cultures. One naturally wants to know the genetic behaviour of monstrous characters, and fortunately some information has already been elicited. Balbiani (1893, p. 56, Pl. II, figs. 44, A—N) was, I think, the first to describe the inheritance of an abnormality. He cut a *Paramecium aurelia* transversely, and the posterior individual of the two so formed regenerated and later divided. "In the course of the fourth generation an abnormal prolongation in the form of a horn developed on the anterior individual." It persisted during seven subsequent generations, passing to the anterior daughter-individual at each fission, but gradually moving further backwards. Finally it passed to the posterior end of its last possessor, which then died. The sister-organisms appear to have been normal and "hornless" in every case.

82. A comparable case was recorded by Simpson (1901). Of four descendants of a normal exconjugant *Paramecium caudatum* three were normal, but the fourth "developed a cleft tail." This animal continued to divide for several generations. The abnormality persisted in the posterior individual at each division, becoming gradually modified from a "cleft tail" into a long "dorsal lobe." After eight divisions, the abnormal animal died. In every case the anterior sister-individuals were normal.

83. More extensive investigations of the inheritance of such abnormalities have been undertaken by Jennings (1908). He isolated abnormal *Paramecia*[2] (with "horns" or "spines," truncated ends, etc.) and studied the fate of the peculiar features during subsequent fissions. In many cases the abnormality gradually disappeared—the normal form being gradually regained in a few generations. Sometimes, however, the animals or their descendants became still more monstrous, and finally died. No permanently abnormal races were ever obtained by selecting monstrous individuals.

[1] Attention was first called, I believe, to ciliate monsters by Tatem (1870) who described two specimens of *Chilodon* and one of *Trachelius* in which the "lip" was abnormally prolonged. His comment is worth quoting: "Malformations such as those I have cited have, in my opinion, a value beyond that of mere curiosities...for may they not help to determine the fixity or otherwise of a species through aberrant forms? and thus a better knowledge of what is to be regarded as essentially specific be ultimately arrived at." Curiously enough, another "monster" described by Tatem is now recognised as a distinct species (*Vorticella monilata*).

[2] The species studied "had the characteristics usually attributed to *Paramecium caudatum*."

84. The behaviour of a monstrous character during fission may be illustrated by the abnormal race *a* of Jennings, which is fully described for 22 generations. An abnormally bent organism divided into a normal posterior daughter-individual and an anterior possessing a dorsal "spine." In subsequent generations this spine persisted, passing sometimes to the anterior, sometimes to the posterior individual. If we write *A* and *P* for anterior and posterior individuals respectively, the transmission of the spine for the 21 generations observed may be represented thus:

A P A P A P A P P P P A A A P A A P A A A.

The last spined animal died. All the sister-individuals were normal, save at the second generation. Here the anterior individual had a small posterior ventral "tooth," which persisted for two further generations, passing each time to the posterior individual, and gradually becoming smaller. At the next division it disappeared completely, two normal individuals being formed.

85. Jennings (1908) gives particulars of other abnormalities in *Paramecium* and their behaviour during fission, with a long discussion of his results. Special mention may be made of a curious "race" in which the individuals showed a tendency to remain united instead of separating completely during fission. This race was "extinguished by natural selection" in competition with free and more active organisms.

86. McClendon (1909) obtained some results like those of Jennings (§ 84), but in a different manner. As a consequence of centrifuging some *Paramecia*[1] he obtained an abnormal individual which divided into two daughter-individuals each possessing a "horn." One of these divided for 7, the other for 5 generations—the horn persisting and passing to one of the daughter-individuals each time, the other being invariably normal. Finally the horned animals died. In position and size the horns differed in different organisms. "After each division the horn is in a different position, and we can predict the position of the horn in each generation by drawing an imaginary line bisecting the animal in the preceding generation transversely."

87. From the observations of Balbiani (§ 81), Simpson (§ 82), Jennings (§ 84), and McClendon (§ 86), the following conclusions may, I think, be drawn. Abnormal growths, however produced, in *Paramecium* may be mechanically handed on for a number of generations. Whether they pass to the anterior or posterior product of division is purely a matter of chance, depending upon the position which the

[1] The species was *P. caudatum*.

structure happens to occupy on the parent at the moment of fission. In some cases the abnormality disappears owing to remodelling during successive generations: in other cases the abnormal forms die. Normal sisters of abnormal forms show no tendency to beget correspondingly abnormal individuals. Such teratological variations are therefore negligible as factors in the production of new races.

88. Additional information about ciliate monsters will be found in the following papers: Balbiani (1891)—double monsters in *Stentor*; Balbiani (1892, 1893)—various monsters resulting from mutilation of *Paramecium*, *Stentor*, etc.; Simpson (1901) and Calkins (1904)—multiple monsters in *Paramecium*; Prowazek (1904)—double monsters produced by cutting *Stentor*, etc.; Prowazek (1904 *a*)—*Stylonychia* monsters with multiple hinder ends, resulting from "degenerative hyper-regeneration"; Calkins (1911) and Peebles (1912)—multiple and other monsters produced by cutting *Paramecia*. Hereditary behaviour of abnormalities is hardly touched on in these papers. Yet an interesting fact is several times reported[1]—namely, that remodelling when it does not take place in a monster itself, may occur in its offspring; so that in certain cases at least monstrosity is a temporary manifestation—the peculiarity of the individual and not of its race. This is in accord with the conclusion reached in the preceding paragraph.

89. Before leaving the events of the asexual period, I would mention an interesting observation by Fermor (1913). The authoress finds that reorganization of the nuclear apparatus may occur in *Stylonychia* during encystment—without any sexual manifestations. In the encysted animal, the meganucleus degenerates and disappears. The micronucleus then separates into two parts—one of the products subsequently forming the new meganuclei, the other forming the new micronuclei. Thus the organism which emerges from the cyst has undergone a nuclear reorganization comparable with that which accompanies conjugation (§ 5). Conjugation was never observed in the race of *Stylonychia* studied. I know of no other similar observation[2].

[1] This occurred, for example, in one of the double *Stentors* described by Balbiani (1891).

[2] It is possible, however, that a similar nuclear reorganization may occur at times in unencysted, asexually reproducing organisms. Hertwig (1889) believed that such a process ("parthenogenesis") occurred in *Paramecium*, and a similar suggestion of the occurrence of "autogamy" in the same form has more recently been made by Woodruff (1908 *a*). The matter requires fuller investigation. [Whilst this article is passing through the press, an important announcement has been made by Woodruff and Erdmann ("Complete periodic nuclear reorganization without cell fusion in a pedigreed race of Paramecium," *Proc. Soc. exp. Biol. and Med.*, Vol. XI. No. 3, 1914, p. 73). The authors state that they

B. The Sexual Period.

90. Turning our attention now to the sexual phase of ciliate life we must consider the causes and effects[1] of conjugation. We may begin with certain preliminaries to the process—considering first "assortative mating." This has been proved biometrically by Pearl (1907) to occur in *Paramecium caudatum*. He found that conjugants are smaller[2], less variable, and more alike than non-conjugants (as regards size and form). The likeness between a pair of conjugants is "not due to any local environmental factor" but to "homogamy"—individuals tending to pair with their likes, not at random. These observations have been confirmed by Jennings (1911 *a*) in both *P. caudatum* and *P. aurelia*. Watters (1912) reports similar conditions in *Blepharisma*.

91. That assortative mating occurs in ciliates generally has not been proved, nor has it been demonstrated that conjugants are always smaller than non-conjugants[3], or that they are usually of the same size. Marked differences in the sizes of a conjugating pair have often been noted—for example, by Mulsow (1913) in *Stentor*. He noticed that conjugants are smaller than non-conjugants, but found that only about half the pairs[4] were composed of similar sized individuals. In other cases one individual was smaller than its partner—the difference being sometimes very considerable. Dotlein (1907) says that "in *Paramecium putrinum* almost half the pairs are composed of distinctly different individuals." Indeed, he was so impressed with the dissimilarities observable between two conjugating individuals in many species that he enunciated a "working hypothesis" to account for them. The frequent suggestion that the differences between the members of a conjugating pair are sexual in nature—the larger being female, the smaller male—is manifestly due to a misunderstanding of the nature of a conjugant (§ 14). For my own part, I do not consider that

have been able to show that "the rhythms in the division rate of *Paramecium* are the physiological expression of profound nuclear changes," which "involve the formation of a complete new nuclear apparatus." "This nuclear reorganization is evidently a normal substitute for typical conjugation."]

[1] I use these elusive terms in their colloquial sense, without prejudice to any conception of causality.

[2] Previously observed by Maupas, Gruber and others.

[3] Maupas (1889) enumerates 10 species in which the conjugants are smaller than the non-conjugants, and 7 in which they are of the same size.

[4] About 3000 pairs were studied—but not biometrically.

"homogamy" in *Paramecium* means much. It is merely an expression of the fact that a population of sexually mature individuals is more uniform in size than is a population of adults, adolescents, and children.

92. Is there a "eugamic period," or period of "karyogamic maturity," in the life-cycle of a ciliate? Many experiments could be quoted to show that there is not. For instance, Calkins (1902) concludes: "'Karyogamic maturity' does not signify much when fertile unions occur with individuals[1] in the 350th, the 410th, the 467th, and 500th generations of the same culture." And Jennings (1913) records an experiment (No. 9) in which he studied two lines of *P. aurelia*, both derived from one original ancestor, but only one of which had been allowed to conjugate. "Under the proper conditions both sets conjugate at the same time, in spite of the fact that one has conjugated at least four times since the other." These and similar observations (especially those on reconjugation—§ 120 *infra*) seem to indicate clearly that a "eugamic period," as conceived by Maupas, does not exist[2].

93. R. Hertwig (1905) states that when *Dileptus* is starved[3], two rapidly succeeding divisions ("hunger divisions") take place, resulting in the formation of four small individuals from each original individual. This is said to occur also in *Didinium* (Prandtl, 1906) and *Paramecium* (Kasanzeff, 1901). "Since Infusoria which have undergone hunger-divisions proceed to conjugate, a causal connexion seems to exist between the two phenomena. The hunger-divisions correspond in this respect with the maturation divisions of multicellular organisms." It is true that these "hunger-divisions" may account for the smaller size of conjugants—when they are smaller: but the remainder of Hertwig's argument seems based on a false analogy. For since the conjugant is not a gamete but a sexual individual (§ 12), it cannot properly be compared with a germ-cell. The phenomena in a ciliate which are homologous with the two meiotic divisions in a metazoon are the micronuclear divisions preceding fertilization (§ 13).

[1] *Paramecium caudatum.*

[2] It is possible, however, that under normal conditions conjugation may occur with fairly regular periodic frequency. Jennings (1910) describes a race of *Paramecium* which, under suitable conditions, will conjugate again 5 days after conjugation: in other races there was an interval of "a year or more." Something like a "eugamic period" may conceivably exist, therefore, though it seems very improbable that this is the "explanation" of these facts.

[3] Hunger, it will be recalled, is a condition conducive to conjugation, according to Maupas (§ 20).

94. Hunger was believed by Maupas (§ 20) to induce conjugation in cultures containing sexually mature individuals. The effects of hunger upon the organism have since been specially studied by Wallengren (1901*a*,—*Paramecium* and *Colpidium*) and Kasanzeff (1901,—*Paramecium*). Wallengren finds that hunger produces degenerative changes in the cytoplasm (vacuolation, etc.) and meganucleus (fragmentation, etc.), but not in the micronucleus. Starved individuals are of conspicuously smaller size. These observations are confirmed by Calkins (1904). Kasanzeff finds that starvation leads to an increase in the size of the meganucleus. And R. Hertwig (1899, 1902, 1905, etc.) has been led by these and similar observations to formulate the following *raison d'être* of conjugation —based upon his hypothesis of the karyoplasmic ratio (§ 30): In the course of normal functional activity of the organism, or as a result of hunger, the meganucleus grows at the expense of the cytoplasm, thus causing an increasing disproportion in the mass-relations of the one to the other. This disproportion may be compensated by a reorganization of the nuclear apparatus: and conjugation therefore takes place in order to bring about this result. Conjugation is thus regarded as a means of regulating the karyoplasmic ratio. Some remarkable experiments inspired by this idea have since been made.

95. It will be recalled that the meganucleus is relatively larger in ciliates kept at a low temperature than in those kept at a high temperature (§ 66). It therefore occurred to Prandtl (1906) that if he were to subject organisms adapted to a low temperature suddenly to a higher, they would find themselves in an abnormal condition in which the meganucleus was too large. In other words, the condition which normally leads to nuclear reorganization through conjugation could be thus brought about experimentally. A sudden change of this sort ought, therefore, to lead to conjugation. The experiment was tried with *Didinium* and *Dileptus*, and with successful results. Conjugation took place at the higher temperature. It should be noted that in these experiments temperature was not the only factor concerned. For Prandtl supplied his animals with plentiful nourishment at room temperature, and then starved them at the subsequent temperature of 25° C. Similar experiments were made on the vorticellid *Carchesium*[1] by Popoff (1908*a*), with a like successful result. He found further that starvation coupled with lowered temperature favoured the production of males: whereas coupled with raised temperature it led to

[1] Wrongly stated to have been *Epistylis* in an earlier paper (Popoff, 1907).

the production of females[1]. In all his experiments, however, starvation introduces a complication: so that after carefully studying the facts which he, Prandtl, and Hertwig have so far recorded, I am still unable to understand the real significance of these interesting observations. They seem to me suggestive rather than demonstrative.

96. The method employed by Maupas (1889) for inducing ciliates to conjugate consisted in the simple procedure of transferring some individuals from a large stock culture to a small culture on a slide. As soon as the food in the smaller culture was exhausted, conjugation occurred—provided the animals were "sexually mature." Essentially the same measure has been frequently used with success by other workers (Hamburger, 1904; Calkins and Cull, 1907; Enriques, 1907; Jennings, 1913; Calkins and Gregory, 1913; Woodruff, 1914; etc.). Jennings (1913) describing his method says: "In the evening large numbers of the animals [*P. caudatum* and *P. aurelia*] were taken from the large cultures and placed in watch glasses; early the following morning they were usually beginning conjugation." He believes that conjugation occurs "not as a result of starvation, but at the beginning of a decline in the nutritive conditions, after a period of exceptional richness that has induced rapid multiplication" (Jennings, 1910). Calkins had earlier (1902) concluded that "hunger is not a pre-requisite for union, it apparently prevents conjugation."

97. Enriques has long maintained that "the necessary and sufficient conditions for conjugation are environmental conditions" (1909 *a*). He states (1907) that *Colpoda steini* will conjugate only when the depth of the culture is 2—3 mm.[2]—never in deeper cultures. He further states that the liquid from a culture in which conjugation is taking place, when added to a non-conjugating culture, brings about conjugations in it: and reversely, liquid from a non-conjugating culture, added to a conjugating culture, causes cessation of conjugation. He concludes that the onset of conjugation "does not depend upon mysterious conditions developing themselves in the infusoria, but the modifications of the circumambient liquid play the chief part."

98. Pursuant of this train of thought, Enriques (1909) has performed a number of experiments with *Cryptochilum nigricans*. He

[1] Popoff incorrectly (§ 14) calls males and females "microgametes" and "macrogametes."

[2] Compare in this connexion the much earlier experiments of Everts (1873) on *Vorticella*. He believed that conjugation was caused by the drying up of the water in which the animals lived.

says that this ciliate will thrive in an infusion of hay in distilled water: but in this medium conjugation cannot occur. Conjugation depends upon the presence of salts. Addition of NaCl, NaBr, or NaI in certain concentrations causes epidemics of conjugation—the efficacy of these salts being in the order given. [That is, the order of efficacy corresponds with the order of the halogens in the periodic system, and is the reverse order of their toxicity.] The effects produced by $CaCl_2$ and Fe_2Cl_6 were surprising. One part (by weight) of either salt in one million parts of medium was sufficient to inhibit conjugation: but stronger doses (1:10,000) caused intense epidemics of conjugation. Addition of iron to the cultures had the most pronounced effect.

99. Similar but more extensive experiments have been made with *Paramecium caudatum* by Zweibaum (1912). Stripped of detail, his results are as follows. If a pure line of *Paramecium* is richly nourished in hay infusion, it will continue to multiply for an indefinite period without conjugating. If organisms are from time to time tested by placing them in solutions free from hay but containing (1) salts[1] in strong or (2) salts in medium concentrations or (3) no salts, then still no conjugations result. If, however, the richly nourished culture is changed—by removing the hay—into a "hunger culture," and if this is similarly tested after the lapse of a considerable time (5—6 weeks), then the trials result thus: in (1) and (3) no conjugations; in (2) abundant conjugations. In other words, the conditions necessary for conjugation in *Paramecium* are—plentiful feeding, followed by starvation, followed by treatment with salts in medium concentration. Conjugation can occur at temperatures from 9° C. to 29° C. (optimum 20—23°). There is an optimum concentration for each salt—which was determined—and successful results can be obtained by substituting glucose for salts. The most effective salt was found to be $AlCl_3$, which in concentrations of N/24000 to N/48000 gave "always almost complete epidemics" of conjugation. As they stand, these experiments appear to be conclusive, though it is difficult to reconcile them with other observations. It is to be hoped that the work of Enriques and Zweibaum will soon be repeated by independent investigators.

100. Some additional evidence of the influence of external conditions in causing conjugation is given by Baitsell (1912). He found that two lines of *Stylonychia*, derived from the same original organism but kept in different media, behaved differently as regards conjugation.

[1] Many different salts were tried, their several effects being described at length and enumerated in 36 tables of experiments.

The line bred in hay infusion refused to conjugate: whereas the parallel line in beef extract conjugated. He concludes that "the determining feature was the medium used." "Conjugation is induced by *external* conditions."

101. Jennings (1910) has come to the conclusion that "the conditions determining conjugation differ greatly in different races of *Paramecium* (*aurelia* or *caudatum*). Some races conjugate frequently, and under conditions readily supplied in experimentation. Others, under the same conditions, conjugate very rarely or not at all." Calkins and Gregory (1913) also share the view that there are conjugating and non-conjugating lines of *Paramecium*. Such a hypothesis would help us to comprehend the extraordinarily different observations made by different workers: but there is a very serious difficulty in the way of accepting it—namely, we have absolutely no proof that any race exists, which, under suitable conditions, is unable to conjugate[1]. Undoubtedly the weightiest evidence for the existence of such a race was that furnished by Woodruff (§ 39), whose pedigree line of *P. aurelia* consistently refused to conjugate for over 4000 generations. We now hear, however, that after more than this number of generations, conjugations have at last taken place (Woodruff, 1914). The likelihood that anybody will ever succeed in demonstrating with any plausibility that any given race of ciliates is incapable of conjugating, seems therefore immeasurably remote.

102. We may now pass to a consideration of the effects of conjugation. We have already seen that Maupas considered the chief result of conjugation to be "karyogamic rejuvenation" (§ 17). His work has frequently been misinterpreted as showing that conjugation reinvigorates the stock in which it occurs—that after a number of asexual generations the stock becomes weakened with age and divides more slowly, but may be restored to its former vigour and rate of reproduction by means of conjugation[2]. The work of Maupas does not show this. Calkins (1904), however, has adopted this standpoint, concluding from his work "that conjugation does actually rejuvenate and overcome the conditions of so-called 'old age'." He appears to be

[1] I do not, of course, hereby controvert the general statement of Jennings (1910) that "the conditions for conjugation are different in different races."

[2] This view was first definitely advocated by Bütschli (1875, 1876) who believed that conjugation resulted in "eine erhöhte Teilungsfähigkeit," which he interpreted as a sign that "Verjüngung" had been brought about. Balbiani's views were—for a time, at least—similar.

still of the same opinion (*vide* Calkins and Gregory, 1913), and many others have at various times concurred.

103. Yet R. Hertwig (1889) clearly showed that such a conclusion is not justified. He found that the rate of fission is not diminished before conjugation, but rather increased. He performed the ingenious experiment of forcibly separating a pair of prospective conjugants soon after they had united, and cultivating them further. Far from dying, they continued to divide normally for many generations—thus showing that they were not incapable of further multiplication, or in need of "rejuvenation" through conjugation. Hertwig's conclusion was the opposite to that generally drawn (§ 102). He believed that the rate of fission becomes abnormally high before conjugation, and that the sexual act has the effect of diminishing and normalizing it.

104. Hertwig's experiment has been repeated by Calkins (1902), and on a large scale by Jennings (1913). The latter has dealt with the facts exhaustively, and his conclusion can hardly be disputed. He writes: "In view of the large number of experiments made by Maupas on this point, the absolute agreement of his results with those of Richard Hertwig; the fact that these men are perhaps the most thorough investigators that have ever worked along these lines; the further fact that there exist no careful experimental results opposed to these; and finally, the very large body of evidence presented in the present paper[1], all giving the same results—is it not time that the statements or implications that in the infusoria conjugation results in increased reproduction should disappear from the literature of science?" The answer is emphatically affirmative. And it should be noted that it sweeps away all arguments that conjugation causes "rejuvenation," "increased vitality," and the like: for this "vitality" itself is ultimately measured by the rate of fission.

105. The effects of conjugation have been studied in elaborate detail by Jennings (see especially Jennings 1911*a*, 1913: Jennings and Lashley, 1913, 1913*a*). The extensive nature of his researches, the large number of details which he has endeavoured to elucidate, the vast array of facts which he has recorded—all these make his work excessively difficult to understand or to summarize. It can be appreciated in the original only. Comparison of experiment with experiment, conclusion with conclusion, leaves me—after devoting much time and attention to the matter—still in doubt as to what all this work really amounts to. Analysis in detail is here impossible, and I must be

[1] Jennings (1913).

content with the baldest statements and criticisms of Jennings's general conclusions. It may be said at once that the programme of his work is admirable. He has studied both "wild" and pure strains of *Paramecium caudatum* and *P. aurelia*, by means of observation and experiment and where possible by biometric methods. Certain characters (fission rate, size, etc.) were studied in individuals or their progeny belonging to the four classes (*a*) non-conjugants, (*b*) conjugants, (*c*) exconjugants, (*d*) "split" or "unpaired" conjugants—i.e. individuals forcibly separated, and bred further, after they had united for conjugation[1]. Knowledge of the behaviour of all these classes of individuals should obviously give definite information concerning the effects of conjugation.

106. The first general conclusion to which Jennings comes is that conjugation causes variation[2]—"Conjugation produces within a pure race heritable differentiations; so that as a result races diverse in their heritable characters arise from a single race with uniform heritable characters." This conclusion is drawn—speaking generally—from the demonstration that, in a given race, the progeny of non-conjugants and split conjugants are alike, but differ from the progeny of exconjugants. There is greater variability among the progeny of the last. Since conjugation is the only known factor which differentially affects the two groups, it seems justifiable to conclude that it is in some way a "cause" of variation. Yet this conclusion is remarkable. We have seen that non-conjugating "pure lines" are constant in character—the differences which the constituent individuals display being temporary and not heritable (§ 65). We have seen further (§ 90) that within the pure line "assortative mating" occurs, so that the members of a conjugating pair of organisms are more alike than those of a non-conjugating pair selected at random. And yet after conjugation these like individuals produce progeny which are unlike themselves and their race.

107. Jennings's second conclusion is at first sight even more strange. It is that "conjugation results in biparental inheritance." (See Jennings and Lashley, 1913, 1913*a*.) The meaning of this misleading expression[3] will be clear from the evidence upon which the conclusion rests. We have seen (§ 106) that the progeny of non-conjugants and split conjugants, within a pure race, are alike: and that the progeny of conjugants

[1] R. Hertwig's experiment (§ 103).

[2] This, of course, is a doctrine long ago promulgated by Weismann.

[3] I say "misleading" although Jennings explains the term (Jennings and Lashley, 1913, p. 457): for nothing is here "inherited" from the "two parents" save the power to *differ from* them in a given respect.

tend to be different. But biometric proof is now offered that the progeny of one member of a pair of conjugants tend to be like the progeny of the other, owing to the hereditary influence of both "parents" (conjugants) on all the progeny. It thus appears that conjugation simultaneously produces uniformity and diversity. I take this paradox to mean that if two similar individuals of the same race conjugate, then the progeny of both will differ from the original race, though the progeny of one will resemble the progeny of the other in whatever respects it differs from the original race. Or, in other terms, a pair of conjugants a_1 and a_2, belonging to a race a, produce after conjugation progeny forming races b_1 and b_2—differing from a, but resembling one another in both being b.

108. Both the fundamental conclusions reached by Jennings appear to me unproved. My chief difficulty is that I cannot find convincing evidence of a single concrete instance in which, from a known race—constant in a certain character—a new race—permanently diverse in this character—has arisen as a result of conjugation. As an abstract biometric proposition, it is no doubt demonstrated "that conjugation among the members of a pure race does result in differentiations that are inherited," so that from a uniform race diverse races might seem to arise. Now the diversity of the new races is manifested mainly in (1) mortality, (2) size, (3) rate of fission. In the first case, Jennings (like others) has found that many exconjugants, or their immediate descendants, die. The consequence of conjugation in these cases is really death. It is true there is increased variation in mortality among the progeny, but the individuals which manifest this diversity merely die out, so that no new races are produced in this fashion. Concerning the second character—size—the evidence[1] seems to me unsatisfactory. All that it appears to show is that although conjugants are smaller than non-conjugants of the same race, their progeny are not: and that there is greater variability in the size of individuals formed by fission from exconjugants *during the first few days after conjugation*, than is seen in the progeny of ordinary non-conjugants. Definite evidence of the production of a new race of permanently different size—as a result of conjugation—I can nowhere find. Perhaps the best is that which can be extracted from the paper of Jennings and Lashley (1913*a*), from which it can be inferred that the descendants of 43 pairs of exconjugants,

[1] Jennings (1911*a*). Further evidence is given in 1913 (expt. 9), but of this Jennings himself says "the results there given are by no means conclusive, the matter requires further study."

measured 25 days after conjugation, all possessed a smaller mean size than that of the race from which they were derived. The number of individuals measured is not great, however, and one may doubt whether *permanently smaller races*[1] had been produced as a result of conjugation.

109. Concerning the third character—rate of fission—the evidence appears at first sight conclusive. The biometric results show clearly that there is a difference (greater variability) in the fission rate of the descendants of exconjugants as compared with those of non-conjugants or split conjugants—all of the same pure line. But as Jennings himself points out "conjugation increases the variation mainly toward the lower extremity of the range"—that is, the effect of conjugation is to retard the rate of fission. Is not this merely another aspect of the same condition which is otherwise manifested as "high mortality" and "loss of vigour" after conjugation? Jennings's "Experiment 6" seems to me the key to the matter. "This experiment as a whole shows the fact that after conjugation the organisms are in a condition such that many may die, while those that have not conjugated live; and the further fact that the rate of reproduction is made slower by conjugation, remaining in this condition for about two months," after which it "has regained about the usual rate." If the result of this experiment may be regarded as typical, then it indicates that the lowering in fission rate following conjugation is transient, recovery occurring sooner or later. It is demonstrated that after conjugation the organism and its progeny are weaker, or less resistant to external conditions (shown by higher mortality, lagging fission rate, unstable size, abnormalities, etc.) for a certain time; and that complete recovery to the normal state preceding conjugation occurs subsequently: but I find no proof that from a race with a given fission rate, another race with a permanently different fission rate has arisen as a result of conjugation[2].

110. Turning now to Jennings's second proposition (§ 107), I can make criticisms of only a very general nature. I am not able to judge of the validity of the biometric methods employed for its demonstration. It appears to me, however, that to prove "biparental inheritance," the

[1] The fact that *all* the races are smaller is significant. One can hardly suppose that conjugation in this race always leads to a permanent reduction of size. It seems much more likely that there was some environmental factor (e.g. food or temperature—cf. §§ 60, 66 *et seq.*) affecting all the cultures alike.

[2] A similar criticism to the above has already been made by Jollos (1913*a*). It seems to me unfortunate that Jennings should have selected for his chief study a physiological character—fission rate—which is so greatly influenced by environmental conditions (§ 51 *et seq.*).

descendants of a pair of exconjugants must form two fairly homogeneous lots—in order that any comparison between them is possible. By taking the mean for some lines descended from each exconjugant, such a homogeneity may be introduced when it does not really exist. For if the progeny of an exconjugant differ from one another—the differences being, *argumenti causa*, caused by conjugation—how can any real comparison be made between all the different organisms which *could* be obtained from one exconjugant—the number is unlimited—and those from its partner? It is physically impossible to study the rate of fission in more than a few lines descended from an exconjugant, for at each fission the number of possible lines to be studied is multiplied by two. Granted that conjugation causes variation, so that the lines derived from different progeny of one individual exconjugant display different fission rates, how is it possible to reach any definite conclusion by studying certain arbitrarily selected lines representing only an infinitesimal fraction of all possible lines? It seems to me that if conjugation gives rise to variations of this sort—as certain experiments seem to show—then any real demonstration of "biparental inheritance" in fission rate is impossible. I cannot comprehend how Jennings's results in this connexion can have any value beyond suggestiveness.

111. Calkins and Gregory (1913) have recently published an account of the variations observable in different lines of *Paramecium* derived from a single exconjugant. In one case as many as 30 lines from one exconjugant were studied—that is, a single line from each of 30 out of the 32 individuals formed by the first five fissions after conjugation. The authors conclude: "The results of this study show that physiological and morphological variations in the progeny of a single exconjugant of *Paramecium caudatum* are fully as extensive as the variations between progenies from different exconjugants. The arguments based upon the latter variations to the effect that conjugation is for the purpose of originating variations cannot therefore be sustained." The authors appear to believe that they have invalidated Jennings's general conclusion that conjugation causes variation. If so, their argument is a palpable *non sequitur*. What they have shown is that the progeny of an exconjugant differ from one another—that there is considerable variability among them. Whether the variability is the effect of conjugation or not, there is no means of judging: for no comparative study of non-conjugants and split conjugants of the same race appears to have been made—a study which is essential if the effects of conjugation are at issue. The real importance of these observations

seems to me to lie in the fact that they afford additional grounds for doubting the validity of Jennings's conclusions concerning "biparental inheritance" (§ 110). It may be noted further that some evidence is given that the differences between the surviving lines derived from the same exconjugant tend to disappear in the course of time—a tendency which is of interest in view of what has previously been remarked (§ 109).

112. It is noteworthy that Pearl's (1907) biometric study of *Paramecium* led him to a conclusion diametrically opposed to that of Jennings (§ 106). He says: "There is no evidence that conjugation tends to produce increased variability in exconjugants. All the evidence indicates, on the contrary, that conjugation serves...to preserve relative stability of type." The same standpoint is taken up by Enriques (1907), though chiefly on *a priori* grounds.

113. A curious observation bearing on the effect of conjugation has been made by Jollos (1913). He states that his arsenic-resistant race of *P. caudatum* (§ 71) has lost its resistance as a result of conjugation[1]. Progeny of exconjugants displayed merely ordinary resistance, whereas progeny of non-conjugants (a parallel line) continued to display their acquired increased resistance for a long period. If this observation is substantiated and confirmed, it appears to indicate that conjugation is a barrier to the transmission of an acquired physiological character—that it eliminates rather than originates variation.

114. It was believed by Calkins (1902) and Cull (1907) that the effects of conjugation are not the same on the two members of a pair of exconjugants—the progeny of one tending to survive, of the other to die out. This was interpreted as evidence of "incipient fertilization" in *Paramecium*—the form studied. When the nature of a conjugant is properly understood, however, it is clear that this is based on a confusion of ideas. The conjugant is neither a male nor a female, nor a gamete of any category (§ 12 *et seq.*). To speak of "incipient fertilization" or "incipient sexuality[2]" in this connexion is meaningless. It is of interest, however, to know whether there is really a difference in the fate of the progenies of a pair of conjugants, and the matter has been exhaustively studied by Jennings and Lashley (1913). They conclude that "if one member of a pair survives, the other member tends to survive also; if one dies out the other tends to die out also." The

[1] After conjugation the resistance is said to disappear "mit einem Schlage": but it is also stated that the progeny of the exconjugants were not tested until two weeks after conjugation, so that the "suddenness" of the loss is hardly demonstrated.

[2] Jennings and Lashley (1913).

effects of conjugation tend to be the same—not different—for both members of a pair. This demonstration furnishes the chief evidence for "biparental inheritance" in *Paramecium*[1] (§ 107).

115. Enriques (1908) states that in *Chilodon*, the two conjugants are alike at the beginning of conjugation: but *during* conjugation they become differentiated into a longer and a shorter. This phenomenon is called "hemisex" Enriques regarding the longer conjugant[2] as "half female," the shorter as "half male." What the significance of this "sexual differentiation as an effect of conjugation" may be, is obscure. It is quite certain, however, that the phenomenon has nothing to do with sexual differentiation properly so called. For from the point of view of sex, the conjugants are identical – both being hermaphrodite (§ 14). It may be noted that Jennings (1911*a*) has observed a tendency to "equalization" during conjugation in *Paramecium*. The two members of a pair of conjugants tend to become more alike—not less alike—during the process.

116. We have seen (§ 20) that Maupas regarded "inbreeding" or "interconjugation[3]" as productive of harmful consequences. Some observations bearing upon this matter have since been made, though they are recorded for the most part incidentally. Cases of apparently successful conjugation between closely related individuals are recorded by Joukowsky (1898), Calkins (1902), Jennings (1913)[4] and others. In other cases, however, such conjugations appear to have been harmful. Baitsell (1912), for example, found that the descendants of a *Stylonychia* interconjugated readily: but all the exconjugants died. Calkins (1912) made similar observations on closely related individuals of *Blepharisma*: "conjugation is equivalent to a death warrant." Nevertheless, if interconjugation really has harmful effects, it is impossible to reconcile this with the facts (1) that conjugation occurs normally between individuals belonging to the same pure line—not between those of different lines

[1] It is important to notice what the characters are which are "biparentally inherited"—namely death, or survival. The characters are not racial characters—save in so far as "survival" is a character of every living race.

[2] Enriques incorrectly—and inconsistently—calls the conjugants "gametes." When he speaks of an organism as being "half male," I do not understand what he considers the other half to be. The term "hemisex" seems to me ambiguous—or else incorrect.

[3] I use this term to denote conjugation between closely related organisms—i.e. between descendants of the same ancestor. The term "inbreeding" is equivocal, and "interconjugation" seems to me a more suitable word. Conjugation is not an act of reproduction (§ 15), and therefore there is really no *breeding* in the process.

[4] In *Paramecium putrinum*, *P. caudatum*, and *P. aurelia* respectively.

(Jennings, 1911 *a*); and (2) that homogamy occurs in addition (Jennings, 1911 *a*). The inference from these two facts seems clearly to be that "inbreeding" is the rule—at least in *Paramecium*.

117. An extreme case of interconjugation has been recorded by Jennings[1]. He obtained no less than nine successive interconjugations in the descendants of the same individual. "The progenitor of the race was a single individual; its progeny conjugated among themselves; from these conjugants a single exconjugant was taken and allowed to multiply till there was conjugation among these." An exconjugant was again isolated and allowed to multiply—and so on, nine times in succession (in the complete experiment). It appears—though Jennings does not emphasize the fact in this connexion—that the mortality among the final descendants was excessively high.

118. A case of conjugation between very closely related individuals is recorded for the colonial Vorticellid *Opercularia* by Enriques (1907). He states that male and female conjugants (§§ 6, 14) are formed by an original "indifferent" individual[2]—incapable of conjugating—dividing into a large and a small product, the latter dividing again into two smaller individuals. The large individual becomes a female conjugant, the two small individuals males. It is stated that conjugation may occur between males and females formed in this manner from the same indifferent individual[3]. Calkins (1912) has even recorded a case of conjugation in *Blepharisma* in which the two conjugants were the products of fission of the same individual—"the closest case of paedogamy in ciliated protozoa on record." Death followed conjugation—as in all cases observed in *Blepharisma*.

119. The general conclusion to be drawn from the recorded cases of interconjugation is by no means clear. It is evident that closely related individuals will conjugate readily with one another: but the ultimate effect of such conjugation on the progeny is not evident,

[1] Jennings (1913, expt. 13). Jennings speaks of this interconjugation as "self-fertilization—which it certainly is not. He obtained eight successive interconjugations—"to avoid, so far as possible, the heterozygotic condition"—and then studied the effects of conjugation at the ninth. Since the general conclusion from this was that conjugation "increases greatly the variability," I cannot understand how previous conjugations are supposed to eliminate "the heterozygotic condition." The mathematical treatment of "self-fertilization" seems to have no bearing on the actual phenomena concerned.

[2] It may be noted that Enriques (1907) states that in the Vorticellid *Carchesium*, the branches of the colony are differentiated as males, females, and "indifferents." This was not confirmed by Popoff (1908 *a*).

[3] I do not know how it was possible to make this extremely difficult observation with any certainty.

because the effects of conjugation with unrelated individuals have never been studied simultaneously.

120. This seems the proper place at which to mention the phenomenon of "reconjugation" discovered by Enriques (1908). He found that exconjugants of *Chilodon*, instead of dividing, sometimes proceed to conjugate again—either with other exconjugants or with ordinary conjugants. The same thing has been recently observed in *Paramecium* by Klitzke (1914). Conjugation may be effected by an exconjugant or by the products of its first fission. These observations clearly show that the stimulus to conjugation—whatever it may be—may exist without a series of asexual fissions having intervened since a preceding conjugation. The fate of the progeny of "ex-reconjugants" has not been adequately described.

121. I may here mention some cases of what may be called "misconjugation." Doflein (1907) says that if conjugating *Paramecia* are forcibly separated, they will reunite with other individuals—either non-conjugants or conjugants of a different stage[1]. Abnormal conjugations may thus be brought about, the results of which are not recorded. Many observers have reported abnormal conjugations of three or more individuals[2]. Several such unions of three individuals were observed by Mulsow (1913) in *Stentor*: but to my knowledge no investigator has yet ascertained the consequences of these misconjugations. Doflein (1907) further describes, in *Paramecium putrinum* and *Stylonychia mytilus*, "agamic fusions" of two individuals, "cytoplasm with cytoplasm, nuclei with nuclei, so that an apparently quite normal, but relatively very large individual resulted." The subsequent behaviour is not described[3].

122. In conclusion, a word may be said about "hybridization" in ciliates—I mean cross-conjugation between two individuals of different species. I know of but a single case in which this is alleged to have happened. Apart from this, there appears to be no recorded case of "crossing" even between individuals belonging to different pure lines

[1] Doflein gives an illustration of this, which, according to Klitzke (1914), really depicts a conjugation between an ordinary conjugant and a "reconjugant" (§ 120). This interpretation certainly appears plausible.

[2] These are described, for instance, by Stein, Engelmann, Jickeli, Gruber, Plate and Maupas—among the older observers—and generally in *Stylonychia* or *P. putrinum*.

[3] But Engelmann said that these compound organisms (*Stylonychia*) can grow and multiply—a statement as yet unconfirmed. Maupas believed that such fusions have nothing to do with conjugation—the fused animals being monstrous, and their divisions irregular and incomplete.

of the same species[1]. The case to which I refer is given by Simpson (1901), who states that twice (out of 21 attempts) he succeeded in getting conjugations between *Paramecium caudatum* and *P. aurelia*. "After separation each of the exconjugants divided once: on the third day they died off." The account is extremely unconvincing, and I think it is infinitely more probable that Simpson was deceived than that cross-conjugation occurred.

CHAPTER IV.

General Conclusions.

123. In this final chapter I propose to consider very briefly certain results of the genetic study of the Ciliata. I would point out that this chapter is not a summary of previous chapters; nor is it intended to be a substitute for them—to enable the reader to dispense with the facts there set forth. Each of the preceding chapters is itself a series of very brief and incomplete summaries, which form—in part—the premisses from which the following conclusions are drawn. If my conclusions appear absurd and wrong, they may nevertheless incite further inquiry into the evidence upon which they rest. This is my desire.

124. It is quite clear to me—and I have every confidence that sooner or later it will be equally clear to others—that many of the problems now associated with the ciliates do not exist in nature. They are really dialectic—not problems of concrete biology. They are offshoots of the fallacies involved in the "cell theory," and of the unbridled academic speculations concerning evolution which were fashionable at the end of last century.

125. The fundamental error in the conceptions of Maupas and his followers is due to the "cell theory." The ciliate has been called a "cell," and certain constituent elements of the metazoan body have been given the same name. Consequently it has been assumed that an exconjugant is the homologue of a fertilized metazoan ovum; and that the whole succession of its descendants is homologous with the entire body of a multicellular animal[2]. It has thus become possible

[1] "Assortative mating has clearly the effect of keeping differentiated races from mixing....When a culture containing two species conjugates, the two as a result of the assortative mating remain quite distinct" (Jennings, 1911*a*).

[2] This view has been advocated most strongly in recent times by Calkins (1909). The reader who wishes for a fuller analysis of the matters here merely touched upon, will find it in an earlier publication (Dobell, 1911).

—and even necessary—to look for periods of adolescence, maturity, senescence, and death, in the successive generations of individuals derived from an exconjugant. But as soon as it is realized that a ciliate is a non-cellular but complete organism, homologous with a whole metazoan: as soon as it is realized that the only resemblance between a ciliate and a metazoan cell was created by biologists when they gave the name "cell" to both: then it will also be realized that there is no reason to expect that successive generations of ciliate individuals will manifest the same series of phenomena as is manifested by a single individual metazoon during its life-time. One of the chief results of the researches recorded in previous chapters is the demonstration that events, whose occurrence we have absolutely no reason to expect, do not, in fact, occur.

126. It has been shown (§ 43) beyond all reasonable doubt that under suitable conditions ciliates are able to live and multiply, in their own fashion, for an unlimited time[1]—like all other organisms that are well adapted to their environment. To ask whether they become "senile" in the course of successive generations, whether "protoplasmic old age" sets in, whether "rejuvenation by fertilization" is a periodic necessity, and so on—to ask such questions is to propound problems which are either unanswerable or meaningless. Among ciliates, offspring are formed by the division, growth and differentiation of the protoplasm of their parents—as in all other organisms. If "immortality" can be predicated of the ciliates, it can also be predicated of all other organisms in the same sense. "Are the descendants of a ciliate older than their progenitor?" is the same question as "Is the child older than its father?" There is no problem here at all, for the answer is self-evident as soon as the questioner intimates what he means by "old."

127. That conjugation is able to "rejuvenate" is a belief whose origin is revealed in the word "fertilization." Conjugation is accompanied by "fertilization," and has consequently become confounded with the original connotation of that term[2]. Probably no living biologist would care to advocate the view that the cytological phenomenon now called "fertilization" is a process of "revitalizing the germ"—though more than one can be found to discuss whether "conjugation results in rejuvenescence." But quite apart from any mental or verbal tangles

[1] I believe there are no *a priori* reasons, or arguments from analogy with other non-cellular organisms, which indicate that the ciliates are incapable of continuing to multiply asexually—in favourable circumstances—for an unlimited number of generations.

[2] This was, of course, pointed out long ago by Weismann.

such as this, it may be stated now with considerable confidence, as a concrete proposition, that conjugation in the ciliates does not result in rejuvenation—no matter whether a literal or metaphorical meaning be attached to the word.

128. Experimental inquiries into the factors which determine division and hereditary transmission of characters; into the factors which determine or inhibit conjugation; into the results which conjugation itself determines, whether in originating or eliminating variations; and into the origin of variations,—all these investigations have yielded a great mass of facts of great interest and suggestiveness. But these facts—in my opinion—throw little light upon the great central problems concerned. Rather do they throw into vivid relief the formidable complexity of all biological problems as presented by the Protozoa. It is safe to prophesy that when the known facts have been doubled or trebled, the ironical statement—which now prefaces so many memoirs on the ciliates—that "the Protozoa are the simplest organisms in which to study the great problems of biology" will disappear from biological literature.

129. The tradition which finds expression in such statements as this is at present almost universally received. It is believed that the non-cellular organisms display vital phenomena in a more elementary and therefore more easily comprehensible form than other organisms. From his earlier physiological studies Jennings (1906) rightly concluded that "the behaviour of the Protozoa appears to be no more and no less machine-like than that of the Metazoa." "Action is as spontaneous in the Protozoa as in man." This truth should never be forgotten. It is vain to seek for simple mechanical factors which "induce conjugation" in ciliates. For conjugation is the resultant of many external and internal factors—environmental opportunities, inherent inclinations and potencies—which are no less complex and no more easily comprehensible than the factors which result in comparable phenomena in man. They are really less easy to understand, because we have no conception of what the "motives" may be which actuate a brainless, non-cellular creature.

130. In the foregoing pages I have confined myself to recording and analysing facts and concrete questions. It is often asked "What is the significance, or meaning, of conjugation?" This question is itself meaningless. As well might one inquire the meaning of the moon. I have purposely avoided all such questions, though they have been freely debated by others. I have also avoided all discussion of

teleological interpretations of conjugation and other phenomena. Whether conjugation is for the purpose of originating variations, or for any other purpose, is to my mind an idle discussion: and I have little liking for the "explanations" which have been given of the phenomenon —explanations which seem to me to explain nothing and lead nowhere[1]. So many real problems which may be attacked by means of experiment are still unsolved, that I think the discussion of more remote problems may be profitably postponed.

131. No new light has been thrown upon the great problems of organic evolution by a study of the ciliates. They have revealed no real indication of the manner in which evolution has proceeded, or is proceeding, within the group[2]: *a fortiori*, they tell us nothing of the process of evolution in general. The facts so far determined could, indeed, be used with far greater force to support the doctrine of the fixity of species. Moreover, even if it were possible to draw any general conclusions from the ciliates themselves, it would not be justifiable as yet to extend them even to the other groups of Protozoa[3]. The ciliates are so curiously organized that in many ways they stand alone among animals. What is true of ciliates is not necessarily—or even probably—true of most other organisms. These will be unwelcome sayings to many, but I believe they are true. And all will admit that it is better to face the facts, however distasteful and unconformable to theory they may be, than to veil them with that former assurance concerning organic evolution which, as is fast becoming evident, was chiefly begotten of ignorance.

[1] Compare for example the following: "Conjugation should be regarded as a set of physico-chemical phenomena resulting in a sort of cellular purification" (Loisel, 1903). "Conjugation appears to us as above all a set of chemical phenomena which counteracts another set of chemical phenomena—senescence" (Loisel, 1903 *b*), etc.

[2] I leave out of account the numerous fanciful speculations concerning the phylogeny of the ciliates which, I know, afford satisfaction to many. I am here considering the facts relating to the ciliates on their merits—apart from any preconceived interpretations.

[3] I believe there are few protozoologists who would endorse the opinion of Calkins (1909) that *Paramecium* is "a typical protozoon."

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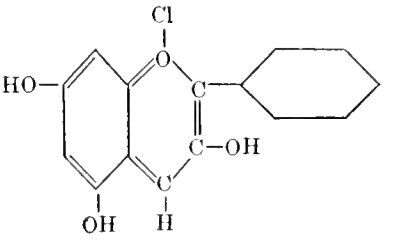
A NOTE ON WHELDALE AND BASSETT'S PAPER "ON A SUPPOSED SYNTHESIS OF ANTHOCYANIN[1]."

By ARTHUR ERNEST EVEREST, M.Sc., Ph.D.

Before the above-mentioned paper of Wheldale and Bassett came to hand, the author had forwarded for publication elsewhere, a paper, the contents of which very largely answer the criticism directed by Wheldale and Bassett against his earlier paper (*Roy. Soc. Proc.* 1914, B, vol. 87, p. 444). There are however a few points to which, in view of Wheldale and Bassett's publication, the author feels it necessary to draw attention.

It must be pointed out that as the result of the recent publication of Willstätter and his collaborators (*Sitzber. K. Akad. Wiss.* Berlin, 1914, xii, 402) wherein the chemical examination of anthocyan pigments (obtained pure and crystalline in every case, both as glucoside and non-glucoside) from no fewer than ten flowers or fruits was briefly described, and in which it was conclusively proved that these pigments were *not*



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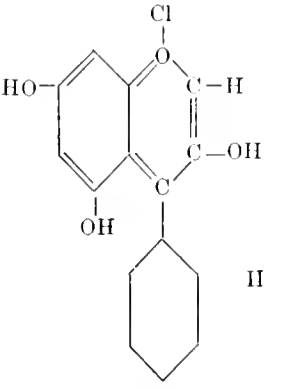

II

[1] *Journal of Genetics*, 1914, Vol. iv. No. 1, p. 103.

oxidation products of flavonols, but were indeed related either to β-phenyl-benzo-γ-pyrone or β-phenyl-benzo-α-pyrone, in the manner suggested by the present author (*loc. cit.*), and were derivatives of either I or II, it becomes necessary that the work of Wheldale and Bassett upon the pigments of Antirrhinum should be continued until crystalline substances having the characteristics of chemically pure compounds are obtained, and these examined, before it can have any weight in favour of the hypothesis which those authors support.

Doubtless Wheldale and Bassett were neither aware of the publication of Willstätter and his collaborators, nor present when Professor Willstätter lectured on this subject at the University College, London, in May last.

It is perhaps advisable to note that in the passage quoted by Wheldale and Bassett, from the author's paper, viz.: "one would expect that by taking the yellow glucoside, hydrolising, then reducing with removal of sugars, the anthocyanidin produced would combine with the sugar present to form an anthocyanin. This is not the case," the original reads: "....., then reducing *without* removal of sugars,....." (*loc. cit.* p. 445).

The author's recent work proves at least that Rutin —a disaccharide of quercetin—passes by reduction, without hydrolysis at all, into a red disaccharide pigment whose properties are those of an anthocyanin. No satisfactory evidence is available to suggest that, in general, higher glucosides of these pigments occur in plants, and until such evidence is forthcoming, hypotheses demanding their existence are not likely to receive much support from chemists.

It appears necessary to point out that the author does not use the solubility or insolubility of these pigments in amyl alcohol as a test to distinguish between anthocyanidins and anthocyanins, the test used depends upon the distribution between dilute aqueous sulphuric acid and amyl alcohol, coupled with the change in such distribution when a glucoside pigment is hydrolysed; this was clearly described in the author's paper (*loc. cit.*).

Chemical Laboratories,
University College,
Reading.

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A GENETIC AND CYTOLOGICAL STUDY OF CERTAIN TYPES OF ALBINISM IN MAIZE.

BY FRANK C. MILES,
United States Department of Agriculture, Washington, D.C.

Previous Investigations.

IT has long been noted that white seedlings, and variegated ones as well, occasionally occur among many of our common plants, but only in recent years has any systematic study of the problem been made.

Baur (1, 2) in 1907 (later, in 1908) reported his study of the yellow-leaved type of *Antirrhinum* and *Pelargonium*. The yellow plants are not capable of assimilating carbon dioxide, and consequently they cannot live. This type of plant can exist only as a hybrid with a green race. The hybrid is an intensive yellow or "Aurea-Farbe," and in the second generation one-fourth of the plants are pure green which breed true in succeeding generations, two-fourths are of the "Aurea" type which segregate again in the next generation, and one-fourth are pure yellow plants which theoretically should breed true, a test, however, being impossible because the yellow plants always die in the seedling stage.

In 1909 Correns (6) reported examples of what has been called the "chlorina" type of plants in *Mirabilis jalapa* and in *Urtica pilulifera*. Baur (3) later reported the same type in *Antirrhinum*. In these cases the colour stuff (including the xanthophyll and carotin as well as the chlorophyllin) seems to be reduced in intensity, and also appears to be diminished in quantity. In inheritance this "chlorina" character behaved as a simple Mendelian recessive.

In the same paper (6) Correns also reported a peculiar manner of inheritance of leaf colour in *Mirabilis jalapa*. He states that the leaves of the plants are irregularly spotted with yellowish white, a

condition which he terms "albomaculata." Occasionally there may appear a plant which is either wholly green or wholly white.

Anatomically it appears that this variegation is due to the fact that in the whitish areas of the leaf the chromatophores are not green, but are more or less bleached. The boundary between the green portion and the white portion of a leaf is not sharp and distinct, but is gradual; the cells on the boundary may contain pale-green chromatophores, and even in the same cell the intensity of colour of the different chromatophores may vary. A pure green branch remains so, and one which is pure white remains white.

Seed from one of the green branches produces green seedlings which in further generations breed true. Seed from white branches produce white seedlings which soon die because of their inability to perform photosynthetic processes. Seed from a variegated branch produces some seedlings which are yellowish-white, some which are green, and some which are spotted or variegated.

In crosses Correns found that this variegated condition was inherited only through the mother. When a flower on a white branch was pollinated with pollen from a normal green plant, only white seedlings were produced. And in the reciprocal cross, when a flower on a green branch was pollinated with pollen from a flower of a white branch of the variegated plant, only green seedlings were produced. But the seedlings were known to be hybrids because of the behaviour of other characters, e.g., colour of the flowers. These green hybrids bred true to the green character in succeeding generations, while the other characters behaved as ordinary hybrids.

Baur (4) believes that in *Primula sinensis* he has a case analogous to that described by Correns in *Mirabilis*, but at the time of his publication he had not fully investigated it.

Soon after the account of Correns concerning the case of non-Mendelian inheritance in *Mirabilis*, Baur (5) described another peculiar form of non-Mendelian inheritance in *Pelargonium zonale*. Among these plants there sometimes occur bud sports which are fully white. Seeds from the flowers on a white branch produce pure white seedlings. Also the white-leaved form remains constant if grafted upon a green plant.

Reciprocal crosses between this white-leaved form and a constant green form gave seedlings which had a mosaic design of green and white. Baur makes it apparent that in the F_1 there was a vegetative segregation, the plants being composed of green and white mosaic areas.

This vegetative segregation concerned only the leaf colour. When a white-leaved type and a green-leaved type were crossed and the parents differed in some other character, e.g., flower colour, then the flower colour Mendelized regularly.

In 1910 Baur (4) reported the occurrence of pure white-leaved plants in *Melandrium*, *Antirrhinum*, and *Phaseolus vulgaris*. He had not fully investigated the case of *Phaseolus*, but in both *Melandrium* and *Antirrhinum* the white condition proved to be a simple recessive character. He believes that in *Antirrhinum* several factors are concerned in the formation of leaf colour.

Analogous examples of the inheritance of the variegated condition of leaves have been reported by Baur (4) for *Aquilegia vulgaris*, and by Correns (6) for *Mirabilis jalapa*. The leaves of the plants have irregular spots of a very light-green, or almost white, colour. Crossed with green plants the F_1 is green and F_2 segregates into approximately three green plants to one variegated plant. The variegated individuals when self-pollinated usually breed true to the variegated condition, but occasionally a fully-green plant occurs. Some of these green plants when self-pollinated yield only green progenies, but others segregate into green plants and variegated plants. The cause of this apparent inconstancy of the variegated segregates is not fully understood.

East and Hayes (9) in 1911 mentioned the occurrence of striped leaves in some of their cultures of maize. The striped plants were apparently heterozygous, and when crossed with normal green plants green was dominant. Progenies from striped plants consisted of green plants, striped ones, and plants lacking chlorophyll. These latter died, but, as the authors suggested, very probably were homozygous recessives.

Emerson (10) in 1912 reported his studies of various degrees of chlorophyll reduction in maize, and described a number of types which he had not fully investigated. He found pure white seedlings void of chlorophyll, and which therefore died at any early age. In inheritance he showed the character to be a simple Mendelian recessive.

In some of his cultures there were individuals which were almost void of chlorophyll, but there was sufficient colouring material to give the seedlings a yellowish-white appearance. Seedlings of this type may turn more or less greenish, but at the time of his publication none of the plants had lived longer than seven weeks. This character also behaved as a simple Mendelian recessive.

The third type which he described concerned the partial reduction of chlorophyll in growing and mature plants, resulting in a distinct

yellowish-green colour of the leaves. Crosses between these yellowish-green individuals and fully-green plants showed that the character was not a dominant one.

Another class included plants which had prominent longitudinal stripes of green, pale green and white. So far as his study had gone this character appeared to be a Mendelian recessive, though he mentioned that in certain cultures it appeared as if it might be due to the absence of two positive factors.

There was also a peculiar, definite, regular striping of the leaf. In the region of the fibrovascular bundles the leaf is dark green, but between the bundles the leaf is pale green. He believed this was also a Mendelian recessive.

He also reported the occurrence of plants the leaves of which showed a few very narrow streaks of white. This type of inconspicuous variegation was noted in a family in which pure white plants also occurred. The character appeared to be a recessive one, but it had not been fully studied.

Gernert (12) in 1912 briefly discussed the occurrence of pure white seedlings in certain of his cultures of maize. He also reported the presence of yellowish-green plants and suggested the possibility that they might be an intermediate stage of albinism. His studies clearly indicate that the albino form is inherited as a simple Mendelian recessive. The data in regard to the yellowish-green condition were rather incomplete, though it is quite likely that this is also a simple recessive.

Davis (7) in 1912 described etiolated rosettes of *Oenothera*, which he discovered in the second generation of a cross between two green plants. The plants later became green and developed to maturity. In a later paper (8) he reported that selfed seed from one of the F_2 plants which had developed from an etiolated seedling gave a progeny consisting entirely of etiolated rosettes. The plants developing from these etiolated rosettes became green, as did those of the preceding generation.

Nilsson-Ehle (13) in 1913 described the appearance and behaviour of pure white and yellowish-white seedlings in some of the common cereals.

In barley he found pure white plants and also some plants whose leaves were yellowish, or sometimes slightly greenish. The individuals of both types die in the seedling stage, though the yellowish ones lived somewhat longer than those which were pure white. Green appeared fully dominant, and in some families which consisted of homozygous

green plants and heterozygous green plants the two classes could not be distinguished by any visible character. Progenies of heterozygous plants consisted of three green plants to one white one.

In rye he noted the pure white-leaved type of plant, and also a distinct yellow-leaved type, which in inheritance is analogous to the white type. The plantlets of both types are incapable of surviving the seedling stage. Green is dominant in both cases, and there is no "aurea" type such as that described by Baur in *Antirrhinum*, and also in *Pelargonium*. He believes that the white condition of the leaves is due to the omission or suppression of some factor, and that the yellowish condition is due to the suppression or omission of a second factor.

He reported having found white plants among his cultures of oats, but no record of the behaviour of the character was given.

The Inheritance of Albinism in Maize.

The experimental work which is here reported was conducted at the Nebraska Agricultural Experiment Station under the direction and supervision of Dr R. A. Emerson, who had begun investigations on these problems, and who kindly placed at the writer's disposal the records of the work so far as it had progressed, together with the seed for continuing it. The writer takes this opportunity to express his appreciation of the gracious assistance and many valuable suggestions which Doctor Emerson has given him. The colour drawings reproduced here were made by Miss Carrie M. Preston of the Nebraska Agricultural Experiment Station, to whom the writer desires to express his thanks for this assistance.

Since Emerson (10) has shown conclusively the recessive nature of the pure white plants (Pl. VIII, fig. 1), little attention has been given to the inheritance of this case of albinism other than to count the white and the green plants in each progeny where both types have occurred. In the fourteen families that have been grown since the spring of 1912 there was a total of 361 green plants and 130 white ones, which approximates very closely the theoretical ratio of three green plants to one white one.

More attention has been given to the case of the yellowish-white individuals (Pl. VIII, figs. 3, 4*a*, 4*b*, 5 and 6). In the twelve progenies which threw both green plants and yellowish-white ones there were 726 green plants, 260 yellowish-white ones, and 5 which were striped. It was possible to self-pollinate two of the striped plants, but

only one of them set an ear. Seed from the self-pollinated ear gave 26 green plants and 16 which were yellowish-white. This is fully in accord with the results obtained by Emerson, for the tests which he made of similarly occurring striped plants led him to the conclusion that the striped individuals were merely heterozygous green plants in which the green was not fully dominant.

Emerson (10) mentioned that these yellowish-white plants sometimes become greenish, and one individual in the greenhouse lived to the age of seven weeks. Perhaps the conditions out of doors were more favourable for the development of these peculiar yellowish-white plants, for during the season of 1912 several of them became fully green. The position of some such plants was so well designated that there was no mistake as to their identity, and two of the individuals were self-pollinated. From the ears of the two plants 278 seedlings were grown in the greenhouse, 277 of which were of the yellowish-white type, and one was green. Undoubtedly the green plant was the result of an accidental foreign pollination, for it is possible that a single pollen grain from some green plant may have come in contact with one of the silks. The fact that the yellowish-white plants breed true affords additional evidence that a Mendelian recessive is being dealt with.

From the study of the pure white plants and the yellowish-white ones it seems that both are simple recessives, yet it appears that more than one factor must be concerned. Believing that there were different factors involved, crosses were made between green plants from a family which had thrown pure white seedlings, and green plants from a family in which yellowish-white plants had occurred. It was hoped thus to secure crosses of heterozygous green plants which, if self-pollinated, would throw 25 per cent. pure white seedlings, with heterozygous green individuals which, if self-pollinated, would yield 25 per cent. yellowish-white seedlings in the next generation. The green plants which were crossed were also self-pollinated. In Table I are indicated only those crosses in which both parents were shown to be heterozygous.

It is interesting to note that the first generation of the cross between the heterozygous green plants of the two categories consisted of 362 fully-green plants and one which was very faintly striped. From this it appears that some factor which was lacking in one parent must have been present in the other parent, in order that all the F_1 plants should be green. Otherwise it would be expected that in such a cross one-fourth of the F_1 plants would not be fully green, owing to the fact that the cross was between two heterozygous plants each of which, when

self-pollinated, yielded a progeny about one-fourth of which was non-green.

TABLE I.

The result of self-pollinating and also of crossing two types of heterozygous green plants.

Pedigree Number	Progeny of selfed parent plants				F_1 progeny when same plants were crossed	
	Green	Striped	Yellowish-white	Pure white	Green	Striped
3008 (33)	28	—	10	—	106	1[1]
3004 (10)	8	—	—	3		
3008 (3)	32	1	10	—	101	—
3004 (18)	8	—	—	6		
3008 (6)	48	—	14	—	58	—
3004 (18)	8	—	—	6		
3008 (19)	36	—	10	—	97	—
3005 (3)	47	—	—	16		

As a working hypothesis it was assumed that the presence of two factors is responsible for the processes which finally result in normal green colour. For convenience, these factors will be designated by "*A*" and "*B*," respectively. Then in the category in which pure white plants occur let a heterozygous green individual be represented by the zygotic formula *AABb*. When a plant of this nature forms its germ cells they would be of two kinds, viz., *AB* and *Ab*. Upon self-pollination the next generation would consist, on an average, of plants in the following ratio: one green plant having the formula *AABB*, and which in later generations would breed true green; two green plants having the formula *AABb*, which in the next generation would segregate into three green plants to one white one; and one white plant having the formula *AAbb*, the white plant soon dying. Here, in the absence of the factor "*B*," the plant is pure white, the factor "*A*" being unable to bring about the formation of any colouring material whatever.

In the category in which yellowish-white plants occur let the zygotic formula *AaBB* represent a heterozygous green plant. If this plant be self-pollinated, the next generation would consist of approximately three green plants to one of the yellowish-white type. One-third of the green plants would have the formula *AABB* and consequently would breed true, while two-thirds of the green individuals would have the formula *AaBB* and in the next generation would segregate in the regular manner. The yellowish-white plant would be represented by *aaBB*.

[1] The striped individual had only a faint white stripe in one of its leaves. Otherwise it was fully green.

It seems that the factor "*B*" differs from the factor "*A*" in that it alone can bring about the changes necessary for the production of a distinctly yellowish colour which later becomes slightly greenish, and in some instances develops into full green.

Continuing the hypothesis that the gametes arising from heterozygous green plants of the category in which pure white plants occur may be represented by *AB* and *Ab*, and the gametes arising from heterozygous green plants of the category in which yellowish-white plants occur may be represented by *AB* and *aB*, then by crossing the two heterozygous green plants one would expect to obtain in F_1 four sorts of green plants in approximately equal numbers. The formulae of the resulting F_1 plants would be:

AABB,
AABb,
AaBB,
AaBb.

By growing the second generation it can be shown experimentally whether or not the first generation plants are of the four sorts enumerated in the foregoing. According to the hypothesis one of every four F_1 plants has the formula *AABB*, and in succeeding generations should breed true green. One of every four has the formula *AABb*, and, this being identical with one parent, it should yield a progeny of green plants and pure white ones in the ratio of three green to one white. One of every four has the formula *AaBB*, and as this one is identical with the other parent the succeeding generation should consist of green plants and yellowish-white ones in the three-to-one ratio. One plant of every four has the formula *AaBb*, and since this one is heterozygous for both factors it would be expected to give a second generation consisting of green plants, yellowish-white plants, and pure white plants in the dihybrid ratio of 9 : 3 : 4. In Table II are shown the hypothetical formulae of the plants in the respective classes predicted in F_2, together with the expected behaviour in the third generation.

In order to make the experimental test, self-pollinated ears were secured from eleven F_1 plants which were grown in the greenhouse. The second generation results are shown in Table III.

It will be noted that the actual experimental results are very closely in accord with the theoretical expectation from the two-factor hypothesis. That the F_1 plants were of four distinct sorts, as regards the factors concerned in formation of leaf colour, is shown by the respective F_2

TABLE II.

The zygotic formulae and colour of plants in the progeny of F_1 plants heterozygous for both factors, together with the expected behaviour in F_3.

F_2 plants			
Number	Zygotic formulae	Colour	Expected behaviour in F_3
1	*AABB*	Green	Should breed true
2	*AABb*	Green	Should throw 3 green, 1 pure white
2	*AaBB*	Green	Should throw 3 green, 1 yellowish-white
4	*AaBb*	Green	Should throw 9 green, 3 yellowish-white, 4 pure white
1	*AAbb*	Pure white	F_2 plants would die in seeding stage
2	*Aabb*	Pure white	F_2 plants would die in seedling stage
1	*aaBB*	Yellowish-white	Survivors (by becoming green) should breed true
2	*aaBb*	Yellowish-white	Survivors (by becoming green) should breed true
1	*aabb*	Pure white	F_2 plants would die in seedling stage

TABLE III.

Results obtained in F_2 of the cross between heterozygous green plants of the category in which pure white plants occur and heterozygous green plants of the category in which yellowish-white plants occur. Hypothetical formulae of the respective F_1 plants shown at the left.

Hypothetical formulae of the respective F_1 plants	Pedigree Number	Number of Plants			
		Green	Yellowish-white	Pure white	Striped[1]
AABB	3402	69	—	—	
,,	3405	6	—	—	—
	Totals	75	—	—	—
AABb	3401	65	—	18	2
,,	3403	28	—	11	—
,,	3411	16	—	5	—
	Totals	109	—	34	2
AaBB	3406	68	26	—	2
,,	3407	31	8	—	—
,,	3410	63	22	—	—
	Totals	162	56	—	2
AaBb	3400	25	5	10	—
,,	3408	52	17	23	—
,,	3409	55	14	23	—
	Totals	132	36	56	—

[1] Previous tests have shown that similarly occurring striped plants were merely heterozygous green plants, and no doubt the striped plants listed in this column should be included with the green plants.

families. Two of these progenies[1] consisted only of green plants; three consisted of green plants and pure white ones in the ratio 3:2:1; three were composed of green plants and yellowish-white ones in very nearly the three-to-one ratio; and three progenies yielded green plants, yellowish-white ones, and pure white ones in a proportion closely approximating the 9:3:4 ratio. Theoretically the individuals of the four classes of F_1 plants should occur in equal numbers. Of the eleven self-pollinated F_1 ears tested, three classes were represented by three each and one class by two. This result is as near the theoretical expectation as would be possible in a test of eleven ears.

Thus from the study of crosses in the foregoing description, between plants of a family in which pure white seedlings occur and those of a progeny where yellowish-white ones are found, it appears that the production of normal green may be due to the presence of at least two factors: in the absence of one the plant is pure white, while if the other be absent the plant is yellowish-white, often becoming greenish and occasionally, after a time, fully green.

Further tests have been made with the yellow-green plants (Pl. VIII. fig. 7) described by Emerson (10). In this category the seedlings have the normal green colour at first, but later the leaves turn a distinct yellowish colour and because of the striking resemblance to the "golden" varieties of some of the horticultural plants the term "golden" will hereafter be used in describing this type.

In the summer of 1912 the F_2 of a cross between a green plant and a golden one yielded 40 green plants and 11 golden ones. Two of the green plants were crossed with pure golden plants and in one of these crosses the green plant was heterozygous, as shown by the fact that the progeny resulting from the cross consisted of 50 per cent. green plants and 50 per cent. golden ones.

None of the F_1 plants showed any of the golden colour, thus indicating that green is completely dominant. Eight of the F_1 plants were self-pollinated. It would be expected that seed from each of the F_1 ears would yield a progeny consisting of 75 per cent. green plants and 25 per cent. golden plants, but the progeny from one ear was composed entirely of green plants. Many seedlings of this progeny were destroyed by mice and only 12 plants grew to the age of eight weeks, or the time when the golden individuals can usually be identified. Since only one out of four plants should be of the golden type, it is

[1] One of these progenies (No. 3405) consisted of too few individuals to show conclusively that it was of the pure green type, yet it is probable that such was the case.

barely possible that there might be 12 green plants without the occurrence of any golden ones. But there is also the possibility that the single grain which gave rise to the F_1 plant was accidentally either self-pollinated (the cross was green ♀ × golden ♂) or crossed by a grain of pollen from a green plant. Seed from seven of the F_1 ears gave 230 green plants and 67 golden plants. This is a ratio of 3·4 : 1 instead of the expected 3 : 1 ratio, but since nearly all of the plants were grown in the greenhouse under rather crowded conditions not all of the golden individuals may have been identified.

A cross of an F_1 plant with one of the golden plants yielded some interesting results. The resulting ear had 195 grains which lacked aleurone colour, and 36 grains which had black aleurone. When seed lacking aleurone colour was planted there resulted 15 green plants and 15 golden ones, just as is expected when an F_1 plant is crossed with the recessive parent, but the seed having black aleurone gave eight plants, all of which were green. It would be interesting to note whether or not there is any coupling between the factors concerned in the development of green in the leaves and the various factors involved in the formation of aleurone colour.

Several of the golden plants were self-pollinated, but only three small ears resulted as this type of plant rarely produces ears. The three ears produced progenies of 4, 20, and 21 plants, respectively, and all 45 plants were of the golden type. This, together with the various other tests herein reported, indicates rather clearly that a simple Mendelian recessive is being dealt with.

The cross between ordinary green plants and striped plants of the race known as *Zea japonica* (Pl. VIII, figs. 8*a*, 8*b*) is affording interesting study. The first generation is green, and when self-pollinated yields a second generation consisting of both green plants and striped[1] plants similar to the japonica parent. The results thus far obtained indicate that the percentage of striped segregates in a progeny depends, to some extent at least, upon whether or not aleurone colour is developed in the grains planted. In the original cross part of the grains lacked aleurone colour, and part of them had dark aleurone. These were separated and F_1 plants were grown from each lot.

In the second generation the family which developed no aleurone colour in the grains consisted of 94 green plants and 30 striped ones,

[1] At first the striped segregates, as well as the individuals of *Zea japonica*, cannot be distinguished from green plants, but about five to eight weeks after planting the new leaves begin to show the prominent longitudinal stripes.

approximating closely the monohybrid ratio. Fifteen of the green plants and five striped ones were self-pollinated. Ordinarily one would expect the striped segregates to breed true, and of the green plants one-third should breed true green, while two-thirds should segregate into green plants and striped plants in the ratio three green : one striped. Of the fifteen progenies grown from self-pollinated ears of the green F_2 plants five consisted only of green plants; nine consisted of both green plants and striped plants, the total numbers being 262 green and 86 striped; and one progeny consisted wholly of striped plants. The fact that one progeny consisted of striped plants can easily be accounted for, because in the original cross one of the parents was a very dark-purple plant and in the F_2 there were a number of purple plants appearing. It was noted during the summer of 1912 that it was often difficult to distinguish the striping on some of the purple plants, and in occasional cases it might have been overlooked. The records show that the individual which gave a progeny of striped plants was a dark-purple plant, and consequently it may have been faintly striped, but the fact was overlooked when notes on plant colour were taken. Of the five progenies grown from the five self-pollinated striped segregates four bred true to the striped condition, there being 288 plants in all four families. But one progeny consisted of 34 striped plants and eight green ones. This condition appears somewhat similar to the results obtained by Baur(4) and Correns(6) in crosses between variegated and green plants, for they reported that not all of the variegated segregates bred true. Emerson(11) reports results of a comparable nature in the case of variegation in pericarp colour of maize grains. As regards plant colour, there is the possibility that the green individuals were the result of accidental cross-pollination of the variegated plants with green plants.

The second-generation family descending from those grains which in the original cross had dark aleurone consisted of 87 green plants and 10 striped ones. Thirteen green plants and five striped ones were self-pollinated. The results from these pollinations are shown in Table IV.

It is seen that too large a percentage of the green plants bred true green for this to be interpreted as a simple case of inheritance. Also, only one of the striped plants bred true to the striped condition, and the fact that four of the striped plants threw some green individuals indicates that the green plants probably were not due to accidental cross-pollination. The peculiar behaviour in this case is not understood

at present, but careful study should be made to determine whether or not some of the factors for aleurone colour may in some way be related to the factor (or factors) concerned in the development of the variegated leaves, for in the instances in which aleurone colour was not involved the variegated condition of leaves appeared to be a simple Mendelian recessive.

TABLE IV.

Colour of F_2 plants and the aleurone colour of grains borne on the self-pollinated F_2 plants, together with results obtained in F_3 of the cross between Zea japonica *and ordinary green maize.*

Colour of F_2 plants	Colour of aleurone in grains of ear of F_2 plants	Results obtained in F_3: Number of green plants	Results obtained in F_3: Number of striped plants
Green	Lacked aleurone colour	30	—
,,	,, ,, ,,	36	—
,,	,, ,, ,,	39	—
,,	,, ,, ,,	39	—
,,	,, ,, ,,	26	11
,,	,, ,, ,,	51	12
,,	,, ,, ,,	25	7
,,	,, ,, ,,	21	6
,,	,, ,, ,,	—	31[1]
,,	3 black grains to 1 non-coloured one	27	
,,		30	6
,,	9 black grains to 7 non-coloured ones	45	16
,,		57	2
,,	Black aleurone colour	35	—
,,	,, ,, ,,	31	—
Striped	Lacked aleurone colour	—	63
,,	,, ,, ,,	13	57
,,	,, ,, ,,	5	32
,,	,, ,, ,,	16	24
,,	9 black grains to 7 non-coloured ones	12	23
,,		27	22

A study has been made of the behaviour of the peculiar green-striped plants (Pl. VIII, fig. 9) described by Emerson (10). When crossed with green plants the F_1 plants are fully green, and in the F_2 there occurs the regular segregation. In a total of 466 plants of nine progenies there were 357 green plants and 109 which had

[1] The parent which yielded this progeny of striped plants was also one of the purplish plants, and if any stripes were present they were so very faint that they were overlooked when notes were taken.

the characteristic green striping. This approximates very closely the 3 : 1 ratio and indicates that a simple Mendelian recessive is being dealt with.

Crosses between plants of the different categories were made as follows:

Zea japonica × golden.

Green-striped × golden.

Green-striped × yellowish-white, later turning green.

In these cases neither parent in any of the crosses was fully green, yet all the F_1 plants resulting from each cross were of normal green colour. The F_2 generations were grown from the respective crosses, and notes were taken concerning the seedlings until five weeks after date of planting. Since the japonica-striped plants, the green-striped ones, and the golden ones cannot be identified with extreme accuracy until they are from eight to ten weeks old, it was unfortunate that no notes could be taken at a later date. But the absence of the writer from the State during the remainder of the growing season prevented such notes being taken.

At the date when the last notes were made, however, one could distinguish individuals of the various categories. In the F_2 of the cross between plants of *Zea japonica* and those of the golden type there were 102 green plants, five of the japonica type, 37 golden ones, and four golden plants striped after the manner of *Zea japonica*. The second generation of the cross between green-striped plants and golden ones consisted of 63 green plants, 14 green-striped ones, 22 of the golden type, and four golden plants which had the peculiar striped pattern of the green-striped category. In F_2 of the cross between green-striped and yellowish-white plants there were 146 green plants, 25 green-striped, and 42 yellowish-white. In these cases there were too few individuals in the various F_2 families for one to place much dependence in the ratios, yet it seems probable that they are merely modifications of the dihybrid ratio. A further study of these crosses must be made, in order to fully understand the relation between the various types.

An Anatomical Study of the Leaves of Certain Types of Maize.

At the suggestion of Professor Emerson the writer undertook a histological study of the leaves of some of the various types of albescent maize. It was soon found that a more satisfactory study could be made

if the specimens were first killed and then prepared according to the paraffin method.

Experiments were made with several different killing reagents, but the most satisfactory method was to allow the leaf sections to remain for about two and one-half hours in a modification of Carnoy's killing fluid composed of absolute alcohol 50 per cent., chloroform 25 per cent., and glacial acetic acid 25 per cent. After killing, the specimens were washed in absolute alcohol, cleared in xylol, infiltrated and embedded in paraffin.

Cross sections of the leaves were made of from three to fifteen microns in thickness, but those sections which were either ten or twelve microns in thickness proved the best for a study of the plastids. Of the stains employed Lichtgrün appeared to be most satisfactory, yet some good results were obtained from Delafield's haematoxylin, and also from acid fuchsin. Camera lucida drawings were made from the prepared slides by Miss Lucille Goodloe, Washington, D.C., to whom the writer desires to express his thanks at this time.

Examination of leaves from the pure white plants showed that the plastids apparently were almost, if not entirely, lacking (Fig. 1). Even leucoplasts could not be differentiated with any of the stains which were used. This is an extreme condition, yet the conclusion that no

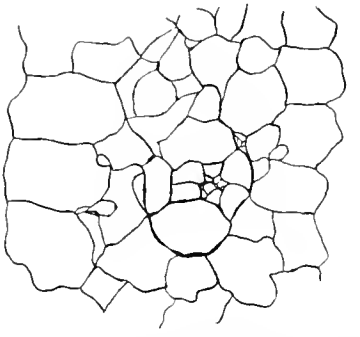

Fig. 1. Cross section through a leaf of a pure white seedling, such as is illustrated in Pl. VIII, fig. 1. No plastids could be differentiated. ×360.

plastids are present is substantiated by the fact that these pure white plants have never been known to turn green, or even greenish, in colour, but always die as soon as the young seedlings have exhausted the food stored in the kernel planted.

The condition found, however, in the leaves of the yellowish-white

plants is somewhat different. Preparations were made of leaves of different ages. Fig. 2 shows the cross section of a leaf of a very

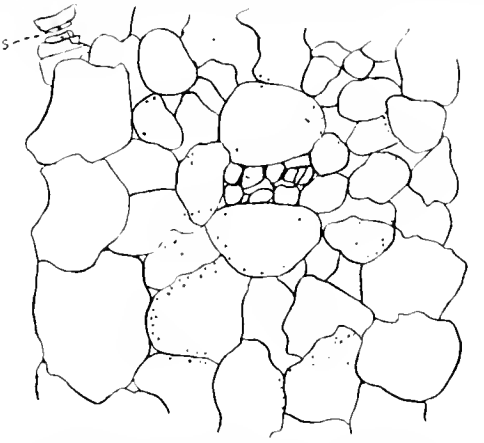

Fig. 2. Cross section through a leaf of a young yellowish-white seedling at the stage illustrated in Pl. VIII, fig. 3. The plastids are small and are comparatively few in number. They were differentiated in the same manner as the plastids of the guard cells of the stoma shown at "S." ×380.

young seedling such as that shown in Pl. VIII, fig. 3. Even though the leaf was only yellowish, there was a differentiation of small granular bodies resembling diminutive plastids. It will be noted that these small bodies react to the stain in the same manner as do the plastids shown in the guard cells of the stoma. The material illustrated in Fig. 3 was taken from an older plant which was turning greenish.

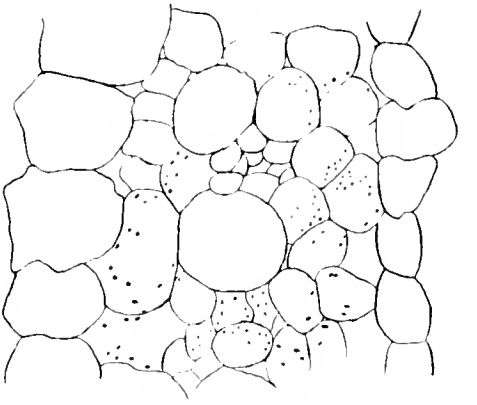

Fig. 3. Cross section through a leaf of an older yellowish-white plant, such as illustrated in Pl. VIII, fig. 4*a*. The plastids have increased in size and number. ×360.

Here it is seen that the granular bodies in the cells have increased both in size and in number. In some cases, perhaps under especially favourable conditions, the plants became almost, if not fully, green (see Pl. VIII, fig. 6). Preparations from the leaves at this time appear as shown in Fig. 4. Here apparently normal plastids are present, and they resemble very closely those found in ordinary green corn leaves (Fig. 5). Thus in the case of the yellowish-white plants which may

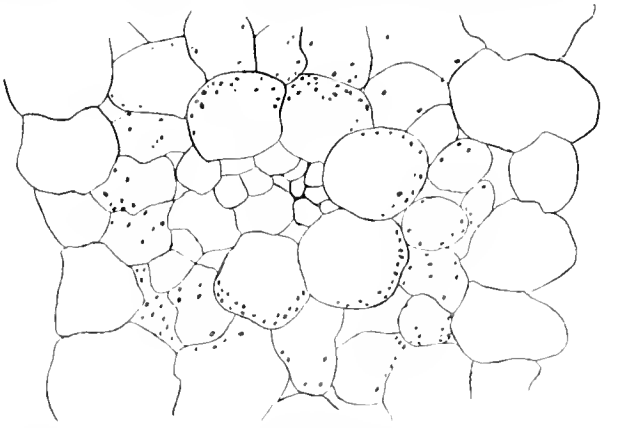

Fig. 4. Cross section through a leaf of a still older yellowish-white plant which is becoming greenish. Leaf from which the section was taken was almost green. × 410.

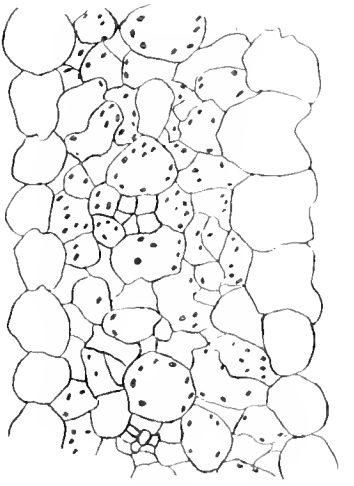

Fig. 5. Cross section through a leaf of a normal green plant. × 380.

turn greenish or green the studies indicate that the gradual development from small and comparatively few granular bodies into apparently normal plastids is synchronous with the changing of the plants from the yellowish-white condition of the young seedlings to the green condition of the older plants.

This condition differs from that in plants which are yellowish-white because of being grown in darkness. Seed which was known to produce only normal green plants was planted in a flat and was germinated in the dark. The young seedlings were yellowish in colour (no green showing), and preparations of the leaves showed that plastids were in abundance (Fig. 6).

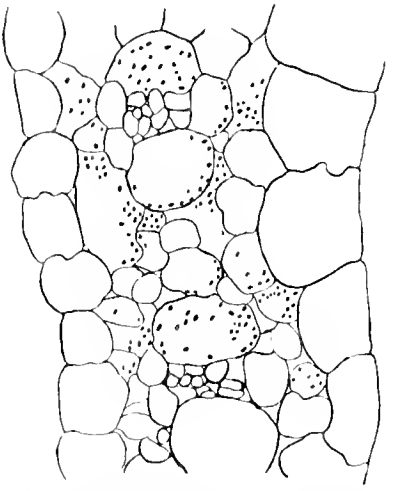

Fig. 6. Cross section through a leaf of a seedling made yellowish by being germinated and grown in darkness. ×380.

The leaves of the race of maize known as *Zea japonica* have stripes of dark green, pale green, and white (Pl. VIII, fig. 8*b*). In the dark-green area of these leaves chloroplasts are present in approximately the same number, and are about the same size as in ordinary green leaves (Fig. 7), while in the white portion there are very few, if any, plastids. There were noted, however, a very few small bodies which may have been leucoplasts. The pale-green stripe afforded an interesting study. Fig. 8 shows the position of the plastids which are present. They are seen to be in only those cells next to the lower epidermis. Since this is the case, the intensity of the green colour is diminished as the

light passes through the several layers which are void of plastids. It was noted that the stripe which appeared a pale green on the upper surface of the leaf was of a more intense green colour on the

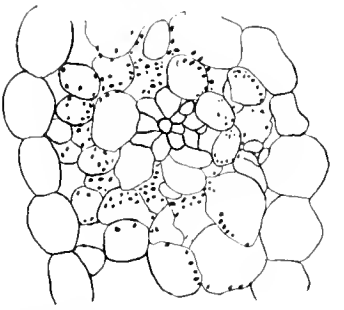

Fig. 7. Cross section through a green portion of a leaf of the striped race *Zea japonica*. Plastids are distributed through the different cell layers. ×350.

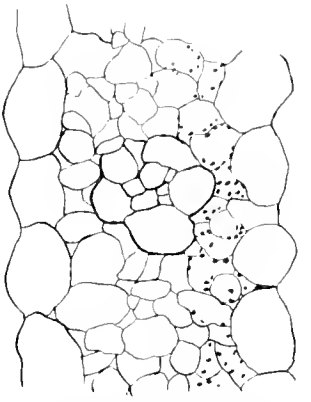

Fig. 8. Cross section through a pale green stripe of a leaf of the striped race *Zea japonica*. Apparently normal plastids are found in the cells near the lower epidermis, and light passing through the colourless layers above causes a reduction of the intensity of the green colour. ×300.

lower surface. This would be expected because if the leaf be viewed from the lower surface the cells containing chloroplasts would be immediately underneath the epidermis, and consequently the green would show more vividly than if the light had to pass through several layers of cells.

Fig. 9 shows a portion of a white stripe and also a portion of a green stripe in a variegated leaf of maize. It is not at all difficult to note the boundary between the white and the green portions, for well-differentiated plastids are present in the green portion and are absent in the white portion.

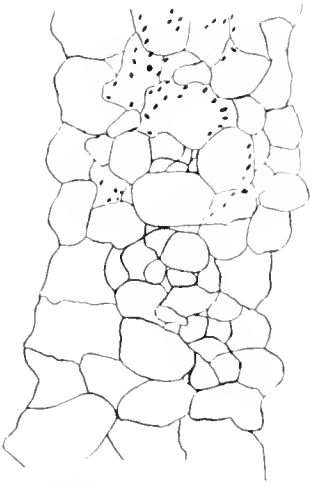

Fig. 9. Cross section through a striped green and white leaf. The boundary between the green and the white stripes is distinct, as is shown by the absence of plastids in the white portion. × 380.

Summary.

From the studies of the various categories it appears that in all cases, with the possible exception of the striped leaves in *Zea japonica*, the several degrees of albinism in corn leaves behave as simple Mendelian recessives: the first generation of a cross with ordinary green races giving fully green plants, and the second generation segregating in the ratio of three green plants to one plant of the particular type which was used in the cross.

The study of the manner of inheritance of variegated leaves of *Zea japonica* in crosses where aleurone colour is involved has not been completed.

A rather definite relation has been pointed out between a pure white type of maize plant and a yellowish-white type, the results indicating that the presence of at least two factors is necessary for the development of normal green in the leaves of maize. In the

absence of one of these factors the plant is pure white and soon dies, while in the absence of the other factor the plant at first is yellowish-white, but is capable of developing into a greenish condition and sometimes into a pure green plant.

Studies of the relation between the other categories have not been completed. Crosses of striped plants of the japonica type with golden plants, and those of the green-striped plants with golden plants, and also the crosses of green-striped plants with yellowish-white individuals which turn green have all resulted in first generation plants which were of the normal green colour. Although it was impossible to note the second generation plants, except during the first five weeks of their growth, it was possible at that time to identify segregates of the respective categories. The results secured in these crosses, however, add further evidence to the hypothesis that more than one factor is concerned in the production of normal green colour in the leaves of maize. Apparently there is lacking in each parent some genetic factor (or factors perhaps) which is concerned in the development of chlorophyll, and, since the F_1 plants are normal green, it appears as if that factor which is lacking in one parent may be present in the other.

In the pure white plants no plastids could be differentiated. In the yellowish-white plants which later may become green plastids apparently are present from the first, although they are few in number and are very small, gradually increasing in number and size as the leaf turns green.

In *Zea japonica* the manner of distribution of plastids may be compared with the condition which Trelease (14) has described in certain variegated Agaves. He found that the normal green condition was due to the presence of plastids in the subepidermal region of the leaf. In variegated leaves, if the stripe was pale greenish, there was found to be a suppression of plastids through several of the subepidermal cells, while in a pure white stripe there was "all but complete suppression of recognizable plastids."

EXPLANATION OF PLATE.

PLATE VIII.

Fig. 1. Pure white seedling.
Fig. 2. Ordinary green seedling.
Fig. 3. Yellowish-white seedling 12 days after planting.
Figs. 4*a* and 4*b*. Yellowish-white seedlings 15 days after planting.

Fig. 5. Yellowish-white seedling which is turning greenish; 20 days after planting.

Fig. 6. Yellowish-white seedling which has become nearly green; 29 days after planting.

Fig. 7. Golden plant. As the plants grow older they become even more golden in colour.

Fig. 8*a*. Striped plant of *Zea japonica*.

Fig. 8*b*. Section of a leaf of *Zea japonica*.

Note the stripes of white, yellowish, pale green and dark green. Figs. 7 and 8 in the text show the distribution of plastids in the dark-green and in the pale-green stripes, "*g*" and "*p*," respectively.

Fig. 9. Section of a leaf of a green-striped plant.

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1

2

4*a*

4*b*

5

7

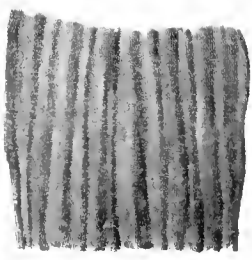
6

8a

8b

g p

3



9

STUDIES IN THE PHYSIOLOGY OF FERTILIZATION.

PART I. ON THE CONDITIONS OF SELF-FERTILIZATION IN *CIONA*.

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CONTENTS.

I. Introduction.

The main problems presented by the fact of self-sterility—that is to say, the difficulty or impossibility of fertilizing the female gametes of certain hermaphrodite animals and plants with male gametes derived from the same individuals—can be divided under five heads. First, the determination of the degree of self-sterility shown by the individuals

of a given race; second, what factors influence the degree of self-sterility; third, the causes of the self-sterility; fourth, the inheritance of self-sterility; and fifth, the effect of self-fertilization on the offspring. The present investigation was commenced in the belief that *Ciona intestinalis* is almost, if not completely, self-sterile, in which case the first, second, and fifth of the problems just enumerated would be non-existent. It was proposed to investigate the inheritance of the self-sterility on the lines of the extremely interesting work by Correns (4) on *Cardamine pratensis*. That is to say, separate cross-fertilized families would be raised to maturity, and then the members of a family be crossed *inter se*, with individuals of the family derived from the reciprocal cross and with unrelated individuals.

Castle (1) originally demonstrated the self-sterility of *Ciona intestinalis* when making fertilizations to obtain stages in the development of the embryo. The animals used were from Newport. A number of specimens were isolated in separate dishes, where they discharged eggs and sperm simultaneously nearly every day. In the majority of cases none of these eggs were found to have been fertilized, although in some as many as 90 % segmented. In contrast with this, when several animals were placed together in an aquarium, usually 100 % of eggs which were discharged segmented. Repeating the observations with eggs and sperm artificially removed from the genital ducts, he found that self-fertilization usually caused few or no eggs to segment, although in one instance 50 % were self-fertilized. Similar observations were made by Gutherz in 1903 (7*a*).

During the course of an extensive investigation on the phenomenon of self-sterility in *Ciona*, Morgan never found such high percentages of eggs self-fertilized as Castle had done. The first part of Morgan's work was done at Woods Hole in 1903, when he states (9, p. 138) with regard to artificial fertilizations, that "I have rarely seen more than from one to ten per cent. of self-fertilized eggs segment, and in the greater number of cases not a single egg segmented." The second part of the work was done at Coronado Beach, California, in 1905. Here, in making fertilizations with eggs and sperm removed artificially from the bodies of the animals, "in only one case out of many hundreds of eggs mixed with their own sperm did fertilization occur." The final part of the investigation was carried out at Woods Hole in 1909 (11), and this time cases of self-fertilization were extremely rare.

Castle himself suggested that when high self-fertilization percentages were obtained, the result may really have been due to the

cross-fertilization of some of the eggs by spermatozoa accidentally present in the water. Morgan (9) thinks this probable, since he never got such high percentages as Castle. Nevertheless, in two cases Castle obtained 90 % of eggs self-fertilized after the animals had been isolated for three days, and it is very unlikely that spermatozoa of *Ciona* can retain their power of fertilizing after having been for such an extended period in sea-water, even if they are, as Morgan suggests, entangled in the branchial basket of the animal.

Apart from experiments to be described below, in which fertilizations were made with spermatozoa that had been in sea-water for varying intervals, and all of which showed a more or less rapid deterioration of the sperm, the following preliminary tests showed me that the activity of the spermatozoa does not last for very long after they have left the vas deferens.

(1) Unfertilized eggs were removed from four animals at 7 p.m. At the same time, sperm was removed from the sperm-ducts of the same individuals and a mixed suspension made up. At 11.20 a.m. on the following morning, some of each of the lots of eggs were fertilized with sperm just removed from another individual, and in all four cases 100 % of the eggs segmented. Other samples of these eggs, however, when inseminated at the same time with the sperm-suspension made on the preceding evening, showed only one irregularly segmenting egg between them. Evidently the life of unfertilized eggs is much longer than that of spermatozoa in sea-water. (2) Eggs were removed from an individual into sea-water at 8 p.m. Fertilized on the following evening at 9.50 p.m., with fresh sperm, 100 % segmented, although irregularly. (3) A comparatively dilute suspension was made of the sperm of one individual at 8 p.m. At 9.50 p.m. on the following evening, this failed to fertilize a single egg of a sample just removed from another animal.

It appears, therefore, that the higher self-fertilization percentages obtained by Castle than by Morgan were probably due to other causes than the presence of spermatozoa of other individuals, since the animals had been isolated for some days.

Preliminary experiments (in which rigid precautions were taken as described in the following section to exclude the presence of "foreign" spermatozoa) soon convinced me that *Ciona intestinalis* at Naples was far from being completely self-sterile, and that the degree of self-sterility varied greatly. In many cases no eggs segmented after insemination with sperm of the same individual, in others comparatively high

percentages of fertilized eggs were obtained. Potts (13, p. 481) has already observed that the *Ciona* at Naples is not completely self-sterile. He obtained "nearly 100 % of embryos" from self-fertilized eggs—an occurrence which was comparatively rare in my experiments, but which would be accounted for if Potts used very concentrated sperm suspensions. Evidently the race of *Ciona* at Woods Hole behaves differently in respect to its capacity for self-fertilization from that at Naples[1].

The first preliminary experiments were made on the lines adopted by Castle. Animals were isolated for a number of days in bowls of filtered water and the proportion of self-fertilized eggs in each lot was counted. Although this was a fairly extensive series lasting over a month, subsequent experience showed that it was valueless except in demonstrating that self-fertilization can occur. The different lots of eggs deposited by a given animal on successive days showed very varying amounts of self-fertilization, a circumstance which undoubtedly depended largely on the different amounts of sperm ejected with the eggs. Further, the varying self-fertilization percentages suggested, but by no means proved, that the eggs of one individual vary from day to day in their capability of being self-fertilized. Subsequent experiments proved this surmise to be true. (See pp. 236 *et seq.*)

The fact that *Ciona* at Naples is far from being quite self-sterile and that the extent of the self-sterility varies in different individuals and in the same individual with successive lots of eggs and sperm produced, appeared at first sight to render the investigation of the inheritance of the self-sterility difficult or impossible. Nevertheless self-fertilization proved always to be very much more difficult to bring about than cross-fertilization. In general a comparatively dilute sperm suspension, but one which was concentrated enough to fertilize 100 % of eggs of another individual, would only rarely fertilize even one to two per cent. of the eggs derived from the same animal as itself. To bring about self-fertilization much more concentrated sperm suspensions were necessary, and even then it by no means always followed. Taking this fact into consideration, it is plain that after the limits and conditions of the occurrence of self-fertilization have been determined, the problem of the inheritance of the *comparative* self-sterility is still a feasible one for investigation.

[1] Whether the form of *Ciona intestinalis* at Woods Hole is really a distinct variety (var. *tenella*) or not seems to be uncertain, especially as the distinguishing characters of var. *tenella* are not very definite (8, 12).

Experiments are now in progress which are designed to give an answer to the question of inheritance; but the object of the work described in Part I of this paper was to investigate thoroughly the conditions under which self-fertilization occurs[1]. Besides this, the present work deals with the effects of self-fertilization on the offspring. The cause or reason of self-sterility is quite another problem and one which is hardly touched upon here, although Section IV deals with a phenomenon which may shed considerable light on the question.

Starting with a race of *Ciona* which was almost completely self-sterile, the main object of Morgan's work was to investigate the cause of this phenomenon and to bring about self-fertilization by artificial means. Besides this he carried out an extensive series of cross-fertilizations to discover whether eggs of one individual can be fertilized equally well by the sperm of all other individuals, provided that it is in good condition. He concluded that this does not take place. A critique of Morgan's methods is given in the following section, showing that the way in which the experiments were carried out does not justify the conclusions drawn from them. It is not denied that there may be different degrees of sterility exhibited in cross-fertilizations, but Morgan's experiments do not at all prove the point. The only undoubted result which Morgan seems to have established with regard to the self-sterility is that self-fertilization can be induced to a certain degree by treatment of the sperm (and possibly also of the eggs) with solutions of ether and alcohol. It is difficult to see, however, what light this sheds on the cause of the self-sterility.

The preliminary experiments of the present investigation brought to light some interesting facts with regard to the manner of egg and sperm deposition. The isolated animals lay some hundred of eggs almost every day for a week or so, after which the frequency of the depositions and the number of eggs laid decreases. Sperm is almost always deposited simultaneously with the eggs, only rarely the one being shed without the other. The manner in which the genital products are ejected is as follows. A small mass of eggs exudes from the aperture of the oviduct into the atrium, and at the same time a small mass of sperm issues from the vas deferens. After about half

[1] In connection with the phenomenon of self-sterility in *Ciona*, the following record of a hermaphrodite Echinoid is of interest. In March 1913 a specimen of *Strongylocentrotus lividus* was kindly given to me by Dr Meyerhof, in which three of the gonads were ovaries and in the remaining two the upper third was testis and the lower two-thirds ovary. Both eggs and sperm were ripe and the specimen proved to be perfectly self-fertile.

a minute, when this mass of eggs and sperm lying at the apertures of the genital ducts has reached a certain size, it is suddenly expelled into the water through the exhalent siphon by a violent contraction of the muscles in the body wall. The process is then repeated. The whole period of egg and sperm deposition usually lasts from five to ten minutes.

Castle (1) first observed that *Ciona intestinalis* deposits its genital products at a definite time of day. His animals always discharged their eggs and sperm an hour or an hour and a half before sunrise. Morgan (9) states that at Woods Hole "the eggs are laid in the early morning, at dawn." Seeliger (14), however, remarks that at Trieste there is no such regularity in the time of deposition. With the *Ciona* at Naples, 131 depositions of genital products were observed by me in a number of isolated animals during the months of December and January. 105 of these took place in the late afternoon after 5 o'clock, that is to say after dusk had set in. Six depositions were in the night (as judged by the state of segmentation of the eggs on the following morning), and 20 in the early morning, about sunrise. Almost all the early morning depositions were by individuals which had been laying regularly for a number of days in the late afternoons, which suggested that morning laying was an effect of the laboratory conditions. As this was not an invariable rule, it is hardly of value to publish the exact records. The only conclusion to be drawn is that in the great majority of cases eggs and sperm were discharged in the late afternoons, and that in no cases did the depositions occur during the period of bright daylight. It is hoped to decide whether the stimulus to discharge the genital products may be given by a change from light to darkness, or from darkness to light, by further laboratory experiments, which will also deal with the mechanism of ejection.

The present investigation was carried out at Naples between December 1912 and July 1913, while I was occupying the Cambridge University Table. I would like to take the opportunity of tendering my very best thanks to the Staff of the Zoological Station, both for the valuable advice and help afforded me throughout the course of the work, and for the trouble taken to procure an abundant supply of material, which was brought in fresh from the sea almost every day. My thanks are also due to Mr R. H. Compton of Gonville and Caius College, Cambridge, who originally suggested to me an investigation of the problem of self-sterility.

II. Methods. Importance of Exactness.

Before detailing the technique employed, the essential nature of the method of experimentation should be explained. Shortly put, it was as follows. Approximately equal numbers of eggs from a single animal were placed in two or more dishes, each of which contained exactly the same quantity of sea-water. Fertilization was then effected by the addition to each dish of exactly the same amount of sperm-suspension from a single animal. The object was to discover the effect on the eggs of different treatments of those in the different dishes, or the effects on the spermatozoa of varying preliminary treatments of the different samples to be added to the dishes. If this preliminary handling consisted in adding a certain solution to the quantity of sperm-suspension to be used for fertilizing the eggs in one of the dishes, the consequent dilution of the suspension was compensated for by the addition to the other lots of sperm of equal quantities of plain sea-water. The criterion of the effects on the spermatozoa of this preliminary handling consisted in a comparison of the percentages of eggs fertilized when the differently treated but equal quantities of sperm-suspension were added to the dishes containing the eggs.

From this outline a number of absolutely necessary precautions can be deduced:

(1) If the experiment consists in the comparison of the effects of a different treatment either of the eggs or of the spermatozoa, the eggs in the different dishes must all be derived from one individual, or no legitimate comparison can be made. Similarly, the sperm used to effect the fertilizations must all be derived from one animal, and moreover the definite quantities used must all be drawn from the *same* suspension. The reason for this is that it is found to be impossible in practice to make up two sperm-suspensions of equal concentrations. The only method of judging the concentration of spermatozoa in a suspension is by the degree of milkiness, as seen with the eye. Differences in the degree of milkiness which are undetectable by the eye may mean considerable variation in the concentration of the spermatozoa (as measured by the percentages of eggs fertilized by equal quantities of the different suspensions).

(2) An experiment can be made to compare the proportions of eggs fertilized when equal numbers are taken from different individuals, and to each lot is added an exactly equal amount of one sperm-

suspension. The reciprocal experiment of fertilizing equal quantities of eggs from one animal with equal amounts of sperm-suspensions from several other individuals does not give a valid result. This again is owing to the fact that it is impossible to make up different sperm-suspensions of exactly equal concentrations.

(3) The way in which the spermatozoa are subjected to the different treatments is as follows. A suspension is made up and afterwards divided among two or more dishes, which can then be subjected to different conditions of temperature, etc., or be kept for different lengths of time before being used. If the treatment consists in the addition of some substance, equal amounts of the one suspension are tried out into several dishes, and to each dish is added an equal volume of liquid containing the substances. One dish is kept as a control of untreated spermatozoa, and to this is added a like volume of plain sea-water. In this way the concentrations of spermatozoa are kept identical in the various subdivisions of the suspension.

(4) In making the fertilizations in the different dishes of an experiment, it is essential that all the eggs in each lot should have an equal chance of being fertilized by the spermatozoa which are added. This is brought about by pouring the water containing the eggs and spermatozoa from one dish to another immediately after the sperm has been added. This pouring was usually done ten times backwards and forwards for each lot of eggs, which ensures a thorough mixing of the water in which the eggs were lying with the sperm-suspension. The definite quantities of the latter were measured out with a graduated pipette, or by a certain number of drops from a pipette of uniform bore closed at the top by the thumb.

(5) Since the experiments consisted in comparing the percentages of eggs fertilized under different conditions, it is obvious that these percentages must be above 0 and below 100. If none or if all of the eggs are fertilized in each of the dishes, no comparisons can be made. In consequence, the concentration of the sperm-suspension employed must be such that some but not all of the eggs are fertilized. Now this is a difficult matter to arrange, since the amount of dilution necessary in making up a sperm-suspension so that some only of the eggs will be fertilized when a definite quantity of the suspension is added to them must be judged by the eye. In practice this can be done with more or less success, but very often the experiment turns out a failure. More than twice as many experiments were made as are recorded below. All those not detailed were failures because the

sperm-suspension was made either so weak that 0 % or less than 1 % (< 1 %), or so concentrated that 100 % of the eggs were fertilized in each of the dishes, thus allowing of no comparisons.

This applies to fertilization in the Echinoids and to cross-fertilization[1] in *Ciona*. In almost every case, provided that none of the eggs are immature or pathological, 100 % fertilizations can be obtained if a sperm-suspension of sufficient concentration is used. With regard to self-fertilization in *Ciona* the matter is simpler, for it is very seldom that 100 % of the eggs can be fertilized by sperm from the same individual, however concentrated the suspension be. On the other hand, a comparatively large number of cases occur in which no self-fertilization at all can be obtained.

The next important point preliminary to an investigation of this nature is a determination of the degree of accuracy of the results. For this purpose an extended series of trials was made, lasting over a month. The object of these trial experiments was to discover the chief sources of error and the ways of overcoming them, together with the extent to which the numerical results were accurate.

It was found that accuracy depends on four main factors, which are as follows:

(*a*) *The mode of making up the sperm-suspension.*

This was found to be the largest source of error. If a suspension be made by taking some of the sperm from the vas deferens, mixing it with sea-water, and then stirring the liquid, the spermatozoa are by no means evenly distributed through the water. In the case of a thick suspension this is easily seen with the naked eye, but with the dilute suspensions used in the cross-fertilization experiments it is not apparent. If equal quantities of liquid are taken from such a suspension and added to equal amounts of eggs, the percentages of the latter which are fertilized are usually by no means the same: the error may even be as high as 20 %. A number of ways were tried of getting a more even distribution of the spermatozoa in the water, such as continued stirring, shaking the suspension in a tube, pouring once or twice from one dish to another, filtering—but all with no certain success. The results could not be depended upon. Eventually, however, it was found that by pouring the liquid to and fro ten times at least, a very uniform suspension could be obtained, so that equal amounts fertilized equal proportions of eggs.

[1] Throughout this paper "cross-fertilization" means the fertilization of Ascidian eggs by spermatozoa of another individual.

(*b*) *Thorough mixing of the eggs before dividing them into different lots.*

The necessity for this precaution will be obvious in the light of experiments to be described below, which show that eggs taken from different parts of the oviduct of an individual *Ciona* have very different capacities of being fertilized.

(*c*) The importance of *the way in which insemination is effected* has already been dwelt upon. A rapid and even distribution of the spermatozoa through the water containing the eggs is essential, so that all the eggs in the different dishes may have an equal chance of being fertilized. The most accurate method was found to be as follows. A certain amount of the sperm-suspension to be used was pipetted into each of a number of dishes containing equal amounts (10—20 cc.) of sea-water. The spermatozoa were thoroughly mixed with the water in each of these dishes by repeated pouring, after which the contents of the dishes were poured on to equal amounts of eggs lying in a drop or two of water in other vessels. The operation was finished by re-pouring each of the lots a number of times. This was the method adopted in most of the experiments, although sometimes the definite amounts of sperm-suspension were simply pipetted straight into dishes in which the eggs were lying in equal quantities of water. After this the liquids were, of course, poured backwards and forwards several times to ensure thorough mixing.

(*d*) *The counting of the percentages of eggs fertilized.*

An investigation of which the results depend on a comparison of the ratio between two classes under different conditions involves, of course, an accurate estimation of this ratio. In our case it is not sufficient to give a rough approximation of the proportion of fertilized to unfertilized eggs as estimated by the eye. The whole investigation is an exact one, and accuracy is as necessary in observing the results as in carrying out the technique of the experiments.

The percentage of fertilized eggs in a given sample was always calculated from counts made when the eggs were in the 4-cell stage. This stage is reached at a convenient time after fertilization, and it is impossible to mistake a segmenting for an unsegmented egg when the former has divided into four cells. A very necessary preliminary to the operation of counting is a thorough mixing of the eggs in each dish. It is frequently found that eggs lying in one part of a dish show a slightly different proportion of fertilized to unfertilized from those in

another part. The actual counting was done by taking a number of eggs up in a pipette and spreading them in a line along a glass slide, which was then passed under a low power of the microscope. *Every percentage recorded is calculated from a count of* 400—500 *eggs.* Since most of the fertilizations were made with less than 2000 eggs, the percentages obtained by counting 400—500 of these should be fairly accurate. Each percentage is reduced to the nearest whole number.

The final result of these trials, in which each experiment was made double or triple in order to test the degree of accuracy attainable, was that the extent of the experimental error in the percentages might be as much as 3 %. It will be seen, however, that in the results of the experiments, the differences between percentages to be compared with one another were almost always considerably greater than the extent of this error. There is one way, of course, in which an absolute control can be kept of any experiment. Each experiment might be duplicated—that is to say, each fertilization be made twice with the same quantities of sperm and eggs—and the results rejected if they did not agree exactly. This method was not adopted because time would not permit of it in all cases, nevertheless a similar control was kept in many of the experiments. Each of the fertilizations was made double, but different quantities of sperm were added in the two cases. By both giving similar differences in the percentages, the weaker-sperm and stronger-sperm series in an experiment confirmed one another. This method had the further advantage that there was much more probability of the experiment being a success. If in the weaker-sperm series the sperm was so dilute that no eggs were fertilized at all, probably the addition of a greater quantity to form the stronger-sperm series would give percentages lying between 0 and 100, and permit of comparison. Similarly, if it happened that the stronger-sperm gave all 100 % fertilizations, the addition of less sperm-suspension to the water with the eggs to form the weaker-sperm series might give a result lying between 0 and 100 %. If, however, both series lay between 0 and 100 %, they were a check on one another.

The above outline gives the way in which the experiments were carried out, and the precautions adopted to ensure the maximum of accuracy in the results. There is another and more obvious source of error which has to be guarded against in all experiments on fertilization. Each experiment is made with the eggs from a single individual and the spermatozoa from a single individual. The presence

of even a minute quantity of spermatozoa of any other individual completely vitiates the results. This applies to cross-fertilizations as much as to experiments on self-fertilization, but in the latter the effects of such contamination are more obvious. It is with comparative difficulty that self-fertilization can be brought about at all in *Ciona*, and when it is found that this difficulty is lessened by the changing of a certain factor, the elementary precaution of ensuring the complete absence of spermatozoa from any other individual must naturally be taken before certainty can be attained that the alteration is really due to the condition which has been changed in the experiment.

The precautions which were taken to ensure the absence of sperm-contamination can be divided under three heads:

(1) *Sterilization of instruments and water.*

All glass-ware was cleaned with hot water before being used. Similarly scissors, forceps, etc. were dipped into hot water after every operation. The sea-water used in the experiments was taken from the circulation in the laboratory, and passed through a Berkefeld filter. The complete absence of spermatozoa from such water was shown by the fact that Ascidian or Echinoid eggs never segmented when left in it without the addition of sperm.

(2) *Method of removing the eggs and sperm from the animals.*

The first source of contamination of the genital products of *Ciona* with the spermatozoa of other individuals is the possible presence in the pharynx, branchial basket and atrium of the animals of spermatozoa taken in with the water. To guard against this, the animals (which were in almost every case brought in from the sea on the day they were used) were treated as follows. The atrial cavity of each was slit up by inserting one point of a pair of scissors into the exhalent aperture. The animal was then pinned down and washed with a copious stream of fresh-water.

The second possible source of contamination is that in removing the eggs from the oviduct some sperm might accidentally be taken with them from the adjacent vas deferens. It is extremely easy to puncture the oviduct and then suck out the contained eggs with a pipette without injuring the adjacent vas deferens at all. Sperm, however, will always exude from the terminal aperture of the vas deferens during this operation. In order to prevent this, the tops of the oviduct and vas deferens were always tightly closed by means of a "bull-dog" clip before the animal was washed under the stream of fresh-water.

After the eggs had been taken from the oviduct, the latter was slit open from end to end and washed out with sea-water to remove all remaining eggs. The vas deferens was then punctured, and a quantity of sperm removed.

(3) *Controls of unfertilized eggs.*

The final check on the possible presence of "foreign" spermatozoa is kept by the unfertilized egg controls, which are a *sine qua non* to all experiments on fertilization. Out of every lot of eggs used in the experiments described below, 1000 or more were kept in a separate dish to which no sperm was added. *In no case recorded did a single egg segment in the control.*

In describing the experiments, the genital products of each animal used are denoted by a single letter, the capital type (e.g. *A*) referring to the eggs of a female and the small type (e.g. *b*) to the spermatozoa of a male. The cross made by fertilizing the eggs *A* of one individual with the sperm *b* of another is then written *A*/*b*. In the case of a hermaphrodite like *Ciona*, *A* eggs self-fertilized is written *A*/*a*, and *A* eggs cross-fertilized with *b* sperm from another individual, *A*/*b*.

The results of the experiments are tabulated, and in the Tables the numbers given always mean percentages of eggs which have been fertilized, unless otherwise stated.

In order to illustrate the most usual method of carrying out the experiments an example is given here, which has the further advantage that it shows up a possible source of error which might easily lead to incorrect conclusions if not taken into account. If sea-water is passed through a filter-paper, the first water to come through is distinctly acid. Whereas normal sea-water gave yellow with neutral red, this filtrate gave a pink colour. It will be shown later that small traces of acid are very injurious to spermatozoa, so that if such filtered water be used in the experiments the results will be quite untrustworthy. If a stream of sea-water is run through a filter-paper for five minutes, after this time the filtrate no longer shows any difference in acidity from normal sea-water when tested with the indicator. The point of the experiments to be described was to discover whether such filtered water still has any effect on spermatozoa, due to the possible presence of some other substance derived from the filter-paper.

The first experiment was made as follows. Eggs *A* were removed from one individual *Ciona* and sperm *b* from another, by the method already described. The eggs were placed in sterilized sea-water and

thoroughly mixed by pouring from one dish to another. A sperm-suspension was made and diluted down to the required amount, after which it was thoroughly mixed by pouring it 20 times from one dish to another. Two small glass dishes were then taken. Into the first (1) was placed 10 cc. of normal sea-water, and into the second (2) 10 cc. of sea-water which had come through the filter-paper. This paper had already been washed for 15 minutes in running sea-water and the water which came through after this interval gave the same reaction as normal sea-water with neutral red. To each of the dishes (1) and (2) were added three drops of the sperm-suspension *b*, so that two sperm-suspensions were obtained, (1) in normal sea-water, and (2) in filter-paper water, and each of identical concentration. The liquid in each dish was thoroughly mixed by pouring. Into each of two other small dishes were then placed approximately equal quantities of *A* eggs in three drops of water. The liquid in dish (1) was then poured on to one of these lots of eggs, and that in (2) on to the other. By this means fertilization was effected, and in order to ensure a quick and thorough mixing of the eggs and sperm, the contents of the two dishes were each poured backwards and forwards into two other dishes several times. When the 4-cell stage was attained, the percentages of eggs which had been fertilized in the two dishes were counted in the way described above.

The experiment was then repeated on exactly similar lines with another cross, *A*/*c*. The results are tabulated below.

TABLE. *To show the effect on the "fertilizing power" of a sperm-suspension of water which has passed through a washed filter-paper.* (44.26.6.)

	(1) Normal water	(2) Filter-paper water
Exp. 1, cross *A*/*b*	100	77
Exp. 2, cross *A*/*c*	95	31

The Table shows that in each of the experiments fewer eggs were fertilized by the sperm-suspension treated with water from the filter-paper than by the sperm not so treated, although the two suspensions were of identical concentrations. In the first case the decrease was from 100 % to 77 %, and in the second from 95 % to 31 %. This is expressed by saying that the "fertilizing power" of the sperm-suspension had been diminished by the treatment with filter-paper water.

The foregoing remarks have emphasized enough the importance on such work as this, where the validity of the results depends on numerical

comparisons, of making the experiments under such conditions that comparisons are strictly possible. In previous work, the results of which have depended on the percentages of eggs fertilized under different conditions, the method of experimentation has in general been far from exact. This is often the case in Echinoderm hybridization work—and numerous other examples might be cited, but the investigation which particularly interests us here is that of T. H. Morgan on fertilization in *Ciona* (9, 10, 11).

A considerable part of the work of this investigator was concerned with the question as to whether the eggs of all individuals are capable of being fertilized by the sperm of all other individuals. This is a problem of peculiar interest, especially in view of recent work on self-sterility in plants. It is far from being an easy point to settle, since the difficulty of making up the sperm-suspensions of the different individuals to be compared of even approximately equal concentrations is great. That this equal strength of the different sperm-suspensions is an absolutely necessary datum for interpreting the results is shown by the fact that in making cross-fertilizations even small differences in sperm-concentration produce large variations in the percentages of eggs fertilized. Morgan's method was to select five or six individuals, make all the possible reciprocal fertilizations between them, and then compare the percentages of eggs fertilized in the different crosses. It is stated (9, p. 148) that "The sperm, *a*, of the first individual was then taken out and put into a small amount of water. It was then distributed to one set of eggs from each of the other individuals, *B*, *C*, *D*, *E*; then the sperm of *B* was taken out and applied to another set of eggs." It is not stated, however, whether sperm *b* was of nearly the same concentration as sperm *a*; nor is there any mention as to whether the eggs and sperm were thoroughly mixed before fertilization, and with one another at the moment of insemination. The observation is made, however (10, p. 321), that in all cases enough sperm was probably used to fertilize all the eggs capable of uniting with that sperm. In the first series (9) several of the combinations were made twice in the same experiment, but frequently gave very divergent results. Thus in Exp XIII, *Ea* (i.e. *E* eggs with *a* sperm) gave first 100 °/₀ and then 85 °/₀ of fertilized eggs. In Ex. XIV, *Ea* gave 0 °/₀ and 4 °/₀: *Ae* 0 °/₀ and 100 °/₀. In Exp. XV, *Ea* gave 2 °/₀ and 0 °/₀: *De* 90 °/₀ and 100 °/₀: *Ae* 0 °/₀ and 100 °/₀. In Exp. XVI, *Ea* 5 °/₀ and 70 °/₀: *De* 100 °/₀ and 30 °/₀: *Ae* 75 °/₀ and 100 °/₀. It seems extremely probable that these divergencies are due to inexact experimentation, although Morgan suggests (10,

p. 320) that the extreme difference of 0 % and 100 % in one combination in Exp. XIV and again in Exp. XV "is due no doubt to the failure to add sperm to the first lot, which might easily occur." How are we to judge of the exactness of the results of the crosses which were not made twice over? The conclusion drawn from 240 combinations was that the sperm of an individual *Ciona* is not capable of fertilizing the eggs of all other individuals to an equal extent, although some of the results, according to Morgan, may have been due to the presence with the eggs of "body-fluids." It would seem, however, that the equality of conditions for each of the crosses should be made very much more exact before this conclusion is justified.

The way in which the percentages of segmenting eggs were counted is not given. Nor are there usually any details as to about how many eggs were used in the experiments, nor how many of these were counted. In some few cases, however, the number of eggs present is given in brackets after the percentages. Apparently these were the cases in which exceptionally few eggs were used, as the following citations would seem to show. From the 1904 paper: p. 145, 0 % (only 3 eggs); p. 149, 0 % (1 egg); p. 150, 10 % (only 10 eggs); p. 151, 25 % (only 4 eggs), 50 % (only 8 eggs), 12 % (only 8 eggs), etc. From the 1910 paper: p. 218, 100 % (3 eggs), 0 % (1 egg); p. 219, 100 % (2 eggs), 50 % (2 eggs), 100 % (4 eggs)[1]. How many eggs were present in the other lots is not stated.

In all work on fertilization under different experimental conditions, the complete absence of genital products of other individuals than those used in a particular experiment is an absolutely essential condition. This is, if possible, more than ever important in work on self-fertilization in comparatively self-sterile organisms, where the presence of the most minute trace of "foreign" spermatozoa upsets the whole results. The importance of this precaution is fully realized by Morgan. He says (9, p. 137), "The individuals to be used were isolated, as a rule, from 24 to 48 hours, and in most cases were rinsed in fresh-water before opening," and that instruments were properly sterilized. Nevertheless, throughout the work the expression of doubt recurs that such and such a result may have been due to contamination with "foreign" spermatozoa. There is no necessity for such doubt at all, for if unfertilized controls are kept of every lot of eggs employed, and unfertilized eggs are left lying in samples of every fluid used, if none of these segment

[1] On the same page is recorded "60 % (no eggs)," but this must be a printer's error.

there can be no reasonable question of the accidental presence of spermatozoa which would vitiate the results.

Experiments were made by Morgan to test the influence of ovary-extract on the extent of self-fertilizations. In two out of eight cases some of the eggs segmented, but "there may have been contamination in the latter case" (9, p. 139). Again, it was tried whether water in which eggs had been violently shaken would favour the self-fertilization of the eggs of another individual. In several cases self-fertilization did occur in the presence of such water (10, p. 325); but "There is, in fact, a source of contamination in this experiment that may fully account for all the cases observed. In removing the eggs from the oviduct some of the sperm from the vas deferens may be accidentally squeezed out and become mixed with the eggs, and remaining in the follicle water fertilize the other eggs." Why were not unfertilized eggs from another individual left lying in the water in which the eggs had been shaken (follicle water) to test whether it contained spermatozoa or not?

Again, the question recurs (11, p. 207) as to whether the few cases of self-fertilization may not have been due to contamination with "foreign" spermatozoa retained in the branchial basket of the animal, which might then come into contact with the eggs removed from the oviduct. But if unfertilized samples of these eggs had been kept as controls the possible presence of such "foreign" spermatozoa would have been made known.

The repetition of such examples serves no further purpose, but in conclusion it should be pointed out that some of the experiments made to yield results by comparison were not comparative at all. For example, eggs were fragmented by pressure under a cover-slip and many of the eggs which had been broken in this way were self-fertilized on the addition of "own" sperm, thus "indicating that the resistance to self-fertilization is due to something in the membranes surrounding the eggs" (10, p. 326). But the second half of the experiment, which would justify such a conclusion, is missing. It is not mentioned whether some of the same lot of eggs, which had not been crushed, showed no self-fertilization when inseminated with an equal amount of the same sperm-suspension. Again, a number of experiments were made to see whether subjection of the genital products to low temperatures would increase the percentages of eggs self-fertilized. In some cases a considerable proportion of eggs showed self-fertilization after such treatment, but apparently the second half of the experiment was again missing, in which some of the same lot of eggs untreated with cold

should have been fertilized with a like amount of the same suspension of "own" sperm, also untreated. Morgan concluded that the variation in the extent of self-fertilization in the different experiments of this series was probably due to contamination with sperm contained in the branchial baskets of the animals from which the eggs and sperm had been removed. But this possibility could have been tested by leaving "foreign" unfertilized eggs in sea-water containing a piece of the branchial basket.

III. Factors influencing the Extent of Self-fertilization.

From the foregoing it is plain that *Ciona intestinalis* at Naples is not absolutely self-sterile, but that self-fertilization occurs to a considerable degree. Moreover the extent to which it takes place is very variable. The experiments described in this section are attempts to analyse the factors which influence the extent of the self-fertilization; that is to say, they are exact comparisons between the proportion of eggs to a given individual self-fertilized under a certain set of conditions, and the proportion when one of the conditions is altered. At the same time a comparison is made between the percentage of some of the same lot of eggs cross-fertilized by sperm of a "foreign[1]" individual, or of eggs of a "foreign" individual cross-fertilized by sperm of the first one, under similar conditions to those used in the self-fertilizations.

1. *Sperm-concentration.*

Each of the first series of experiments made to test the influence of sperm-concentration on self-fertilization was carried out as follows.

Approximately equal amounts of eggs to a given individual were placed in equal quantities of water in four dishes. Fertilization was effected by the addition of sperm as follows: to dish (1), five drops of a milky suspension of "own" sperm; to dish (2), 4 cc. of ditto; to (3), five drops of a milky suspension of "foreign" sperm of approximately

TABLE I. (7.8.1.)

	Eggs selfed			Eggs crossed	
Cross	5 drops sperm	4 cc sperm	Cross	5 drops sperm	4 cc. sperm
Exp. 1. *A/a*	0	58	*A/m*	100	100
Exp. 2. *B/b*	0	22	*B/m*	100	100
Exp. 3. *C/c*	12	100	*C/m*	100	100
Exp. 4. *D/d*	0	56	*D/m*	100	100

[1] "Own" sperm is used to denote sperm taken from the same individual as the eggs; "foreign" sperm is that from another individual.

the same concentration as the suspension of "own" sperm; to (4), 4 cc. of ditto. The results of these experiments are shown in Table I.

The Table shows that in each of the experiments an increase in the concentration of "own" sperm caused an increase in the number of eggs self-fertilized, the percentage in Exp. 3 being raised to 100, a proportion which subsequent experiments proved to be uncommon. The fertilizations of the eggs with "foreign" sperm demonstrate the ease with which cross-fertilization is effected as compared with self-fertilization by an approximately equal concentration of sperm, 100 % of the eggs segmenting in each experiment, even with the more dilute sperm-suspensions.

Further experiments were then carried out confirming this conclusion. The results are shown in Tables II, III and IV. The method used in the experiments of Table II, which was the same as that used in those of Tables III and IV, was as follows.

Six dishes were prepared, containing equal amounts of water. To the first three were added approximately equal amounts of eggs *E* to be selfed, and to the last three, approximately equal amounts of eggs of another individual *F* to be crossed. Fertilization was effected by the addition to each dish of the amounts of sperm-suspension *e* given in the Table. In this case the comparison between the proportion of eggs selfed and the proportion crossed with the same amount of sperm was *exact*, since the *same* sperm-suspension was used in each fertilization.

TABLE II. (8.9.1.)

	Self-fertilization				Cross-fertilization			
Cross	2 drops sperm	25 drops sperm	10 cc. sperm		Cross	2 drops sperm	25 drops sperm	10 cc. sperm
E/*e*	2	24	100		*F*/*e*	100	100	100

TABLE III. (8.9.1.)

	Self-fertilization				Cross-fertilization				
Cross	1 drop sperm	5 drops sperm	20 drops sperm	100 drops sperm	Cross	1 drop sperm	5 drops sperm	20 drops sperm	100 drops sperm
G/*g*	0	1	3	11	*H*/*g*	100	100	100	100

TABLE IV. (10.14.1.)

	Self-fertilization					Cross-fertilization				
Cross	3 drops sperm +10 cc. water	1 cc. sperm +10 cc. water	5 cc. sperm +10 cc. water	10 cc sperm	Cross	3 drops sperm +10 cc. water	1 cc. sperm +10 cc. water	5 cc. sperm +10 cc. water	10 cc. sperm
J/*j*	<1	<1	47	89	*K*/*j*	19	77	100	100
N/*n*	0	0	0	43	*O*/*n*	100	100	100	100

2. *Length of time that eggs and sperm lie in water before fertilization.*

The next factor investigated was the length of time the eggs and sperm lay in water before they were brought together. The eggs and sperm of one individual and the eggs of another were removed separately from the genital ducts and fertilizations were made at definite intervals after these genital products had been brought into the sea-water.

The exact procedure in Exp. 1 was as follows:

The genital products were removed at 2.55—3.10 p.m.: at 3.45—3.50[1] the first fertilizations were made. A certain quantity of eggs ***A*** was placed in water, after which 3 cc. of a thick sperm-suspension *a* was added. At the same time an approximately equal quantity of eggs *D* was placed in 10 cc. water in another dish and 1 cc. of a dilute sperm-suspension *a* was added. In the previous section it was shown that a considerably greater concentration of sperm is necessary to effect self-fertilization than would cross-fertilize 100 % of foreign eggs, and therefore the sperm *a* used for cross-fertilization with eggs *D* had to be made much more dilute than that used to self-fertilize eggs *A*. The details just given were repeated exactly at each subsequent fertilization, the times of which are given in the Table below. The same sperm-suspensions were of course used to effect the self- and cross-fertilization after each interval.

The other four experiments were carried out on the same lines as Exp. 1. The details of the quantities of sperm and water used and the intervals at which the fertilizations were made are given in Table V.

As in all the experiments dealing with self-fertilization, more trials were made than the successful ones recorded below, owing to the fact that there are always a certain number of individuals in which *none* of the eggs will self-fertilize. In this series, five successful experiments are recorded, but besides these there were three in which the self-fertilization percentages were nil throughout.

The first point brought out by the experiments of Table V is that in every case the percentages of eggs self-fertilized rose with succeeding fertilizations. In four cases it fell again after having reached a maximum, but in Exp. 3 at 4½ hours after removing the eggs and sperm from the animal the maximum had not yet been reached. The corresponding cross-fertilizations with the sperm from the same animals showed in no case a rise in percentages with successive fertilizations. In Exps. 2

[1] The fertilization times given in Table V were taken at the end of each operation, which occupied 3—5 minutes.

and 3 there was an excess of sperm throughout in the cross-fertilizations, 100 % of eggs segmenting every time. In Exps. 1, 4 and 5, however, the percentages fell—in the last two, rapidly.

TABLE V.

Cut out[1] 2.55—3.10 p.m.		Fert. 3.50 (19·5° C.)	Fert. 4.50 (20° C.)	Fert. 5.45 (20° C.)	Fert. 6.50 (20·5° C.)
Exp. 1	Self-fert. *A/a*, 3 cc. thick sperm, 10 cc. water	4	6	9	0
	Cross-fert. *D/a*, 1 cc. dilute sperm, 10 cc. water	100	100	79	89[2]
Exp. 2.	Self-fert. *C/c*, 3 cc. thick sperm, 10 cc. water	21	23	—	12
	Cross-fert. *F/c*, 1 cc. dilute sperm: 10 cc. water	100	100	—	100

Cut out 1.0—1.10 p.m.		Fert. 1.45	Fert. 2.30	Fert. 3.15	Fert. 4.0	Fert 4.44
Exp. 3.	Self-fert. *G/g*, 3 cc. thick sperm, 10 cc. water	4	23	75	78	83
	Cross-fert. *H/g*, 1 cc. dilute sperm, 10 cc. water	100	100	100	100	100

Cut out 11.45—11.55 a.m.		Fert. 12.20 (18° C.)	Fert. 1.20 (18° C.)	Fert. 2.20 (18° C.)	Fert. 3.20 (18° C.)	Fert. 4·20 (18° C.)	Fert. 5·20' (18·5° C.)
Exp. 4.							
Self-fert.	*I/i*, 3 cc. thick sperm, 10 cc. water...	0	<1	<1	0	0	0
Cross-fert.	*J/i*, 4 drops dilute sperm, 10 cc. water	97	96	80	23	2	0

Cut out 11.30—11.45 a.m.		Fert. 12.20 (18° C.)	Fert. 1.20 (18° C.)	Fert. 2.20 (18·5° C.)	Fert. 3.20 (18·5° C.)	Fert. 4.20 (19° C.)
Exp. 5.						
Self-fert.	*K/k*, 4 cc. thick sperm, 10 cc. water...	6	10	29	1	<1
Cross-fert.	*L/k*, 2 drops dilute sperm, 10 cc. water	100	65	23	8	0

From the experiments already described on the length of time that sperm and eggs remain capable of fertilizing, it appeared that the sperm loses its capability rapidly, while the eggs "go off" much more slowly. From this it would seem that the falling off of the cross-fertilization percentages in Exps. 1, 4 and 5 is to be attributed to the failure of the sperm rather than of the eggs.

From a comparison of the corresponding self- and cross-fertilizations in Exps. 1, 4 or 5 it seems that the self-fertilization percentages continue to increase until the sperm begins to fail (as indicated by the decreasing cross-fertilization percentages) after which they decrease again. In Exp. 3, where the cross-fertilization percentages show that the sperm did not commence to lose its fertilizing power during the progress of the experiment, the proportions of eggs self-fertilized continued to rise steadily.

[1] By "cut out" is meant the time at which the genital products were removed from the ducts.

[2] This last fertilization showed much polyspermy, which may account for the irregularity in the percentages. In all other cases recorded in this paper segmentation was regular, unless otherwise mentioned.

In Exps. 1, 4 and 5 the temperatures of the water at the moments of fertilization are recorded. Table V shows that the maximum variation is only 1 C., and as there is no rise and fall correlated with the rise and fall of the fertilization percentages, there can be no connection between the two.

The conclusion can thus be drawn from the experiments that lying in water increases the self-fertilizing capacity of eggs and sperm. This increase continues up to a maximum, after which it falls off again, probably due to the loss of both self- and cross-fertilizing power by the sperm.

Although it is certain that there is an increase in the extent to which self-fertilization takes place after the eggs and sperm have been for some time in sea-water, yet the experiments do not decide whether it is due to a change in the eggs or in the sperm. It was thought that further experiments to decide this point could be made as follows:

About half the eggs contained in the oviduct are removed and placed in sea-water. The hole in the oviduct wall through which the eggs were removed is then clipped and the sperm removed from the sperm duct to make up a suspension. At regular intervals equal amounts of this sperm-suspension are used to fertilize (1) some of the eggs already lying in water, and (2) some more eggs, just removed from the oviduct. If the eggs (1) showed the typical rise and fall in self-fertilization percentages, while the eggs (2) did not, the phenomenon would be due, at any rate in part, to an alteration in the eggs during their stay in sea-water.

The experiment was tried and was a complete failure. The eggs which had been for varying lengths of time in sea-water showed the usual increase and decrease in fertilization percentages, but the proportions in eggs taken from the oviduct immediately before each fertilization were quite irregular. The reason for this will be apparent when the results of experiments to be described below are seen; for it will be shown that eggs and sperm taken from different parts of the genital ducts of a given animal behave quite differently in their capacity for self-fertilization. Now, whereas the eggs lying in water are thoroughly mixed together before being used, and are thus homogeneous material, every time that eggs are removed from the oviduct for comparison, a sample is obtained having a quite different behaviour.

The reverse experiment, namely that of removing all the eggs and half of the sperm from an animal and then taking samples of the remainder of the sperm from the vas deferens at each fertilization, is

impracticable for a further cause. It is impossible to make up sperm-suspensions of identical concentrations each time that sperm is removed from the duct.

Thus it cannot be decided whether the change in the capacity for self-fertilization shown by the genital products on lying in water is due to a change in the eggs or in the sperm.

3. *Position of genital products in their ducts.*

The irregular variation in the self-fertilization percentages obtained on successive days when an isolated animal is allowed to lay eggs and sperm naturally must be explained in part at least by the differences in the concentration of sperm, depending on the amount ejected on each occasion. The importance of the sperm-concentration has already been shown (pp. 232–233 above), but this may not be the whole reason for the differences in the percentages just referred to. The phenomenon suggests that the genital products of a given animal vary in their capacity for self-fertilization from day to day.

This hypothesis can be tested as follows. The eggs lying at the inner end (base), in the middle and at the outer end (top) of the long oviduct are removed separately from the animal and brought into sea-water. The sperm is then removed from the sperm duct and used to fertilize the three lots of eggs separately.

If equal amounts of the three lots of eggs showed the same amounts of self-fertilization with equal concentrations of the sperm, it would argue a uniformity of behaviour in one animal: although the possibility would not be excluded that the eggs given off from the ovary at a later occasion might act differently. If, on the other hand, the three lots gave irregular differences in the percentages, it would be clearly shown that eggs produced at one time have a different capacity for self-fertilization from those produced at another time, since eggs lying at the top of the oviduct are older than those lying at its base. There is also a third possibility, namely that a regular gradation in percentages from base to top would be found.

Experiments were made on this plan, and also the reverse ones of testing samples of eggs, taken from the whole oviduct and well mixed together, with sperm from different positions in the vas deferens. The details of the first series of experiments were as follows:

The oviduct of an animal was clipped in one or more places and the eggs removed separately from the different lengths thus divided off. Comparative tests were made of the self-fertilizing capacities of these

different lots of eggs, and other tests of their cross-fertilizing capacities. For the former, sperm was removed from the sperm duct of the same animal from which the eggs were derived, and a suspension of it made up. Approximately equal quantities of the different lots of eggs were put into dishes containing 10 cc. of water, and to each 1 cc. of a thick "own" sperm-suspension was added. For the cross-fertilizations a dilute sperm-suspension of another individual was made up. Fifty drops of this were added to each of a number of dishes containing approximately equal amounts of the different lots of eggs in 10 cc. water. The results of the experiments are shown in Table VI.

TABLE VI. (7.6.1 and 8.11.1.)

		Part of oviduct from which eggs were taken		
		Base	Middle	Top
Exp. 1.	*A* eggs selfed, *A/a*	4	—	12
	A eggs crossed, *A/b*	100	—	100
Exp. 2.	*C* eggs selfed, *C/c*	3	1	—
	C eggs crossed, *C/d*	100	100	—
Exp. 3.	*E* eggs selfed, *E/e*	93	90	—
	E eggs crossed, *E/f*	5	20	—
Exp. 4.	*G* eggs selfed, *G/g*	89	85	—

		Part of oviduct from which eggs were taken			
		Base	Lower middle	Upper middle	Top
Exp. 5.	*H* eggs selfed, *H/h*	0	0	0	0
	H eggs crossed, *H/i*	60	13	17	1

The Table shows at a glance that there is no uniformity in the self-fertilization percentages of eggs taken from the base and from the top of an oviduct of a given animal. Nor do the different animals agree in the eggs from the top being always either more or less readily self-fertilizable than those from the base. Exps. 3 and 5 show that the same conclusions apply to the cross-fertilization percentages (in Exps. 1 and 2 an excess of sperm was present and no comparisons are possible). Finally there is no correspondence between the self-fertilization percentages of eggs of a given animal, taken from the base and from the top of the duct, and the cross-fertilization percentages of these eggs. In Exp. 3 eggs from the base were more readily self-fertilizable than those from the top, while the reverse held for the cross-fertilizing capacities.

Besides the above, further experiments were made in which, not only the eggs were removed separately from the different regions of the

duct, but the sperm was treated similarly. It must be pointed out, however, that comparisons between the effects of sperm from the base and from the top of the vas deferens on any given lot of eggs are necessarily very inexact. This is owing to the fact that it is impossible to make up two different sperm-suspensions of exactly equal concentrations, since the latter can only be judged by the eye, a comparatively inaccurate method.

In each experiment the eggs were removed separately from the different regions of the oviduct. Approximately equal quantities of each of the lots of eggs were fertilized (1) by equal concentrations of sperm from the base of the sperm duct, (2) by equal concentrations of sperm from the top of the sperm duct, (3) by equal concentrations of a sperm-suspension of another individual. Besides this, equal amounts of the sperm-suspensions from the base and from the top of the sperm duct were tested with "foreign" eggs. See Table VII.

TABLE VII. (12.16.1.)

		Part of oviduct from which eggs were taken		
		Base	Middle	Top
Exp. 1.	Eggs *A* selfed with sperm from base of duct, A/a_1	0	0	0
	Eggs *A* selfed with sperm from top of duct, A/a_2	18	4	13
	Eggs *A* crossed, A/n	100	100	100
		(116)[1]	(116)	(116)
	Eggs *C* crossed with sperm a_1 from base of duct		7 (116)	
	Eggs *C* crossed with sperm a_2 from top of duct		74 (116)	

		Part of oviduct from which eggs were taken			
		Base	Lower middle	Upper middle	Top
Exp. 2.	Eggs *B* selfed with sperm from base of duct, B/b_1	6	1	<1	<1
	Eggs *B* selfed with sperm from top of duct, B/b_2	0	0	0	0
	Eggs *B* crossed, B/n	97	81	85	81
		(87)	(87)	(87)	(87)
	Eggs *D* crossed with sperm b_1 from base of duct ...		...	...	91
	Eggs *D* crossed with sperm b_2 from top of duct ...		...	...	42
	Eggs *E* crossed with sperm b_1 from base of duct ...		...	...	90
	Eggs *E* crossed with sperm b_2 from top of duct ...		...	...	31

These experiments are a further confirmation of those of Table VI in showing the irregularity in behaviour of eggs from different parts of an oviduct both in respect to self- and cross-fertilizing capacities—see

[1] The figures in brackets are the number of minutes after fertilization when the 4-cell division was completed. See p. 245.

Exp. 1, A/a_2, Exp. 2, $B\,b_1$, and B/a. The further point which these two experiments were made to test, namely the behaviour of sperm from different regions of the vas deferens, must remain undecided for the reason that the different suspensions cannot be made up to exactly equal concentrations. In Exp. 1 sperm from the base failed to self-fertilize, while that from the top fertilized the eggs of the same animal to varying degrees. In Exp. 2 exactly the reverse was the case. Further, the tests of the two sperms in each experiment with "foreign" eggs showed that the self-fertilizing power went hand in hand with the cross-fertilizing power. Whether, however, the behaviour of the two sperm-suspensions means that there is a real difference between them or whether it is due to a slight difference in the concentrations of the suspensions must remain unsettled.

The final conclusion to be drawn from the experiments described in this section is that eggs from different regions of the oviduct have quite different self- (and cross-) fertilizing capacities, when treated with equal concentrations of sperm. Whether or not there is a similar difference in the behaviour of the sperm is undecided. The varying behaviour of the eggs is in no way correlated with their position in the oviduct, i.e. with their age, and we must therefore conclude that an animal produces eggs which are at one time more and at another less prone to self-fertilization.

IV. The Effect of a Sperm-Suspension on the Eggs of the same Individual.

As soon as it was found that *Ciona intestinalis* at Naples is not totally self-sterile, but is self-fertile to a very varying degree in different individuals, the proposed heredity experiments referred to in the introduction had to be postponed until the limits and conditions of self-fertility had been settled. In the preceding sections some of the main factors have been described which influence the degree of self-fertility, and although the latter is very variable, it has been shown that a very much greater concentration of sperm is always necessary to bring it about than is required to effect cross-fertilization. Moreover a considerable proportion of individuals are always present, the eggs of which cannot be self-fertilized at all.

The causes of this self-sterility have been investigated before, but although the problem has been narrowed down considerably, they remain fundamentally as obscure as ever. It was with a view to

attacking the problem of the means by which the immunity of eggs to fertilization by "own" sperm is brought about that experiments on the influence of egg-secretions on fertilization were begun. This investigation forms Part II of this paper. As the work progressed, so many preliminary questions with regard to the effects of the egg-secretions on normal cross-fertilizations in *Ciona* and other forms had to be settled, that, at the time of writing, not enough experiments on the influence of the secretions of "own" and "foreign" eggs on self-fertilization had been made to justify publication.

In Part II it will be shown that the eggs of *Ciona* secrete a substance into the water which stimulates spermatozoa, both of the same and of other individuals, to effect cross-fertilization. Whether this secretion fails to have the same effect on the self-fertilizing power of spermatozoa of the same individual, or whether some other substance is secreted which actually inhibits self-fertilization has not as yet been settled. But whether such a possibility is true or not, there seems to be another factor intimately connected with the difficulty of self-fertilization, and that is a change brought about in the eggs by the presence of "own" sperm. The experiments which will be described in this section seem to show that if eggs be brought into contact with a sperm-suspension of the same individual, their capability of being subsequently fertilized by "foreign" spermatozoa is diminished as compared with that of eggs not so treated.

The first series of experiments was carried out as follows. In each instance the eggs of an individual were divided into two lots, which were placed respectively in (1) plain water, (2) an opalescent suspension of "own" sperm. At definite intervals 1 cc. of a "foreign" sperm-suspension was pipetted into each of two dishes containing 22 cc. water. The liquids so prepared were then poured separately on to (1) a drop of eggs from the water, and (2) a drop of eggs from the suspension of "own" sperm. These fertilizations were repeated at intervals given in Table VIII, so that the effects on the eggs of remaining for different lengths of time in "own" sperm could be compared.

The percentages of segmenting eggs were counted 80 minutes after each fertilization, and at the same time the percentages of self-fertilized eggs lying in "own" sperm-suspension (2) were counted. Table VIII gives the results of the experiments.

It should be noted in the first place that the Table shows a decrease in the percentages at each subsequent fertilization, a fact which, as has already been pointed out, is almost certainly due to a gradual decline

in the fertilizing power of the sperm. The striking point in the results is however that, for each pair of fertilizations, the percentages are

TABLE VIII. (44.27.6 *et seq.*)

Time of fertilization, after mixing eggs and "own" sperm			Percentage fertilization in eggs from water	Percentage fertilization in eggs from "own" sperm	Percentage selfed
Exp. 1.	*B/c*	5 minutes	97	84	1
		25 ,,	100	97	2
		45 ,,	100	95	3
		65 ,,	96	87	3
		85 ,,	94	?	3
		105 ,,	72	66	3
		125 ,,	47	38	3
		145 ,,	21	14	3
		165 ,,	6	5	3
Exp. 2.	*A/b*	10 minutes	100	100	—
		30 ,,	100	100	—
		50 ,,	100	95	10
		70 ,,	98	58	13
		90 ,,	51	39	16
		130 ,,	6	20 (i.e. all selfed)	20
Exp. 3.	*D/e*	5 minutes	84	79	5
		25 ,,	13	9	7
Exp. 4.	*F/g*	5 minutes	75	13	—
		35 ,,	40	30	28
Exp. 5.	*H/i*	5 minutes	16	6	0
		35 ,,	4	< 1	< 1
Exp. 6.	*J/k*	5 minutes	17	9	0
		35 ,,	5	< 1	0
Exp. 7.	*L/m*	10 minutes	90	68	0
		40 ,,	0	0	0

lower with eggs which have been previously treated with "own" sperm than with those not so treated. In most cases, moreover, the cross-fertilization percentages of the former should be still lower than those actually counted and recorded in the Table, since during their stay in "own" sperm-suspension a certain number of the eggs had already been self-fertilized. In order to allow for this factor the proportion of eggs remaining in "own" sperm, which had been self-fertilized, was calculated at the same time as the cross-fertilization percentages were counted. These figures are given in the last column of the Table. In the last fertilization of Exp. 2, the treated eggs show an apparently higher cross-fertilization percentage than the untreated; but on examining

the last column it is seen that all these eggs had already been selfed. Again in Exp. 4, although the treated eggs show for both fertilizations lower percentages than the untreated, in the previously treated eggs the second percentage is higher than the first. This is, however, really due to the presence in the treated eggs of 28 % already self-fertilized.

When the presence of self-fertilized eggs in the lots which have been treated with "own" sperm is allowed for, the fall in cross-fertilization percentages of the latter is even more marked than appeared at first sight. This might be due to one of two causes. Either the presence of "own" spermatozoa calls forth a reaction on the part of the eggs hindering the entrance of the former, and also—although to a much lesser degree—hindering the entrance of "foreign" spermatozoa: or the presence of the small amount of sperm-suspension which is necessarily carried over with the drop of eggs removed for cross-fertilization itself inhibits the "foreign" spermatozoa. For spermatozoa in the vas deferens are motionless, and this must be due either to the absence of sea-water, or to the presence of a substance inhibiting movement. If the latter exists, it must be present in a dilute form in the suspension of "own" sperm, and might conceivably act on the "foreign" spermatozoa when these are added to the drop of eggs taken from this suspension. In order to test whether this is the explanation of the diminished cross-fertilization percentages of the treated eggs, or whether the latter have really been altered in their capacity for cross-fertilization by their sojourn in "own" sperm, further experiments were made. Before being cross-fertilized, the treated eggs were thoroughly washed in a comparatively large volume of water.

As in the previous experiments, the eggs of each individual were divided into two lots, and placed respectively in water and in opalescent "own" sperm suspension. After a definite interval (10 mins. in Exps. 1 and 2; 15 mins. in Exp. 3) 1 cc. of "own" sperm containing eggs was removed to 100 cc. of plain water in a finger-bowl, in which the eggs were allowed to settle in order to remove excess of sperm. A definite quantity of "foreign" sperm-suspension (given in Table IX) was pipetted into each of two dishes containing 10 cc. water. These were then poured on to separate approximately equal quantities of eggs (1) from plain water, (2) from the finger-bowl.

In these experiments fertilizations were not made at different intervals of time as in the former ones, but each was made double, two different amounts of sperm being used. The results of the experiments are recorded in Table IX.

TABLE IX. (16.2.7.)

			Eggs from water	Eggs from "own" sperm, washed
Exp. 1.	*N/o*	{ 3 drops sperm ...	95	70
		{ 1 cc. sperm ...	100	100
Exp. 2.	*P/q*	{ 3 drops sperm ...	3	1
		{ 1 cc. sperm ...	9	4
Exp. 3.	*R/s*	{ 3 drops sperm ...	10	18
		{ 1 cc. sperm ...	82	61

The Table shows that the eggs which have been in the presence of "own" sperm behave in subsequent cross-fertilization in the same way after the "own" sperm has been removed by washing as they did in the previous experiments when this was not done. This makes it extremely probable that the real reason for the diminished capacity for cross-fertilization is a change brought about in the eggs by the sperm of the same individual.

An examination of Tables VIII and IX shows that the change in the eggs, as indicated by the lowered cross-fertilization percentages, is comparatively small, and that when an excess of "foreign" sperm is present, as in Table VIII, Exp. 2, and Table IX, Exp. 1, 100% of these eggs can be fertilized. That the eggs can be cross-fertilized after the treatment, although with rather less ease, does not mean that there may not have been a large change in them, as regards their receptivity to their "own" sperm. Whether this is so or not cannot be tested, as our only criterion is that of comparing the extent to which they can be *cross*-fertilized, with and without the previous treatment. The facts certainly point, however, to the sperm having caused an alteration in the eggs of the same individual, and to this alteration being at any rate one of the means by which self-fertilization is effected.

V. Comparison of the Subsequent Development of Eggs self- and cross-fertilized under Various Conditions.

1. *Eggs cross-fertilized at different intervals after the removal of the genital products from the body of the animal.*

The foregoing investigations, concerning some of the conditions which favour and limit the extent of self-fertilization, and the reasons for the self-sterility itself, bring the main part of this division of the work to a conclusion. A considerable number of observations were, however, also made on the subsequent development of eggs self- and

cross-fertilized under different conditions. It was thought that these experiments were worth recording, and the details, together with the conclusions to be drawn from them, are given in the following sections.

The first series of observations concerns the comparative rates of development and the condition of the larvae hatched out when different lots of eggs are cross-fertilized at regular intervals after the genital products have been removed from the body of the animal into sea-water. The fertilizations were made in exactly the same way as those described on p. 234 above, and indeed several of the experiments in this section are identical with those recorded in Table V, the subsequent development of the eggs having been noted. The method it will be recalled, was briefly as follows. For each experiment eggs were removed from one individual and sperm from another. At definite intervals (given in the Table below) approximately equal quantities of the eggs were fertilized by the addition each time of exactly equal amounts of the dilute sperm-suspension.

The subsequent observations were as follows:

In the first place, the percentages of eggs fertilized were counted as usual.

Secondly, the rates of segmentation of the eggs were compared by noting the length of time after fertilization at which the 4-cell division took place. This stage was fixed upon as it is the easiest to observe rapidly and accurately under a low power of the microscope. Now, although all the eggs in a given lot do not complete the 4-cell division at a given moment, yet in general the majority divide almost simultaneously, and for this reason the criterion adopted for the comparison of the rates of segmentation was the time at which the first few eggs in a given sample were seen to have completed their second division. This naturally involves a certain latitude of experimental error, and in consequence the figures given in the Tables below are not correct to a minute. The extent of this error, however, does not exceed two minutes, and it will be seen that the differences between the times taken to complete the second divisions in the different fertilizations in a given experiment are usually considerably larger than this.

The rapidities of development of the embryos up to hatching were compared by noting the lengths of time after fertilization at which the first larvae emerged from the eggs in the different dishes. It is usually about half an hour after the first has come out before the majority have emerged from the eggs, and when the larvae are weakly, this time is considerably longer. The comparisons were made by observing when

the first larva of each lot hatched out. In the experiments in which the times of hatching were noted the conditions of the resulting larvae were also recorded.

The figures for these experiments are given in Table X.

TABLE X.

Explanation of Table.

"Time of fertil." The approximate time in hours, after the genital products had been removed from the body of the animal, at which the fertilization was made.

"Percent fertil." Percentage of eggs fertilized.

"Time of 4-cells." Time in minutes after fertilization at which the first four-cell division was completed.

"Time of hatching." Time in hours and minutes after fertilization at which the first larvae hatched out of the eggs.

	Time of fertil	Percent. fertil.	Time of 4-cells	Time of hatching	Condition of larvae
Series A. (9.13.1.) (Temp. 19·5 —21·5 C.) Exp. 1. *A/m*	1	100	115	22, 8	Good
	2	100	108	22, 3	Good
	3	100	101	22, 20	Good
	4	100	100	23	Fair
Exp. 2. *C/m*	1	100	110	22, 48	V. weak, few hatched
	2	100	98	21, 43	Good
	4	100	95	22, 10	V. weak, few hatched, malformed
Exp. 3. *F/c* (=Table V, Exp. 2, *F/c*)	1	100	113	22, 48	V. weak, few hatched
	2	100	98	22, 28	Good
	4	100	95	21, 40	Good
Exp. 4. *B/m*	1	100	108		
	2	100	98		
	4	100	95		

	Time of fertil	Percent fertil	Time of 4-cells
Series B. (11.15.1.) Exp. 5. *A m*	1	96	105
	1¾	25	100
	2½	1	(88)
Exp. 6. *C/a*	1	100	99
	1¾	100	96
	2½	100	81
	3¼	100	90
Exp. 7. *G/n*	¾	93	97
	1½	19	98
	2¼	5	89
Exp. 8. *H/g* (=Table III, Exp. 3. *H/g*)	¾	100	90
	1½	100	91
	2¼	100	80
	3	100	87
	3¾	100	76

TABLE X (*continued*).

	Time of fertil.	Temp.	Percent. fertil.	Time of 4-cells
Series C. (13.17.1.)	1	18° C.	100	82
	2	do.	100	86
Exp. 9. *A/m*	3	do.	100	87
	4	do.	85	88
	5	do.	3	93
	1	18° C.	96	83
Exp. 10. *C/a*	2	do.	3	86
	3	do.	<1	(92)
	½	18° C.	100	84
	1½	do.	100	86
Exp. 11. *I/o*	2½	do.	100	85
	3½	do.	84	89
	4½	do.	12	88
	½	18° C.	97	78
	1½	do.	96	85
Exp. 12. *J/i*	2½	do.	80	87
(=Table V, Exp. 4. *J/i*)	3½	do.	23	88
	4½	do.	2	87
Series D. (14.18.1.)	1⅓	18° C.	100	88
	2⅓	do.	78	91
Exp. 13. *B/m*	3⅓	18·5° C.	16	91
	4⅓	do.	4	86
	1⅓	18° C.	4	87
Exp. 14. *D/b*	2⅓	do.	15	89
	3⅓	18·5° C.	<1	(95)

Considering first the four experiments forming Series A, it will be noticed that in each case the eggs fertilized later completed the 4-cell division in a shorter time than those fertilized earlier. Thus in Exp. 1, eggs fertilized one hour after being removed from the animal into sea-water took 115 minutes to complete the 4-cell division, those fertilized after two hours in sea-water took 108 minutes, those after three hours 101 minutes, and those after four hours only 100 minutes. The figures for the other three experiments show a similar increase in rate. This point has already been recorded by Morgan (9), although he made no exact observations on the times of segmentation. With regard to the *later* development, the hatching rates of this series show that the second fertilization continued to have a more rapid rate than the first ones. In Exps. 1 and 2, the parallel with the earlier segmentation ceased here, for in the latest fertilized eggs the rate of development of the embryo slowed down again. In Exp. 3, on the other hand, the

eggs fertilized after a previous stay of four hours in sea water hatched quickest of all, just as the 4-cell division of these eggs had been completed in the shortest period.

In comparing the last column of Table X. which gives the condition of the larvae, with that in which the times of hatching are recorded, it is seen that those larvae which emerge from the egg in the shortest time from fertilization are the healthiest.

In the four experiments of Series B the rates of early segmentation were noted, but not the times of hatching. The general result is the same as that of Series A, namely that the later fertilized eggs segment quicker, but Exp. 8 shows a marked irregularity difficult to account for. There is a fall from the 91 minutes of the $1\frac{1}{2}$-hour eggs to 80 minutes of the $2\frac{1}{4}$-hour eggs, after which the next fertilized lot give 87 minutes, and then the final batch drop to 76 minutes again. Finally in Exp. 6, there is a decrease in the times at which the 4-cell stage was attained from the 1-hour lot (99 minutes) to the $2\frac{1}{2}$-hour lot (81 minutes), after which there is a rise to 90 minutes again. This is a phenomenon similar to the change in the *hatching* rates in Series A. The segmentation time of the last fertilization in Exp. 5 is given in brackets, as it is not really justifiable to compare this observation, made on the very few eggs that were fertilized in this case, with the large numbers in the other batches.

Exps. 5 and 7 show that the increase of segmentation rates with succeeding fertilizations went hand in hand with a decrease in the percentages of eggs fertilized.

The experiments of Series C show an exactly reverse phenomenon to those of Series A and B. In every case the segmentation rates become *slower* with successive fertilizations. This is especially marked in Exp. 9, where there was a gradual rise from 82 minutes of the 1-hour fertilization to 93 minutes of the 5-hour lot. The two experiments forming Series D behaved similarly, although there was a final increase in the rate for the last fertilization of Exp. 13.

It was thought that there might be a connection between the change in temperature during the progress of the experiments and the alterations in the rate of segmentation. The evidence, however, seems to point rather against this. In Series A the temperature rose from 19·5° to 21·5° C. during the experiments. In Series C it remained constant, and in Series D rose from 18° to 18·5° C. If the rise of two degrees in the experiments of Series A will account for the increase in the segmentation rates, there is no fall in temperature in the later

series correlated with the decrease in the latter. Moreover in the first series, although the segmentation became more rapid in later fertilizations, the time taken to complete the 4-cell division was longer than that of the slowest segmentation in Series C. Nevertheless the temperature during the latter was throughout lower than during the former series of experiments. The possibility is, however, not excluded, and unfortunately time would not allow of a further investigation. Nevertheless the difference in behaviour of the eggs would seem to depend rather on some condition of the animals from which they are taken. The four series were made on four different days, and each with a different batch of animals brought into the laboratory. This, taken together with the fact that the experiments of each series agreed in general with one another, seems to support this view.

2. *Eggs and sperm from different parts of genital ducts.*

During the experiments made to investigate the effect of the "age" of eggs and sperm, as indicated by their relative position in the genital ducts, on the extent of self- and cross-fertilization, the segmentation rates of the fertilized eggs were noted. The lengths of time after fertilization at which the first 4-cell divisions took place are recorded in Table VII (p. 239) for some of the lots of eggs.

A comparison between the times taken by *C* eggs cross-fertilized: (1) by sperm a_1 from the base of the sperm duct, and (2) by sperm a_2 from the top of the sperm duct, in Exp. 1, shows that the segmentation rates were identical, although the percentages of eggs fertilized were very different in the two cases. Again, eggs from (1) base, (2) middle, and (3) top of the oviduct cross-fertilized by the same sperm, all segment at the same rate, although the percentages fertilized may be different. This is shown by the segmentation times in Exp. 1, cross A/m and Exp. 2, cross B/n of Table VII.

Thus, neither the position in the genital duct of the sperm used to make a cross, nor that of the eggs crossed causes any variation in the early rate of division of the segmenting eggs.

3. *Comparison of development after self- and cross-fertilization.*

The first experiment consisted in a comparison of the segmentation rates of (1) eggs self-fertilized (K/k), (2) eggs of the same individual cross-fertilized (K/p), and (3) eggs of another individual cross-fertilized by the same sperm as that used for the self-fertilization (L/k). In each case the eggs were fertilized at four different intervals after the genital

products had been removed into the sea-water. The details and results of the experiment are recorded in Table XI, and it will be noticed that the experiment is the same as that given in Table V, Exp. 5. In addition, the cross *K p* was made, and is tabulated here only, as the percentages of eggs fertilized were immaterial to the matter under discussion in the previous section.

TABLE XI. (14.18.1.)

For explanation of headings of Columns see Table X.

	Time of fertil.	Temp.	Percent. fertil	Time of 4-cells
Eggs *K*, cross-fertilized, *K/p*	$\frac{3}{4}$	18° C.	100	90
	$1\frac{3}{4}$	do.	86	92
	$2\frac{3}{4}$	18·5 C.	30	92
	$3\frac{3}{4}$	do.	7	91
Self-fertilization, *K/k* ...	$\frac{3}{4}$	18° C.	6	87
	$1\frac{3}{4}$	do.	10	87
	$2\frac{3}{4}$	18·5 C.	29	87
	$3\frac{3}{4}$	do.	1	91
Sperm *k*, cross-fertilized, *L/k*	$\frac{3}{4}$	18° C.	100	87
	$1\frac{3}{4}$	do.	65	87
	$2\frac{3}{4}$	18·5° C.	23	87
	$3\frac{3}{4}$	do.	8	85

The variations in the segmentation times of the eggs fertilized at different intervals in any of the three crosses were small. It is seen by comparing *K/k* with *L/k* that there is practically no difference in the rate of early segmentation of eggs self- and cross-fertilized. The four fertilizations *K/p* took rather longer to divide than the other two combinations. The results further emphasize the fact that the rates of segmentation do not depend on the percentages of eggs fertilized.

As was explained in the Introduction, the original scheme of work has not yet been carried out, namely that of rearing reciprocal cross-fertilized families to maturity, in order to find the degree of cross-fertility of sisters *inter se* and of members of one family with those of the reciprocal. This has not yet been attempted owing to the many preliminaries to be settled first, the investigation of which forms the substance of this paper. A number of families were, however, reared, partly in order to settle the optimum conditions of food, etc., (6), but more especially to compare the later development of animals derived from cross- and from self-fertilized eggs. The details of two typical experiments are given below.

It should be noticed in the first place that, whereas in all previous experiments in cross-fertilization very dilute sperm-suspensions were used so that the percentages of eggs fertilized should lie between 0 and 100, thus allowing of comparison, the fertilizations in the following cases were all made with an *excess* of sperm. The reason for this was, of course, that the object of the experiments was not to compare percentages of eggs fertilized under different conditions, but to obtain the maximum number (100 %) of developing eggs for rearing purposes.

In the first experiment (Table XII) *A* eggs were self-fertilized (*A*/*a*) and *B* eggs were self-fertilized (*B*/*b*). At the same time the reciprocal cross-fertilizations were made between these two individuals (*A*/*b* and *B*/*a*) and eggs of a third individual *C* were cross-fertilized with *a* sperm (*C*/*a*).

TABLE XII. (15.20.1.)

		Percent. fertil.	Time of 4-cells	Time of hatching	Settled down	Alive, 8 days old	Alive, 20 days old
Self-fert.	*A*/*a*	7	86	19, 37	V. few	None	—
	B/*b*	<1	83	19, 30	None	—	—
Cross-fert.	*A*/*b*	100	83	19, 15	Most	Equal number of *A*/*b* and *B*/*a*, more of *C*/*a*	Fewest of *A*/*b*, medium number *B*/*a*, most *C*/*a*
	B/*a*	100	83	19, 27	Many		
	C/*a*	100	86	19, 26	Most		

The Table shows that the excess of sperm gave in each cross-fertilization 100% of segmenting eggs. The second column of the Table shows that there was very little difference in the early segmentation rates of the five fertilizations. Both when *b* sperm was used to self-fertilize *B* eggs and to cross *A* eggs, the 4-cell stage was reached at the same time—83 minutes. Again, when *a* sperm was used to self *A* eggs and to cross *C* eggs the segmentation rates were identical (in this case 86 minutes for 4-cell stage), but when the *a* sperm fertilized *B* eggs the latter segmented a little quicker (83 minutes).

Thus the early segmentation was not slower in the self-fertilized eggs than in the cross-fertilized, but the times of hatching (given in the Table in hours and minutes after fertilization) show that the segmentation rate of the former slowed down a little in the later stages.

In the column headed "Settled down" is given the proportion of larvae in each case which fixed themselves after their brief free-swimming period. The proportions were roughly estimated by eye—the only method practicable—and are independent of the absolute numbers of larvae present in the different cultures, which varies, of course, with the percentages of eggs fertilized. It is striking that none

of the few larvae present in the *B/b* culture settled down, and very few in the *A/a*, whereas a large proportion fixed themselves in the cross-fertilized lots. The next examination was made eight days afterwards, when no individuals derived from self-fertilized eggs were found to have survived, while those from cross-fertilizations were growing rapidly.

The second experiment, made on the same lines as the first, is recorded in Table XIII.

TABLE XIII. (15.22.1.)

		Percent. fertil.	Time of 4-cells	Time of hatching	Condition of larvae	8 days old	20 days old
Self-fert.	*B/b*	< 1	—	—	—	—	—
	C/c	41	81	21, 48	Good, but not so vigorous as crosses	One or two alive	No survivors
Cross-fert.	*B/c*	100	82	21, 29	V. good	Many alive	Many alive
	C/b	100	82	21, 31	V. good	Many alive	Many alive
	C/a	100	80	21, 39	V. good	Many alive	Few alive
	A/c	100	80	21, 19	V. good	Many alive	Few alive

In this case, of the self-fertilizations only *C/c* gave enough segmenting eggs to make observations on. The rate of early segmentation was here slightly slower than in the crosses, and the time of hatching was considerably later. Moreover the *C/c* larvae hatched out were not so vigorous as those of the crosses, which were about equal to one another in this respect. Eight days later one or two only of the self-fertilized individuals which had fixed themselves were surviving, and after 20 days these were found to have died off.

It should be mentioned here that after the majority of the larvae had settled down in their different dishes, the latter were washed in a stream of sea-water to remove all larvae which had failed to fix themselves together with all that had settled on the surface-film[1]. The dished were then sunk in a large tank of water, so that the different cultures should be exposed to identical conditions during the growth of the young animals.

In the last column of Table XIII it will be noticed that after 20 days fewer of the crosses *C/a* and *A/c* were living than of *B/c* and *C/b*. Nevertheless, the former had shown a slightly quicker rate of early segmentation than the latter, and the time of hatching of *A/c* had

[1] In later experiments, where a strict comparison between the states of development in the various cultures was not wanted, the animals which settled on the surface-film were not discarded. They were indeed in most cases found to grow more rapidly than those on the walls of the dishes.

been the earliest in the crosses, although that of *C/a* had been the latest. There seems to be little or no correspondence between the relative rapidity of segmentation and the subsequent development.

The larvae of each cross which settled down on the first, on the second and on the third day after hatching were reared separately. Those from the first and second days developed equally well, but those from the third much worse in each case.

Besides the two experiments given in Tables XII and XIII, a considerable number of other similar ones were carried out. All agreed in showing that the early segmentation of self-fertilized eggs is little if at all slower than of cross-fertilized, but that the larvae of the former hatch out somewhat later. Very frequently, however, the larvae from the self-fertilizations do not fix themselves at all, but die off. When they do settle down they fail to develop further, perishing in the course of a few days. One exception only was found to the last statement. A self-fertilized culture (*A/a*) made on June 20th gave a low percentage of fertilization. Some of the larvae fixed themselves, and on June 22nd —more than a month later—four individuals were still alive. They had attained the length of 1 cm. and appeared to be very healthy. Young from both of the cross-fertilizations, *A/b* and *B/b*, however, grew much more rapidly.

VI. Summary of Experimental Results.

(1) A greater concentration of sperm is usually necessary to bring about any self-fertilization than would cross-fertilize 100% of "foreign" eggs.

(2) An increase in the concentration of the sperm-suspension causes an increase in the number of eggs self-fertilized.

(3) The proportion of eggs self-fertilized increases with the length of time the eggs and sperm have been in sea-water before fertilization is effected. The percentages rise to a maximum and then decrease again, the time of the maximum being different for each individual. The subsequent decrease in the percentages is probably due to a falling off of the "fertilizing power" of the sperm-suspension. It cannot be determined whether the rise in self-fertilization percentages is due to a change in the eggs or in the sperm. The *cross*-fertilization percentages decrease as the time the eggs and sperm lie in sea-water before fertilization increases.

(4) There is no uniformity in the fertilization percentages of eggs from different parts of an oviduct, fertilized with equal amounts of a given sperm-suspension. Sometimes eggs from the outer end, at others eggs from the inner end are more readily self-fertilizable. Nor is there any correspondence between the relative ease of self-fertilization and that of cross-fertilization of eggs from different parts of the duct. Probably the same applies to sperm taken from different regions of the vas deferens. Thus the degree to which eggs of a given individual can be self-fertilized varies with each batch produced.

(5) Contact with a suspension of "own" sperm decreases the ease with which eggs can subsequently be cross-fertilized.

(6) The rates of segmentation of eggs cross-fertilized at increasing lengths of time after the eggs and sperm have been brought into sea-water either increase or decrease.

(7) Cross-fertilized eggs from different regions of the oviduct segment at the same rates. This is also true for eggs cross-fertilized by sperm from different parts of a vas deferens.

(8) The rates of segmentation are independent of the percentages of eggs self- or cross-fertilized.

(9) The early segmentation rate is not slower in self- than in cross-fertilized eggs, but the former hatch out a little later. Many of the larvae from the self-fertilizations fail to settle down, and those which do almost always die off in the course of a few days.

(10) There is little or no relation between the relative rapidities of segmentation and the subsequent development of different lots of cross-fertilized eggs. In a given culture, however, the larvae which settle down last develop worst.

VII. Conclusion.

In comparing Castle's and Morgan's work with the present results, it is evident that there are races of *Ciona intestinalis* which differ considerably with regard to their capacity for self-fertilization. This is not exactly the same phenomenon as that originally discovered by Darwin in *Reseda* and recently reinvestigated from a hereditary standpoint by Compton (2). Here there are individuals which are completely self-sterile and others which are completely self-fertile. Compton's work is not as yet finished, but the results so far obtained indicate that in

Reseda self-fertility is a Mendelian dominant to self-sterility. For *Ciona*, however, in those individuals where self-fertilization can most easily be brought about, it takes place to a very much lesser degree than does cross-fertilization. A much greater concentration of sperm is needed to induce a comparatively high percentage of self-fertilized eggs than would easily cross-fertilize all the eggs of another individual. On the other hand many individuals were experimented upon at Naples in which no eggs at all could be fertilized with sperm from the same animal, although in general the Naples *Ciona* seems to be much more self-fertile than the races used by Morgan. Again, in *Reseda* apparently an individual is either self-fertile or self-sterile. This is not at all the case with *Ciona* at Naples, where a given animal may vary widely in its capacity for self-fertilization with each lot of eggs and sperm produced[1].

The question arises as to whether the sterility of hermaphrodite animals is confined to the genital products of the same individual; whether all individuals are equally fertile when crossed with one another, presupposing of course that the eggs and sperm are mature and in good condition. Morgan attempted to investigate this question in *Ciona* and concluded that all individuals are not equally fertile *inter se*. A discussion of Morgan's methods has already been given (Section II) and it is considered that his conclusions are unjustified. The question is not an easy one to attack owing to the difficulty of making different sperm-suspensions of nearly equal concentrations; for a small difference in the strength of a sperm-suspension makes a large difference in the proportion of eggs cross-fertilized. Nevertheless if there be considerable variations in the degree of cross-fertility, variations at all comparable with the extent of self-sterility, they would certainly be detected. It was not attempted to investigate the question in the present work, but it can be stated that in practically every case 100% of segmenting eggs could be obtained as a result of cross-fertilization, provided that enough sperm was used. There were of course a few cases in which some of the eggs were obviously pathological and could therefore not be fertilized, but this does not affect the question. It is possible that the degree of cross-fertility may be much less in nearly related individuals (cf. Correns (4)), but this naturally cannot be decided with material taken from the sea. It is hoped that the laboratory cultures now being reared will shed further light on the subject.

[1] This may have been something like the same phenomenon in Correns' *Cardamine*, for repeated pollinations with the same plant did not always give the same result.

The fact that self-fertilization can usually only be brought about at all in concentrated sperm-suspension suggests that in the water containing the crowded spermatozoa there may be a substance which favours the self-fertilization. This cannot be connected with the reaction of the water, however, as the following test shows. A milky suspension of *Ciona* sperm was filtered. Five minutes after the suspension had been made the filtrate gave the same reaction as normal sea-water with neutral red and with phenolphthalein. An hour and a half afterwards the latter indicator showed that a freshly filtered sample of the same thick suspension was very slightly more acid than normal sea-water. But, as will be shown in Part II, acid diminishes, not increases, the fertilizing power of a sperm-suspension. Experiments also will be made in the following way to test whether the thick sperm-suspension contains substances which favour fertilization. A concentrated sperm-suspension will be made up and then filtered through a porcelain filter which keeps back spermatozoa. Exact comparative experiments will then be made to compare the effect of this filtrate with that of ordinary sea-water on the proportion of eggs fertilized by a definite concentration of sperm. It may be, however, that self-fertilization takes place only with concentrated sperm-suspensions because only one spermatozoon out of a very large number has the power of fertilizing eggs derived from the same individual.

The usual (though not invariable) progressive increase in capacity for self-fertilization shown by eggs and spermatozoa as they lie in sea-water suggests a connection with the maturation of the eggs. Golski (7) has shown that in the unfertilized egg of *Ciona* the first maturation spindle is already formed, but the first polar body is not given off until the spermatozoon has entered[1]. It might be that the increase in self-fertility is connected with the preparation for the first maturation division. At all events some change must come about in the eggs or spermatozoa or both after they have been shed into the sea-water. It is very interesting to note in this connection that self-fertilization can only take place to a very small extent in nature. For the only time that the eggs are in the presence of concentrated sperm of the same individual is for the few moments after the genital products have been exuded from the ducts before they are ejected through the atrial aperture: but at this time the eggs are usually not yet in a condition to allow of any self-fertilization. When they have reached this condition

[1] I have not been able to obtain a copy of Golski's paper, but believe this statement to be correct.

some time after having been deposited, the eggs are no longer in the presence of the concentrated "own" sperm, which has become diffused in the surrounding water and washed about by waves and currents. In allowing an animal to deposit eggs and sperm when isolated in a small dish, the conditions are quite otherwise, for the water into which the eggs are deposited becomes at the same time charged with spermatozoa. The latter can subsequently self-fertilize the eggs, to a greater or less extent according to concentration and individual capacity, when the optimum time has been reached.

The cause of self-sterility is hardly touched upon in this investigation. The search for a cause led to the investigation of substances secreted by eggs into sea-water. The results of this work form the substance of a separate Part, which, however, deals with cross-fertilization alone. So many points had to be settled with regard to the effects of the egg-secretions on spermatozoa used to effect ordinary cross-fertilization in *Ciona* and other forms, that hardly any self-fertilization experiments had been made. It is therefore really premature to discuss possible means by which self-sterility is brought about. The experiments detailed in Section IV above indicate, however, that one of the factors may be a change set up in the egg by contact with spermatozoa of the same individual—a change which inhibits the entrance of such spermatozoa, and also, although to a much lesser degree, hinders the entrance of "foreign" spermatozoa.

As is well known, Darwin (5) first made a thorough investigation of the effects of self-fertilization on the offspring, showing that in flowering plants such offspring are not in general so vigorous as those derived from cross-fertilization. In *Ciona* this effect of self-fertilization was very striking. As has been shown above, the early segmentation is not usually slower in self- than in cross-fertilized eggs. It is interesting to note also that there is no difference in the segmentation rates of self-fertilized eggs from different parts of an oviduct, although the various lots may show quite different capacities for self-fertilization. The later segmentation of self-fertilized eggs is usually a little slower than that of cross-fertilized. In a few cases the larvae failed to hatch well, that is to say, they had difficulty in breaking out of the egg membranes, but this was not usually the case. In many cultures derived from self-fertilizations the larvae failed to settle down after their free-swimming period, but in most instances they settled down in the same way as those from cross-fertilizations. It was after the settling down and metamorphosis, however, that the marked effect usually showed itself.

With one exception, in which four individuals were reared for over a month, in all the cultures derived from self-fertilizations the young metamorphosed animals died off during the first week. This was very striking in contrast with cultures from cross-fertilizations in which the animals grew rapidly from metamorphosis onwards. Potts (13) states that "The pathological development which Castle found characteristic of self-fertilized embryos did not occur in my experiments." It is plain, however, that the effect of self-fertilization does not usually manifest itself in the embryo, but at, or more usually after, metamorphosis.

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PART II. THE ACTION OF EGG-SECRETIONS ON THE FERTILIZING POWER OF SPERM.

CONTENTS.

I. Introduction.

The investigations described in this paper arose out of experiments on self-fertilization in *Ciona intestinalis*, an account of which is given in the previous part of this paper. The present experiments were carried out in part over the same period as those on self-fertilization, namely during the spring and summer of 1913. The whole of the work was done at the Zoological Station, Naples, and to the staff of this institution I owe my very best thanks for the willing advice and help given to me throughout my stay.

The fact of self-sterility in *Ciona*, whether comparative or absolute, suggested that some substance may be exuded by the eggs which paralyzes the spermatozoa of the same individual, but does not affect those of other individuals—or there might be something within the egg envelope which acts in the same way. To test this hypothesis experiments were commenced by trying the action of suspensions of crushed eggs in sea-water on the spermatozoa. It was soon found that there was a very large number of questions with regard to the action of such egg-extracts on spermatozoa used to effect ordinary cross-fertilization which must be investigated and cleared up before the more special question of the mechanism of self-sterility could be attacked. The outcome of these investigations with cross-fertilization is recorded in this part of the paper.

The results obtained are all based on *numerical* data. They depend on the comparison of the proportions of eggs fertilized by sperm-

suspensions after either the eggs or the sperm have been subjected to certain preliminary treatments. Thus the validity of the results depends altogether on the way in which the experiments were carried out; that is to say, on whether the conditions of experiment allowed of any such numerical comparison of the after-effects and of deductions to be drawn from them. For a detailed description of the precautions taken to ensure that the experiments should be of an *exact* nature the reader is referred to the Part on "The conditions of self-fertilization in *Ciona intestinalis*" since the methods used were identical with those employed in this case. A short résumé of the technique is, however, given in the following section. It is thought that these methods will break a new road for the investigation of fertilization phenomena, for work hitherto done on this subject, in which the results have been based on numerical counts, seems to have been carried out under such comparatively inexact conditions that it is usually extremely difficult to judge the extent to which the results are valid.

II. Methods.

The method of experimentation was the same as that employed in investigating the conditions of self-fertilization in *Ciona intestinalis*. For a detailed account of these methods reference should be made to Part I.

Essentially the method consisted in an exact comparison of the percentages of eggs fertilized by sperm-suspensions of identical concentrations, after either the eggs or the sperm had been treated in different ways prior to the fertilization. The results of such treatment were judged by the relative ease or difficulty with which fertilization could subsequently be brought about. The sperm-suspensions used were always so dilute that they would not cause the fertilization of all of the eggs to which they were added. In this way comparisons could be instituted, since the percentages of eggs fertilized in the different lots lay between 0 and 100.

The term "fertilizing power" of a sperm-suspension is employed in the following sense. If a sperm-suspension of a certain concentration can bring about the fertilization of say 40 °/₀ of the eggs after it has been treated in a definite way, whereas another suspension of the same sperm, of identical concentration but not having been subjected to the treatment, only causes 20 °/₀ of the eggs to be fertilized, it is said that the "fertilizing power" of the sperm has been increased by this treatment.

A capital letter (e.g. *A*) is used to denote the eggs of a certain female, and a small letter (e.g. *b*) the sperm of a certain male. An experiment to test the effect of solution X on the fertilizing power of sperm *b* would be carried out as follows. The object is to inseminate approximately equal quantities of *A* eggs with (1) a plain dilute suspension of *b* sperm, and (2) a suspension of *b* sperm of identical concentration as suspension (1), but to which solution X has been added. To obtain (1) and (2), one sperm-suspension is made up and diluted to the required amount by the further addition of sea-water. Equal quantities are pipetted into two dishes. To the first dish is added a certain quantity (*n* cc.) of sea-water; to the second an equal quantity (*n* cc.) of solution X. These two suspensions (1) and (2) are then added to the two lots of eggs and the effect of solution X is judged by the relative numbers of eggs fertilized in the two dishes.

It is obvious that the whole validity of this method depends on the degree of exactness with which all other conditions are kept identical for the two fertilizations, while the one factor alone—here the presence or absence of solution X in the sperm-suspension—is altered. Of especial importance is the identical concentration of the two (or more) sperm-suspensions, and the complete mixing of the eggs and spermatozoa in making the different fertilizations, so that all eggs have an equal chance of being fertilized. The precautions taken to ensure this exactness, that is to say, the method of making up the sperm-suspensions, of subdividing and treating the eggs and sperm, the way in which insemination is effected, together with the method of counting the proportions of fertilized and unfertilized eggs, are given in full in the Part on the "Conditions of self-fertilization in *Ciona*."

The results of each series of experiments are tabulated, the numbers in the Tables being the percentages of eggs fertilized, unless otherwise stated.

Eggs from a single individual and sperm from another single individual were used in each experiment. It goes without saying that the presence of genital products of any other individual was always rigidly excluded. All glassware and instruments were sterilized with hot water before each operation, and the hands were dipped into hot water. All sea-water employed was taken from the circulation and passed through a Berkefeld filter. The method employed in removing the eggs and sperm from *Ciona intestinalis* has already been described in the part (Part I) on self-fertilization in this form. Similar methods were used in taking the genital products from the other Ascidians used

in the investigation, namely, *Ascidia mentula* and *Phallusia mamillata*. Besides these forms, four species of Echinoids—*Strongylocentrotus lividus*, *Sphaerechinus granularis*, *Echinus microtuberculatus*, and *Arbacia pustulosa*—were made use of, together with one Asteroid, *Asterias glacialis*. The procedure in obtaining the eggs and sperm of the sea-urchins was this. Each individual was washed under a stream of fresh-water to destroy any adherent spermatozoa, after which it was opened by an equatorial cut. The aboral half was then reversed over a dish of sea-water, so that the genital pores just dipped beneath the surface. If the animal was mature, large quantities of eggs or sperm as the case might be were shed into the dish of water, with very little admixture of body cavity fluid[1]. The eggs of *Asterias* were obtained by cutting open the body wall, after the animal had been washed in fresh-water, and removing portions of the ovary. These were placed in sea-water, which was presently filtered through bolting silk to strain off the pieces of ovarian tissue from the eggs.

The efficacy of these precautions was tested in every case by keeping a sample of eggs separate, to which no sperm had been added. In no experiment recorded here did a single egg in the control segment.

By "egg-extract" is meant a suspension of crushed eggs in sea-water. The eggs were crushed between two sterilized glass plates and the substance so obtained washed off the glass into sea-water. By this means a very finely divided suspension can be made, and presumably soluble substances present in the eggs go into solution in the sea-water. The suspension was examined after having been made up, to be certain that no uncrushed eggs were lying in it, the subsequent fertilization of which would destroy the value of the percentages. Throughout the work the egg-extract was made up of more or less the same concentration, although it is impossible to make up two samples of absolutely identical strength. For every 12 cc. of suspension, two drops of eggs were crushed between the plates and then added to the water.

"Ovary-extract" of *Ciona* was made up in the same way as the egg-extract. In this case special precautions are necessary that none of the spermatozoa from the testis have been removed with the ovary. To check this possible source of error, unfertilized eggs from another individual were in each case left lying in a sample of the ovary extract and afterwards examined to see that none had segmented.

"Egg-water" is used to designate sea-water in which eggs have been lying for a longer or shorter interval.

[1] I am indebted to Dr Otto Koehler for bringing this method to my notice.

Ciona blood was removed from the body in the following way. An animal was washed in a stream of fresh-water. After this an incision was made in the body-wall in the region of the heart, which then bulged out through the cut. The animal was held over a small glass dish with the cut downwards, and the projecting heart was then punctured, so that the contained fluid flowed out into the dish. The comparatively small volume of blood obtainable from one animal was usually insufficient for carrying out an experiment, and in such cases the volume of liquid was increased by the addition of sea-water. The possible presence of spermatozoa in the blood was checked in the same way as with the ovary-extract. Unfertilized eggs from another individual were placed in a small quantity of the blood and later on examined to make sure that none had segmented.

III. Effects of Egg- and Ovary-Extracts on Fertilization Percentages.

1. *Cross-fertilization*[1] *of Ciona in egg-extract.*

The first experiments were made to try whether, when cross-fertilization of *Ciona* was brought about in the presence of crushed eggs of the same species, the percentage of eggs fertilized was raised or lowered. As in all subsequent experiments the method was strictly *comparative*. In each experiment, approximately equal numbers of eggs of one individual were placed in equal quantities of (1) sea-water, and (2) extract of the eggs of another individual. After a certain interval equal quantities of well-mixed dilute sperm-suspension from a third individual were added simultaneously to the two lots of eggs and mixed. When in the 4-cell stage, the percentages of eggs fertilized in each lot were counted[2].

TABLE I.

(The bracketed numbers give the time in minutes after fertilization at which the first 4-cell division was completed.)

In each experiment the eggs lay in (1) water, (2) extract for forty minutes before fertilization.

		Water	Egg-extract
Exp. I.	(16.27.1.) ...	37 (87)	100 (87)
Exp. II.	(16.28.1.) ...	12 (89)	95 (89)
Exp. III.	(16.29.1.) ...	3	19

[1] The term "cross-fertilization" is here used to mean the fertilization of the eggs of a hermaphrodite animal by the spermatozoa of another individual. No self-fertilizations were made in this investigation.

[2] As in all the experiments described in this paper, samples of the lots of eggs were kept unfertilized as controls, to guard against accidental contamination with foreign sperm. This is to be taken as granted in the descriptions of subsequent experiments.

The Table shows that in each of the experiments more eggs were fertilized in the water containing the egg-extract than in plain sea-water. The suggestion at once arose—were the eggs in the egg-extract developing in part parthenogenetically? This was ruled out of court by controls of unfertilized eggs in egg-extract, none of which segmented.

The egg-extract present at fertilization and during the early stage seems to have no baneful effect on development. Table I shows that in Exps. I and II the eggs in plain water completed the 4-cell division simultaneously with those in the egg-extract. Moreover, in a further experiment (17.29.1) in which the eggs *B* were fertilized by sperm *a* in (1) water, (2) *B* egg-extract, (3) *A* egg-extract, (4) *C* egg-extract, the resulting larvae all developed equally well.

Evidently some substance extracted from the eggs by breaking them up in sea-water so affects the eggs or the sperm or both that more eggs are fertilized than is the case in plain sea-water, when other conditions are equal.

An experiment (22.7.2) was then made, in which equal quantities of *A* eggs were put simultaneously into (1) four dishes, each containing 12 cc. sea-water, (2) four dishes, each containing 12 cc. *B* egg-extract. At given successive intervals one drop of *b* sperm was added, at the same moment, to (1) a dish of water containing *A* eggs and (2) a dish of *B* egg-extract containing *A* eggs. Each was then well mixed.

TABLE II.

(The numbers in brackets give the time in minutes after fertilization at which the first 4-cell division was completed.)

Eggs tried out into dishes containing (1) water, (2) extract at 12.20.

		(1) Water	(2) Extract
Cross: *A*/*b*, *B* extract.	Fertilized 12.20	62 (88)	91 (88)
	Fertilized 12.40	53 (89)	57 (89)
	Fertilized 1·0	31 (89)	57 (89)
	Fertilized 1.20	24 (84)	52 (84)

This experiment was, in the first place, a confirmation of the previous ones, in that the eggs fertilized in the egg-extract all showed higher fertilization percentages than those fertilized after a corresponding length of time in water. In this case the egg-extract was obtained from the same individual from which the sperm was used.

The Table also shows that although the fertilization percentages decreased with each fertilization, the length of time taken to attain the 4-cell stage became shorter in the last fertilization (see Part I). As

in the last experiments, the segmentation rates were identical in each pair of fertilizations for eggs in water and in egg-extract.

Finally the experiment shows that the extract has an *immediate* effect in raising the percentage, since the first pair of fertilizations was made as soon as the lots of eggs had been tried out into water and extract. The falling off of the percentages in subsequent fertilizations was less rapid in the eggs in extract than in those in water.

2. *Cross-fertilization of Ciona in boiled egg-extract.*

An experiment was made (21.3.2) to discover whether heating the egg-extract altered its effect on fertilization.

Extract was made of *C* eggs and a portion heated to boiling-point. Approximately equal numbers of *A* eggs were placed in (1) 12 cc. water, (2) 12 cc. unboiled egg-extract, (3) 12 cc. boiled egg-extract. After 33 minutes one drop of diluted sperm-suspension *b* was added to each.

TABLE III.

	Water	Extract	Boiled extract
Cross: *A*/*b*, *C* extract	1	9	8

The counts of eggs fertilized show that both boiled and unboiled extracts raised the percentages to an almost equal extent. The difference of 1 % between the two lies within the limits of experimental error.

Thus the value of the egg-extract is not destroyed by boiling for a short time. No further experiments were made on the stability of the extract as there were more important questions to be decided first.

3. *Cross-fertilization of Ascidia in egg-extract.*

The question then arose as to whether the effect of egg-extract on fertilization percentages was confined to *Ciona* or was to be found in other forms as well. The next animal to be tried was *Ascidia mentula*.

Approximately equal numbers of eggs *D* were placed in each of (1) two dishes containing 17 cc. of sea-water, (2) two dishes containing 17 cc. *C* egg-extract. Thirty-three minutes afterwards, two drops of a weak sperm-suspension *c* were added (1) to a dish of water containing eggs, (2) to a dish of egg-extract containing eggs. At the same time five drops of a stronger sperm-suspension *c* were added (1) to a dish of water containing eggs, (2) to a dish of egg-extract containing eggs. The experiment was thus double, the comparison being made first with weaker and then with stronger sperm.

TABLE IV. (1.2.2.)

		(1) Water	(2) Extract
Cross: *D/c*, *C* extract	Weaker sperm	0	8
	Stronger sperm	<1	36

In both parts of the experiment the fertilization percentage was greater in egg-extract than in water. It will be shown later on that this is not only the case for *Ciona* and *Ascidia*, but is the same for all the forms tried.

4. *Cross-fertilization of Ciona in ovary-extract.*

In the following experiment, a comparison was made between the percentage of eggs fertilized (1) in plain sea-water and (2) in ovary-extract, instead of egg-extract.

Approximately equal amounts of *A* eggs were placed in (1) 12 cc. water, and (2) 12 cc. *C* ovary-extract. After 38 minutes each was fertilized by the addition of one drop of *b* sperm-suspension. Besides the usual control of unfertilized eggs *A*, some of the latter were also put into *C* ovary-extract to guard against the accidental presence in this of sperm from the testis of the same animal[1].

TABLE V. (20.1.2.)

		(1) Water	(2) Ovary-extract
Cross: *A/b*, *C* extract	...	86	98

As shown by Table V, the presence of the ovary-extract caused an increase in the percentage of eggs fertilized.

The next experiment was made on similar lines, but three different concentrations of ovary-extract were used.

Approximately equal amounts of eggs *E* were tried out into four dishes containing respectively equal quantities of water and of three different concentrations of *D* ovary-extract, as is shown in Table VI. After 34 minutes, each was fertilized by the addition of one drop of *f* sperm-suspension.

TABLE VI. (20.1.2.)

	16 cc. water	15 cc. water 1 cc. extract	8 cc. water 8 cc. extract	16 cc. extract
Cross: *E/f*, *D* extract	68	91	92	94

As is seen from the above Table, this experiment was a confirmation of the last. (The weakest extract was here almost sufficient to raise the percentage of fertilization to the maximum.)

[1] This was done in all other experiments in which ovary-extract was used.

5. *Comparison of the effects of egg- and ovary-extracts on the fertilization in Ciona.*

The increase in the number of eggs fertilized in the presence of the extracts might be due to some substance contained in ripe eggs alone. In the case of the ovary-extract, it would then be derived from ripe eggs present in the lumen of the ovary. Whether this is so, or whether the substance is contained in the ovarian tissue itself, can be determined by making a comparison of the effects of equal concentrations of egg- and ovary-extracts on the fertilization. The following experiment was made to determine this point.

Approximately equal numbers of *B* eggs were placed in 16 cc. of (1) water, (2) *A* egg-extract in three concentrations, (3) *A* ovary-extract in three corresponding concentrations. The strongest egg- and ovary-extracts were made up in approximately equal concentrations by taking as nearly as possible equal volumes of eggs and ovary, and washing the juices into equal quantities of water. Fertilization was effected by the addition of one drop of *c* sperm-suspension to each of the dishes.

TABLE VII. (20.4.2.)

Cross: *B*/*c*, *A* extract	16 cc. water 0 cc. extract	15 cc. water 1 cc. extract	8 cc. water 8 cc. extract	0 cc. water 16 cc. extract
Egg-extract	0	2	13	33
Ovary-extract		3	21	36

The Table shows that an increasing number of eggs were fertilized in increasing concentrations of both egg- and ovary-extracts. The numbers were, however, not identical in corresponding concentrations of the two extracts, so that the presence of the substance which aids fertilization in equal amounts in both ripe eggs and ovary is not proved. Indeed the ovarian extract had a greater effect than that derived from the eggs. It must be remembered, however, that it is exceedingly difficult to obtain egg- and ovary-extracts in exactly equal concentrations, and to such a difference in concentration the differences in effect shown in the Table might easily be due.

6. *Comparison of the effects of "foreign" and "own" egg-extracts on the cross-fertilization percentages in Ciona*[1].

The experiments with egg-extracts were originally made to test whether there is some substance in the eggs of *Ciona* which hinders

[1] "Foreign" means from another, and "own" from the same individual.

fertilization by their "own" sperm, i.e. by sperm from the same individual. It was thought that if there were such a substance, its presence in the sea-water should prevent sperm taken from the same individual as itself from cross-fertilizing the eggs of another individual. That such is not the case is demonstrated by the experiments shown in Tables II and IV, in which the extracts were taken from the same individuals as those from which the sperm was derived. These extracts caused an *increase* in the number of eggs fertilized in their presence.

It might be, however, that an egg-extract from the same individual as the sperm had a lesser stimulating effect than one from a "foreign" animal. In the much smaller concentration in which the substance must be present at the surface of eggs in the sea than in artificial egg-extracts, "own" extract might have no perceptible favouring influence on fertilization, while the influence of that from another individual would be great enough to be effective. In this way, the difficulty of effecting self-fertilization could be accounted for.

The hypothesis was put to the test in the following experiment. Approximately equal amounts of *A* eggs were put into (1) 10 cc. water, (2) 10 cc. of egg-extract derived from the same animal as the eggs (*A*), (3) 10 cc. of egg-extract derived from the same animal as the sperm (*B*), and (4) 10 cc. of extract derived from a "foreign" animal (*C*). After 35 minutes, one drop of *b* sperm-suspension was added to each dish. It should be noted that the three extracts were made as nearly as possible of equal concentration.

TABLE VIII. (17.29.1.)

	Water	*A* extract	*B* extract	*C* extract
Cross: *A*/*b* ...	21	63	62	63

The Table shows that the extracts derived from the three individuals had practically the same stimulating effect on fertilization. The difference of 1% lies within the limits of experimental error.

The foregoing experiments show that self-sterility in *Ciona* cannot be accounted for by the presence of a substance in the eggs of an individual *B*, which not only prevents the union of *B* eggs with *b* sperm, but also of "foreign" eggs (*A*) with *b* sperm. Nor is it due to something contained in *B* eggs which favours fertilization of eggs *A* by *b* sperm relatively less than does a substance contained in the "foreign" eggs. At any rate, if such an inhibiting substance be present, its effect is masked by stimulating substances contained in the egg-extracts.

The most important experiments in regard to this question, however, are those to find the influence of egg-extracts, both from "own" and "foreign" eggs on self-fertilization itself. Some such experiments have been made already, but these must be extended and confirmed before being published.

IV. Does the Extract affect the Eggs or the Sperm?

The experiments already described have shown that if a given number of eggs be mixed with a certain concentration of sperm-suspension in sea-water, fewer eggs segment than if approximately the same number of eggs are inseminated by exactly the same concentration of sperm in egg- or ovary-extract, other factors remaining the same. Now this result might be due to a "stimulating" effect of the extract on the spermatozoa alone, or to some change brought about in the eggs so that they can be more easily fertilized, or to a combination of both causes. The experiments described in the following sections were made to settle this question.

1. *Effect of extract on the sperm of Ciona.*

In each of the experiments recorded in Table IX, a sperm-suspension was made up in the usual way. 5 cc. of this was mixed with 5 cc. of water (= Sperm α), and at the same time 5 cc. was mixed with 5 cc.

TABLE IX. (23.10.2.)

Sperm in each experiment: { α = 5 cc. sperm-suspension + 5 cc. water. / β = 5 cc. sperm-suspension + 5 cc. extract.

	(1) α sperm	(2) α sperm + 1 drop extract added to water at fertilization	(3) β sperm
Exp. 1. *E/f*, *D* extract ...	<1	<1	6
Exp. 2. *H/i*, *G* extract ...	0	0	14
Exp. 3. *J/l*, *J* extract ...	0	0	17
Exp. 4. *N/o*, *M* extract ...	0	0	11
Exp. 5. *N/q*, *M* extract ...	5	5	12

of the egg-extract (= Sperm β), suspensions α and β being thus of identical concentrations. The eggs to be fertilized were divided into three equal lots, each in 16 cc. sea-water. Thirty minutes after the sperm mixtures had been made up, fertilization of the three lots of eggs was effected as follows:—(1) addition of one drop of Sperm α; (2) addition of one drop of Sperm α, and at the same time one drop of egg-extract; (3) addition of one drop of Sperm β. The fertilization (2) was made to

test whether or not the drop of egg-extract necessarily introduced with Sperm β itself influences the fertilization, or whether Sperm β had been altered by its treatment with extract.

The experiments all agreed in showing that if two suspensions of sperm from a given animal are made up of equal concentrations in (a) water and (b) egg-extract, suspension (b) is capable of fertilizing more eggs than is (a). The addition of a drop of extract to the eggs at the same time as the sperm from the water-suspension (a) caused no increase in the percentage of eggs fertilized (see column (2) of Table), proving that the presence of this small quantity of extract at the moment of fertilization was not reason for more eggs being fertilized by (b). Evidently the sperm which had been treated with egg-extract before being added to the eggs was stimulated in such a way that more spermatozoa were capable of fertilizing eggs than was the case when the sperm had previously been in sea-water alone.

Two more experiments were made confirming the above conclusions. In each case the sperm was treated, before being used to fertilize the eggs, with different concentrations of egg-extract. Moreover, instead of lying in the extract for half an hour before being used, as was the case in the last experiments, the suspensions were added to the eggs as soon as they had been well mixed by pouring. The results showed that in the short time necessary for the latter operation, the sperm was already stimulated. Increasing numbers of eggs were fertilized by sperm from increasing strengths of extract.

The details of the experiments are as follows:

In Exp. 1 the sperm-suspension was made up and then three drops were added to each of four dishes containing (1) 10 cc. sea-water, (2) 8 cc. water + 2 cc. extract, (3) 4 cc. water + 6 cc. extract, (4) 10 cc. extract. These four liquids, containing identical concentrations of sperm, were poured on to four equal quantities of eggs in separate dishes.

In Exp. 2, three drops of sperm-suspension were added to (1) 10 cc. water, (2) 7 cc. water + 3 cc. extract, (3) 10 cc. extract. These were then poured on to three equal quantities of eggs.

Table X gives the result of the experiments, which confirm those of Table IX, and also show the effects of varying concentrations of extract on the sperm.

TABLE X. (34.16.4 and 34.21.4.)

	Sperm (1)	Sperm (2)	Sperm (3)	Sperm (4)
Exp. 1. *A/b*, *C* extract	8	12	74	82
Exp. 2. *D/e*, *F* extract	< 1	52	69	—

2. *The effect of extract on the eggs of Ciona.*

The experiments described in the preceding section showed clearly that treatment with egg-extract increases the fertilizing power of a sperm-suspension. That is to say, such a suspension is capable of fertilizing more eggs than sperm of equal concentration made up in plain sea-water, other conditions remaining unchanged. This does not prove, however, that the increased percentages of eggs which segmented in the initial experiments when fertilized in the presence of extract, over those fertilized in plain water with the same amount of sperm, were to be attributed *only* to the action of the extract on the spermatozoa. The substances in the egg-juice might also have influenced the eggs, making them more receptive to spermatozoa. In the experiment to be described next, the action of the extract on the spermatozoa was as far as possible eliminated. Eggs were treated with extract, and the proportion subsequently fertilized by the addition of a given amount of sperm was compared with that in eggs not previously so treated.

The eggs of an individual were divided into two lots and placed in (1) water, and (2) ovary-extract, and left for 43 minutes. After this interval, approximately equal numbers of eggs were tried out as follows:

(a) Eggs in 1 cc. water, from (1).
(b) Eggs in 1 cc. extract, from (2).
(c) Eggs in 1 cc. extract, from (2).

Immediately before fertilization a sperm-suspension was made up, and equal amounts were dropped into (α) 30 cc. water; (β) 30 cc. water; (γ) 30 cc. extract. Fertilizations were effected by pouring (α) on to (a); (β) on to (b); and (γ) on to (c). The large amount of water (30 cc.) added to the eggs with the sperm was to dilute the extract in (b) as much as possible, in order to prevent action on the sperm.

The experiment was made double, the first series being when three drops of sperm were added to (α), (β), and (γ), and the second when ten drops were added to each.

TABLE XI.

	I Eggs (a) from water Sperm (α) from water	II Eggs (b) from extract Sperm (β) from water	III Eggs (c) from extract Sperm (γ) from extract
B/c, *A* extract.			
3 drops sperm ..	1	2	52
10 drops sperm ...	5	6	89

The comparison of the percentages of eggs fertilized in I and II in the Table shows whether or not the extract had affected the eggs, which

had lain in it before being fertilized. III was made to show the effect of extract on the sperm, for comparison.

The Table shows that in both parts of the experiment—with the stronger sperm and with the weaker sperm—the percentages in II were 1 % higher than those in I. This fact may be due to one of three causes. It may be experimental error; but the fact that the difference is the same in both parts of the experiment argues against this. Again, it may be that the eggs (*b*) were affected by their stay in the extract previous to fertilization, so that they became more easily fertilizable—although to a very slight extent. Lastly, the increase may have been due to the action of the 1 cc. of extract introduced with the eggs (*b*) on the sperm, at the moment of fertilization. A comparison of these figures with those in III, which shows the very large increase in the fertilizing power of the sperm caused by a previous treatment with extract of the full strength, shows that the last of the three possible explanations given above is almost certainly the correct one.

Thus from the experiments recorded in Tables IX, X and XI, we can draw the conclusion that the excess in the number of eggs fertilized by a given amount of sperm in the presence of extract over the number fertilized in plain water is due to an action of the extract on the sperm and not on the eggs.

3. *Effect of egg-extract on the fertilizing power of the sperm of Strongylocentrotus.*

The experiments already recorded on the influence of egg- and ovary-extracts on the proportion of eggs fertilized by a given concentration of sperm-suspension have, with one exception, all been made with *Ciona intestinalis*. In the following section, experiments are described on the effect of egg-extract on the fertilizing power of the spermatozoa of *Strongylocentrotus lividus*.

The first experiment is a plain comparison of the effects of fertilizing with sperm-suspensions of equal concentration, made up in (1) plain sea-water, and (2) egg-extract. The experiment was made for four different strengths of sperm.

A sperm-suspension was made up, and a given number of drops, added to (1) 10 cc. sea-water, (2) 10 cc. extract. Each of these were then poured on to approximately equal numbers of eggs.

The Table shows that the extract had the same effect on the percentages of eggs fertilized as was the case with *Ciona* and *Ascidia*.

If equal amounts of the same sperm-suspension be added to equal

quantities of (1) plain sea-water and (2) egg-extract, and a drop of each compared under the microscope, the spermatozoa are very much more active in the extract suspension. Moreover, if eggs be put in

TABLE XII. (1.26.2.)

		Water	Extract
Cross: *A/c* *A* egg-extract	1 drop sperm	0	7
	4 drops sperm	1	42
	8 drops sperm	1	49
	12 drops sperm	1	86

each of the drops, the number of spermatozoa which collect round the eggs is considerably greater in the latter suspension. These effects, which are very obvious in *Strongylocentrotus*, are hardly noticeable in *Ciona*, where the sperm is always much more sluggish in its movements.

The greater activity of the spermatozoa of *Strongylocentrotus* in the presence of egg-extract suggests that the greater proportion of eggs fertilized by such suspension is due to more spermatozoa coming into contact with eggs, owing to their quicker movement. This is, however, at all events not the whole explanation, since Lillie (3) has shown that the egg-extracts have a positive chemotactic effect on the spermatozoa, as well as an activating influence.

The following two experiments were made to determine the effect on the fertilizing power of the sperm of treatment for varying lengths of time with egg-extract.

In each experiment a sperm-suspension was made in sea-water and 1 cc. was pipetted into each of (1) four dishes, each containing 5 cc. of plain sea-water, and (2) four dishes, each containing 5 cc. of egg-extract. These suspensions of equal concentration were used for fertilization immediately and at ten minute intervals. At each fertilization, 5 cc. plain water was added to (1) a dish of water sperm-suspension, and (2) a dish of extract sperm-suspension. After the usual thorough

TABLE XIII. (4.7.6.)

	Times of fertilization after making sperm suspensions (1) and (2)	(1) Water	(2) Extract
Exp. 1. *A/b*	Immediately	61	69
	10 minutes	10	53
	20 "	4	27
	30 "	0	8
Exp. 2. *A/c*	Immediately	89	93
	10 minutes	17	72
	20 "	4	32
	30 "	1	10

mixing, these were poured separately on to approximately equal amounts of eggs.

In the two experiments the fertilizing power of the sperm-suspension decreased rather rapidly, both in water and extract, but at each succeeding fertilization more eggs were fertilized by the extract-sperm than by the water-sperm. These experiments are a parallel to that recorded in Table II for *Ciona*.

F. R. Lillie (15, p. 558) states that "the powerful effect of the egg-extract on spermatozoa of the same species may be shown by a complete loss of motility, as we have already seen, and also by a corresponding loss or diminution of the fertilizing power." He then describes an experiment illustrating this. To five watch glasses containing each eight drops of water or of different concentrations of egg-extract were added three drops of "opalescent" sperm-suspension. After 12 minutes "a drop of a suspension of fresh eggs was added to each." 5 % of the eggs in the water segmented, but none of those in the four different concentrations of extract.

That this was quite contrary to my own results is clearly seen in the experiments recorded in Table XIII. I determined, however, to repeat exactly the experiment of Lillie's described above. I made the fertilizations in the very small amounts of liquid which Lillie used, although my preliminary trials had shown that this is an inaccurate method, as, under these conditions, the eggs cannot be rapidly and uniformly mixed in the different vessels.

The details of the experiment are as follows. To each of four watch glasses, containing respectively eight drops of water and eight drops of different concentrations of extract as given in Table XIV, were added three drops of a sperm-suspension. 12 minutes later, one drop of eggs was added to each watch glass. Two such experiments were made.

TABLE XIV. (4.24.6.)

	8 drops water	5 drops water 3 drops extract	3 drops water 5 drops extract	8 drops extract
Exp. 1. *A/c*, *A* extract	0	<1	4	8
Exp. 2. *A/d*, *B* extract	5	13	35	57

Thus, in both experiments, treatment for 12 minutes in all three concentrations of egg-extract *increased* the fertilizing power of the sperm. The most important points in which these two experiments differed from that of Lillie quoted above, are the following: (1) Lillie's experiments were made with *Arbacia*, mine with *Strongylocentrotus*; (2) Lillie's extract was probably stronger; (3) I used a weaker suspension of sperm.

Another experiment was then made, on exactly the same lines as the last, but in which the extract was made up of eight times the usual strength. This experiment was made double, two different strengths of sperm being used to each concentration of extract. The result was exactly parallel to those of the preceding experiments. It is given in Table XV.

TABLE XV. (1.24.6.)

		8 drops water	3 drops water 5 drops extract	8 drops extract
B/d, *B* extract	Weaker sperm	2	32	35
	Stronger sperm	3	68	92

Finally the experiment was repeated with thick sperm. As was to be expected, 100 % fertilized in water as in all the concentrations of extract, but the sperm-halos round the eggs were progressively larger in increasing concentrations of extract.

Thus I cannot agree with Lillie's conclusion that the fertilizing power of sperm which has stood in egg-extract is decreased. On the contrary, the experiments just described, together with others recorded in this paper, show clearly that treatment with egg-extract not only has an immediate effect in increasing the fertilizing power of the sperm, but suspensions in the presence of extract *keep* the power of being able to fertilize more eggs than suspensions of the same sperm of the same concentration in plain sea-water.

V. Effects of Egg-waters on the Fertilizing Power of the Sperm.

The experiments described up to now have shown that a substance or substances can be artificially liberated from the eggs—by breaking them up in sea-water—which stimulates spermatozoa. It remains to be seen whether or not such a substance is normally secreted by the eggs into the sea-water. If not, we have been dealing with a purely artificial phenomenon, but if there is a normal secretion of a sperm-stimulant by the eggs, its investigation must be of the highest importance for the understanding of the fertilization process. In the experiments recorded below, eggs were allowed to remain in a relatively small amount of water for some time, and this water was then used in the same way as the extract had been in previous experiments. Such sea-water, in which eggs have been allowed to stand, I call "egg-water" for the sake of brevity.

1. *Experiments with Ciona intestinalis.*

In Exps. 1 and 2 (Table XVI), eggs from three individuals[1] were kept in 3—4 times their volume of water for $5\frac{1}{2}$ hours, to obtain the "egg-water." Given amounts of sperm-suspension were dropped into (1) 10 drops of plain water, (2) 10 drops of the egg-water. 10 cc. water was then added to (1) and (2), and they were poured on to approximately equal amounts of eggs from a given individual.

In Exp. 3, eggs from several individuals lay in 3—4 times their volume of water for two hours. The rest of the treatment was the same as in Exps. 1 and 2.

For Exp. 4 an individual was found with an exceptionally large number of eggs. Some of these were allowed to stand in 10 times their own volume of water for two hours to obtain the egg-water. Given amounts of sperm-suspension were dropped into (1) 1 cc. of water, (2) 1 cc. of egg-water. (1) and (2) were then poured on to equal amounts of eggs from the same individual from which the egg-water had been obtained.

The results of the experiments are given in Table XVI.

TABLE XVI.

			Plain water	Egg-water
Exp. 1. *A/b*,	Mixed egg-water	3 drops sperm ...	2	11
		6 ,, ,, ...	3	18
Exp. 2. *C/d*,	Mixed egg-water	2 drops sperm ...	0	0
		4 ,, ,, ...	<1	23
Exp. 3. *E/f*,	Mixed egg-water	2 drops sperm ...	2	5
		5 ,, ,, ...	22	42
Exp. 4. *G/h*,	*G* egg-water ..	3 drops sperm ...	0	2
		10 ,, ,, ...	11	26

These experiments show that for *Ciona*, water in which eggs have lain has the same stimulating effect on spermatozoa that artificial egg-extracts have.

2. *Experiment with Arbacia pustulosa.*

This experiment was made on exactly the same lines as those just described with *Ciona*. Egg-water experiments are, however, much more easily made with Echinoderms than with *Ciona*, since a much greater volume of eggs can be obtained, and hence more water containing the egg-secretions for experimentation.

[1] I need hardly repeat that as in all other experiments recorded in this paper, controls were kept. Unfertilized samples of all the eggs used for egg-waters were put on one side and afterwards examined to see that none had segmented.

Eggs *A* were washed twice by allowing them to settle in finger-bowls full of water. They were then placed in a tube in 3—4 times their volume of water. After 35 minutes, this water was drawn off and a second lot added to the eggs. When the latter had been in this water for one hour, it was drawn off and used for the experiment. Given amounts of sperm-suspension *c* (see Table XVII) were added to (1) 2 cc. plain sea-water, and (2) 2 cc. egg-water. To each of these 10 cc. of plain sea-water was added, and they were poured on to separate equal lots of eggs *B*.

TABLE XVII. (1.2.5)

		Plain water	Egg-water
B/c, *A* egg-water	3 drops sperm ...	35	89
	10 ,, ,, ,,	91	99

Thus *Arbacia* eggs behave as *Ciona* in secreting a substance into the water, which increases the fertilizing power of the sperm of the same species.

3. *Experiments with Strongylocentrotus lividus.*

The four experiments given in Table XVIII were all made with the same egg-water. Eggs were taken from three females and washed twice to remove all coelomic fluid and ovarian tissue, by allowing them to settle in finger-bowls full of water. They were then put in about five times their volume of water, which was drawn off at varying intervals (given in the Table) to be used for the experiments.

Just before the fertilizations were made, given amounts of sperm-suspension were added to (1) 2 cc. plain water, and (2) 2 cc. egg-water. After 1—2 minutes 10 cc. plain water was added to each, and they were then poured on to approximately equal lots of eggs.

TABLE XVIII. (3.14.5.)

		Plain water	Egg-water
Exp. 1. *A/b*, Egg-water 55 minutes on eggs	3 drops sperm	48	78
	1 cc. sperm	97	100
Exp. 2. *A/c*, Egg-water 65 minutes on eggs	3 drops sperm	45	58
	1 cc. sperm ...	96	100
Exp. 3. *A/e*, Egg-water 100 minutes on eggs	3 drops sperm	18	30
	1 cc. sperm ...	59	84
Exp. 4. *A/f*, Egg-water 120 minutes on eggs	3 drops sperm	8	17
	1 cc. sperm ...	35	53

Table XVIII shows that the eggs of *Strongylocentrotus* give off a substance, or substances, into the sea-water which stimulates the sperm of the species in the same way as we saw the case for *Ciona* and *Arbacia*.

VI. Effect of *Ciona* Blood on the Fertilizing Power of *Ciona* Sperm.

Trials were next made to see whether there is an agent which increases the fertilizing power of the sperm in the blood, as well as in the eggs of *Ciona*. Owing to lack of time no other tissues of the body were tested, but it will be important to do so in the future.

The blood was removed from the body by the method already given (see p. 264). In each experiment the blood of one particular individual (given in the Table) was used. After removal from the body it was diluted to varying extents, according to the amount obtained. Given amounts of sperm-suspension were added to (1) $2\frac{1}{2}$ cc. water, and (2) $2\frac{1}{2}$ cc. blood. To each was added 10 cc. water, after which (1) and (2) were poured on to approximately equal numbers of eggs. Each experiment was made double, two different amounts of sperm being used. The results of the experiments are given in Table XIX.

The Table shows that this series is an extensive one, there being 22 double experiments, or 44 comparisons in all. The reason for this is not that this investigation is more important than previous ones, but that, as will readily be seen from the Table, the results of each of the Series A, B, C, D and E contradict one another, although the experiments within each series agree. For the experiments in Series A and D show an increase in the number of eggs fertilized as a result of the treatment of the sperm with blood, while in Series B, C and E the percentages are decreased. The only exceptions to this statement are that in Series D, all other experiments of which showed an increase in the percentages as the result of the blood treatment, the second parts of Exps. 10 and 15 gave no change[1]. Owing to the contradictory results of the different series, the experiments were continued until a reason for this behaviour was found.

At first it was thought that an explanation of the different effects of the blood on the sperm might be that the behaviour of the former differed according as it was derived from the same animal as the eggs used in the same experiment, or as the sperm, or from a third individual. That this is not the explanation is seen from the fact that Series D, in which the percentages were raised by treatment of the sperm with blood, there are experiments in which the blood was derived from (1)

[1] The first parts of Experiments 9 and 13 allow of no comparisons, as too little sperm was used. The same is the case with the second parts of Experiments 20 and 21, where too much sperm was used.

TABLE XIX. (41.4.6 *et seq.*)

				Water	Blood
Series A.	Exp. 1.	*A/b*, *A* blood	3 drops sperm ...	41	74
			1 cc. sperm ...	85	88
	Exp. 2.	*C/d*, *C* blood	3 drops sperm	0	1
			1 cc. sperm	<1	10
	Exp. 3.	*E/f*, *F* blood	3 drops sperm	0	<1
			1 cc. sperm	0	6
Series B.	Exp. 4.	*A/b*, *B* blood	3 drops sperm	39	5
			1 cc. sperm .	89	7
	Exp. 5.	*C/d*, *D* blood	3 drops sperm	46	23
			1 cc. sperm .	84	68
	Exp. 6.	*E/f*, *F* blood	3 drops sperm	44	27
			1 cc. sperm ...	96	75
	Exp. 7.	*G/h*, *H* blood	3 drops sperm	14	5
			1 cc. sperm	22	10
Series C.	Exp. 8.	*D/k*, *K* blood	3 drops sperm	16	<1
			1 cc. sperm ..	62	23
	Exp. 9.	*L/m*, *L* blood	3 drops sperm ...	<1	1
			1 cc. sperm ..	10	6
Series D.	Exp. 10.	*A/b*, *C* blood	3 drops sperm .	8	27
			1 cc. sperm . .	71	70
	Exp. 11.	*A/d*, *D* blood	3 drops sperm ...	31	65
			1 cc. sperm .	85	89
	Exp. 12.	*E/f*, *G* blood	3 drops sperm	12	26
			1 cc. sperm ...	37	48
	Exp. 13.	*E/h*, *J* blood	3 drops sperm	0	0
			1 cc. sperm	0	2
	Exp. 14.	*E/k*, *J* blood	3 drops sperm	1	21
			1 cc. sperm	7	27
	Exp. 15.	*M/n*, *M* blood	3 drops sperm ..	3	8
			1 cc. sperm	14	14
	Exp. 16.	*M/o*, *O* blood	3 drops sperm ...	18	33
			1 cc. sperm	19	74
	Exp. 17.	*M/p*, *P* blood	3 drops sperm	<1	8
			1 cc. sperm	4	19
Series E.	Exp. 18.	*Q/r*, *S* blood	3 drops sperm	33	<1
			1 cc. sperm	95	5
	Exp. 19.	*R/q*, *S* blood	3 drops sperm	7	5
			1 cc. sperm	57	9
	Exp. 20.	*Q/t*, *T* blood	3 drops sperm	100	93
			1 cc. sperm	100	100
	Exp. 21.	*V/w*, *W* blood	3 drops sperm	100	94
			1 cc. sperm	100	100
	Exp. 22.	*X/r*, *X* blood	3 drops sperm	77	51
			1 cc. sperm	100	89

the same animal as the eggs, (2) the same as the sperm, and (3) a third animal. Similarly in Series E, where the blood caused a decrease in the fertilization percentages, all three types are present.

What must be the true explanation of the phenomenon is seen from the following list giving the times after they were brought into the Aquarium at which the animals were used for experimentation.

Series A. Animals brought in from the sea the same day as the experiments were made.

Series B. Animals had been in the laboratory two weeks.

Series C. The same lot of animals as Series A, but used the following day.

Series D. Animals brought in from the sea on the same day as they were used.

Series E. The remainder of the same lot of animals used for Series D, but experimented with two days afterwards.

For Series A and D, in both of which the blood caused an increase in the percentages of eggs fertilized, the animals were brought in fresh from the sea on the same day. For Series B, C, and E, on the contrary, the animals used had been in the Aquarium circulation for lengths of time varying from one day to two weeks, and it was in these experiments that the blood produced a decrease in the percentages.

Evidently, then, even one night in the Aquarium water caused some change in the composition of the blood of *Ciona*, causing it to reverse its effect on the fertilizing power of the spermatozoa. It can hardly be that this change is the first stage in the death of the animal, since all unhealthy looking individuals were discarded, and animals such as were used for the experiments were capable of living in the Aquarium circulation in apparently good condition, for weeks after they were brought in. That physiological changes occur in these animals, however, is seen not only in the different behaviour of the blood described above, but in the progressive decrease in egg-production, and in the fact that the eggs are less and less pigmented each day[1].

For the experiments on egg-extracts and egg-waters of *Ciona*, which have been described in the preceding sections, animals were used which had been in the circulation for varying lengths of time after they were brought in from the sea, although *most* were used fresh the same day. Results were, however, uniform, and the following experiment, made with animals which had been five days in the Aquarium is further proof that there is no change in the eggs as there is in the blood.

[1] The eggs of *Ciona* which has been reared in the Aquarium are always more or less unpigmented.

Given amounts of sperm-suspension (see Table XX) were added to (1) 5 cc. water, and (2) 5 cc. egg-extract. To each was then added 5 cc. of water and they were poured on to separate lots of eggs. The experiment was made with two different amounts of sperm. Table XX gives the result.

TABLE XX. (42.4.7.)

		Water	Egg extract
Cross: *A/b*	13 drops sperm ...	1	3
	11 cc. sperm .	2	10

VII. Influence of Egg-waters and Egg-extracts of Other Animals on the Fertilizing Power of *Ciona* Sperm.

The reason why eggs can, in general, only be fertilized by sperm of the same species has always been a puzzle to physiologists. The experiments on egg-secretions suggest that the action on the spermatozoon of a substance given off by the egg is a necessary preliminary to fertilization. Now, if the secretions of the eggs only affected the sperm of the same species, but had no action on the sperm of other species, this might be an explanation of the specificity of the fertilization process. To test this hypothesis, the actions of the egg-extracts and egg-waters of *Phallusia*, *Arbacia* and *Strongylocentrotus* on *Ciona* sperm used to cross-fertilize *Ciona* eggs were tried.

1. *Phallusia egg-extract.*

This experiment was made before it had been settled that the action of extracts was on the sperm and not on the eggs. In consequence the eggs of *Ciona* (*A*) were allowed to stand in five dishes, the first of which contained 16 cc. of sea-water, and each of the others 16 cc. of different concentrations of *Phallusia* egg-extract, for 36 minutes. After this interval a drop of *Ciona* sperm-suspension (*b*) was added to each of the dishes. The result was as follows:

TABLE XXI. (21.6.2.)

Cc. sea-water . ..	16	15	10	5	0
Cc. *Phallusia* egg-extract	0	1	6	11	16
Cross: *Ciona A/b*	1	8	20	21	27

2. *Arbacia egg-extract.*

In the next series of three experiments, the *Ciona* sperm was treated with *Arbacia* egg-extract before being used to cross-fertilize *Ciona* eggs.

Given amounts of *Ciona* sperm-suspension (see Table XXII) were added to (1) 3 cc. plain sea-water, and (2) 3 cc. *Arbacia* egg-extract. 10 cc. water was then added to each, and they were poured on to approximately equal amounts of *Ciona* eggs. Each experiment was double, two different quantities of sperm being used.

From Tables XXI and XXII it is seen that percentages of eggs fertilized in *Ciona* by a certain concentration of sperm is raised by treatment of the sperm with *Phallusia* or *Arbacia* extract, in the same way as it was by treatment with extract of the eggs of the same species.

TABLE XXII. (38.6.5.)

		Water	*Arbacia* egg-extract
Exp. 1. *A/b*	3 drops sperm	76	88
	12 ,, ,,	90	97
Exp. 2. *C/d*	3 drops sperm	2	15
	12 ,, ,,	25	46
Exp. 3. *C/e*	3 drops sperm	0	2
	12 ,, ,,	< 1	10

3. *Arbacia egg-water.*

To obtain the egg-water, the eggs of *Arbacia* were allowed to stand in little more than their own volume of water for $4\frac{3}{4}$ hours. Given amounts of *Ciona* sperm-suspension (see Table XXIII) were added to (1) 1 cc. plain sea-water, and (2) 1 cc. *Arbacia* egg-water. The latter contained the *Arbacia* eggs, and was not drawn off free of them, as in previous egg-water experiments. To each of the dishes was then added 10 cc. water, and they were poured on to equal amounts of *Ciona* eggs. The experiment was double, being made for two different concentrations of sperm.

TABLE XXIII. (35.25.4.)

		Water	Egg-water containing *Arbacia* eggs
Cross, *A/b*	3 drops sperm ..	67	0
	10 drops sperm ...	70	0

The complete inhibition of cross-fertilization of *Ciona* by sperm treated with *Arbacia* egg-water, as seen in Table XXIII, was unexpected, more especially as *Arbacia* egg-extract had had the opposite effect. It was thought that the result might be due to the presence of numerous *Arbacia* eggs in the sperm-suspension which was used to effect the fertilization. The *Ciona* spermatozoa might conceivably have become entangled in the jelly surrounding these eggs. A second

possibility was this. The comparatively small volume of water in which the *Arbacia* eggs had stood for a relatively long time may have become acid enough to inhibit the fertilizing power of the spermatozoa (see p. 294). The acidity of this egg-water was not tested when it was used for the experiment, but $1\frac{1}{2}$ hours afterwards it gave a pink colour with α-Naphtholphthalein, whereas normal sea-water gives green.

Owing to these two possibilities of error, the experiment was repeated. After removal from the ovary, the *Arbacia* eggs were washed by allowing them to settle twice in finger-bowls of water. They were then placed in 3—4 times their volume of water for 55 minutes only. When this egg-water was drawn off the eggs, it was still green to α-Naphtholphthalein.

Given amounts of sperm-suspension were added (see Table XXIV) to (1) 3 cc. plain sea-water, and (2) 3 cc. *Arbacia* egg-water, which was quite free from *Arbacia* eggs. 10 cc. of water was added to each, after which they were poured on to separate equal quantities of eggs. The experiment was double as last time.

TABLE XXIV. (39.6.5.)

		Water	*Arbacia* egg-water
Cross, *F/g*	3 drops sperm ...	10	42
	12 drops sperm ...	13	77

The Table shows that, under the conditions of this experiment, the *Arbacia* egg-water increased the fertilizing power of *Ciona* sperm in the same way as the *Arbacia* egg-extract had done in the experiments of Table XXII.

4. *Strongylocentrotus egg-extract.*

The two experiments recorded in this section were made to test the action of *Strongylocentrotus* egg-extract on *Ciona* sperm, used to cross-fertilize eggs of the same species. Definite amounts of *Ciona* sperm-suspension (see Table XXV) were added to (1) 10 cc. plain sea-water,

TABLE XXV. (31.25.3.)

		Water	*Strongylocentrotus* egg-extract
Exp. 1. *B/c*	3 drops sperm ...	27	62
	7 ,, ,, ...	55	96
	20 ,, ,, ...	81	100
Exp. 2. *C/d*	5 drops sperm ...	0	83
	12 ,, ,, ...	0	97
	20 ,, ,,	0	100

and (2) 10 cc. *Strongylocentrotus* egg-extract. Each was then poured on to approximately equal quantities of *Ciona* eggs. Each experiment was made triple, three different strengths of sperm being used.

In both experiments, involving six comparisons between the effects of plain water and of the egg-extract on the fertilizing power of the *Ciona* sperm, the percentages of eggs fertilized were raised by treatment with the extract. The results are especially marked in Exp. 2.

5. *Strongylocentrotus egg-water.*

The first experiment with *Strongylocentrotus* egg-water was made in the same way as that with *Arbacia* egg-water recorded in Table XXIII. *Strongylocentrotus* eggs (previously washed twice in finger-bowls of water) were kept in little more than their own volume of water for $2\frac{1}{2}$ hours. Five drops of *Ciona* sperm-suspension *b* were added to (1) 1 cc. normal sea-water, and (2) 1 cc. egg-water, *containing Strongylocentrotus* eggs. 10 cc. of water was then added to (1) and to (2), and each was poured on to separate equal amounts of *Ciona* eggs *A* (see Table XXVI).

TABLE XXVI. (35.30.4.)

	Water	Egg-water containing *Strongylocentrotus* eggs
Cross, *A/b*	27	26

The Table shows that the egg-water had practically no effect on the fertilization percentages. The conditions of the experiment were the same as those for the experiment shown in Table XXIII, in which the *Arbacia* egg-water inhibited the fertilizing power of the *Ciona* sperm. That is to say, the egg-water was prepared by keeping the eggs for a comparatively long time in a relatively small volume of water, and *Strongylocentrotus* eggs were present in the sperm-suspension used to effect the fertilization. At the time of fertilization the egg-water gave a pink colour with α-Naphtholphthalein, instead of the green of normal sea-water.

For the reasons already given on p. 283 it was thought that there was in this experiment some other factor influencing the *Ciona* sperm than the *Strongylocentrotus* egg-secretion. A further series of experiments was therefore made, in which the acidity of egg-water which has stood for some time on a relatively large volume of eggs was eliminated, and in which the egg-water used to influence the sperm did not contain eggs.

Eggs from two females of *Strongylocentrotus* were washed twice in finger-bowls of water, after which they were placed in tubes with 4—5

their own volume of water. At lengths of time varying from 65—120 minutes portions of this water were drawn off and used in the experiments. At the time of the last fertilization made, this water was still green to α-Naphtholphthalein. Definite equal amounts of *Ciona* sperm-suspension (see Table XXVII) were added to (1) 2 cc. of plain sea-water, and (2) 2 cc. of the *Strongylocentrotus* egg-water, this time not containing any *Strongylocentrotus* eggs. 10 cc. water was then added to each[1], and they were poured on to separate equal amounts of *Ciona* eggs. The experiments were made double.

TABLE XXVII. (40.16.5.)

				Water	*Strongylocentrotus* egg-water
Exp. 1.	*A/b.*	Egg-water 65 minutes on eggs	3 drops sperm	23	77
Exp. 2.	*B/c.*	Egg-water 80 minutes on eggs	3 drops sperm	20	73
			1 cc. sperm	74	96
Exp. 3.	*C/d.*	Egg-water 95 minutes on eggs	3 drops sperm	28	65
			1 cc. sperm	31	99
Exp. 4.	*E/f.*	Egg-water 120 minutes on eggs	3 drops sperm	60	86
			1 cc. sperm	91	100
Exp. 5.	*F/g.*	Egg-water 120 minutes on eggs	3 drops sperm	1	73
			1 cc. sperm	8	79
Exp. 6.	*H/i.*	Egg-water 120 minutes on eggs	3 drops sperm	80	100
			1 cc. sperm	94	100

In all the experiments of Table XXVII the *Ciona* sperm which had been treated with *Strongylocentrotus* egg-water had its fertilizing power increased to a marked extent.

As has already been stated, there are two possibilities to account for the absence of this effect in the experiments of Tables XXIII and XXVI. It would seem, however, that the possible entanglement of the spermatozoa in the jelly of the Echinoid eggs present, although it might conceivably account for the complete inhibition of fertilization in Table XXIII, could hardly cause no change in the percentages, as seen in Table XXVI. Considering the strong inhibitory effect of the presence of small quantities of acid on the fertilizing power of spermatozoa, as will be shown on p. 294, it is extremely probable that the cause of the anomalous results obtained in these two experiments was the presence of acid in the egg-water. It was shown that the egg-water of Table XXVI gave a pink colour with α-Naphtholphthalein at the moment when it was used. Thus, presumably owing to the fact that it had

[1] In Experiments 5 and 6 the sperm was added to 5 cc. (1) water, (2) egg-water, after which 5 cc. water was added to each.

stood for a considerable time with a large volume of eggs, this egg-water had an acid reaction, and it is not to be wondered at that this acidity counteracted the stimulating effect of the egg-water on the sperm, which latter is clearly shown in the other experiments (Tables XXIV and XXVII)[1].

The end result of the experiments described in the preceding five sections is therefore that treatment of the spermatozoa of *Ciona* with egg-extracts of *Phallusia*, *Arbacia* or *Strongylocentrotus* or with the egg-waters of the two latter, increases its fertilizing power.

VIII. Further Experiments to test the Specificity of the Egg-secretions.

The experiments described in the last sections seem to show that the general specific capability of spermatozoa for fertilizing eggs of the same species only is not due to their being unstimulated by the egg-secretions of other species. There remains, however, one other possibility in this connection. It might be that the specificity of fertilization phenomena depends on the relatively greater excitation of the spermatozoa by secretions of the eggs of the same species than by those of others. This suggestion can be tried by making strict comparisons between the effects on the fertilizing power of sperm-suspensions produced by extracts from the eggs of the same and of other species. The hypothesis was thoroughly tested in the experiments described below.

1. *Comparisons of the effects of Strongylocentrotus and of Ciona egg-extract on the fertilizing power of Strongylocentrotus sperm.*

The chief difficulty encountered in making these experiments is the practical impossibility of making two different egg-extracts of exactly equal concentrations. They were made as nearly as possible of the same strength by taking approximately equal quantities of the eggs of the two species to be experimented upon, crushing them to the same extent, and then washing off the juices into exactly equal quantities of water. Differences of one or two per cent. in the fertilization percentages of eggs fertilized with sperms treated with two different extracts are probably to be accounted for by slight differences in the concentrations of the extracts.

[1] It should be noted that all other experiments with egg-waters described in this paper were made under the same conditions as those of Tables XXIV and XXVII, thus eliminating the acidity factor.

In the first experiment three drops of *Strongylocentrotus* sperm-suspension were added to each of three dishes containing (1) 5 cc. water, (2) 5 cc. *Strongylocentrotus* egg-extract, and (3) 5 cc. *Ciona* egg-extract. 5 cc. water was then added to each and they were poured on to three separate equal lots of *Strongylocentrotus* eggs (see Table XXVIII).

TABLE XXVIII. (4.23.6.)

	Water	*Strongylocentrotus* extract	*Ciona* extract
Cross: *Strongylocentrotus A/b* ..	68	87	88

Both extracts increased the fertilizing power of the sperm to the same extent.

2. *Comparison of the effects of Strongylocentrotus and Sphaerechinus egg-extracts on the fertilizing power of Strongylocentrotus sperm.*

In each of the two experiments made (Table XXIX), definite equal amounts of *Strongylocentrotus* sperm-suspension were added to (1) 5 cc. water, (2) 5 cc. *Strongylocentrotus* egg-extract, and (3) 5 cc. *Sphaerechinus* egg-extract. To each was then added 5 cc. water, and they were poured on to three approximately equal quantities of *Strongylocentrotus* eggs. Each experiment was made double, two different strengths of sperm-suspension being used.

The results of the experiments are given in Table XXIX, which shows that both extracts increased the fertilizing power of the sperm equally.

TABLE XXIX (1.30.5.)

		Water	*Strongylocentrotus* extract	*Sphaerechinus* extract
Exp. 1. *Strongylocentrotus B/c*	·6 cc. sperm	22	85	87
	2 cc. sperm	49	100	100
Exp. 2. *Strongylocentrotus B/d*	·6 cc. sperm	61	100	100
	2 cc. sperm	88	100	100

3. *Comparison of the effects of Strongylocentrotus and Echinus egg-extracts on the fertilizing power of Strongylocentrotus sperm.*

In each experiment definite amounts of *Strongylocentrotus* sperm (see Table XXX) were added to (1) 5 cc. water, (2) 5 cc. *Strongylocentrotus* egg-extract, (3) 5 cc. *Echinus* egg-extract. To each was then added 5 cc. water, after which they were poured on to three approximately equal lots of *Strongylocentrotus* eggs.

The results shown in this Table are not so uniform. In the first part of Exp. 1 the *Echinus* egg-extract raised the fertilization percentage

7 % more than did the *Strongylocentrotus* extract. As the other three comparisons in the Table show an equal effect of the two extracts this difference is probably due to an error in the experiment.

TABLE XXX. (2.30.5 and 3.9.6.)

		Water	*Strongylocentrotus* extract	*Echinus* extract
Exp 1. *Strongylocentrotus A/b*	1 cc. sperm	40	88	95
	3 cc. sperm	62	96	95
Exp. 2. *Strongylocentrotus C/d*	3 drops sperm	13	25	26
	1 cc. sperm	29	78	77

4. *Comparison of the effects of Strongylocentrotus and Echinus egg-extracts on the fertilizing power of Echinus sperm.*

In the following experiment *Strongylocentrotus* and *Echinus* extracts were again used, but in this case a comparison was made between their effects on the fertilizing power of *Echinus* sperm. Definite equal amounts of *Echinus* sperm (see Table XXXI) were pipetted into (1) 5 cc. water, (2) 5 cc. *Strongylocentrotus* egg-extract, and (3) 5 cc. *Echinus* egg-extract. To each was then added 5 cc. water, and they were poured on to separate equal quantities of *Echinus* eggs. Table XXXI shows that both extracts had the same effect on the *Echinus* sperm.

TABLE XXXI. (2.31.5.)

		Water	*Strongylocentrotus* extract	*Echinus* extract
Echinus B/c	·6 cc. sperm ...	41	79	78
	2 cc. sperm ...	66	84	83

5. *Comparison of the effects of Strongylocentrotus and Echinus egg-extracts on the fertilizing power of Strongylocentrotus sperm, used to fertilize Echinus eggs.*

The experiments detailed in the preceding sections have shown clearly that the fertilizing power of sperm-suspensions is as much increased by treatment with egg-extract from another species as it is by an approximately equal concentration of the egg-extract of the same form. Evidently then the specificity of fertilization can in no way be due to any differential effect of egg-secretion from the same and from other species, and the cause of the general difficulty of effecting inter-specific hybridizations must be sought for elsewhere.

Now, suppose two species, X and Y, which hybridize with comparative ease. From what has just been said, it would be expected

that the percentage of X eggs fertilized by Y sperm would be increased to an equal extent by previous treatment of this sperm (1) by X egg-extract, and (2) by an equal concentration of Y egg-extract. That such is the case is proved by the following three experiments in which the cross *Echinus microtuberculatus* ♀ × *Strongylocentrotus lividus* ♂ was tested, the actions of egg-extracts of these two species being compared.

Although *Strongylocentrotus* sperm of considerably greater concentration is required to effect hybridization with *Echinus* eggs, than to fertilize eggs of the same species, it is by no means necessary to make use of milky suspensions. Indeed, if such are employed it often happens that 100 % of the *Echinus* eggs are fertilized, which is of course quite useless for these comparative experiments.

In each experiment 5 cc. of *Strongylocentrotus* sperm-suspension was pipetted into each of three dishes containing (1) 5 cc. water, (2) 5 cc. *Strongylocentrotus* egg-extract, (3) 5 cc. of an approximately equal concentration of *Echinus* egg-extract. Each was then poured on to separate approximately equal quantities of *Echinus* eggs. Each experiment was made double, weaker and stronger sperm-suspensions being used. Table XXXII shows the results of the experiments.

TABLE XXXII. (3.1.6.)

			Water	*Strongylocentrotus* extract	*Echinus* extract
Exp. 1.	*Echinus A*/*Strongylocentrotus m*	Weaker sperm ...	22	32	31
		Stronger sperm...	58	71	70
Exp. 2.	*Echinus A*/*Strongylocentrotus n*	Weaker sperm ...	12	40	39
		Stronger sperm...	65	68	70
Exp. 3.	*Echinus A*/*Strongylocentrotus p*	Weaker sperm ...	40	70	70
		Stronger sperm ..	72	71	73

In all the experiments except the second part of Exp. 3, both extracts raised the percentages of eggs hybridized by an equal amount, allowing for 2 % experimental error. That there was no raising of the percentages in the second part of Exp. 3 is explained by the fact that a sample of the *Echinus* eggs *A*, fertilized with *Echinus* sperm in plain water, showed only 74 % of segmenting eggs. Evidently there was a considerable number of unfertilizable eggs present, and the maximum possible percentage had been touched on in the fertilization by untreated sperm in the second part of Exp. 3.

Although the experiments described in the last five sections have shown that the hypothesis which they were made to test is untenable, the results given in Table XXXII indicate a valuable method of

increasing the number of eggs fertilized in inter-specific hybridization, namely, the preliminary treatment of the sperm with egg-extracts.

As has already been mentioned, the sperm-suspension used in the hybridization experiments of Table XXXII were not very concentrated. Nevertheless, they were strong enough to show under the microscope halos of spermatozoa collected round the eggs in the second parts of the last two experiments. A comparison of the size of these halos is of interest. In Exp. 2, halos were not visible round the eggs fertilized by the water-suspension of sperm. They were present, however, both round the eggs inseminated by the *Strongylocentrotus* extract suspension and by the *Echinus* extract suspension, and they were of equal size in each. In Exp. 3 there was a slight zone of spermatozoa visible round the eggs fertilized with the water-suspension, and strong, equally large ones surrounding the eggs in the sperm-suspensions made up in the two extracts.

6. *Comparison of the effects of Strongylocentrotus and Asterias egg-extracts on the fertilizing power of Strongylocentrotus sperm.*

Von Dungern (1, p. 36) found that a substance can be obtained from the eggs of *Asterias* by breaking them up, which in small doses is poisonous to the spermatozoa of Echinoids, but which does not affect the sperm of the same species. The Echinoid spermatozoa could be seen under the microscope to be killed by this substance. The latter is given off by the eggs into the sea-water, and owing to its presence the spermatozoa of Echinoids cannot enter the eggs of *Asterias*.

This discovery appeared to me to be the more surprising as my previous experiments had shown no such phenomena for the forms experimented with. It was desirable, therefore, to repeat Von Dungern's experiments with the Asteroid extract. This was carried out, using the same strictly comparative method employed in the other experiments described in this paper.

The first trials were made with egg-extracts obtained in the usual way. Definite equal amounts of *Strongylocentrotus* sperm-suspension (the quantities are given in Table XXXIII) were added separately to

TABLE XXXIII. (3.20.6.)

		Water	*Strongylocentrotus* extract	*Asterias* extract
Exp. 1. *Strongylocentrotus A/b*	3 drops sperm	8	68	0
	1 cc. sperm ...	62	100	0
Exp. 2. *Strongylocentrotus A/c*	3 drops sperm	28	89	0
	1 cc. sperm ...	84	100	0

(1) 5 cc. water, (2) 5 cc. *Strongylocentrotus* egg-extract, (3) 5 cc. *Asterias glacialis* egg-extract. 5 cc. water was then added to each, and they were added to separate equal amounts of *Strongylocentrotus* eggs. Each of the two experiments was double, two strengths of sperm being used.

The results given in the Table show that the *Asterias glacialis* egg-extract had the effect described by Von Dungern. Whereas the *Strongylocentrotus* extract increased the fertilizing power of the sperm of that form (as was previously shown in Table XII), the *Asterias* extract completely paralyzed the *Strongylocentrotus* sperm, so that it was incapable of fertilizing a single egg.

7. *Comparison of the effects of Strongylocentrotus and Asterias egg-waters on the fertilizing power of Strongylocentrotus sperm.*

It remained, finally, to see whether this poison for *Strongylocentrotus* sperm is given off by the eggs into the water or not. Egg-waters of *Strongylocentrotus* and *Asterias* were made up of approximately equal concentrations as follows. The eggs of the two forms were washed thoroughly by allowing them to settle four times in finger-bowls full of water. They were then placed in tubes in seven times their volumes of water. After 1½ hours the waters were drawn off the eggs and used in the following experiment.

Definite equal amounts of *Strongylocentrotus* sperm-suspension were added to (1) 2 cc. water, (2) 2 cc. *Strongylocentrotus* egg-water, and (3) 2 cc. *Asterias* egg-water. 10 cc. of water was then added to each and they were poured separately on to approximately equal lots of *Strongylocentrotus* eggs. The experiment, the result of which is detailed in Table XXXIV, was made with two different amounts of sperm.

TABLE XXXIV. (4.23.6.)

		Water	*Strongylocentrotus* egg-water	*Asterias* egg water
Cross: *Strongylocentrotus* ♂/♀	3 drops sperm ...	4	18	17
	1 cc. sperm ...	30	70	71

The result shows clearly that the poison is not given off by the *Asterias* eggs into the water. On the contrary, the *Asterias* egg-secretion stimulates the *Strongylocentrotus* spermatozoa to just the same extent as does the *Strongylocentrotus* egg-secretion itself. I can only explain Von Dungern's statement to the contrary on the supposition either that broken eggs were present among those from which he obtained the egg-water, or that some of the mucus from the outside of the body of the Asteroid was present with the eggs. Von Dungern

himself has shown that the latter also contains a poison for Echinoid spermatozoa.

The result of the experiment described last shows that the effect of *Asterias* egg-secretions on the sperm of other species is no different from that of the other forms experimented with. The presence of a substance in the Asteroid eggs which is poisonous for Echinoid spermatozoa is interesting in itself, but it cannot be an example of the general way in which eggs protect themselves against fertilization by sperm of other species. For if this were so, the extract of *Ciona* eggs would not have an equally stimulating effect on the spermatozoa of such a widely different form as *Strongylocentrotus* as has the egg-extract of the latter species itself.

IX. Effects of the H-ion Concentration of the Water on the Fertilizing Power of Sperm.

In the experiments to be described in the two following sections comparisons were made between the fertilizing powers of sperm-suspensions of *Ciona* of equal concentrations made up (1) in normal sea-water, and (2) in sea-water of altered H-ion concentration. The experiments were carried out on exactly the same lines as those already described on the effects of egg-secretions on the sperm. It may be stated at once that the results showed an increase in the fertilizing power in the presence of a slight decrease in the H-ion concentration, and a very marked inhibition for a small increase in the latter.

1. *Effect of a decrease in the H-ion concentration.*

In each of the two experiments, the results of which are shown in Table XXXV, two drops of a sperm-suspension of *Ciona* were added to each of the two dishes containing (1) 100 cc. normal sea-water, (2) 100 cc. alkaline sea-water. The latter was made up by the addition of 1 cc. N/10 NaOH to 200 cc. sea-water. 10 cc. of each of the suspensions (1) and (2) were then poured on to separate approximately equal quantities of eggs from another individual of *Ciona*.

TABLE XXXV. (30.1.3.)

		Normal water	Alkaline water
Exp. 1.	*A/b*	0	9
Exp. 2.	*C/d*	0	24

Thus in both cases the effect of the alkaline water was to increase the fertilizing power of the sperm-suspension.

2. *Effect of an increase in the H-ion concentration.*

In the first series of experiments equal quantities (given in Table XXXVI) of a *Ciona* sperm-suspension were pipetted into (1) 100 cc. normal sea-water, (2) 100 cc. acid sea-water, the constitution of which is given below. 10 cc. of each of the liquids (1) and (2) were then poured on to separate approximately equal amounts of eggs of another individual *Ciona*. Exp. 1 was made for two, and Exp. 3 for three different strengths of sperm.

The acid sea-waters (2) were made up as follows:

In Exp. 1, ·2 cc. N/10 HCl added to 100 cc. sea-water.
In Exp. 2, ·3 „ „ „ „
In Exp. 3, 1 „ „ „ „

Each of these solutions was tested previously, using the colorimetric method elaborated by Sörensen (4). The addition of eight drops of α-Naphtholphthalein to test-tubes containing 10 cc. of the liquid gave the following colours:

Normal sea-water ..... Green.
Acid water of Exp. 1 ..... V. light green.
Ditto of Exp. 2 ..... Light greenish brown.
Ditto of Exp. 3 ..... Pink.

The results of these experiments are given in Table XXXVI.

TABLE XXXVI. (30.11.3.)

			Normal water	Acid water
Exp. 1.	*Eff*	·6 cc. sperm	0	0
		20 cc. sperm ...	100	8
Exp. 2.	*Cfd*	5 cc. sperm ..	3	0
Exp 3.	*Afb*	·6 cc. sperm ...	45	0
		2 cc. sperm ..	97	0
		5 cc. sperm ..	100	0

In the first part of Exp. 1 too little sperm was added to give a comparison. In the second part of the same experiment the effect of the acid water on the sperm was to cause a great fall in the fertilization percentage. In Exps. 2 and 3, of which Exp. 2 was made with a slightly greater and Exp. 3 with a considerably greater concentration of H-ions than Exp. 1, the effect of the acid waters on the spermatozoa was to inhibit fertilization completely.

One further experiment was carried out, in which a comparison was made between the effects of normal water and of three increasing concentrations of acid water on the fertilizing power of a sperm-suspension of *Ciona*, used to effect a particular cross. 1 cc. of sperm-suspension *h* was pipetted into each of four dishes containing (1) 100 cc. normal sea-water, (2) 100 cc. acid water A, (3) 100 cc. acid water B, (4) 100 cc. acid water C. 10 cc. of each was then poured on to separate approximately equal quantities of *Ciona* eggs *G*.

The acid waters were made up as follows:

A. ·2 cc. N/10 HCl in 100 cc. sea-water (V. light green with α-Naphtholphthalein).

B. ·4 cc. N/10 HCl in 100 cc. sea-water (Light brown with α-Naphtholphthalein).

C. ·6 cc. N 10 HCl in 100 cc. sea-water (Light pinkish brown with α-Naphtholphthalein).

The result of the experiment is given in Table XXXVII, which shows that in this case even the least increase in the H-ion concentration of the water completely inhibited the fertilizing power of the sperm-suspension.

TABLE XXXVII. (31.15.3.)

	Normal water	Acid water A	Acid water B	Acid water C
Cross: *G/h*	89	0	0	0

The first experiment on the effect of *Arbacia* egg-water on *Ciona* sperm (Table XXIII) showed inhibition of the fertilizing power of the latter, and the first experiment on the influence of *Strongylocentrotus* egg-water on *Ciona* sperm (Table XXVI) showed no change in the fertilizing power. It was seen that on the repetition of these experiments with egg-waters which had stood for a shorter time with a relatively smaller quantity of eggs, the fertilizing power of the sperm was increased in the same way as it was by treatment with the egg-extracts. It was suggested that the results obtained in the experiments of Tables XXIII and XXVI were due to the presence of acid in the egg-waters. The egg-water used in Table XXVI gave, at the time fertilization was made, a pink colour with α-Naphtholphthalein (p. 285), whereas the normal sea-water gave green. Tables XXXVI and XXXVII show that a lesser increase in the concentrations of H-ions in the water than is enough to give a pink colour with the indicator will quite inhibit the fertilizing power of sperm-suspension of *Ciona*,—at any rate of suspensions of about the strength of those used in the experiments described

in this paper. Thus it is extremely probable that the results of Exps. XXIII and XXVI are to be put down to this cause.

3. *The reactions of Ciona and Asterias egg-extracts and of Ciona blood.*

Since spermatozoa show themselves to be so very sensitive to small alterations in the H-ion concentration of the water, it is necessary to test the reactions of the egg-extracts and egg-waters. For if the latter showed a lesser H-ion concentration than that of normal sea-water, their stimulating effects on spermatozoa might be due to this cause.

With a view to settling this, comparative colour tests were carried out with normal sea-water and *Ciona* egg-extract, the latter of about the same concentration as that used in the experiments. Three indicators were used[1], as is shown in Table XXXVIII.

TABLE XXXVIII. (2.11.6.)

	Normal water	*Ciona* extract
α-Naphtholphthalein, 8 drops in 10 cc.	Green	Green
Neutral Red, 2 drops in 5 cc. ...	Yellow	Yellow
Phenolphthalein, 12 drops in 5 cc. .	Pink	V. faint pink

The first two indicators showed no difference between the reactions of the normal water and of the water containing egg-extract. The phenolphthalein, however, indicated that the extract had a slightly greater concentration of H-ions than the water. If this difference had been in the other direction, it might have explained the effect of the extract on the fertilizing power of sperm. As it is, however, the cause of this must be sought in some other factor, which remains at present undetermined.

As the extract of the eggs of *Asterias* had a reverse effect on sperm-suspensions from the other extracts examined, its reaction was specially investigated with the colour indicators. The results of the tests are given in Table XXXIX.

TABLE XXXIX.

	Normal water	*Asterias* extract
α-Naphtholphthalein ...	Green	Faint green
Neutral Red	Yellow	Pink

[1] Too much reliance cannot be put on the colour reactions given in this section, since Sörensen, *Ergebnisse der Physiologie*, states that the indicators used, namely Neutral Red, α-Naphtholphthalein and Phenolphthalein, are not always trustworthy in the presence of proteins. Neutral Red is said to be "kaum brauchbar," and the other two "nur selten."

Both indicators show that the water containing the *Asterias* extract has a considerably greater concentration of H-ions than normal sea-water. The colour given by the *Asterias* extract with *a*-Naphtholphthalein approximates to that given by the "acid water" of Exp. 1, Table XXXVI, and by "acid water A" of Table XXXVII, both of which inhibited the fertilizing power of *Ciona* sperm-suspensions. If one assumes a like action on *Strongylocentrotus* sperm, the "poison" for the latter which is contained in *Asterias* eggs may be the presence of an acid. This is, however, only a suggestion which remains quite unproved.

Finally, colour tests were made with the blood of *Ciona* to see whether the change in its effect on the fertilizing power of *Ciona* sperm, according to the time the animals from which it is taken have been in the Aquarium, is correlated with any change of reaction. It will be remembered (see p. 281 *seq.*) that the blood of animals taken fresh from the sea increased the fertilizing power of sperm-suspensions, while a sojourn of the animals for even one night in the Aquarium water reversed the effect of the blood on the sperm.

For the colour tests the blood was first centrifuged in order to obtain the liquid fairly clear and colourless. The plasma showed a red colour with Neutral Red, yellow with Methyl Orange and pink with α-Naphtholphthalein. There was *no alteration* in the reaction when the animals had been kept in the Aquarium, the latter experiments being made with animals from the lot used in Series D and E, Table XIX.

The fact that the blood gave a pink colour with α-Naphtholphthalein shows that it has a concentration of H-ions considerably greater than that of normal sea-water. Tables XXXVI and XXXVII showed that if acid is added to the latter until it gives a pink with this indicator, the fertilizing power of sperm-suspensions made up in it is completely inhibited. From this it follows that there must be something in the blood of fresh *Ciona* which counteracts the effect of this acidity as well as stimulates the spermatozoa. It might be added as a suggestion that if this stimulating substance disappeared from the blood after the animal had been kept in the Aquarium water, the acidity of the blood would have the effect of paralyzing the spermatozoa, which would be a possible explanation of the observed phenomena.

X. Conclusion.

Soon after the present work was commenced a preliminary account of an investigation by F. R. Lillie published some months previously (2) came to my notice. Before my experiments were completed the full account of this very important work was published (3). It consisted of a study of the reactions of spermatozoa to substances secreted by the eggs, and was thus concerned with the same general problem as the one I was attacking. Lillie's methods, were, however, quite different from mine. The main results were based on observations of the activity of the spermatozoa made both with the naked eye and more especially under the microscope, when the spermatozoa were treated with egg-extracts, substances secreted by the eggs, and chemical agents. Besides this, the effects of the treatments on the subsequent capability of the sperm for fertilizing eggs were tried. Thus, whereas Lillie's results depended mainly on direct observation, mine were based on indirect methods. The effects of the different factors on the spermatozoa were in my case investigated by comparing the subsequent fertilizing powers of the suspensions of sperm experimented upon. The main result of Lillie's work was to show that substances are secreted by the eggs of *Arbacia* and of *Nereis* which cause intense activity of the spermatozoa, agglutinate them in masses and to which the spermatozoa are positively chemotactic.

Now Lillie found evidence for a specificity in the egg-secretions. The *Nereis* "agglutinin" did not agglutinate *Arbacia* sperm, but the *Arbacia* substance was agglutinative for *Nereis* sperm. The *Arbacia* extract, however, probably contained two agglutinins, one specific for the *Arbacia* sperm and the other not specific; for if the substances from *Arbacia* eggs were kept for some days, *Nereis* sperm was no longer agglutinated, while *Arbacia* sperm was. Moreover, the coelomic fluid of *Arbacia* contained an agglutinin for *Nereis* sperm, which did not affect *Arbacia* sperm.

It will be remembered that I could find no evidence for specificity in the effects of egg-secretions on the fertilizing power of the sperm. On the contrary, in all the forms tried, the fertilizing power of a sperm-suspension was increased to an exactly equal extent by equal concentrations of the egg-secretions of the same and of another form, the two

forms being sometimes as widely different as Ascidians and Echinoderms. The factor in the egg-secretions, then, which increases the fertilizing power of the sperm, has no connection with the specificity of the fertilization process. It may well be that this specificity is due to the "iso-agglutinins" of Lillie—that for a spermatozoon to fertilize an egg, there must be a reaction between it and the iso-agglutinin of the egg, the visible effect of which is agglutination.

In the one point in which Lillie's experiments and my own coincide there is complete disagreement. All my experiments have shown that treatment of a sperm-suspension with egg-secretions, or with substances artificially extracted from the eggs, *increases* the fertilizing power of the suspension—that after this treatment more spermatozoa can fertilize eggs than before the treatment. Lillie, however, states that there is a loss or diminution of the fertilizing power of the sperm as an effect of the egg-extract. Special experiments were made on the exact lines as those of Lillie (see p. 275), but they too gave the invariable result,—the fertilizing power was increased. I am therefore at a loss to explain this after-effect on the sperm described by Lillie for his experiments.

The end result of my own investigations is that eggs of Ascidians and of Echinoids secrete substances into the sea-water in which they lie. These substances increase the fertilizing power of sperm-suspensions. If a sperm-suspension of a certain concentration be taken and divided into two equal portions, to one of which is added some plain sea-water, and to the other an equal amount of sea-water containing egg-secretions, the latter portion can fertilize more eggs than can the former. Further, the egg-secretions of a foreign species increase the fertilizing power to an exactly equal extent as those of the same species, provided they are equally concentrated. This increased fertilizing power of sperm-suspensions was shown for a number of ordinary intra-specific cross-fertilizations and for one case of hybridization.

In conclusion, it should be pointed out that the conditions in nature must be somewhat different from those in the laboratory. For as the substances in question are secreted by the eggs lying in water, the secretions must be continually washed away by currents and wave action. It follows that at a small distance from the surface of the egg the secretions will be present in the water only in minimal concentrations. The action on the spermatozoa must therefore take place on, or at a very small distance from, the surface of the egg.

XI. Summary of Experimental Results.

1. If a certain number of eggs of an individual *A* of *Ciona*, in a given amount of plain sea-water, are fertilized by the addition of a certain quantity of a sperm-suspension *b* of another individual, fewer eggs segment than when approximately the same number of *A* eggs in the same amount of sea-water, but this time containing egg-extract, are fertilized by an equal amount of sperm-suspension *b*.

2. The rate of segmentation of the eggs is the same in plain water and in egg-extract.

3. The extract has an *immediate* effect in raising the fertilization percentage.

4. The phenomenon described in (1) is the same in the case of *Ascidia*.

5. Ovary-extract of *Ciona* has the same effect as egg-extract.

6. In *Ciona* the fertilization percentage is raised by exactly the same amount by equal concentrations of extract derived from the same animal as the eggs used in the cross, from the same animal as the sperm, and from a third animal.

7. The extract acts as a *sperm* "stimulant" in *Ciona* and does not affect the eggs. Its presence in a sperm-suspension of a certain concentration causes more spermatozoa to fertilize eggs than is otherwise the case, i.e., it increases the "fertilizing power" of the sperm.

8. The sperm of *Strongylocentrotus* is affected by the egg-extract of the same species in exactly the same way as is that of *Ciona*.

9. In *Strongylocentrotus* the stimulating action of the extract continues as long as the sperm-suspension is capable of effecting fertilization.

10. The eggs of *Ciona*, *Arbacia* and *Strongylocentrotus* secrete a substance or substances into the water which increase the fertilizing power of the sperm of the same species, in the same way that the egg-extracts have been shown to do.

11. The effect of the blood of *Ciona* on the fertilizing power of the sperm depends on the time that the animals from which the blood is taken have been in the Aquarium. The blood of freshly caught animals increases the fertilizing power of the sperm, while that of

animals which have been in the Aquarium for one night or longer decreases it.

12. Egg-extracts of *Phallusia*, *Arbacia* and *Strongylocentrotus* and egg-secretions of the two latter increase the fertilizing power of sperm-suspensions of *Ciona*.

13. The fertilizing power of a *Strongylocentrotus* sperm-suspension used to fertilize eggs of the same species, is increased to the same extent by egg-extracts of *Strongylocentrotus*, *Sphaerechinus*, *Echinus* and *Ciona*. The fertilizing power of an *Echinus* sperm-suspension used to fertilize the eggs of that form is increased to an equal extent by the extracts of both these forms.

14. *Asterias* egg-extract completely inhibits the fertilizing power of a *Strongylocentrotus* sperm-suspension. The egg-secretion of *Asterias*, however, increases the fertilizing power of the latter to the same extent as does *Strongylocentrotus* egg-secretion itself.

15. A small rise in the H-ion concentration of the water gives an increase in the fertilizing power of a sperm-suspension of *Ciona*; while a small fall causes a very marked decrease in the latter.

16. Water containing *Ciona* egg-extract has a very slightly greater concentration of H-ions than normal water. *Asterias* extract, however, has a considerably greater concentration. The blood of *Ciona* shows no change in reaction correlated with its changed effect on the fertilizing power of sperm-suspensions.

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NOTE ON THE INHERITANCE OF HETEROSTYLISM IN *PRIMULA ACAULIS* JACQ.

BY R. P. GREGORY, M.A.

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THE experiments with the wild Primrose, which it is the purpose of this note to record, were begun by Mr Bateson and the writer concurrently with our experiments on *Primula sinensis*[1]. The results obtained in the two species are exactly similar: the inheritance of the characters of short and long style is of a simple Mendelian type, the short style being dominant, the long style recessive.

The two forms of the wild Primrose are about equally numerous in nature[2]. Among the wild plants used for experiment were nine short-styled plants, all of which proved to be heterozygous. Darwin[3] found the "illegitimate" mating *short-style* × *short-style* to be relatively even less fertile in the Primrose than it is in *P. sinensis*; my experiments have given a similar result, and from numerous matings of this kind only five families have been obtained[4]. Two of these families were the offspring of wild plants; they consisted of 13 short-styled, 4 long-styled plants. Of these 13 short-styled plants, only two produced any offspring; one of them shewed itself to be heterozygous, giving 11 short-

[1] Bateson and Gregory, *Roy. Soc. Proc.*, B. Vol. LXXVI. p. 581, 1905; Gregory, *Journal of Genetics*, Vol. I. p. 73, 1911.

[2] Darwin, *Forms of Flowers*, p. 34. I have counted the two forms in several localities where the plant grows wild, without finding any significant departure from equality of numbers.

[3] *L.c.*, p. 37.

[4] The plants used for experiment were grown out-of-doors, in pots covered with muslin bags, so that flowers which had been operated upon were exposed to the weather at a time of year when frosts are common. As a consequence a great number of the experiments were unsuccessful, and the whole of the crosses made in 1905, and again those made in 1907, were lost.

style and 4 long-style when self-fertilized, and 9 short-style, 11 long-style, when crossed by the recessive. The other short-styled plant, when crossed by long-style, gave 4 short-style, 0 long-style; other experiments with this plant failed, so that it remains doubtful whether it was pure or heterozygous. It was the only short-styled plant, from which seeds were obtained, which was not definitely shewn to be heterozygous. Altogether, the heterozygous short-styled plants, self-fertilized, gave 39 short-style, 13 long-style. The crosses of heterozygous short-style ♀ × long-style ♂ gave 119 short, 138 long; the reciprocal crosses gave 110 short, 96 long; or a total, for the matings in both forms, of 229 short, 234 long. The results of my experiments are shewn in tabular form below.

Form of Mating	Number of Families	Short-style	Long-style	Expectation
Long × Long	21	0	199	All Long
Short × Short (heterozygous) ..	5	39	13	3*D* : 1*R*
Heterozygous Short ♀ × Long ♂	17	119	138	1*D* : 1*R*
Long ♀ × Heterozygous Short ♂	15	110	96	1*D* : 1*R*

ON VARIEGATION IN *PRIMULA SINENSIS*.

By R. P. GREGORY, M.A.

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CONTENTS.

Introduction.

THE purpose of this paper is to describe some observations upon a race of *Primula sinensis*, in which it has been found that the alternative characters of normal green, variegated or pale yellowish-green leaves (and other organs containing chloroplasts) are transmitted from parent to offspring through the egg-cells only, the male gamete playing no part in determining the nature of the zygote in respect of these characters.

The experimental results which have been obtained are similar to those which have already been described by Correns[1] in *Mirabilis Jalapa albomaculata*, and by Baur[2] in *Antirrhinum majus albomaculatum*, the principal difference being that, whereas in *Mirabilis* and

[1] "Vererbungsversuche mit blass (gelb) grunen und buntblättrigen Sippen bei *Mirabilis Jalapa*, *Urtica pilulifera* und *Lunaria annua*," *Zeitschr. f. Induktive Abstammungs- u. Vererbungslehre*, I. p. 291, 1909; "Zur Kenntnis der Rolle von Kern und Plasma bei der Vererbung," *Ibid.* II. p. 331, 1909.

[2] "Untersuchungen über die Vererbung von Chromatophorenmerkmalen bei *Melandrium*, *Antirrhinum* und *Aquilegia*," *Zeitschr. f. Induktive Abstammungs- u. Vererbungslehre*, IV. p. 81, 1910. In this paper (p. 99) Baur records the fact that he has observed the maternal inheritance of variegation in *Primula sinensis*, without, however, studying it fully.

Antirrhinum the yellowish-white plants, which are entirely devoid of normal chloroplasts, do not survive beyond the seedling stage, in *Primula* it has been found possible to raise a few examples of the corresponding pale-coloured type to maturity and to use them both as the female and as the male parents in crosses.

The pale yellow or yellowish-green plants of *Primula* differ from the normal green type in having smaller chloroplasts, which are of a pale yellowish-green colour, instead of being bright green. There is some variation among the yellow-leaved plants as regards the degree of chlorosis[1]; some are very pale, others are a greenish-yellow, the differences in appearance being due to difference in the size and pigmentation of the chloroplasts. The variegated plants consist of a patchwork of cells of two kinds, containing respectively bright green and pale-coloured chloroplasts. In the mature organs of the variegated plants, the individual cells, at any rate in the great majority of cases, contain chloroplasts of one kind only, but evidence has been obtained that in the very young leaves two kinds of chloroplast may be present together in the same cell. The bearing of this observation on the genetic problem of the maternal transmission of variegation is discussed on pp. 314–317.

As in *Mirabilis* and *Antirrhinum*, the variegated plants of *Primula*, when self-fertilized, give three kinds of offspring, namely, self-coloured green, variegated and self-coloured yellow, in irregular proportions. The green offspring of a variegated plant give only green progeny in succeeding generations, the variegated continue to give all three types. Most of the yellow-leaved plants die at an early stage, but a few individuals have been brought to flower; I have not succeeded in obtaining seeds from the self-fertilization of these plants, but they have been crossed with normal green plants. The result of these experiments is to shew that, apart from any question of variegation, the character of the chloroplast is transmitted through the egg-cell only; when the yellow plant is used as the female parent all the offspring are yellow; when it is used as the male parent, in a cross with a green plant, both the F_1 *and the succeeding generations* consist entirely of self-coloured green plants, the yellow character not having been transmitted to the

[1] As the term "chlorosis" in some sense connotes disease, it should be said that the term is used in this paper in a purely descriptive sense, applied to a condition of the plants. The chlorosis in *Primula* is inherent in the plant and is not due to methods of culture. Nor has it anything to do with the "infectious chlorosis" of some plants (see Baur, *loc. cit.*).

offspring through the pollen of the male parent. This result corresponds with the similar results obtained by Correns and Baur when crosses were made with flowers taken from the pure white (yellowish-white) shoots sometimes borne by the variegated plants of *Mirabilis* and *Antirrhinum*.

It is to be noticed that crosses between green and yellow plants do not give variegated heterozygotes; all the offspring are pure for the character borne by the female parent used in the cross. The original variegated plant, from which my variegated race has been bred, appeared in the F_2 of a cross between two normal green races, Ivy-leaf and Snow-drift[1]; with this exception, variegated plants have invariably been the offspring of a variegated mother.

As might be expected, the character of the chloroplast is found not to be affected by the presence or absence of anthocyanic pigment in the cell-sap. The original variegated race was without sap-colour; it has, however, been brought in through matings with other races, and the various combinations of green and of yellow plastids with sap-colour have been obtained in the progeny.

The variegated character of the stems and leaves, again, is quite independent of the flaked or striped flowers, which result from the development of sap-colour in some cells and its absence from others. The two characters, in fact, stand in contrast to one another, for the flaked character of the flowers is inherited through the male, as well as through the female parent. Here again my results agree with those described by Correns in *Mirabilis*[2].

The plastids of the green and of the pale yellow or yellowish-green cells.

In the fully grown organs of the plant, the plastids contained in any individual cell are, in general, of one kind only; those of the chlorotic cells are smaller than those of the green cells and are of a pale yellowish-green colour, instead of being bright green. The difference between the normal and chlorotic plastids is very strikingly shewn if a variegated plant be examined after exposure to bright sun-light; the plastids of the normal cells are then packed with rounded or oval starch-grains, while those of the neighbouring chlorotic cells contain only a few small granules of starch (Pl. X, fig. 7). The differences in the

[1] A description of these races is given in *Journ. of Genetics*, Vol. I. p. 102, 1911.

[2] *Zeitschr. f. Induktive Abst.- u. Vererbungslehre*, I. p. 322, 1909.

size of the plastids persist after the starch has been removed by keeping the plants dark for some days (Pl. X, figs. 3—6).

In the young leaf of the variegated or yellow plants the plastids of the chlorotic cells are very small and almost colourless (Pl. X, figs. 9, 13); in the older leaves they have increased in size (Pl. X, fig. 2) and their pigment is obvious, though they always remain smaller and paler than the typical green plastid. Cells have been found containing plastids which exhibit intermediate degrees of chlorosis (Pl. X, figs. 8, 10); plastids of this kind are rather larger than in the extreme case and they form larger quantities of starch, though not so much as do the normal green plastids.

The variegated and yellow-leaved plants.

The variegated plants of *Primula sinensis* shew very much the same series of forms as those described by Correns in *Mirabilis Jalapa albomaculata*. The green and yellow cells are mingled in a mosaic, which may be finely divided, small groups of cells of one kind forming tiny flecks scattered among cells of the other kind; or may be coarse, cells of one kind forming patches of considerable size, or even whole leaves or sectors of the plant. When the mosaic is coarse, the component parts may be quite irregularly arranged, but generally there is a tendency towards the formation of a pattern, which, however, is usually not very definite. The rather rare case of a more or less definite sectorial arrangement is illustrated in Pl. IX, fig. 2. More commonly there is a tendency for cells of one kind, either green or yellow, to be distributed about the median line of the leaf, the peripheral parts consisting of cells of the other kind; the boundary between the two parts is nearly always irregular. A plant in which the green tissue occupies the middle of the leaf is shewn in Pl. IX, fig. 1. In such a plant, yellow cells constitute the whole of the peripheral parts of the leaf, and very often the sub-epidermal layer in the green region is also yellow, only the internal layers being green. The plants are not, however, true periclinal chimaeras; the yellow cells are not confined to the peripheral layers, but occur also in other layers and may be scattered sporadically among the green cells, while, conversely, isolated groups of green cells may occur among the yellow cells. As is the case in most plants, the cells of the epidermis (other than the guard cells of the stomata) contain only colourless plastids, even in the normal green leaves; one curious exception to this rule has, however, been found. The case was that of

a variegated plant, in which the central parts of the leaves were green. In one very young leaf, besides the central patch of green, there was a faint green stripe along the margin of the leaf, and in this region it was found that the epidermal cells alone contained definitely coloured plastids (Pl. X, fig. 9), those of the deeper layers being nearly colourless, as they usually are in the very young stages of the yellow tissues.

In most of the variegated plants there occur patches which are intermediate in colour between the full green and the clear yellow (Pl. IX, figs. 1—3). Gradations of this kind are due to the presence of more or fewer layers of normal green cells in particular regions. In one variegated plant the mosaic consisted entirely of patches of full green and lighter green; that is to say, green cells were present in some layers, at least, in all parts of the plant.

In the variegated plants, the stems, sepals and other organs, the cells of which contain chloroplasts, have a structure similar to that of the leaves.

In the pure yellow-leaved plants (i.e. plants which have no normal green plastids at all), the very young stems and leaves are always of a pale yellowish-white colour. The rate of growth of these plants is very slow as compared with that of the variegated plants, and still more so as compared with that of the pure green plants (see Pl. IX, fig. 3, in which three sister plants of the same age are shewn). Most of the yellow-leaved plants die at an early stage; in such as survive, the pigmentation of the plastids increases somewhat in the older leaves, which become yellowish-green. Apart from this change with age, various yellow-leaved plants may also shew some slight gradation, from a less to a more pronounced greenish tint, which is no doubt due to different degrees of chlorosis of the constituent cells. In some cases, plants, which have no normal green cells, have, side by side, cells of different degrees of chlorosis (Pl. X, fig. 8); thus the plant is built up of a patch-work of different kinds of cells, and in that respect is comparable with a variegated plant, although it is, nevertheless, chlorotic throughout.

So far as the fully grown organs of the variegated plants are concerned, no exception has been found to the rule that any particular cell contains chloroplasts of one kind only, though there may be minor variations in the size of the individual chloroplasts. But in the very young, actively growing leaves, evidence has been obtained of the existence, side by side in the same cell, of chloroplasts of different kinds, which differ from one another in the same way as do the chloro-plasts of the normal and of the yellow cells in the mature organs.

Cells containing different kinds of plastids have been found to occur along the lines of junction between groups of green and yellow cells. In such cells, as seen in sections taken from fresh material, the differences are readily recognizable, not only in the size, but also in the colour and starch-content, of the chloroplasts. Fig. 10, Pl. X, is from a fresh preparation. In this case, the cells *C*, *D*, *E* and *F* contained only chlorotic plastids, but each of the cells *A* and *B* contained both large bright-green chloroplasts of the normal type and also smaller pale-coloured plastids (*c*, *c*, *c*), indistinguishable in appearance from the pale-coloured plastids of the chlorotic cells. The chlorotic plastids of the cells *C*, *D*, *E* and *F* were not all exactly alike, the majority of those contained in the cell *C* being definitely of a pale yellowish-green colour, while most of those contained in the cell *D* were nearly or quite colourless; in each cell, however, both kinds of chloroplast were represented, *C* containing a few colourless plastids (*d*, *d*, *d*), *D* containing a few pale-coloured plastids (*c*, *c*, *c*). The plastids of *E* were pale-coloured, those of *F* colourless. The difference between these two kinds of chlorotic plastid is, of course, by no means so sharp as the difference between the chlorotic and the normal plastids; too much stress should not be laid upon it, but it suggests that the plastids of the cells *C* and *D* represent a mixture of two kinds of chlorotic plastids, analogous to the mixture of normal and chlorotic plastids found in *A* and *B*.

The use of fresh material for observations of the kind just described is open to certain objections, chiefly on account of the risk of confusion due to the displacement of chloroplasts from the cells to which they rightly belong. The observations have, therefore, been checked by the examination of fixed material, cut in paraffin and stained. By the use of this method under suitable precautions the risk of error due to displacement of the chloroplasts can be almost eliminated, and systematic searching is much facilitated. The method suffers from the disadvantage that differences of colour between the chloroplasts are no longer recognizable and the only distinction is one of size. Even in the very young leaves examined, the enormous majority of the cells contain only one kind of plastid, either large or small, but a certain number of cells have been found, always near the boundary between patches of normal and chlorotic tissue, in which there is no doubt that chloroplasts of different sizes exist side by side. Some of these cells are illustrated in Pl. X, figs. 11—18.

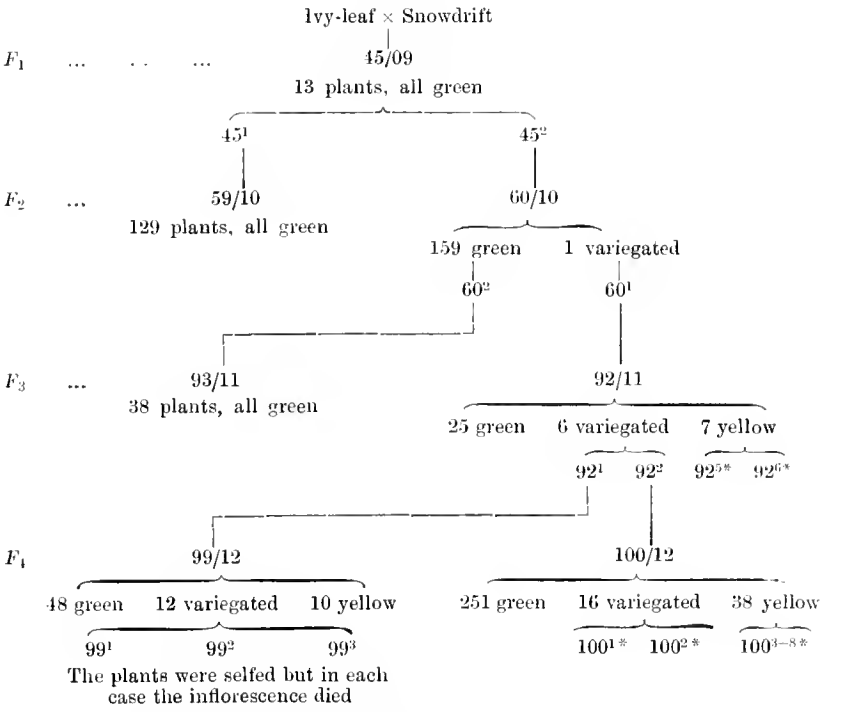
In view of these observations, and their probable significance in relation to the maternal inheritance of variegation, it is desirable that

investigations should be made, with the object of tracing back these differences between the plastids to the earliest stage in development at which they can be recognized. At the present time no material is available for this purpose, but I hope some may be obtained next year.

Breeding Experiments.

The original variegated plant, from which my variegated race has been bred, appeared in the F_2 from a cross between the two normal green races Ivy-leaf and Snowdrift[1]. The F_1 from this mating consisted of normal green plants, two of which were selfed; one of them gave a large family, consisting of 160 plants, one of which was variegated. The variegated plant was selfed and gave a family containing green, variegated and yellow plants. Two of these variegated plants again were selfed, and gave families containing the same three classes of offspring. The foregoing results are set out in detail in Table I.

TABLE I.



* Used in crosses; see Tables II and III.

[1] For a description of these races see *Journ. of Genetics*, Vol. I. p. 102, 1911.

There is one further point in connexion with these experiments which should be mentioned, namely, the fact that the flaked type of flower-coloration was contributed to the original mating by the Ivy-leaf parent, and this character appeared in each of the subsequent generations. In families containing green, variegated and yellow plants, both self-coloured and flaked flowers occurred in each of the three classes of offspring. The flaked character of the flowers is seen, therefore, to be quite independent of the variegation of the leaves, and it is, in fact, a character of a different nature from that of variegation, for it is inherited through the male, as well as through the female, gamete[1].

The extracted variegated and yellow-leaved plants have been used both as male and as female parents, in crosses with normal green plants. The results of these experiments are shewn in Tables II and III. It will be seen that when the yellow-leaved plant is used as the male parent, both the F_1 and the succeeding generations consist entirely of normal green plants; neither variegated nor yellow-leaved plants appear among the progeny of these matings. When, on the other hand, the yellow-leaved plant is used as the female parent, the F_1 is yellow. Owing to the slow growth of the yellow-leaved plants, and the consequent lapse of time before they bear seed, I have only just obtained the seedling F_1-plants from crosses in which the yellow-leaved plants were the female parents; but there can be no doubt, I think, that the yellow-leaved plants can only give yellows in succeeding generations. It will be noticed that matings between green and yellow, whichever way they are made, do *not* lead to the production of variegated plants; the progeny are all either self-coloured green or self-coloured yellow, according to the character of the mother.

For the sake of simplicity, I have omitted from the Tables any reference to characters other than those of green, variegated and yellow leaves. Each of the F_1's was, however, heterozygous in respect of a series of factors, some of which were derived from the male, some from the female parent. All these factors underwent normal segregation in the F_2's shewn in Table II. This result is quite in accordance with anticipation and the only point upon which it is necessary to remark is the fact that the flaked coloration of the flowers, observed in the families 106/13 and 107/13, was derived from the male parent used in the first cross.

[1] See also Correns, *Zeitschr. f. Ind. Abst.- u. Vererbungslehre*, 1. p. 322, 1909.

TABLE II.

Matings of Normal Green ♀ × Variegated or Yellow-leaved ♂.

Mating			F_1		F_2		F_3
Rosy-magenta* Normal Green	×	92⁵/11 Yellow-leaved	28/12	24 plants. All Green	11/13	78 plants. All Green; no Variegated; no Yellow	—
ditto	×	ditto	29/12	17 plants. All Green		—	—
ditto	×	92⁶/11 Yellow-leaved	30/12	2 plants. All Green	12/13	(not sown)	—
Crimson King* Normal Green	×	92⁶/11 Yellow-leaved	159/12	27 plants. All Green	106/13	114 plants. All Green	161/14 28 plants. All Green 162/14 40 plants. All Green
					107/13	85 plants. All Green	— 163/14 5 plants. All Green

* For a description of these races, see *Journal of Genetics*, Vol. I. p. 102.

TABLE III.

Matings of Variegated or Yellow-leaved ♀ × Normal Green ♂.

Mating			Number of flowers pollinated	F_1	
100/12 Variegated	×	4/12 Normal Green	5		No seeds
100/12 Variegated	×	ditto	1		No seeds
100/12 Yellow-leaved	×	Crimson King Normal Green	7	2Y/14	5 plants. All Yellow
100/12 Yellow-leaved	×	ditto	2	4Y/14	5 plants. All Yellow
100/12 Yellow-leaved	×	ditto	2	3Y/14	1 plant. Yellow
100/12 Yellow-leaved	×	32/12 Normal Green	2		No seeds
100/12 Yellow-leaved	×	ditto	1		No seeds

Discussion of an hypothesis.

The work of Correns on *Mirabilis*[1] has given results of particular interest, because of the relations which the form *albomaculata* has been shewn to possess, not only with the normal green type, but also with the *chlorina* races. The form *chlorina* is distinguished from the normal green type by the pale green colour of its leaves, which is due to the relatively small amount of pigment developed in the chloroplasts. In crosses between the normal green and the *chlorina* form, the normal is dominant to the *chlorina* character, and segregation takes place in the

[1] *Zeitschr. f. Ind. Abst.- u. Vererbungslehre*, I. p. 291, 1909; and II. p. 331, 1909.

usual way. When the *chlorina* ♀ is crossed with *albomaculata* ♂, the hybrids so formed are indistinguishable from hybrids between the *chlorina* and the normal green races, both in appearance and in the progeny to which they give rise. That is to say, the pollen grains of the *albomaculata* race, even when they are produced by flowers borne on the white branches, nevertheless carry the factor for normal green, as distinguished from the pale green of the *chlorina*. In these races there are, therefore, two distinct characters of the chloroplasts, one of which is inherited through the mother only, while the other is inherited in the usual manner. Correns suggests[1] that these facts may be explained by means of an hypothesis based on the assumption, which has received some degree of support from cytological observations, that in the process of fertilization in the higher plants the nucleus of the male gamete passes over to the egg-cell alone and unaccompanied by any cytoplasm, so that the cytoplasm of the zygote is entirely of maternal origin. The germ-cells of the *albomaculata* race are regarded as possessing nuclei which are perfectly normal and carry the factor for the typical green leaf-colour; the cytoplasm of the germ-cells, however, in correspondence with the mosaic of which the plant consists, is either normal or chlorotic ("gesund oder chlorotischkrank") and, accordingly, either permits or prevents the development of normal green chloroplasts. Assuming the cytoplasm of the zygote to be entirely of maternal origin, the offspring of a variegated *albomaculata* plant are, therefore, green, variegated or yellow, according as the cytoplasm of the egg-cells is entirely normal, a mosaic of normal and chlorotic components, or entirely chlorotic.

In formulating this hypothesis, Correns[2] leaves it an open question whether the seat of the abnormality in the chlorotic plastids is to be looked for in the plastids themselves, or in the cytoplasm which surrounds them. Baur, in discussing his experiments on *Pelargonium*[3], inclines to the former alternative; Correns regards the latter as at least equally possible in *Mirabilis*.

In *Primula* no form is yet known corresponding with the *chlorina* races of *Mirabilis*, but the maternal inheritance of the variegated and yellow-leaved characters in *Primula* corresponds exactly with that of the *albomaculata* character in *Mirabilis* and is, I think, to be explained on lines similar to those put forward by Correns. But the evidence

[1] *Zeitschr. f. Ind. Abst.- u. Vererbungslehre*, II. p. 331, 1909.

[2] *L.c.* p. 332, footnote (2).

[3] *Zeitschr. f. Ind. Abst.- u. Vererbungslehre*, I. pp. 348 ff., 1909.

which I have obtained, in the very young leaves of variegated Primulas, of the existence of normal and chlorotic chloroplasts side by side in the same cell, appears to me to afford definite support for the view that the abnormality is localized in the chloroplasts themselves, and is not a function of the cytoplasm as a whole.

In the present state of our knowledge of the functions of cytoplasm and nucleus, the question is one of some importance. If the maternally inherited character pertains to the cytoplasm in general, as contrasted with the nucleus, the consequence is, as Correns has remarked, strongly to emphasise the importance of the part played by the nucleus, at the expense of any part the cytoplasm might be supposed to play, in the transmission of characters which are inherited through the male and female equally. The hypothesis that this function is limited to the nucleus is, on diverse grounds, regarded favourably by many cytologists, but there are still difficulties to be solved before its complete acceptance can pass unquestioned. Moreover, for the present purpose, no assumption in this respect need be made, if the view be justified that the abnormality of the chloroplast, which is inherited through the egg-cell only, is localized in the chloroplast itself. Such a view permits of a modification of Correns' hypothesis, which would serve to account for the maternal inheritance of the character with which we are dealing, while leaving untouched the question as to the relative functions of nucleus and cytoplasm (apart from the chloroplasts) in the transmission of characters which are inherited in the usual way.

Since the work of Schimper[1] and others thirty years ago, the view has gained general support and acceptance that the plastids of a plant-cell are persistent cell-organs, in the sense that they are invariably formed by the division of previously existing plastids and are handed down from mother to daughter cell. It is, then, reasonable to suppose that an abnormality inherent in the plastid itself would be handed on to the products of its division[2], so that abnormal plastids would give

[1] *Jahrb. f. wissensch. Bot.* xvi. pp. 1—247, 1885. References to recent literature connected with this subject are given by Cavers, *New Phytologist*, xiii. pp. 96—106 and 170—180, 1914.

[2] If it be granted that the abnormality, with which we are dealing, is inherent in the chloroplast itself, the genetics of the normal, variegated and chlorotic plants provide what amounts to a proof of this proposition. The original variegated plant, which was the offspring of pure green parents, is an exceptional case. But the rarity of such exceptions—only one has occurred among many thousands of plants—and the fact that the partially or completely chlorotic progeny of that plant has continued invariably to throw plants with chlorotic plastids, is additional support for the view that normally the products of the division of a plastid are like the plastid to which they owed their origin.

rise by division to further abnormal ones, while the products of the division of a normal plastid would be normal. In order to explain the maternal inheritance of this abnormality, it is necessary to adopt the hypothesis, which has met with general acceptance as an extension of the general theory of the persistence of plastids, that the plastids of the zygote are derived solely from those present in the unfertilized egg. Thus we should reach an explanation of the fact that the progeny of pure-green normal and pure-yellow chlorotic plants are, respectively, all green and all yellow, no matter what was the character of the male parent. The variegated plants are invariably the offspring of variegated mothers and they give rise to green, variegated and yellow offspring. The green and the yellow offspring may be explained as originating from egg-cells formed in the pure green or pure yellow patches of tissue which occur in the variegated mother-plant. The variegated offspring must be supposed to have their origin from egg-cells, which are endowed at their formation with a mixture of plastids of different kinds, such as has been found in the cells of the young leaves of the variegated Primulas. The segmentation of a fertilized egg containing different kinds of plastids, each giving rise by its division to plastids of its own kind, would tend to a gradual sorting out of the different kinds of plastids into different daughter cells. Eventually, as Baur has pointed out[1], the great majority of the cells would contain plastids of one or other kind only, and the adult tissues would be a mosaic of cells, the pattern of which would depend upon the distribution in the embryonic cells of the different kinds of plastids.

The foregoing hypothesis rests primarily upon the general theory of the persistence of plastids from cell to cell in a series of cell-divisions, and the extension of that theory to the effect that the plastids of the adult are genetically derived from those present in the egg. Both the theory and its extension have formed the subjects of an extensive literature with which it is impossible to deal in detail here. Suffice it to say, that the theory of the persistence of the plastids has met with very general support and acceptance for many years. The question has, in some respects, been re-opened by recent investigations on chondriosomes in plants[2], but the work which has been done in this direction has not as yet yielded any well-established results: such as they are,

[1] *L.c.* p. 349.

[2] A general review of this work has recently been given by Cavers, *New Phytologist*, XIII. pp. 96—106 and 170—180, 1914. I am greatly indebted to Dr Cavers for his kindness in giving me an advance proof of the second part of his article.

they tend, I think, not so much to call in question that aspect of the theory with which we are concerned, as to support the view that the plastid-origins are handed on from parent to offspring as definite bodies.

Granting that the plastids are persistent cell-organs, the existence of mixtures of plastids of different kinds in certain cells renders it difficult to resist the inference that the abnormality lies in the plastid itself and not in the surrounding cytoplasm. The inference is supported, I think, by the fact that different degrees of chlorosis may occur in the same plant, and by the fact that some entirely chlorotic plants have been found to be mosaics of cells, the plastids of which exhibit well-marked differences in the degree of chlorosis.

The hypothesis that, in the higher plants, the plastids of the zygote are genetically derived from those present in the unfertilized egg-cell has, like the general theory of which it forms an extension, been widely accepted: but it is obvious that it remains an assumption for any particular species, until that species has been the subject of special investigation. In any case, however, the assumption that no plastids (or plastid-origins) pass over in fertilization, from the male to the female gamete, demands less than does the alternative assumption that no cytoplasmic structures of any kind accompany the male nucleus into the egg-cell. In many cases the nucleus of the male cell has been described as becoming disengaged from the cytoplasm, which does not enter the egg-cell: on the other hand, there are cases among the higher plants, in which the male generative cell as a whole enters the egg, and, in some cases, bodies resembling leucoplasts have been observed in the mass of cytoplasm brought into the egg with the male cell[1]. This may, however, be compared with the behaviour of the chloroplasts in certain species of the alga *Spirogyra*. In this genus, the two gametes contribute almost equally to the cytoplasm of the zygote, the chloroplast (or chloroplasts) of the male gamete passing into the zygote along with the other structures of the male cell; but Chmielewsky[2] and Tröndle[3] have shewn that the chloroplast of the male gamete degenerates after entering the zygote, while that of the female gamete alone persists and becomes the chloroplast of the zygote.

[1] For instance in *Pinus*. See V. H. Blackman, *Phil. Trans. Roy. Soc.* B. Vol. 190, 1898, pp. 395—426.

[2] *Bot. Zeit.*, Jahrg. XLVIII. pp. 773—789, 1890.

[3] *Bot. Zeit.* Jahrg. LXV. pp. 187—216, 1907; *Zeitschr. f. Bot.* III. pp. 593—619, 1911.

In the present connexion, the recent investigations on chondriosomes in plants, to which reference has already been made, require some consideration, which, however, need only be of the briefest, as the subject has recently been dealt with fully in a general review by Cavers[1]. The chondriosomes are bodies, the presence of which in the cytoplasm of many organisms has been demonstrated by means of appropriate methods of fixation and staining. They have been described as persistent cell-organs and are regarded by some writers as homologous with the mitochondria of animal cells, to which Meves and others have attributed important functions in the determination of heritable characters. In another direction, the suggestion has also been made that the plastids of plants are derived from the chondriosomes present in the embryonic cells. At the present time, however, our knowledge of chondriosomes has by no means reached the stage at which any definite conclusions can be drawn as to their significance. The real nature of the bodies which have been described under this name is still a matter of considerable uncertainty[2]: whether they are persistent cell-organs, and whether the chondriosomes of plants are homologous with those of animals, still remains to be proved, while the suggested relations between the chondriosomes of plants and plastids are very much open to question[3]. Moreover, apart from the uncertainty attaching to the foregoing points, there is, so far as I know, no definite evidence of the transference of chondriosomes from the male generative cell to the egg-cell, in the fertilization of higher plants, even in those cases in which chondriosomes have been described as occurring in the developing gametes of both sexes.

So far, then, the study of chondriosomes has not afforded any results sufficiently well established to be adduced either in support of, or in opposition to, the hypothesis which has been put forward in explanation of the maternal inheritance of certain forms of variegation and chlorosis. It is much to be desired that investigations should be made on variegated plants, by means of the improved methods employed in the study of chondriosomes, with the object of tracing back the differences between the plastids, which have been observed in the cells of the young leaf, to the earliest stages in development at which they can be recognized. It

[1] "Chondriosomes (Mitochondria) and their significance," *New Phytologist*, XIII. pp. 96–106 and 170—180, 1914.

[2] See Cavers, *l.c.* p. 175, on the resemblance of chondriosomes to myelin forms.

[3] See, for instance, the recent paper of Scherrer, "Untersuchungen über Bau und Vermehrung der Chromatophoren und das Vorkommen von Chondriosomen bei Anthoceros," *Flora*, N. F. LXX. pp. 1—56, 1914.

may be hoped that such investigations will lead to opportunities for observing the distribution of plastids to the daughter cells, during the division of cells containing mixtures of plastids of different kinds. Investigations should also be made into the development and structure of the egg-cells formed in the green, variegated and yellow parts of the plant, and into the process of fertilization.

In the hypothesis which has been put forward above, the suggestion has been made that the character, which is inherited through the egg-cell only, is inherent in the plastid itself and is therefore handed on to the products of its division; in other words, that the plastids are self-determining in respect of the character under consideration. But if this is so in regard to characters of a particular class, there are other characters in which it is not the case. The *chlorina* character of *Mirabilis*, and the plastid-colours of many flowers, are inherited through both sexes equally and undergo normal segregation. Their inheritance may be expressed in terms of Mendelian factors, and it may be presumed that the means by which they are transmitted is the same as that of other Mendelian characters, whatever that may be. The hypothesis of Correns would assign to the nucleus the function of transmitting the factor, which distinguishes the normal green from the *chlorina* races of *Mirabilis*; at the expense, however, of making the assumption that the nucleus alone passes over from the male cell to the egg-cell in fertilization. The modification of the hypothesis indicated above would suggest that this assumption is unnecessary, in order to explain the purely maternal transmission of certain characters of the chloroplast, such as those with which we are dealing. On the other hand, it leaves untouched the question as to the relative parts played by nucleus and cytoplasm in the transmission of ordinary Mendelian characters, among which certain other characters of the plastids are included.

Part of the expenses of the experiments described in this paper was defrayed by means of grants from the Royal Society and from the British Association. A large number of plants were grown for me at the John Innes Horticultural Institution and I desire to express my great gratitude to the authorities for the facilities which they have continued to extend to me.

DESCRIPTION OF PLATES.

PLATE IX.

Fig. 1. A variegated plant with the green tissue occupying the central regions of the leaves, the yellow tissue occupying the peripheral part.

Fig. 2. A variegated plant shewing a sectorial arrangement of the pure yellow tissue.

Fig. 3. Three sister plants from the family 99/12. The plants were of the same age and were grown together under the same conditions. They illustrate the difference in the rate of growth between the pure green (on the left), the variegated (on the right) and the pure yellow-leaved (above) plants.

In the foregoing figures, the arrows point to lines of junction between full green and paler green areas. This difference in colour is due to the presence of more layers of normal green cells in the full green areas than are present in the areas of a lighter green colour.

PLATE X.

Figs. 1—8 and 10 are from sections of fresh material. Figs. 9 and 11—18 are from stained preparations. All the figures were drawn with the aid of a camera lucida.

Fig. 1. Chloroplasts from the palisade cells of the leaf of a normal green plant, after exposure to bright sunlight, shewing the starch grains. ×650.

Fig. 2. Chloroplasts from the palisade cells of a yellow-leaved plant, similarly treated. The plastids are much smaller and contain much less starch. ×650.

Fig. 3. A palisade cell from the leaf of a normal green plant, shewing the chloroplasts after the starch had been removed by keeping the plant dark for some days. ×440.

Fig. 4. A cell from the mesophyll of the same plant. ×440.

Fig. 5. A palisade cell from the leaf of a chlorotic plant for comparison with fig. 3. ×440.

Fig. 6. A cell from the mesophyll of the same chlorotic plant. ×440.

Fig. 7. Cells from the mesophyll of the leaf of a variegated plant after exposure to sunlight. Two of the cells shewn contain normal green chloroplasts; the others contain plastids exhibiting various degrees of chlorosis. ×440.

Fig. 8. Palisade cells from a pure yellow-leaved plant. This yellow-leaved plant consisted of a mosaic of cells of different degrees of chlorosis, and in that respect was comparable with a variegated plant, though it was, nevertheless, quite without normal chloroplasts. Of the three cells shewn, the middle one contained plastids exhibiting an extreme degree of chlorosis; in the plastids of the other two cells the chlorosis was of a less extreme type. ×650.

Fig. 9. Section through a very young variegated leaf, in a region in which the epidermis alone contained coloured chloroplasts. The well-developed plastids of the epidermal cells are shewn; all the other cells in this region of the leaf contained only small, chlorotic plastids (see p. 309). ×650.

Fig. 10. Section of a very young variegated leaf, examined in the fresh state. The section represents a group of cells at the junction between a patch of green tissue and the surrounding chlorotic tissue. The cells *A* and *B* contained (1) bright green chloroplasts of the normal type, with well-developed starch-grains, and also (2) smaller plastids, *c*, *c*, *c*, of a pale colour and with little starch, which were indistinguishable from the pale-coloured plastids of the cell *C*. In most of the plastids of the cell *C* the pigment was readily recognizable, but in a few, *d*, *d*, *d*, no colour could be detected with certainty. The plastids of the cell *D* were, for the most part, nearly colourless in appearance, but three or four, *e*, *e*, *e*, could pass for the pale-coloured kind found in *C*. The cells *C* and *D*, therefore, contained plastids differing from one another in the degree of chlorosis. *E* and *F* were also chlorotic cells, as were all the cells on this side of the section. The plastids of *E* were of the pale-coloured type; in none of those of *F* could any colour be detected with certainty. × 650.

Figs. 11—18 are drawn from very young variegated leaves, examined in fixed and stained preparations. The material was cut in hard paraffin, so as to avoid, as far as possible, the risk of crushing the cells and consequently displacing the chloroplasts from the cells to which they properly belonged. No indication was obtained that any displacement had taken place, and all cells, the walls of which were ruptured, were rejected. The sections were cut so as to pass through adjacent patches of normal and chlorotic tissue. The stains employed were Carbolic Fuchsin and Light Green. This method did not give any definite differential coloration as between the normal and chlorotic plastids, so that it was necessary to rely upon differences in size as the distinguishing feature. The figures represent cells containing chloroplasts of different sizes; all these cells were found at the junction between the normal and the chlorotic tissues. In certain cases, where two smaller chloroplasts lie close together in a pair, it was not always possible, with the method of staining employed, to say whether they were chlorotic plastids, or whether they were the products of a recent division of a normal plastid; but it is clear that the possibility of accounting for some of the small plastids in this way only applies in the minority of cases. This sort of difficulty is much less in preparations of fresh material, where the differences in colour form an additional guide. Figs. 11—18 are all × 650.

Fig. 11. The chloroplast, *x*, is a large one seen in end view. Near it is another large one and also a small one, both seen in face view. The two smaller chloroplasts to the left of the cell may be the products of the recent division of a normal one.

Fig. 12. Cell containing two large chloroplasts and several small ones. The two chloroplasts at (*f*) were in different planes, near the upper and the lower walls of the cell respectively.

Fig. 13. The palisade cells (above) contain only small chloroplasts. Below, to the right, are three cells containing large chloroplasts, some of which are seen sideways or end-on. To the left are two cells each containing both large and small chloroplasts.

Fig. 14. Cell containing several large and one small chloroplast. All the chloroplasts contained in this cell are shewn in the figure.

Fig. 15. The four chloroplasts shewn were in focus together.

Fig. 16. Cell containing four large and five small chloroplasts.

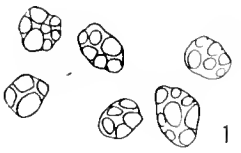
Fig. 17. Cell with three large chloroplasts and several small ones.

Fig. 18. The cell shewn in the middle had chloroplasts of different sizes; those above had chlorotic plastids, those below large ones only.

Fig. 1.

Fig. 2.

Fig. 3.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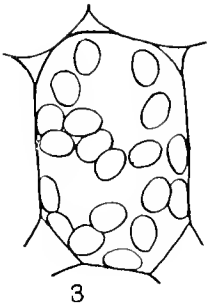
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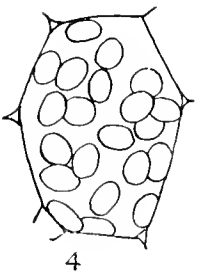
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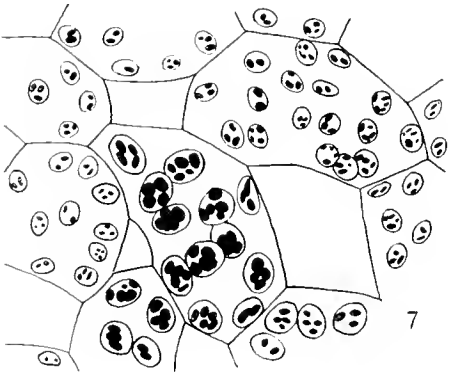
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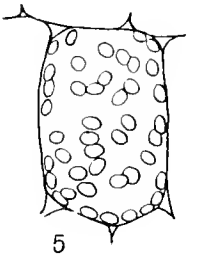
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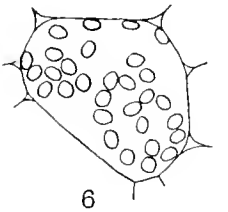
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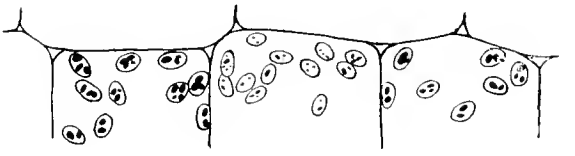
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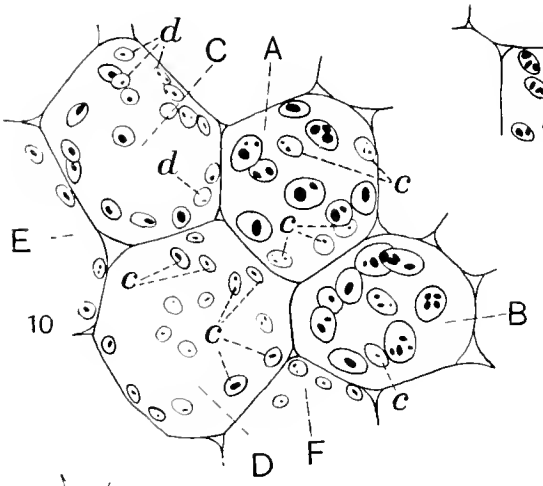

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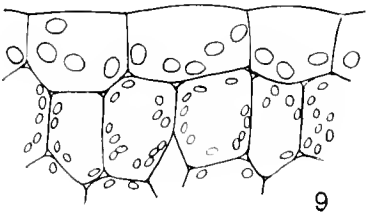
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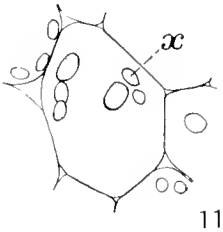

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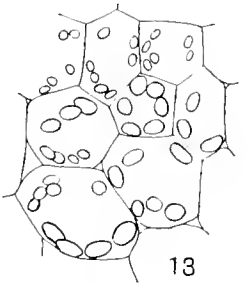
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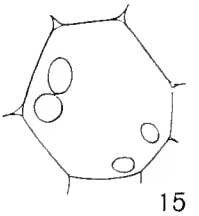
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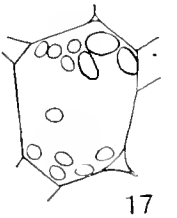
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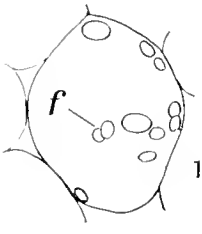

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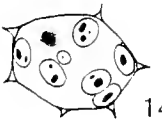
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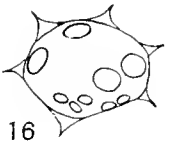
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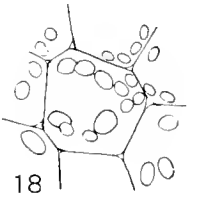

18

A SECOND BRACHYDACTYLOUS FAMILY.

By H. DRINKWATER, M.D., F.R.S. (Edin.), F.L.S.

I HAVE already published accounts of three families showing an inherited symmetrical shortness of the fingers and toes in individuals who are also below the average in stature. In one—the "Brachydactylous Family[1]"—the fingers are reduced to about half the normal length: in the other two families they are intermediate in length between these very short ones and the fingers of average normal individuals, a condition which I have designated by the term "Minor-Brachydactyly[2]."

In both types, the shortening was shown to be due chiefly to an abortive condition of the middle phalanx. The main features are represented in the outline illustrations in Fig. 1, where *A* shows the bones of the middle finger of a normal adult, *C* the Brachydactylous condition, and *B* the Minor-Brachydactylous.

In *C* the middle phalanx (2) is seen to have become ankylosed to the terminal phalanx (3).

In the Summer of 1913, my friend Dr J. D. Lloyd informed me that he knew of some people resident in his neighbourhood whose hands closely resembled those of one or other of the above-mentioned families; and he not only afforded me an opportunity of examining some of them at his own surgery, but very generously consented to allow me to carry out whatever investigations I might be disposed to undertake with regard to them.

Examination at once made it evident that the abnormalities in these new cases are identical with those described in my paper on "Brachydactyly"; there is, namely, the maximum amount of shortening of the digits, and the same characteristic shortness of stature. The essential peculiarities are so exactly similar that one cannot resist the

[1] "An account of a Brachydactylous Family," *Proc. Roy. Soc.* Edin. Vol. XXVIII. Part I.

[2] "Account of a Family showing Minor-Brachydactyly," *Journal of Genetics*, February, 1912. "Minor-Brachydactyly" No. 2, *Journal of Genetics*, February, 1914.

conviction that the two families must have descended from the same original stock.

The connection between them unfortunately cannot now be established, for there is not a single surname common to the two families. This difference in names may be accounted for by the

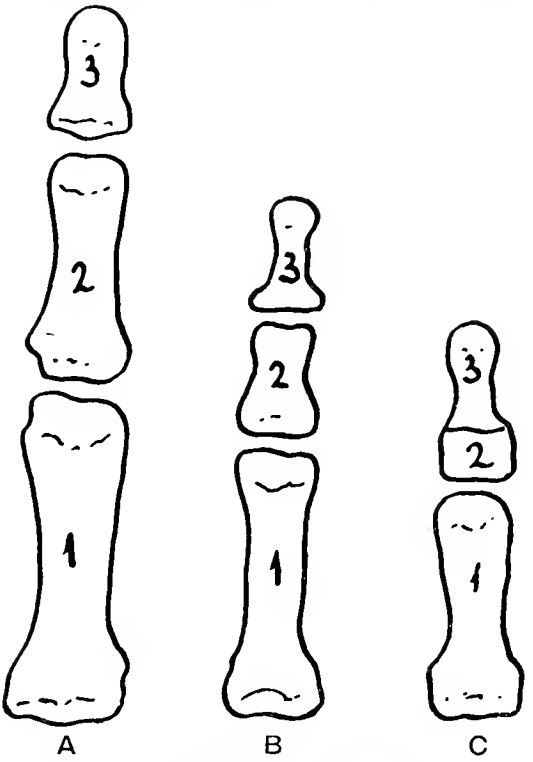


Fig. 1. Normal and Brachydactylous Phalanges. (Natural size.)

A. Normal. B. Minor-Brachydactylous. C. Brachydactylous.

fact that the inheritance in the second family has been almost exclusively through the female line, so that the surname has changed (through marriage) at each generation.

Both families reside in the same part of the country: a fact which would seem to give some support to the theory of their common origin.

Dr W. C. Farabee was the first to describe the condition of Brachydactyly in a family that he studied in 1903[1] in North America. Is

[1] Published in March 1905 by the Peabody Institute, Harvard, U.S.A.

there any blood relationship between this American family and either of the English families? The cases observed by Farabee were apparently identical as regards the anatomical and hereditary features with those described in my first paper in August 1907. I was not aware of Farabee's work until my own investigations were nearly completed, and there was not time to decide the question of relationship before presenting my paper to the Royal Society of Edinburgh. His reply to my enquiries did not come for several months afterwards, for he happened to be in some out-of-the-way part of Peru engaged on a scientific expedition. However, it was found that no surname was common to the two families; nor was it possible to make my chart fit in with his: so that, assuming that there had been a common ancestor of the Brachydactylous type (and this hardly admits of doubt), he or she must have lived prior to the earliest member now traceable in either of the two families. A study of Farabee's account made me think it likely that his family had descended from "an abnormal member of the English family, four generations back, who had migrated to America, but of whom no tidings have since been received by his relatives in this country," but as this man's name does not occur in Farabee's family, he was obviously not the connecting link. So far, therefore, I have not been able to prove any blood relationship as existing either between my two families or between the first of these and Farabee's.

There still remains the question whether the second family (about to be described in the present communication) is related to Farabee's. Farabee informed me that the name amongst his people which most nearly approached any name in the English family was *Hyde*. Now the man marked 2 in the chart on page 327 was named *Benjamin Hyde*. In November of 1913 when the chart was almost complete, I forwarded Dr Farabee a copy of the earlier generations, numbering each individual 1, 2, 3 etc. My letter was a long time in reaching him, for again he was away from home on another scientific expedition—this time in Brazil. I asked if there was a *Benjamin Hyde* in the American family? At the end of May I received a letter from him from Barbados, dated May 16th, 1914, in which he says:

"Your letters of November 26th, 1913, and February 9th, 1914, have just reached me. For the past ten months I have been in the interior of Northern Brazil and Southern British Guiana, out of touch with the rest of the world.... *You have settled the whole question. 2 is Benjamin Hyde.* His mother had short fingers, and she was the only one of her family who had. She had eleven children, but I was

unable to learn even the sex of three of them marked ??? as they died young. I had hoped to look up the English branch of this family, but am glad to know you have found it. You will have pleasure in the comparative study...."

Now, as the name *Benjamin Hyde* is a very rare combination, there can be little doubt that Farabee's family and this second English family are blood relations and belong to the same stock; the only difficulty with regard to it is the fact that in the English there is one generation more than in the American family. This may be owing to the fact that it is eleven years since the American chart was constructed, and also because marriage may not have occurred at such an early age over there as in this country, for in the English family many of the women have married whilst still "in their teens."

Moreover the relative proportion of abnormal males and females is exactly the same in these two families, viz. 61 per cent. of women in the American family and 61 per cent. in the English family. This may be a mere coincidence, but nevertheless it seems rather remarkable, and differs from the proportions found in my first family, where there were 19 females to 25 males.

Fig. 2 shows Farabee's chart of the American family, and Fig. 3 the English family.

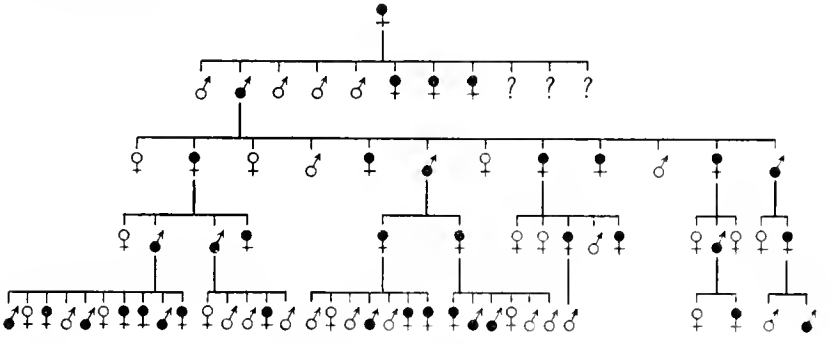


Fig. 2. Farabee's Chart.

It will be observed that the second generation in the English family is incomplete compared with the second generation in Farabee's chart, but there is no real discrepancy. It was Benjamin Hyde's sister, who removed from America and settled in this country, who became the ancestor of the English branch. My informants know that her maiden name was Hyde, that she had a brother named Benjamin Hyde, and that their mother had *several* other children, but do not know their type or sex. As the rest of the family remained in America and lived in

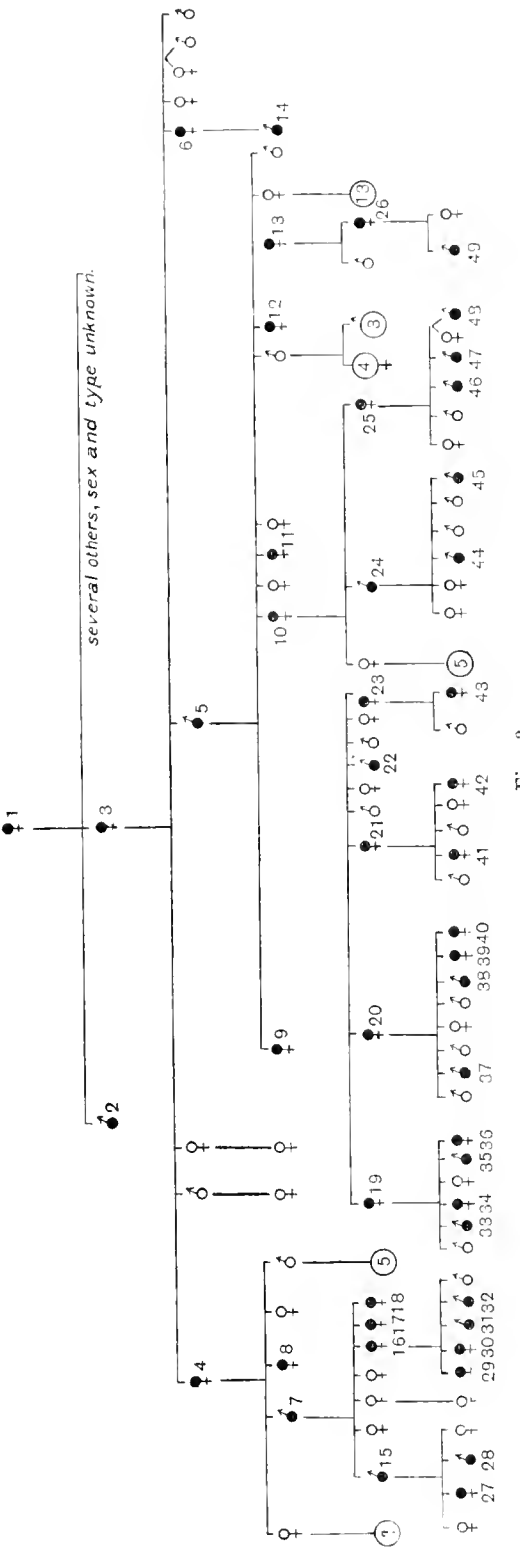


Fig. 3.

Farabee's neighbourhood, he would be likely to get a more complete record than I could obtain, and hence the difference in the second generation in the two charts is accounted for.

It will be observed that Farabee has here indicated the descendants of *abnormals* only; my chart includes the offspring of *normals as well as abnormals.*

The rest of this paper refers exclusively to the English family which I have recently studied.

The abnormality, wherever it exists at all, affects *both* hands and *both* feet, as well as the stature. Both hands and both feet are always affected symmetrically.

The chart includes 50 abnormal individuals, of whom 34 are living. Through the great kindness of these people I have been able to obtain photographs of the right hand of 30 of them, and radiographs of the hand and foot of 29, as well as a few full length portraits;—a most satisfactory record, when one considers their very natural reluctance to do anything calculated to draw attention to them individually. In fact there are only two adults whom I have failed to persuade to pay a visit to the photographer and radiographer.

An Example of Mendelian Inheritance.

Students of Mendelism will at once recognise that Brachydactyly as illustrated by this family conforms in a remarkable degree with certain laws enunciated by Gregor Mendel. One of these laws states that a "dominant" character is transmitted only by a member showing that character, and not by a member showing the "recessive" character, and that the recessives always breed true to the recessive character.

In this family the normal individuals are "recessives," and according to the theory *cannot* bear abnormal (short-fingered) children; and the chart shows that the normals have had normal children only. In other words, every abnormal child has an abnormal parent. As there has been no inter-marrying of abnormals, it follows that each abnormal child has had one abnormal and one normal parent—one showing Brachydactyly, and one with the normal type of hand and foot.

When abnormal individuals—the offspring of parents of two distinct types—marry *normals*, the offspring (in conformity with Mendel's Law) should be of both types, in approximately equal numbers—50 per cent. of normals and 50 per cent. of abnormals. That is what Mendel found

in the case of two types of sweet-pea, and his results have been repeatedly confirmed by other observers. The normals (recessives) should produce normals only: the abnormals (dominants) should produce normals and abnormals in about equal numbers. The chart at once shows that the *types* produced accord with this theory. What about the relative proportion of the two types among the children of the abnormals? The short-fingered children of abnormal parents ought to be equal or very nearly equal in number to the normal-fingered children of the same parents.

As a matter of fact this equality is not usually *exact*, and theory does not require it to be so, for there is a *chance* element which comes into play which makes this precise number uncertain and variable. According to Mendel the germ cells of any individual, such as one of these abnormals, are of two kinds. One kind of cell carries the factor which can produce the abnormality, and is inherited from the abnormal parent; the other kind of cell, being inherited from the normal parent, lacks this factor: and these two kinds of germ cells are present in the individual *in equal numbers*. If this be true, it must of necessity follow that the particular kind of germ cell which will take part in any given fertilization, will depend, so far as we can tell, upon a chance meeting; just as there is a chance of drawing a black or a white marble out of a hat which contains an equal number of each. It is a lottery with equal chances for both kinds. Sometimes one kind will predominate, sometimes the other, but in the long run the numbers will be approximately, if not exactly, equal.

In this family there are 50 abnormals, so that according to Mendel's theory there ought to be *about* 50 normals. The actual number of normals is 48.

Thus, instead of the exact 50 per cent. of abnormals which *might* occur in strict accordance with theory, we have 51·02 per cent. This is sufficiently near to constitute this family a striking instance of Mendelian inheritance in the human subject. This remark applies also to the other instances of Brachydactyly published by Farabee and myself, as well as to my illustrations of Minor-Brachydactyly.

If a normal member of this family were to ask whether if married to another normal (relative or non-relative) there would be any risk of having Brachydactylous children, one would be justified in replying that there was no risk whatever of such children resulting from the union, for, not in a single case has a short-fingered child been born of two normal parents. This abnormality thus differs in a remarkable

manner from certain hereditary diseases (such as colour-blindness and haemophilia) which are known to appear in the male children of a woman, who, though showing no sign of these diseases herself, is yet able to transmit them from her affected male parent to her children.

There are six generations shown in the chart. All the individuals of the first, second and third generations are dead: of the abnormals of the fourth generation, only two are still living (Nos. 8 and 9); of the fifth and sixth generations, nearly every member (of both types) is still alive.

Of the 34 normals living at the present time, I am able to give fairly complete details of 30, and very incomplete details of the remaining four.

I am greatly indebted to Dr Geoffrey Williams of Wrexham, Dr John of Stoke-on-Trent, and Drs Bythell and Boydell of Manchester for the excellent radiographs, without which it would have been impossible to arrive at a correct interpretation of the exact nature of this interesting abnormality.

"The hands[1] and feet, as already stated, are abnormal in each affected individual, and the feet are, if anything, more abnormal than the hands, at least as regards their digits. The middle phalanx is practically or virtually—though not actually—absent from each finger and toe. The metacarpal bones are short and otherwise abnormal, but the metatarsus is scarcely, if at all, affected. Nor is the variation limited to the hands and feet, for *all* the individuals, with the exception of young children.....are below the average stature, as shown by a reference to the table of measurements" (page 338). It will be well to study each feature in turn in the following order: hands, feet, stature, etc., as measured and as revealed by photography and radiography.

Facts revealed by Photography.

A. *Hands: External aspect.*

Length. "The most conspicuous feature is the shortness, especially of the fingers; these are only slightly more than *half* the normal length, sometimes even less than half, whilst the hand looks abnormally broad. The middle finger, measured on the palmar surface from the

[1] All quotations such as this, where the source is not mentioned, are from my paper on Brachydactyly read before the Royal Society (Edin.) and serve to show features common to the two families.

base or metacarpo-phalangeal crease to the tip, is normally as long as the width of the palm at the knuckles: in these people it is approximately half," the average length in the adults being $\frac{5}{8}$. "Sometimes it is shorter than the first finger." The extreme shortness in one or two cases is due chiefly to the metacarpal bone. The hand, measured from the carpal end of the radius to the tip of the first finger, has an average length of about 5 inches. The width of the hand exceeds the normal in numerous instances.

Markings. "The skin creases are peculiar. Each finger shows only one crease, corresponding to the space between the first and third phalanx[1]. It is *single*, like the one present in ordinary fingers opposite the second joint." The crease "opposite the first joint is double in most normal hands."

"Whilst it would be difficult to tell that there are only two phalanges in each finger, from an inspection of its dorsal aspect, the palmar view shows clearly that such is the case; and this is confirmed by radiography in the majority of instances."

"The palm shows two peculiar lines." "One runs straight across the hand transversally...it appears to be a union of the palmist's lines of "heart" and "head."

"The second line, often the deeper of the two, starts from the middle of this transverse line and runs to the space between the first and second fingers." This is shown very distinctly on Nos. 20, 38, and 39 (Plates XII and XIII). It is occasionally faintly visible in normal hands, but as a rule is quite absent.

"The skin is loose, and the whole hand is soft and flabby."

"The palm is very compressible, owing to the wide intermetacarpal spaces." The fingers are all "double jointed," i.e. they can easily be flexed dorsally by slight pressure applied directly to their palmar surfaces.

"A slight lateral pressure makes the palm half an inch narrower, without altering the plane of the metacarpals. The narrowing is not due to a transverse folding of the hand."

"On flexing and extending a finger at the basal (metacarpo-phalangeal joint) *slowly*, one can feel that the opposing surfaces are not uniformly curved as in the normal hand. At certain points the phalanx seems to slide over a ridge. This is what one would anticipate from an examination of the radiographs." "The ring-finger in several instances is bent at the middle, so that the tip points towards

[1] A bone of the finger.

the middle finger." This inclination is sometimes so marked that, in the position of rest, the ring-finger lies partly in front of the middle one. Strangely enough the little finger tends to project away from its neighbour, so as to leave a large gap between the two (Pl. XII, Nos. 27, 23, 41).

The skin of the back (dorsum) of the hand is very coarsely reticulated.

The mouth of the sweat glands is conspicuous.

The nails are well formed in every individual.

Strength of Grip. It is considerably below the normal average.

B. *The Feet.*

"Here the one main peculiarity is the shortness of the toes—each one apparently having only two phalanges. They are also broad and straight, with very little tendency to the extreme flexion of many ordinary toes."

In the photograph of the plantar aspect of the foot of the child (Pl. XII, No. 43), the toes are shown fully extended. Frequently the length scarcely, if at all, exceeds the width, as in the three smaller toes in this child.

Facts revealed by Radiography.

A. *Hands.*

The X-rays show that the middle phalanx is not really absent; a fact which could not possibly have been ascertained from the most careful ocular and digital examination. Without the aid of radiography one would conclude that there is an absence of the middle phalanx, and even the X-rays might lead to the same conclusion if the hands of *some* adults were the only ones examined. The young man, Pl. XIV, No. 37, for instance, has only two bones in each finger, and at first sight it looks as though the middle bone—the second phalanx—is altogether missing, whilst the other bones appear about normal; but if the terminal phalanx is carefully compared with the corresponding bone in a normal hand it will be seen that the base is much thicker than it should be. In most of the abnormal fingers, this thickened base is seen to form almost a perfect cube, being as broad as long. Whenever this condition is present, it will be found that there is no vestige of the *separate* middle phalanx.

What is the nature of this cubical basal portion of the terminal phalanx? and what has become of the second phalanx?

In the *middle* finger of every abnormal adult member of this family, the second phalanx is always present as a *separate* bone, but invariably it is so far reduced in length as to become a cube; and the terminal (third) phalanx is of the normal pyramidal shape. The ring-finger also, in certain individuals, resembles the middle finger in these particulars, having a normal-looking third phalanx, and a separate, short, cubical middle phalanx. Whenever the terminal phalanx has a cubical base, this base corresponds in shape with the second phalanx present as a separate bone in the middle finger. In fact "this cubical basal portion is the second (middle) phalanx that has become ankylosed[1] to the terminal phalanx. The pyramidal distal portion of these bones corresponds to the ungual phalanx and the basal cubical portion to the middle phalanx."

"It does not exist as a separate bone in either the index or little finger in a single adult."

"What, then, has happened to the middle phalanx? It varies in two respects from the normal:

1. It is always very short.
2. It generally becomes ankylosed to the base of the terminal phalanx.

The fact that the middle phalanx is abortive, but not completely absent, is proved conclusively by an examination of the radiographs of the hands of young children, before ankylosis has occurred, and in whom the middle phalanx is seen to be invariably present, but at the same time abnormal. Each phalanx, in the ordinary (normal) hand, consists at first of cartilage (gristle) and is gradually transformed into bone, or, as it is termed, becomes ossified, such transformation being limited for some time to two parts, one forming the great bulk or *shaft* of the phalanx, and the other a thin plate or disc at the base. This disc is termed the *Epiphysis* of the phalanx.

That narrow strip of the cartilage which is situated between the bony shaft and the bony epiphysis usually remains unossified until about the twentieth year in each phalanx. The epiphysis of each phalanx is seen to be situated at its base; but the epiphysis of each metacarpal (palmar) bone is at the distal extremity. So long as the strip of cartilage between the shaft and the epiphysis remains

[1] Joined by bony union.

unossified, the bone can increase in length, but after its ossification further growth does not occur.

The disc seen in some of the radiographs at the base of the terminal phalanx *looks* very much like an epiphysis, but is really the second phalanx in a very abortive condition.

In some instances in children there is only one piece of developing bone visible between the shafts of the third and first phalanges; normally, three can be seen: namely, (1) the epiphysis of the third, (2) the shaft of the second, and (3) the epiphysis of the second phalanx, and it is only possible by a comparison of several hands to say what this single bone represents. I believe it always represents the shaft of the second phalanx.

In no single instance does the radiograph show the presence of an epiphysis at the base of the second phalanx.

It is thus clear that there is no real absence of the second phalanx in any individual, but merely a rudimentary condition, and that at a certain stage of development there is a union of this with the terminal phalanx.

"The essential feature of the abnormality apparently consists in an absence of the epiphysis at the base of the second phalanx. It is possible that the epiphysis is also missing, in some instances, from the third phalanx, and that the two phalanges (second and third) consist at first of a single piece of cartilage."

Premature union of shaft and epiphysis is of general occurrence in this family. Such union should not occur until about 20 or 21 years of age. In No. 41, a girl of 15, it is already complete, and the radiograph would pass for that of an adult hand. In this instance, moreover, the union is not recent, for the epiphyseal lines are already almost entirely obliterated. The hand of the boy (Pl. XV, No. 31), aged 8 years and 4 months, shows a very interesting peculiarity. The epiphysis of the third phalanx, though still separate from its own shaft, has already united with the shaft of the second phalanx in the first and little fingers. This seems to point strongly to the opinion expressed above, that these two phalanges are sometimes represented by only one piece of cartilage. *This premature union of the shaft and epiphysis is no doubt one great factor in producing the characteristic shortening.*

"The index and fourth fingers seem more aberrant than the second and third, as they (the former) never show the middle phalanx as a separate bone in the adult." In Pl. XV, No. 27 (*aet.* 19), the union has occurred in the little finger: in the index finger the union is not yet complete, but it is evidently taking place.

"Functionally, the fingers are all reduced to the bi-phalangeal condition, and thus come to resemble thumbs."

The Thumb. "The change in the *thumb* consists of a shortening of the first phalanx which is reduced to a cube. There is no attempt at ankylosis however." In some children the epiphysis is present, but in others it is apparently absent, for if present union must have occurred before the seventh year. The terminal phalanx has the epiphysis in every individual.

"The so-called metacarpal bone of the thumb is in reality the first phalanx, for here the epiphysis is clearly seen at the *base*, whereas in the other metacarpals the epiphysis is seen at the *distal* end of the bones."

"The Metacarpals vary more or less in different individuals, but, as a rule, where ossification is complete, they are abnormal." "The head of each metacarpal is distinctly nodulated in many cases....The middle one is the shortest of the four," in No. 25, and in this woman the whole palm shows the maximum degree of shortening. In Pl. XV, No. 27, the metacarpals of the middle and little fingers are much shorter than the other two.

"*Sesamoid* bones are well shown in several cases."

Spaces. The intermetacarpal spaces are generally increased in width, especially between the *heads* of the bones. Sometimes it is about three times the normal width. This accounts for the easy compressibility of the palm laterally.

MEASUREMENTS: AVERAGE IN INCHES.

The following table gives the average length of the middle finger, the hand, and the stature in eleven abnormal adult women (8 married and 3 spinsters).

Middle Finger	Hand	Stature
$1\frac{5}{8}$	$4\frac{7}{8}$	$57\frac{1}{2}$

In normal women these measurements are about 3, $6\frac{3}{4}$ and 64 respectively.

There is only one abnormal adult male member of the family now living, and I have not yet seen him. The only measurement he has sent me is that of the middle finger, which he says is $1\frac{5}{8}''$ in length.

There are three abnormal males between 17 and 21 years of age, and their averages are as follows[1]:

Middle Finger	Hand	Stature
$1\frac{7}{8}$	$5\frac{5}{8}$	$61\frac{1}{2}$

[1] In the first family: these measurements were

1—15/16	$5\frac{1}{2}$	61.

The longest middle fingers measure 2 inches (Nos. 8 and 28). The shortest measures $1\frac{1}{4}$ inches (No. 21), and three measure $1\frac{1}{2}$ inches (adults).

The tallest male is $64\frac{1}{2}$ inches in height (No. 28): the tallest woman is 59 inches (No. 26): the shortest woman 52 inches (No. 9).

B. *Feet.*

The shortness of the feet is "due solely to the abortive middle phalanx, and the somewhat stunted growth of the first."

"The so-called first phalanx of the big-toe is considerably shortened"; in most cases it is reduced to a cube, and in some the shortening goes even further.

"The middle phalanx in the other toes has become ankylosed to the terminal one in every adult." This ankylosis "occurs at an earlier age in the foot than in the hand, though the hand as a whole is more abnormal."

"There is an absence of the basal epiphysis of the first phalanx of the big-toe, though it is conspicuous in the first phalanx of each of the other toes (in children), so that this bone appears to be really homologous with the second phalanx of the other toes."

Metatarsus. There is very little wrong with the metatarsal bones, in which respect they differ from the metacarpals. In two cases (Pl. XIV, Nos. 27 and 28) the third and fourth are the shortest of the series.

Thigh and Leg. These are shorter than normal, but the numbers especially of the normals are too few from which to calculate a reliable average. There is no doubt, however, that the short

Stature is entirely accounted for by the shortness of the lower limbs. When the adults are seen seated they do not strike one as being particularly short people, but on standing up it is at once apparent that they are considerably below the average height. The spinal column is therefore approximately of the normal length.

This fact seems fairly clear from the photographs of the two brothers (Pl. XI): the one on the right (No. 37) is $17\frac{1}{4}$ years old, $59\frac{1}{2}$ inches in height, and is Brachydactylous: the other one is 14 years old, 3 inches taller, and is not Brachydactylous. The difference in the length of the legs is very obvious, which no doubt accounts for their difference in stature.

The infant (No. 48) is three-quarters of an inch taller than his normal twin sister, in this respect presenting a striking and unexpected

contrast to the rule that holds good in adults and youths, so that the arrest of growth occurs in some later period of life, probably beginning at or soon after the age of 2 years.

The shortness of hands is well shown in Pl. XII, Nos. 23, 37 and 41, where they are represented with normal relatives for the sake of comparison.

Symmetry.

"In every instance the hands are exactly symmetrical, as shown both by photography and radiography (Pl. XV); and I believe the same rule holds true with regard to the feet, though *both* feet were not examined in all cases." This remarkable symmetry obtains even in those cases that show special peculiarities of any of the bones, either in shape or ossification, or in the presence and time of union of the epiphysis.

Persistence.

There is no apparent tendency to revert to the normal type, for the hands and feet of No. 9 are no more abnormal than those of her grandchildren; neither is there any other indication of the abnormality disappearing: on the contrary the numbers seem to be on the increase, the abnormals in the last four of the six generations in the English branch being as follows:

3	in the	third	generation.
8	„	fourth	„
12	„	fifth	„
23	„	sixth	„

One would expect *a priori* that individuals whose fingers are short, stumpy and below the average in strength would be so much handicapped in the "struggle for existence" that they would be swamped by the general population, and in an uncivilized state of society this surely must have occurred; but the conditions of modern society have afforded them such chances of survival that their numbers are steadily increasing, yet it seems clear that they *are* handicapped to some extent, for all the employed men and women are engaged in occupations where there is no great need for manual dexterity: their social position is below that of their normal relatives.

Marriage.

Marriage is the fashion amongst these short-fingered people and they "go off," as one of the women expressed it, before their normal relatives. The great majority of those shown in the chart as not having had children died in infancy. Several adult normals remained unmarried. Why should this be? There must be some special fascina-

Measurements of Abnormals in inches.

Initials	Number in Chart	Sex	Age	Middle finger	Hand: Length	Hand: Width	Ulna	Tibia	Height
Mrs E. T. ...	9*	female	64	1½	5	3	7¾	10	52
Mrs M. ...	16	,,	37	1⅝	5¼	3⅛	--	--	58⅝
M. H. ...	17	,,	33	1¾	5	3⅛	--	—	58½
M. H. ...	18	,,	31	1⅞	5¼	3¼	9	11½	60½
Mrs M. A. L....	19*	,,	43	1¾	5¼	3¼	7⅝	—	55¼
Mrs R. F. ...	20*	,,	41	1½	4⅝	3⅛	9	13	59½
Mrs S. E. J....	21*	,,	39	1¼	4	2¾	6¼	9	56
Mrs O. R. ...	23*	,,	26	1⅝	4⅝	3½	7½	10½	57½
W. H. ...	24	male	39	1⅝	--	—	—	—	—
Mrs O. G. F....	25	female	37	1½	4½	3⅛	8	11¾	57
Mrs R. T. ...	26*	,,	28	1⅝	5	3½	7⅝	—	59
H. H.	27*	,,	19	1⅜	5	3½	8½	11¾	59
S. H.	28	male	18	2	6	4	—	-	64½
E. M.	29*	female	12¾	2	5¼	3	—	-	58
E. M.	30	,,	10½	1 13/16	4 1/16	2⅝	-		52¾
J. M.	31*	male	8 1/16	1½	4⅛	2⅛	-		47½
P. M.	32	,,	5 1/16	1¼	3⅛	2⅛	-		42
W. L.	33	,,	17	1¾	5½	3⅝	9½	—	60½
E. L.	35	,,	10 1/16	1½	4½	2½			13
L. L.	36*	female	7	1¼	4	2½	5⅓	8½	43
C. F.	37*	male	17¼	1⅝	5½	3¼	9	13	59½
W. F.	38	,,	8	1 1/16	4	2½	6⅛	9	45½
E. F. ...	39*	female	6	1 1/16	3⅛	2⅛	6	7⅛	42½
A. F.	40*	,,	2½	⅝	2¼	2	1½	6	32¼
S. J.	41*	,,	15	1⅝	5	3	7½	11	54½
A. J.	12	,,	3¾	1⅛	3	2 1/16	5	6⅛	32
E. R.	43	,,	4½	1⅛	3½	2¼	5⅛	8	38¼
W. H.	44	male	8	1½	—	—	—	—	--
D. H.	45	,,	1 1/12	1	—	—	—	—	
J. A. F.	46	,,	12	1⅛	4¼	2¾	7½	11	54½
A. F.	47	,,	6¼	1 1/16	3⅛	2¼	6¼	8	41
A. F.	48	,,	1 1/12	⅝	2⅛	1¼	3½	4¼	28¼
C. C. T.	49*	,,	7	1⅝	4	2½	5¾	9¼	44¼

* Individuals marked by an asterisk are illustrated on Plates XII—XV.

tion that compensates for the manual defects, but what it is I am unable to say. It might be suggested that their amiability and obliging disposition (of which this paper is sufficient evidence) will afford an explanation, but I should not like to say that the normal members fall short of them in this respect.

Of the first family there are 27 or 28 abnormals now living: so that altogether there are at least 60 people of the Brachydactylous type in England at the present time.

The normals do not appreciably differ in the above dimensions from the general population, and I give the details of only a few children and adolescents for comparison with their short-fingered relatives.

Measurements of Normal Members of the Family.

Initials	Sex	Age	Middle finger	Hand		Ulna	Tibia	Height
				Length	Width			
A. J. ...	male	17	$3\frac{1}{8}$	$7\frac{1}{2}$	$3\frac{1}{2}$	$9\frac{1}{2}$	$12\frac{1}{4}$	$67\frac{1}{2}$
W. J. ...	,,	12	$2\frac{1}{2}$	6	$2\frac{3}{4}$	$7\frac{1}{4}$	$10\frac{3}{8}$	51
A. F. ...	female	16	$2\frac{1}{2}$	6	$2\frac{7}{8}$	9	12	$58\frac{3}{4}$
F. W. F. ...	male	14	$2\frac{3}{4}$	6	3	$8\frac{1}{4}$	$11\frac{1}{2}$	$54\frac{3}{4}$
L. O. F. ...	female	$1\frac{5}{12}$	$1\frac{1}{2}$	3	$1\frac{3}{4}$	$3\frac{3}{4}$	$5\frac{1}{2}$	$27\frac{1}{2}$
E. F. ...	male	19	$2\frac{3}{4}$	$6\frac{3}{4}$	$3\frac{3}{8}$	9	12	$60\frac{1}{2}$
J. F. ...	,,	14	3	$6\frac{1}{2}$	$3\frac{1}{2}$	$9\frac{1}{2}$	$14\frac{1}{4}$	$62\frac{1}{2}$
R. F. ...	female	10	$2\frac{1}{2}$	$5\frac{3}{4}$	$2\frac{3}{4}$	$7\frac{1}{2}$	11	50
E. H. ...	,,	20	$3\frac{1}{8}$	7	$3\frac{1}{4}$	—	—	$61\frac{1}{2}$
H. H. ...	,,	17	3	7	$3\frac{1}{2}$	—	—	$59\frac{1}{2}$
S. M. ...	male	$2\frac{1}{2}$	$1\frac{3}{4}$	—	—	—	—	$33\frac{1}{4}$

Part of the expense of this investigation has been defrayed by the "Earl of Moray Fund for Original Research" of the University of Edinburgh. I very gratefully record my thanks to the Trustees for their generous help.

EXPLANATION OF PLATES.

The numbers are those given in the chart on p. 327 and also in the Table on p. 338.

PLATE XI.

Fig. 1. Shows three brachydactylous females; a child, its mother and grandmother (No. 43, 23 and 9). The short stature of the women is evident when compared with that of the author which is 5 feet 8½ inches.

Fig. 2. Shows two brothers. The one on the right is brachydactylous and two years older than the one on the left, who is normal.

PLATE XII.

Photographs of hands and a foot. The numbers refer to those in the chart. Their ages and other particulars are given in the table of measurements on page 338. The same applies to the following plates.

PLATE XIII.

All these hands are brachydactylous.

PLATE XIV.

Radiographs of hands and feet, all abnormal.

PLATE XV.

Radiographs of hands. Both hands are shown in order to illustrate the bilateral symmetry.

Fig. 2.

Fig. 1.

No. 23 and normal.

No. 37 and normal.

No. 20.

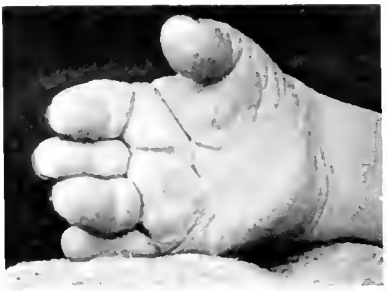
No. 43.

No. 30.

No. 27.

No. 41 and normal.

No. 9.

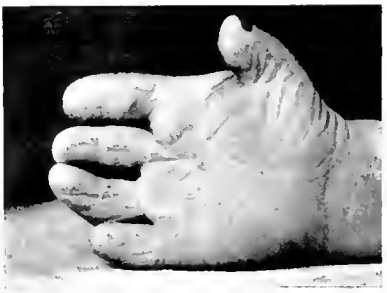


No. 21.

No. 17.

No. 23.

No. 18.



No. 25.

No. 19.

No. 26.

No. 32.

No. 42.

No. 36.

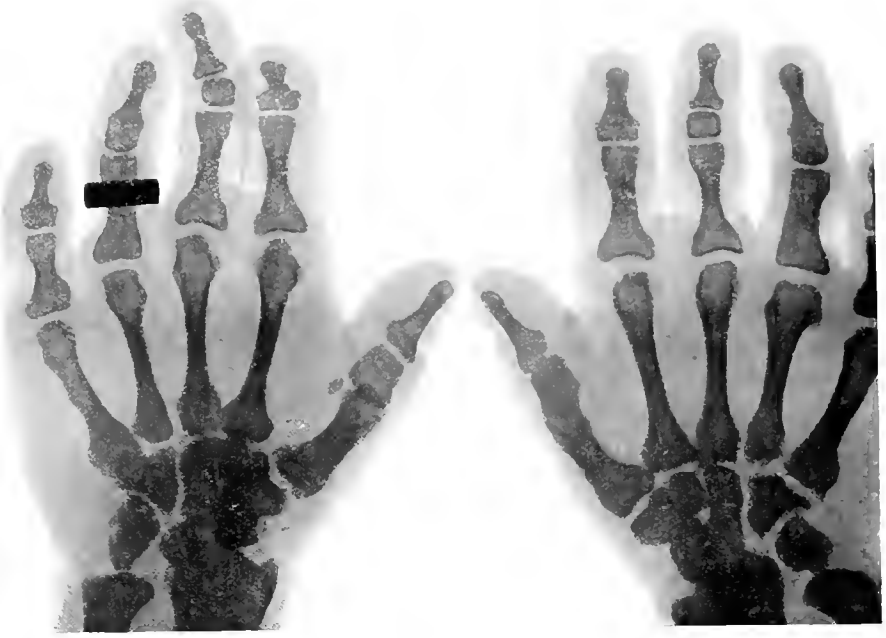
No. 43.

No. 38.

No. 47.

No. 40.

No. 49.



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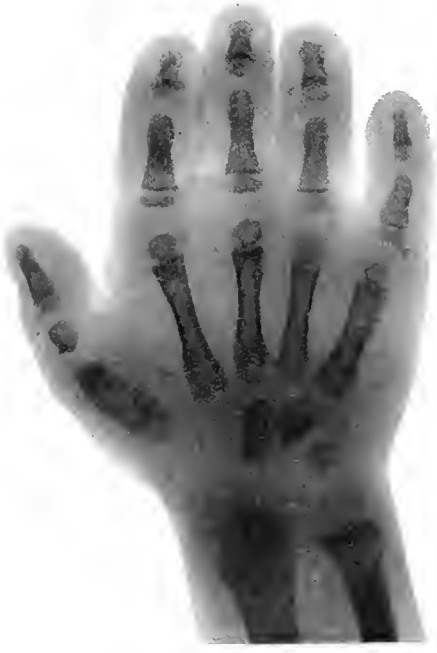

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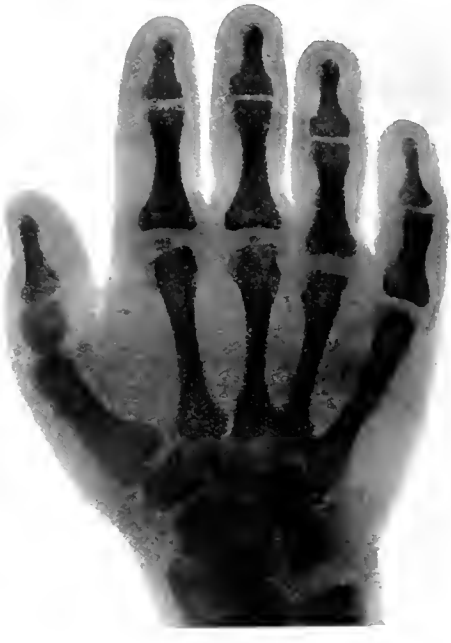

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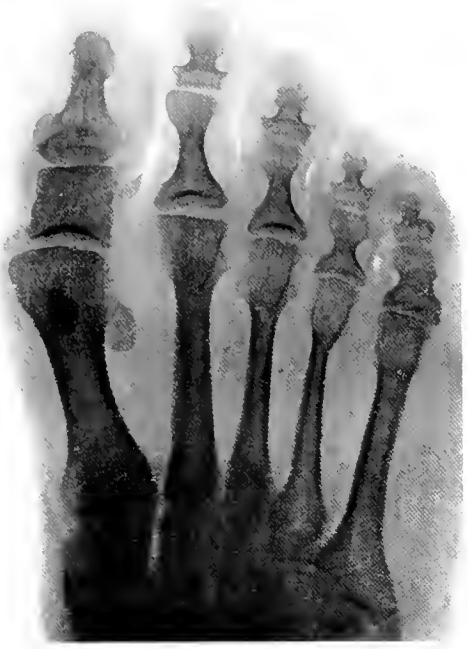

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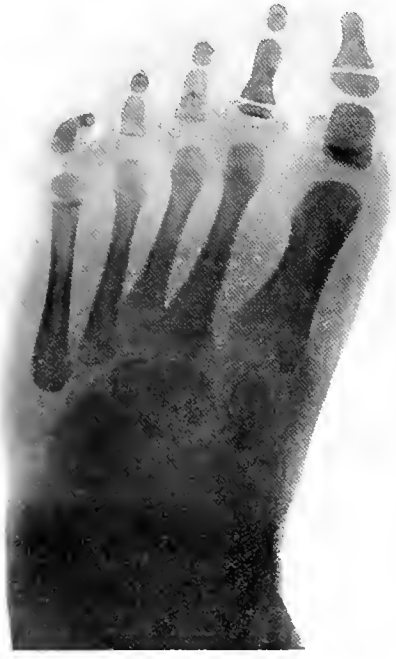
No. 27.



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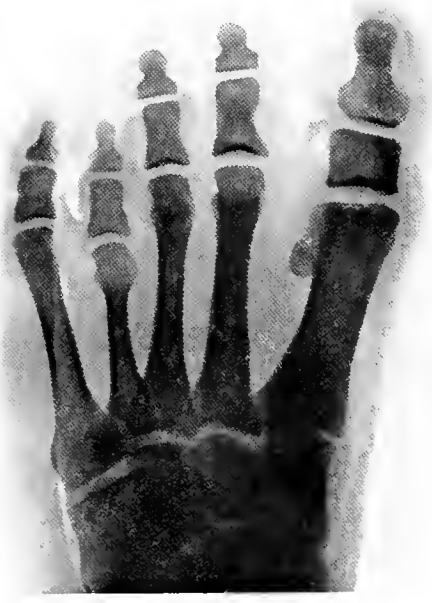
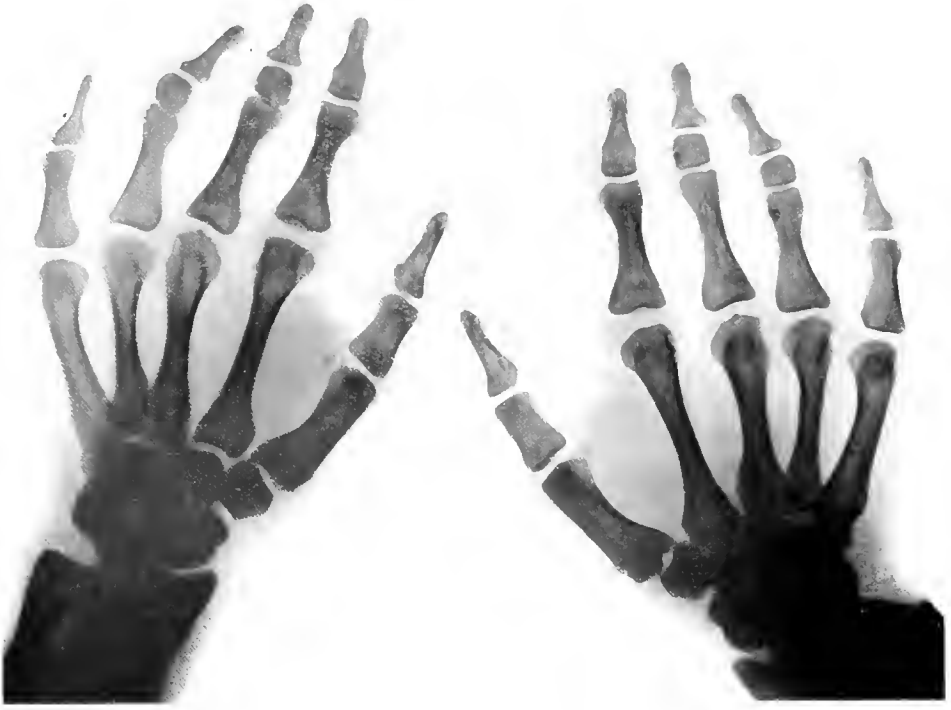


No. 41.



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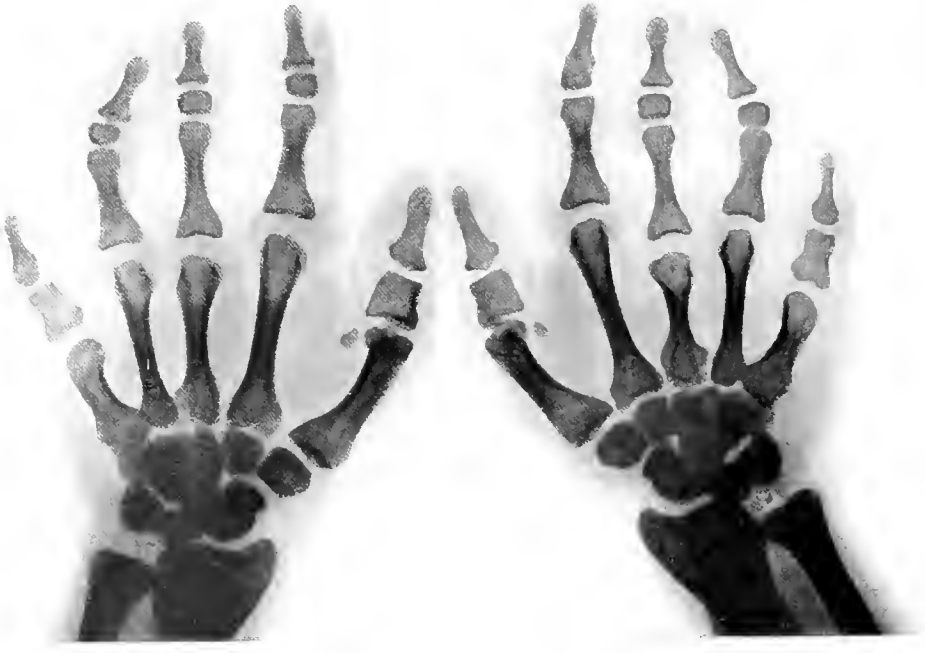
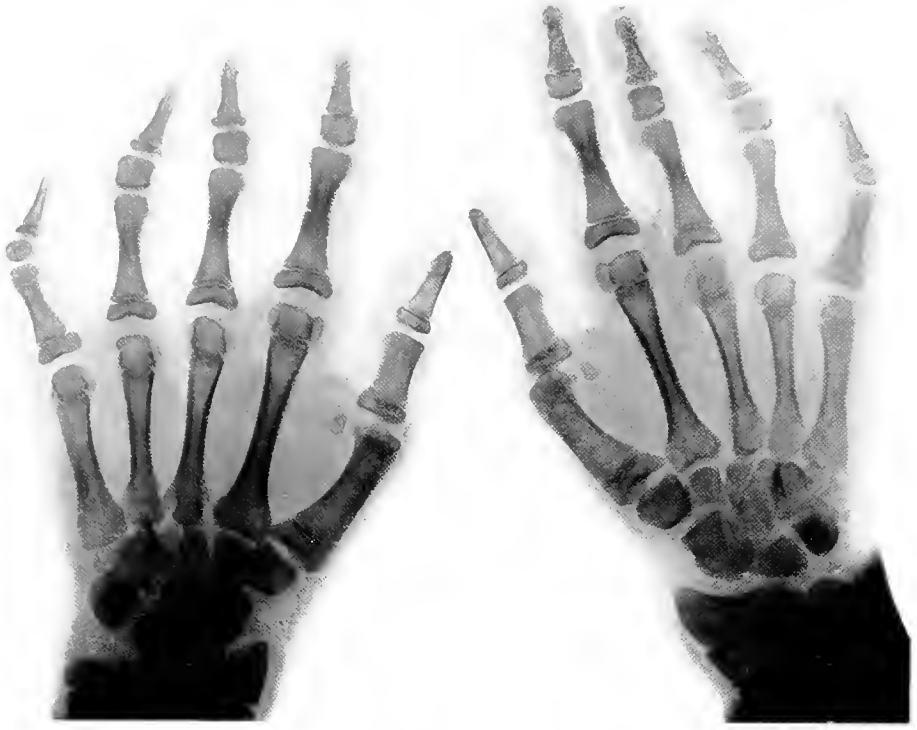


No. 27.



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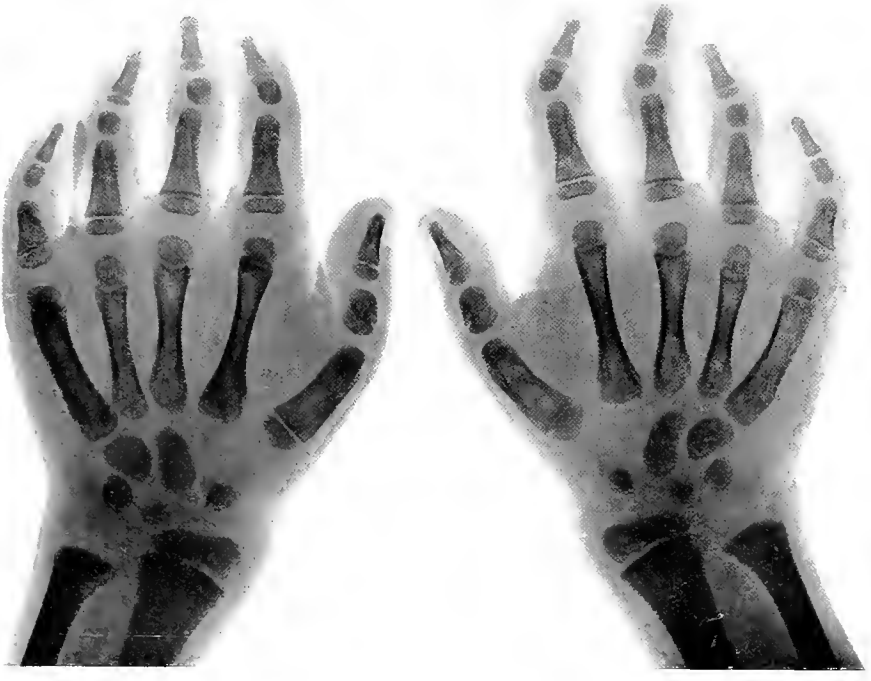


No. 31.

ON THE PRIMARY AND SECONDARY SEX CHARACTERS OF SOME ABNORMAL BEGONIA FLOWERS AND ON THE EVOLUTION OF THE MONOECIOUS CONDITION IN PLANTS.

By C. J. BOND.

PART I.

My attention was first directed to the problem of Sex Dimorphism in the Begonias when noticing that certain flowers in which the process of Sex Differentiation was incomplete were almost invariably accompanied by an abnormal or supernumerary Floral Bract. This accessory and asymmetrical Floral Bract occurs on the pedicle which bears the abnormal flower. It seems to mark the situation in the growth of the flower stalk where under normal conditions the Male and Female Sex Organs are segregated out into flowers of opposite sex. (Pl. XVI, fig. 1.)

The importance of this abnormal floral bract lies in the indication it affords of the fact that the differentiation of the Sex Character is a matter of qualitative cell division in somatic as well as in germinal cells.

The next point is that partial or complete failure to undergo qualitative cell division in these abnormal flowers is associated with abnormality of another kind. This secondary abnormality may take the form of modification in, or increased number of, accessory floral parts, or elongation of the staminal axis with increased number of stamens, or petallody of stamens (doubleness in the male flower). In the female flower it may take the form of an increased number of carpellary leaves, as indicated by an abnormal number of ovarian loculi, or (by failure of these carpellary leaves to close) an exposed condition of the ovules, a partial return in fact to the Gymnospermous condition.

Any of these changes may afford the earliest indication in male and female flowers respectively of a disturbance of sex equilibrium, in other

words a disturbance in the regularity of the qualitative cell divisions by which the male and female sex organs are normally differentiated out into different flowers.

A further stage in abnormality may be the appearance of male gamete bearing organs on female flowers, or more rarely the appearance of female gamete bearing organs on male flowers.

Finally, a complete ring of stamens, functionally active, may surround the gynoecium of a flower of female type. (Pl. XVI, fig. 7.)

This association in the same flower between abnormalities of accessory and of essential floral parts is important because it shows that instability of equilibrium in the primary sex elements carries with it in nearly all cases instability of equilibrium of somatic tissues also.

The association between sexual instability or hermaphroditism and modification of accessory floral parts is also shown genetically thus:— The F_1 seedlings of a cross between a Begonia with double male flowers as the seed parent and a single flowered variety as the pollen parent are for the most part single flowered plants. A certain number, however, show indications of a tendency to doubleness by the presence of one or two extra petals in the male flowers, while others exhibit an elongation of the axis which bears the otherwise normal stamens. In other words singleness is only partially dominant over doubleness in the F_1 generation of such a cross.

Of the seedlings of the F_2 generation so raised an abnormal percentage shows a tendency to hermaphroditism and abnormal flowers.

It is more difficult by cross fertilization to transfer the double character to female flowers, that is to convert a sex limited character like doubleness in the Begonias, into a specific character. In the female as in the male flower however the earliest indication of sexual instability or hermaphroditism is often the presence of an increased number of pistils and carpellary leaves. Thus in plants (as in animals) any disturbance in the structural if not in the functional characters of the primary sex organs is usually associated with changes in the secondary floral characters.

Primarily Male and Primarily Female Flowers.

The question now arises whether the arrangement and distribution of the male and female sex organs in these hermaphrodite Begonia flowers throw any light upon the central position of the Gynoecium in our modern hermaphrodite flowering plants.

It is a matter of considerable interest to find that two types of abnormal flowers exist in the Begonias:—

1. The common type in which, round a central more or less perfectly developed gynoecium, rudimentary organs of the male type occur. (Pl. XVI, fig. 7.)

2. A far less common type in which rudimentary female organs are gathered round a centrally placed androecium. (Pl. XVI, figs. 3, 4, 5, 8.)

Flowers of the first type are here called primarily female. Flowers of the second type are called primarily male. The criterion being the relative position of the male and female sex organs to each other on the common floral axis.

It is here suggested that the almost universal central and terminal position of the female organs in hermaphrodite flowers is a fact of considerable phylogenetic importance.

It would seem that the female portion of the flower represents the undifferentiated reproductive rudiment from which the male portion has during evolution segregated off.

This suggestion receives some support from the frequent (though not invariable) occurrence of vestigial remains, or rudimentary organs of the opposite sex, in the female rather than in the male flower in monoecious and in some dioecious plants. It would be interesting to know how many of these cases in which vestiges of the opposite sex organs are found in the male and not in the female flower represent a return to, rather than a step forward towards, the hermaphrodite form. Further the question of the homozygous or heterozygous nature of the male and the female plant, in respect of sex in the Mendelian sense, assumes additional importance from this point of view.

Bearing in mind the fact that sex differentiation is one of the earliest stages in plant, as in animal evolution, and that the differentiation of male and female sex organs on different individual plants or on different parts of the same individual plant, was probably already established in the Cycads, Ginkgos, Sphenophylls and other possible ancestors of our flowering plants, the problem of the sex evolution of the hermaphrodite flower is probably not so much a question of differentiation of sex organs into male and female, as of bringing together into close juxtaposition on one common floral axis, male and female sex organs formally located in different plants or in different parts of the same plant.

Though the active factor in bringing about the evolution of the hermaphrodite flower was probably a necessity for adaptive capacity on

the part of plants to undergo fertilization by means of insect instead of wind agency, in association with the great evolution of insect life which occurred in cretaceous and precretaceous times, the essential factor which determined this adaptive change along primarily[1] female rather than primarily male lines was probably, not any superior capacity for fertilization so obtained (though this may have been a point in its favour), but the greater facility afforded by the central position of the gynoecium on the floral axis in providing for the longer retention of the embryo in contact with the tissues of the parent plant.

This growing tendency to retain the embryo in contact with the mother plant, and thus provide it with a better start in the world, forms a striking feature of plant development in recent times, just as the tendency to retain the embryo in contact with the maternal tissues is a marked characteristic of the mammalia, the highest order of animals.

The ultimate aim, if one may be allowed the teleological expression, has been the production of the largest number of offspring with the best chances of successful separate existence, along with the greatest economy of material, and the chief steps towards this end have been:—(1) The replacing of the naked ovule by the ovule enclosed in an ovary, i.e. the evolution of the Angiosperms. (2) The provision of nourishment in an available form to as late a stage as possible in the growth of the embryo, and this was secured by placing the ovary in the centre of the floral axis.

The provision for the passing of the prothallus or asexual reproductive stage while the embryo remains in contact with the maternal tissues is also in harmony with this scheme.

The ultimate test of the respective value of the unisexual as against the hermaphrodite type of flower, is capacity to ensure, not merely the fertilization of a large number of ovules but the survival of a large number of embryos, and the central position of the gynoecium in the hermaphrodite flower favours such survival.

In this connection one is reminded of Baur's conception of hybrids as Clinal and Periclinal Chimaeras. It is possible to apply this conception to the hermaphrodite flower which would then be a sex-chimaera built up on a clinal or central female basis with periclinal male accessory organs.

The problem of the central position of the gynoecium in the hermaphrodite flower when reduced to cytological terms becomes a question

[1] "Primarily" in the sense described above in reference to the position of the gynoecium.

of the relative position assumed by the factors for maleness and femaleness in those qualitative cell divisions which control the segregation of the sex organs. Thus if maleness passes into the lateral daughter cells, while femaleness is retained in the cells which continue the axial line of growth the flower although hermaphrodite is primarily female. If on the other hand (as in these abnormal Begonia flowers) femaleness is thrown off into the lateral daughter cells and maleness remains in the central cells then the flower although still hermaphrodite is primarily male.

It is in fact even more true of plants than animals that sex is a function of the position of the factor or factors which control it.

These abnormal Begonia flowers, primarily male and primarily female, also show that under certain conditions the qualitative cell divisions which determine sex may be imperfect or incomplete. Thus if the type of the division has been settled by the passage of the factor for maleness into the central daughter cells, then any remnant of the female factor if present must apparently pass into the lateral daughter cells. This fact suggests some association between the volume of the factor and the position assumed by that factor in cell division. The conception is indeed forced upon us that the sex of a unisexual flower on a monoecious plant partly depends on the relative volume of the sex factors present in that flower. Whether the male organs shall occupy a central or a circumferential position on the floral axis (in other words whether the flower shall be male or female) seems to depend partly on whether the factor for maleness is present in greater volume than the factor for femaleness. If it is, then maleness is handed on to the daughter cells in the direct line of growth and the flower is primarily male, whereas if the volume of the female factor is greater than that of the male factor, then femaleness continues in the line of growth and maleness is cast off into lateral daughter cells.

The importance of this matter lies in the fact that sex segregation in the flowers of monoecious plants seems to be associated with a definite position assumed by the alternative factors which control sex either in one or in a series of qualitative cell divisions.

One may perhaps hazard the suggestion that if future cytological research should associate the male (or the female sex) in the Begonias with the presence of an accessory chromosome, then the position taken up by that accessory chromosome in the qualitative cell divisions which divide the growing cell into end on and lateral daughter cells will determine the primary sex of the flower, just as the passage of such

an accessory chromosome into one of two alternative germ cells determines the sex of the individual. At present we are ignorant as to the stage in the growth of the floral parts at which this segregating division occurs. Neither do we know the difference in nuclear or factorial constitution between the primarily male and the primarily female flower or whether this difference is of a genetic or a somatic character.

Primarily Male and Primarily Female Inflorescences.

This conception of a flower as primarily male or primarily female may also be extended to the Inflorescence.

In the normal inflorescence of the tuberous Begonia the flower which terminates the floral peduncle is a male flower. A few abnormal examples occur in which on cursory examination it appears that a female flower terminates the inflorescence, but careful inspection will show that even in these cases a rudimentary or abortive male terminal flower really exists. If in some varieties with double male flowers the inflorescence is apparently entirely composed of one male flower, careful examination will reveal however rudimentary or abortive female flowers in the axils of the bracts of the floral peduncle which carries this flower. Further, in some varieties both in the tuberous and fibrous kinds (e.g. Mrs J. Heal, Beg. Socotrana, Gloire de Lorraine, and others) only male flowers are present. In these cases the inflorescence is not symmetrical and tripartite, but asymmetrical with male flowers given off on one side only. In some climbing Begonias the male flowers normally appear at the fourth or fifth dichotomous division of the floral peduncle while female flowers appear later at the sixth or seventh division; further, as the male flowers fall early the result is that the inflorescence appears to be composed of female flowers only. The point of physiological and cytological interest about the inflorescence thus becomes (as in the flower) a problem of the relative position of certain organs. In other words the disposition of sex organs in flowers of opposite sex like the disposition of sex organs on a common floral axis in the hermaphrodite flower is a problem of qualitative cell division. In the inflorescence, as in the flower, we find two types:—

(*a*) A male type in which the terminal flower is a male flower and the female flowers are thrown off laterally, as in the Begonia.

(*b*) A female type in which the terminal flower is a female flower and the male or hermaphrodite flowers are thrown off laterally, as in some caryophyllaceous plants.

The type of inflorescence and the type of flower may vary in the same individual plant, and the question arises whether the Begonia (and other monoecious plants) may not have attained their present monoecious condition in two ways: (1) by diverging from the hermaphrodite stage along primarily female lines as far as the flower is concerned, and (2) by developing along primarily male lines as far as the inflorescence is concerned. In the walnut and some other monoecious trees the occasional occurrence in some individual trees of a rudimentary gynoecium in a central position in a male floret, suggests that these plants have passed through a hermaphrodite stage in which the flowers were built up on a primarily female type.

Sex Dimorphism thus becomes a problem of (1) the kind of units among which the sex differentiating process takes place, and (2) the period in the life history of these units at which this process occurs. If the differentiating cell division occurs in the germ cell stage the dioecious variety of sex dimorphism results, if it occurs a little later during the development of the flowers the monoecious form results, while if it occurs at a still later stage during the development of the sex organs on the common floral axis the hermaphrodite flower is formed. We are ignorant of the factors which determine the stage at which this all-important segregation shall occur, but of the three stages at which it has up to the present taken place in the history of plant evolution, the middle or monoecious stage seems to have been the most unstable of the three. For it is in monoecious plants that we find the greatest number of vestigial remains or rudimentary organs of the opposite sex. In some monoecious plants, e.g. the Box, indications exist that the plant is now on the way to or away from the hermaphrodite condition.

It is a matter of considerable interest to find that in plants as in animals a close association exists between a sex abnormality like hermaphroditism and a delayed occurrence of those differentiating cell divisions which determine sex. Postponement of the sex differentiating cell division from the germ cell to the zygote stage may not only bring about hermaphroditism in the individual, but the actual period in the development of the zygote at which the differentiation of sex organs occurs also influences the type of hermaphroditism which results, for instance, if the differentiation of the factors for maleness and femaleness is postponed to the stage of blastomeric division, in which the right and left halves of the animal body are laid down, then a lateral gynandromorph is produced. Many such cases have been recorded among insects, and quite a number have now been recorded among the vertebrates,

especially in birds. (See H. Poll, "Zur Lehre von den sekundären Characteren" (*Stzgsber. Ges. Naturf. Freunde zu Berlin*, 1909); C. J. Bond, "On a case of Unilateral Development of Secondary Sex Characters in a Pheasant" (*Journal of Genetics*, Feb. 1914).) In this connection also the limitation of paternal and maternal characters to opposite sides of the body in the larvae of some sea urchin hybrids reared by Herbst as the result of artificial fertilization is of great interest. (See J. Loeb, *Artificial Parthenogenesis.*) Although I am not aware of any recorded observations of the limitation of paternal and maternal characters to opposite halves of the individual in the cotyledonary stage in seedling plants, yet such limitation does undoubtedly occur at a later stage of growth, for both individual leaves and opposite branches may sometimes show a hemilateral arrangement of paternal and maternal characters.

Certain conditions in animals suggest that in addition to the bilateral type of architecture, the individual organism is also built up on a serial or segmental plan, thus sex abnormalities like hermaphroditism may show a segmental distribution in birds. (C. J. Bond, *Journal of Genetics*, Feb. 1914.) In plants also the distribution of the unisexual florets in the compound inflorescence follows in some abnormal cases a distinctly segmental or periodic type. This is well seen in the irregular inflorescence of some abnormal maize plants. Plate XVII, fig. 1, shows a serial alternation on the same peduncle of male and female florets. Plate XVII, fig. 2, shows a magnified view of the same thing.

PART II.

Secondary Sex Characters in Begonias.

It has already been pointed out that the normal male flower of the monoecious Begonia has four petals and the normal female flower five. Although this association between the sex of the flower and the number of petals seems characteristic of most Begonias under normal conditions, yet it is not an absolutely constant feature. For instance a flower may develop an outer whorl of pistillate structures on its staminate floral axis. It may even show exposed ovules on some of these pistillate leaves, it may in fact be partly a female flower and yet retain the four petals characteristic of the male flower. (See Pl. XVI, figs. 3, 4, 8.) In the same way the primarily female flower may have a row of stamens

outside the central pistil and yet retain the five petals characteristic of the female flower. (Pl. XVI, fig. 6.) Although doubleness chiefly affects male Begonia flowers, and is therefore a sex limited character, it is possible under certain conditions, by cross pollination, to transfer this character of doubleness to the female flower. Double female flowers so produced, however, retain secondary male characters. The structure and arrangement of the accessory floral parts are not influenced by being linked up with maleness. In fact a primarily female flower with a well-formed ovary and a central gynoecium may develop a complete ring of stamens and yet retain the five petals characteristic of the female flower. (Pl. XVI, fig. 6.) From these facts we are I think justified in concluding that the presence of male organs does not necessarily modify the secondary sex characters of female flowers. The same is true of male flowers which develop female organs. The secondary characters seem to depend on the primary sex of the flower as shown by the central position of the gynoecium or androecium on the floral axis. The accidental occurrence, so to speak, of rudimentary, or even well developed, organs of the opposite sex, does not affect the growth of the secondary sex characters. That this should be so is not surprising when we recall the apparent absence in plants of any secretion corresponding to the sex hormones in animals.

There is, however, evidence pointing to the existence of enzymes in plants.

Keeble (*Plant Animals*, 1910) suggests that the cambium cells influence the neighbouring cortex cells and stimulate them to undergo cell division by means of some secretory substance. He also suggests that the green algal cells in *Convoluta roscoffensis* supply the biochemical stimulus on which the later development of that organism depends, and in the absence of which it fails to grow.

There are also the important facts concerning the influence of fertilization on the growth of accessory floral structures. Darwin showed long ago that some kinds of foreign pollen can act as a poison to the stigmata and pistils of certain flowers. The rapid wilting and the falling within 36 hours of the petals of a successfully fertilized female flower provides us with a practical indication as to whether the pollen artificially introduced has "taken," and whether the flower is *adequately* fertilized in any given case. But these latter examples are examples of an inhibitory rather than a stimulatory influence. In the other direction we have the stimulating effect of the fertilized and growing ovule on the tissues of the floral peduncle, and this influence is

transmitted through the tissues which connect the ovule with the mother plant.

In the dioecious *Lychnis* if pollination and fertilization of the ovules be prevented the female flower falls on the fifth or sixth day after opening. This falling of the unfertilized flower (like the falling of the autumn leaf) is brought about by a degenerative change in the collar cells which form the neck of the floral peduncle at the point where this swells out into the thalamus or cone on which the ovules are imbedded. If on the other hand the ovules have been fertilized active growth occurs in these cells. Either the absence of a stimulus derived from the growing ovules or possibly the presence of an inhibitory substance formed by the atrophying and degenerating ovules brings about the death and degeneration of these collar cells, and this results in the fall of the unfertilized or insufficiently fertilized flower.

In the falling corolla after pollination, and the falling flower in the absence of pollination, a quantitative relationship can be observed between the number of pollen grains which gain access to the pistil and the rapidity of the wilting of the stigma and corolla in the one case, and the number of ovules which undergo fertilization and the rapidity of the falling of the flower in the other. If only a few pollen grains are allowed to gain access to the pistil the petals may remain erect and vigorous for several days, while if the stigmata be dusted with a larger quantity of pollen they fall in 24 to 48 hours. In the case of the flower, if only 5 or 6 ovules (in *Lychnis*) undergo fertilization the degenerative change in the collar cells may not be prevented and the flower may fall although it has been partly fertilized. A minimal number of ovules must in fact undergo fertilization and begin to grow in order to prevent those changes in the cells at the root of the thalamus which lead to the falling of the unfertilized flower.

This fact, viz. that a quantitative relationship exists between the factor which stimulates or arrests cell growth and the stimulated or arrested cell activity which results from its influence is an important one. It is unlike the behaviour of an animal enzyme which when introduced into a fermentable solution serves to hydrolyse its whole volume.

It is also unlike the method of action of specific sex hormones, where the smallest quantity of the substance, if present in the blood stream, is capable of influencing the metabolism of the whole body.

In fact these chemical substances which stimulate and arrest cell growth in plants seem to be incapable of self multiplication and seem to exercise only a local influence on the tissues of the plant.

PART III.

The question now arises whether the factor, or factors, which control sex segregation in the monoecious plant are the same as those which determine sex in dioecious plants and in animals.

In animals and dioecious plants segregation of the factors controlling sex takes place among germ cells either before or during fertilization, whereas in the flowers of monoecious plants segregation takes place among cells which present the external characters of somatic cells although they no doubt retain germinal material capable not only of forming new individual plants by bud formation, but also of acting as the carriers of hereditary characters.

But both processes depend essentially on qualitative cell division. In one case this takes place in the meristematic cells of the plant stem and results in maleness passing into the terminal and femaleness into the lateral daughter cells, or vice versa; in the other case it occurs amongst male and female bearing gametes at an earlier stage of their life history, probably at the period of maturation.

The chief distinction between the two processes is one of time of occurrence rather than one of kind. One affects the germ cell at or before fertilization, the other affects the zygote which results from the repeated division of this germ cell after fertilization.

It seems clear that the association between the segregation of primary and the segregation of secondary sex characters is less intimate in plants than in animals. Both depend on qualitative cell division, but the primary sex organs do not exercise such continuous control over and are not so indispensable to the growth of the corresponding secondary sex characters in plants, as in animals. In the higher animals, unlike some invertebrates, a very elaborate system of interdependence and control is present between those cell divisions which represent primary maleness and femaleness and those metabolic processes which underly the production of male and female secondary sex characters. The functional activity of the latter is dependent on the functional activity of the former, whereas in the case of the monoecious plant the determination of the sex of the flower, and the number and arrangement of its petals, although both may be primarily under the control of a common genetic factor, are more or less independent processes.

EXPLANATION OF PLATES.

PLATE XVI.

Fig. 1. Shows to the right an abnormal hermaphrodite flower in association with an asymmetrical floral bract.

Fig. 2. Shows "exposed ovules" in a partially hermaphrodite flower.

Fig. 3. Shows female sex organs, ovules, in a peripheral position on a primarily male flower. (See also Fig. 8.)

Fig. 4. A primarily male abnormal flower with a ring of pistillate structures peripheral to the central androecium. This flower, although partly hermaphrodite, preserves the four petals characteristic of the male flower.

Fig. 5. A primarily male flower partly hermaphrodite with one or two pistillate structures peripherally situated on the floral axis. This flower had four petals only.

Fig. 6. Abnormal primarily female flower with a peripheral ring of stamens round a central gynoecium and the five petals characteristic of the female flower.

Fig. 7. Two primarily female hermaphrodite flowers. A peripheral ring of stamens surrounds the gynoecium in each case.

Fig. 8. Terminal primarily male flower with abnormal stamens, multilocular anthers, and ovules peripherally placed on the petals. Though partly hermaphrodite it has the four petals characteristic of the male flower.

PLATE XVII.

Fig. 1. Inflorescence of maize plant showing a serial alternation on the same peduncle of male and female florets.

Fig. 2. Magnified view of same.

Fig. 1.

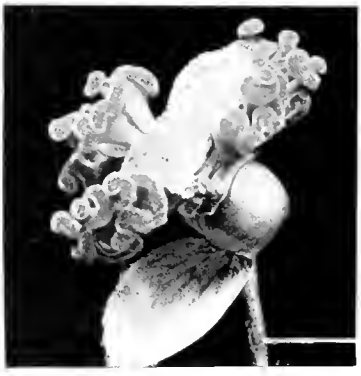


Fig. 2.

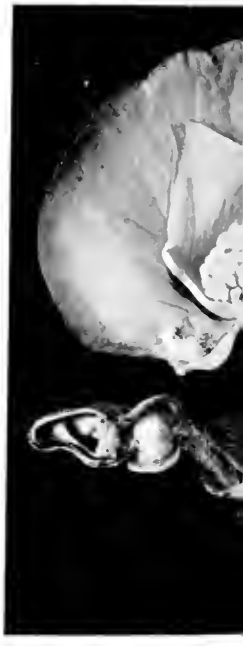


Fig. 3.

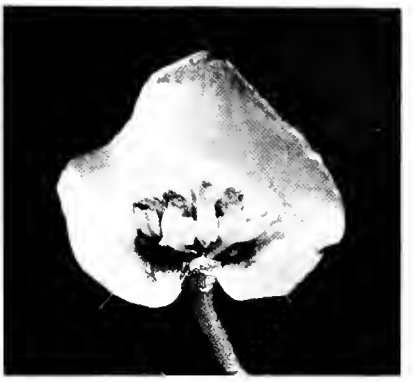
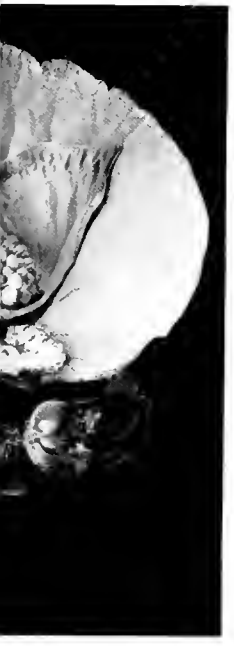


Fig. 4.

Fig. 5.



8.

Fig. 6.

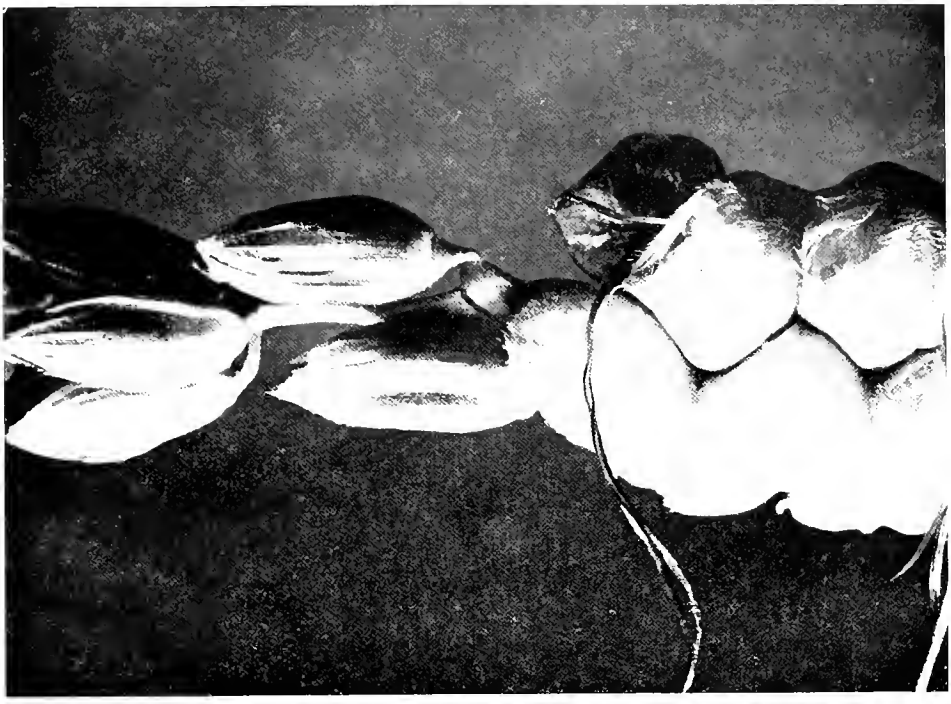


Fig. 2.

Fig. 1.

ON THE ORIGIN AND BEHAVIOUR OF *OENOTHERA RUBRICALYX*.

By R. RUGGLES GATES, Ph.D., F.L.S.,
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In a recent paper which purports to deal with F_1 hybrids of *Oe. rubricalyx*, Shull (1914) takes occasion to attack my conclusions regarding the origin and hereditary behaviour of this mutant. I may remark that I have been studying the hereditary behaviour of *Oe.* mut. *rubricalyx* ever since it appeared in my cultures in 1907. The results of these studies, portions of which have been published in several papers (1909—1914), are perfectly clear and definite, and the main conclusions to which they have led are, in my opinion, irrefutable. Among my crossing experiments is an extensive series of F_1 and F_2 hybrids between *rubricalyx* and *Oe. grandiflora*, Solander (Gates, 1914) numbering nearly 3000 plants. I have also grown a still more extensive series of F_3 families, whose results confirm the previous conclusions from the F_2 families and will be summarized in a book which is now in press.

Before entering further into the evidence on which my conclusions rest, it may be well to examine for a moment the nature of the evidence which Shull brings forward as the basis for his criticism and for conclusions which are contrary to mine. In the first place, the internal evidence from the author's own paper, as well as other facts, shows that his supposed pure *rubricalyx* was in reality *Oe. rubricalyx* × *grandiflora*. This in itself of course vitiates *in toto* the "results" stated in his paper, for in every cross mentioned one must read "*rubricalyx* × *grandiflora*" instead of "*rubricalyx*." For example, when he describes what he states is *rubricalyx* × *rubrinervis* F_1 he is really describing (*rubricalyx* × *grandiflora*) × *rubrinervis*. What must one think of observations which pretend to be critical, and yet which make the fundamental error of

failing to distinguish between pure *rubricalyx* and its hybrid with a wholly distinct species? These two things are so different that the veriest amateur could have no difficulty in distinguishing between them, either as rosettes or as mature plants.

Such looseness of observation as Shull's paper displays is inexcusable when put forward with such a show of confidence in the soundness of his results. I have stated repeatedly in previous papers that *rubricalyx* is morphologically identical with *rubrinervis*, of which it is in fact a very marked and striking colour variety. Photographs have also been published (see Gates 1911, Figs. 4 and 5) which prove these assertions. However, Shull evidently had occasional misgivings regarding the real nature of his supposed *rubricalyx* rosettes, as when he says (footnote, p. 84): "Several features of my plants suggested a relationship to *O. grandiflora*, particularly the rather lax rosettes, strong red spotting of the young rosette-leaves, and the development of buds more slender and rounder than in my *O. rubrinervis* cultures." As I pointed out several years ago (1909), these are all conspicuous features of *Oe. grandiflora*, and this knowledge should have given him pause, particularly when he was aware that his "*rubricalyx*" plants were from unguarded seeds.

In several places through his paper Shull wavers between the assumption (which he knew was doubtful) that his "*rubricalyx*" was pure, and the admission that it may have been a cross with *grandiflora*. Yet he must have realized that his results were almost valueless if the latter was the case, and quite so if he was unable to determine between these two alternatives. It is difficult to understand how the paper could have been brought forward as a contribution to genetics under these circumstances. Since the author states that it was originally written before my last paper (Gates, 1914) dealing with *rubricalyx* and its hybrids was published, the references to that paper, as well as certain other changes, must have been made later.

The plants which Shull assumed to be pure *rubricalyx* were obtained by him from Dr A. F. Blakeslee, of Storrs, Connecticut, who grew them from unguarded seeds of *rubricalyx* received from me. Dr Blakeslee has kindly sent me the original seed packet, from which I find that the seeds in question were collected from a plant grown in 1909. I grew several families of *rubricalyx* in that year, and one of these was adjacent to a culture of *grandiflora*. These unguarded seeds were not intended for genetic experiments, and were sent (marked "open-pollinated") in answer to a request for any plants which would show the red-budded

character for demonstration purposes. In using them for breeding experiments, knowing they were unguarded, Shull took his own chances, with unfortunate results. Though I did not record on the packet the pedigree number of the plant from which these seeds came, I have other reasons than the subsequent results for believing that they were from the culture next to the *grandiflora* plants, where the opportunities for crossing with that species were of course greater. So far as can be learned, Shull's plants were probably derived from seeds directly from this packet, in which case they would be *rubricalyx* × *grandiflora*, F_1. I have already (1914) published an extended account of several years' experiments with this cross and the reciprocal in F_1 and F_2, as well as sesquireciprocal and double reciprocal crosses. Although the same strain of *Oe. grandiflora* was not available for all these crosses, yet all the features of buds, foliage and development were the same in the F_2 families from *rubricalyx* × *grandiflora* and *grandiflora* × *rubricalyx*, so that any disadvantages from this source are more theoretical than real. A glance at Shull's published figures of his "*rubricalyx*" shows clearly by their bright red spots that they have been crossed with *grandiflora*, and the leaf-shape also shows some effect of this cross, even in the young rosettes.

Since the differences between Shull's conclusions and mine result from the fact that I was dealing with *rubricalyx* while he experimented with *rubricalyx* × *grandiflora* under the misapprehension that it was *rubricalyx*, his conclusions obviously fall to the ground, and his criticisms of my experiments on the basis of his "results" are worth nothing.

Nevertheless, the origin of *rubricalyx* is a matter of such significance and has been so much discussed that it seems worth while to restate the evidence on which rests my conclusion that *rubricalyx* originated from *rubrinervis* through a single unit-change. The ultimate nature of this change will be discussed in my forthcoming book.

In his anxiety to find a basis for doubting that *rubricalyx* originated as a simple Mendelian dominant, Shull does not treat the observed data fairly. Instead of considering all the data, he omits entirely certain of the ratios which disprove his unsupported assumption that *rubricalyx* can have originated through the union of two independent factors for red. The data, if all taken into account, are however ample to show that *rubricalyx* when it appeared did actually differ from *rubrinervis* in a single Mendelian factor. It is, indeed, decidedly amusing to find a Mendelian repudiating his own method of argument when it happens

to lead to conclusions which he does not wish to accept. But if the Mendelian argument holds in other cases (as I think it does), then it will have to be admitted to hold also in this case. The significance of mathematical ratios can no more be suspended for the convenience of some one, than can the law of gravitation. I hold no brief for Mendelism, but when the facts point so clearly to a 3 : 1 ratio in the offspring of the *rubricalyx* mutant, and a 1 : 1 in the cross of a heterozygous *rubricalyx* individual with another species, I am not loath to draw the inevitable conclusion that the mutation originated through a single dominant unit-change and that heterozygous plants were producing two types of germ cells in equal numbers.

The evidence is as follows: The offspring of the original *rubricalyx* mutant which were not accidentally destroyed numbered only 12. Of these, 10 came into bloom, and 9 of them were shown by their buds to be *rubricalyx* and 1 *rubrinervis*. Two other plants remained rosettes, and I formerly classed them as undetermined or doubtful because they showed only a small amount of "ventral" red. Subsequent examination of many rosettes, however, has shown that whenever there is even a trace of ventral red on the rosettes the plants invariably develop red buds. Hence all rosettes which were formerly classed as doubtful because they showed only small amounts of ventral red, were really *rubricalyx*. The above ratio in the F_1 of the *rubricalyx* mutant was therefore 11 *rubricalyx* : 1 *rubrinervis*. This number is of course too small to determine a ratio. But three of the *rubricalyx* plants in this culture were selfed and yielded F_2 families containing *rubricalyx* and *rubrinervis* in the ratios respectively of 10 : 5, 14 : 6 and 33 : 11. These three ratios are all very closely in accord with 3 : 1, and even the 11 : 1 ratio is not a wide departure. Adding these four ratios we have a total ratio of 68 : 23, which is extremely close to a 3 : 1 ratio, while the chances against it representing a 15 : 1 are enormous.

This evidence in itself is clearer and more indubitable than that on which many conclusions from Mendelian ratios rest. But there is still more evidence, which Shull omits to quote. Two of the *rubricalyx* plants in the F_2 culture which gave the ratio 33 : 11 and thereby proved the monohybrid character of *rubricalyx*, were crossed reciprocally with *Oe. grandiflora*. They both proved to be heterozygous in regard to a single character-difference, for in both cases the F_1 contained about 50% red-budded and 50% green-budded plants. Thus in *rubricalyx* × *grandiflora*, of 67 plants 58 came into bloom, and of the latter 30 were red-budded and 28 green-budded. This is a far

closer approach to equality than Mendelians frequently regard as sufficient to prove their point. In the reciprocal cross, *grandiflora* × *rubricalyx*[1], the F_1 contained 147 plants, of which 58 bloomed. The latter were in the ratio 34 red-budded to 24[2] green-budded. Anyone can decide for himself whether this ratio is nearer 1 : 1, as it should be on my view, or 3 : 1 as it should be on Shull's view. The full data from this family, however, prove the situation still more conclusively, for all the plants were carefully examined in the rosette stage and 42 were found to show clearly red on their ventral surfaces, 71 were without any trace of red, and 37 were doubtful, i.e. showing the red faintly. We now know that the latter must be classed with the first, for all alike develop red buds, and plants with very little red on the rosette have the usual deep red buds. In this family there were therefore 79 with the *rubricalyx* character (*R*) to 71 without it (*r*), a close approach to equality.

These ratios are exceptionally close to expectation and, taken together, they furnish abundant evidence as will, I think, be agreed by all Mendelians, to establish the very high probability that a single unit-difference was concerned in the origin of *rubricalyx* from *rubrinervis*. On the other hand, the evidence is sufficient to establish absolutely the impossibility that two Mendelian factors could have been concerned. These facts are shown in the following Table:

	Actual ratios	Expectation on author's view	Expectation on Shull's view
	11 : 1	9 : 3	11·25 : 0·75
	10 : 5	11·25 : 3·75	14·06 : 0·94
	14 : 6	15 : 5	18·75 : 1·25
	33 : 11	33 : 11	41·25 : 2·75
Total ...	68 : 23	68·25 : 22·75	85·31 : 5·69
	30 : 28	29 : 29	43·5 : 14·5
	79 : 71	75 : 75	112·5 : 37·5

From this Table it will be seen that there is not an atom of evidence for the presence of duplicate factors in *Oe. rubricalyx*, while the evidence for a single factor is of the kind which is universally regarded by Mendelians as conclusive.

In addition to the records presented above, several other ratios were given in my paper (1914, Pedigree 1). These are of families

[1] A full account of these crosses is given in my former paper (1914).

[2] In a previous paper (Gates, 1914, Pedigree 1) this was given as 23. I have not the original records at hand for determining the cause of this discrepancy, but the difference is immaterial to the argument anyway.

descended from *rubricalyx* individuals in the culture having a ratio of 33 : 11. They were all observed only as rosettes, and as they were not observed with the same care as the family of 147 above-mentioned it is obviously unsafe to attempt to draw any conclusions from them. It is certain, however, that the number of *rubrinervis* rosettes was overestimated, for the reasons given earlier in this paper. As recorded, the seven cultures total 97*R* : 78*r*. In any case the numbers do not support Shull's hypothesis of duplicate factors.

Turning now to certain other statements in Shull's paper, we find (p. 85) the statement "Gates (1914) wavers between the treatment of the *rubricalyx* type of pigmentation as a Mendelian and as a non-Mendelian character. His most positive declarations on the subject are that it is non-Mendelian; but if he sincerely (*sic*) holds to this conviction it is strange that he should continue to treat the genetic behavior of this character as if it threw valuable sidelights on Mendelian phenomena." It is difficult to realize the frame of mind in which such a statement could be written, showing as it does entire failure to grasp the points of view developed by me in the paper in question. One can only conclude, as other statements also indicate, that it was not carefully read or digested.

Among the "facts" presented in Shull's paper we find the statement that the offspring of his "*rubricalyx*" (which we now know to be *rubricalyx* × *grandiflora*) yielded (in F_2 or a later generation) 117 individuals, of which 107 were red-budded and 10 green-budded. This ratio, 10·7 : 1, is rather close to the ratio 9·5 : 1 obtained by me (1914, p. 235) in an F_2 family of *grandiflora* × *rubricalyx* numbering 157 plants, and confirms my results in obtaining various aberrant ratios from these crosses. It is astounding to find that although Shull is unwilling to recognize 33 : 11 as a 3 : 1 ratio, yet he is capable of suggesting that 107 : 10 represents a 15 : 1 ratio!

Without entering further into the extensive data in my paper (1914), it may be remarked that two things appear to be clearly proven; (1) that *Oe.* mut. *rubricalyx* originated through a single unit-change producing a new character-difference which is dominant; (2) that when the new form is crossed with a distinct species—*Oe. grandiflora*—having a different physiology, the red-budded feature comes out in F_2 in varying ratios in different families, 3 : 1, 4 : 1, 5 : 1, and about 10 : 1. Not only is this the case, but occasionally intermediate individuals as regards bud-pigmentation occur, and it has since been found that these when selfed breed true to their intermediate

condition, without reversion to either the *rubrinervis* or the *rubricalyx* type of pigmentation. Moreover, back-crosses of the original F_1 hybrids with *rubricalyx* intensify the pigmentation, while back-crossing with *grandiflora* dilutes and modifies it, producing a spotted condition of the sepals in certain cases. I may perhaps be pardoned for maintaining that all these facts confirm my view that, (1) the *rubricalyx* pigmentation originated through a unit-change, as demonstrated by the ratios before crossing and in the F_1 hybrids; (2) this sharp unit-character may be modified and broken up by crossing with a physiologically diverse species. An adequate explanation has not yet been found for the F_2 ratios, such as 5 : 1 and 10 : 1, found in these hybrids, but a little thought will show that any argument is fallacious which attempts to argue backwards, as Shull has done, from these ratios to the original condition of the unit-character *R* before it was crossed.

In Shull's paper he devotes considerable attention to the red spotting of the rosette leaves in his hybrids. This is a conspicuous feature of *Oe. grandiflora*, and I have devoted much time to the study of its inheritance in hybrids with *rubricalyx*. There is an evanescent stage in the development of the rosettes of these F_2 hybrids, when on superficial examination it seems easy to classify them into red-spotted and non-red-spotted. But more intensive study shows that every degree of spotting exists, from rosettes in which all the leaves are well spotted to those in which only one or two tiny spots appear on the whole rosette. In other words, there is a continuous series from rosettes with a large amount of spotting to those with none at all. This feature was not found to be correlated with any well-marked condition of the adult plants (as is the case with the ventral red of *rubricalyx* rosettes), and as the classification of the rosettes is necessarily more or less arbitrary and hence not dependable, it was not considered worth while publishing ratios in which there was such a large margin of error.

When Shull speaks of "negative correlation" between the pigmentation of the buds and of the stems and rosettes in his triple hybrids, he is observing complex phenomena which have not been analyzed. I have observed a large number of similar phenomena in F_2 and F_3 hybrids of *grandiflora* and *rubricalyx*, but have awaited further data on the subject before publishing. It means, of course, that red pigmentation in different parts of the same plant may be separately inherited, as is known to be the case in various other plants. Shull's "negative correlations" appear to depend, in part at least, upon

the fact that *grandiflora* frequently has dark red stems while in *Oe. Lamarckiana* and its derivatives (except *rubricalyx*) the stems have very little diffuse red, though they are covered with red papillae. I have not, however, found this "negative correlation" to be universal, since for example in some families the hybrid plants have red buds and red stems as well. It is probable that the alternation in Shull's hybrids is really between the dark red stems of some *grandiflora* races and the green or nearly green stems of forms in the *Lamarckiana* series.

Finally, it must be made clear that Shull's results, such as they are, can only be used for reference if in every case, for *rubricalyx*, we read *rubricalyx* × *grandiflora*.

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RECENT CHEMICAL INVESTIGATIONS OF THE ANTHOCYAN PIGMENTS AND THEIR BEARING UPON THE PRODUCTION OF THESE PIGMENTS IN PLANTS.

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The relationship—if any—which exists between the red, purple and blue plant pigments (Anthocyans) and those of the yellow plant pigments which are of the flavone or flavonol class is of considerable interest to students of genetics.

Until quite recently all ideas upon this subject were based of necessity upon evidence obtained from botanical investigation, as reliable chemical investigation upon this subject was entirely lacking. At last however definite chemical evidence bearing upon the relationship existing between these two important classes of naturally occurring pigments has been obtained, and in the present paper the author summarises briefly the important points that have been investigated, and points out their bearing upon the theories previously put forward.

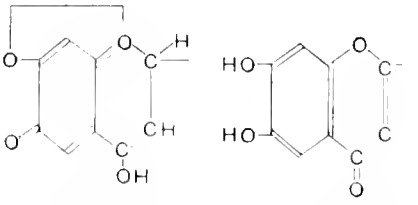
Up to the present the consensus of opinion has been in favour of the theory that the anthocyans are produced by oxidation of yellow pigments of the flavone or flavonol series. The fact that these ideas have failed to stand the test of chemical investigation—in every case that has been examined in a chemically satisfactory manner—must now be realised.

Attacking the problem with a view to establishing the chemical nature and structure of the anthocyan molecule, Willstätter and Everest (*Liebig's Annalen*, 1913, **401**, 189) investigated a number of these pigments, and in particular that of the corn-flower. Their results contain

much that is of interest to those studying these pigments from the standpoint of genetics.

The corn-flower pigment, which was obtained pure and crystalline, was proved to be a glucoside, which on hydrolysis with acids yielded two molecules of glucose and one molecule of the true pigment—a fine crystalline substance—and it was also shown that in all the cases examined the pigments occurred in the plants only as glucosides. These results have been fully confirmed by later work, and the idea, so frequently put forward, that the pigments occur together in plants both as glucosides and non-glucosides, must as a result be abandoned. In cases where previous workers obtained mixtures of glucoside and non-glucoside, e.g. Heise, Glan, it was due to partial hydrolysis during the processes of isolation. This generalisation simplifies somewhat the problems to be dealt with when the processes going on within the plant tissues come to be considered, but by no means so much as the proof that the blue anthocyan pigments are alkali salts of compounds which in the free state are violet or violet-red in colour, and which are capable of uniting with acids to form oxonium salts which are red in colour. Beyond these results the same authors demonstrated that, at least in the case of the corn-flower pigment, the decolourisation which so readily takes place in aqueous solution was not due to reduction as has been assumed so frequently. It was further shown that by *oxidation* the pigment of the corn-flower readily passed to a yellow pigment which had properties similar to those of the flavone or flavonol derivatives.

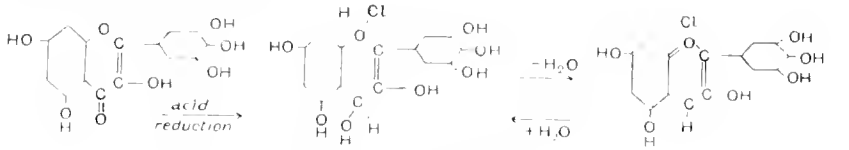
This work brought the present author to the opinion that the anthocyans were reduction products of the flavone or flavonol compounds, whereas Willstätter favoured rather the idea that they were related to some unknown flavone or flavonol derivatives in which a hydroxyl group occurred in the benzopyrone nucleus in the para position to the linkage of the oxygen atom of the pyrone ring. The relationship is clearly shown by the formulae I and II, and it will be seen that this makes the anthocyan an oxidation product of the compound II.



I. Anthocyan. II. Flavone derivative.

The present author's idea that the anthocyans were reduction products of flavone derivatives was deepened by consideration of the work of Keeble, Armstrong and Jones (*Roy. Soc. Proc.* 1913, B, 113).

Working upon these ideas he carried out experiments that clearly showed that by the careful reduction of flavone derivatives a series of red pigments having the properties of anthocyanidins could readily be obtained; moreover, contrary to the almost generally accepted idea, he showed that quite similarly glucosides of the flavone series yielded—*without intermediate hydrolysis*—glucoside red pigments in every way similar to anthocyanins. From these investigations the present author put forward the scheme:

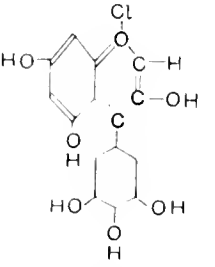


III. Flavone derivative. IV. Colourless or faintly coloured intermediate product. V. Anthocyan pigment.

to represent the change from flavone derivative to anthocyan, and the formula V as that of a typical pigment of the latter group (Everest, *Roy. Soc. Proc.* 1914, B, 444). As further support for these conclusions the work of Watson and Sen (*J. Chem. Soc.* 1914, 389) and Combes (*Compt. Rend.* 1913, **157**, 1003) were cited.

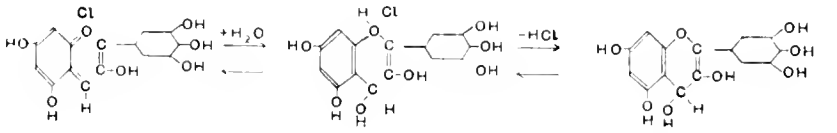
Shortly after the appearance of the above mentioned paper by the present author, Willstätter and his collaborators published a short but important paper (*Sitzber. k. Akad. Wiss.*, Berlin, 1914, XII. 402) describing the continuation of the work on the isolation of pure anthocyans from flowers and fruits. They had obtained in a chemically pure and crystalline condition the anthocyan pigments from no fewer than nine flowers or fruits, all of which pigments proved to be glucosides, and by hydrolysis of the glucosides obtained the colour components also chemically pure and crystalline. They described the decomposition of the non-glucoside pigments (anthocyanidins) by the action of warm alkalis, and the products produced thereby. As the result of their work they put forward for the anthocyan molecule a formula identical with that previously proposed by the present author. They pointed out however that their results did not allow them to finally decide between it (V) and VI. They further concluded that the scheme previously put forward by the present author (see above) should represent the passage of a flavonol derivative to an anthocyan pigment, and additional support to this was

brought by their showing that the decolourisation of anthocyans in aqueous solution was produced by the addition of a molecule of water



VI.

to the anthocyan molecule, the addition causing loss of a molecule of hydrochloric acid; addition of hydrochloric acid to the decolourised product causes reversal of these changes, thus:



Despite this they criticised the present author's conclusions that the pigments obtained by him by the reduction of flavone derivatives and their glucosides were true anthocyanidins and anthocyanins. They stated that such pigments were soluble in ether, and also differed in stability from the natural anthocyans.

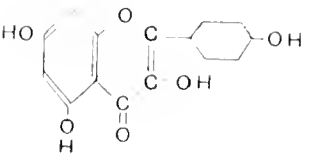
To this criticism, Wheldale and Bassett (*Journal of Genetics*, 1914, IV. No. 1, p. 103) added their support, but in a paper recently read before the Royal Society (forwarded for publication July 15, read Nov. 12) and in a note in the *Journal of Genetics* (1914, IV. No. 2, p. 191) the present author has shown that these criticisms are not well founded.

Since these papers were sent for publication, a further paper by Willstätter and collaborators has come to hand (*Sitzber. k. Akad. Wiss.*, Berlin, 1914, 769), in which, after repetition of the present author's experiments, they withdraw their criticism of his conclusions, for they find indeed that by reduction Quercetin really does produce the anthocyan pigment Cyanidin. Their experiments show that whilst at temperatures below 0°C., the hydrochloride of a new substance—which they term Allocyanidin—is produced, at higher temperatures the product of reduction of quercetin consists of a mixture of allocyanidin chloride and *cyanidin* chloride, and moreover they were able to show that the

latter is in every way identical with the natural cyanidin chloride obtained from the corn-flower or rose.

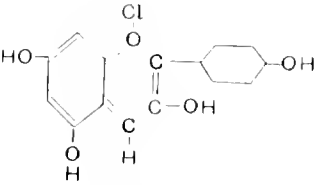
Thus the structure of all the pigments of this group at present known in a pure condition becomes evident, and the manner in which they are related to naturally occurring flavone derivatives is clearly shown by the following formulae:

Flavone derivative.

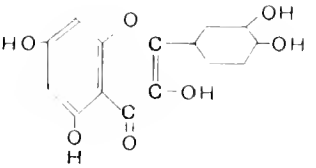


Kaempferol.

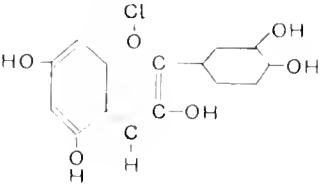
Anthocyan.



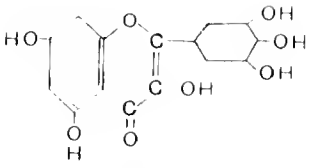
Pelargonidin chloride.



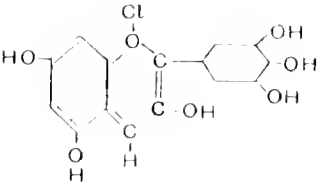
Quercetin.



Cyanidin chloride.

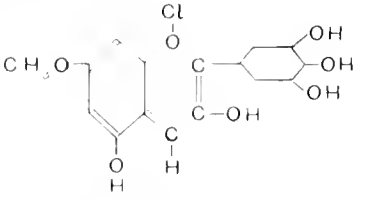


Myricetin.

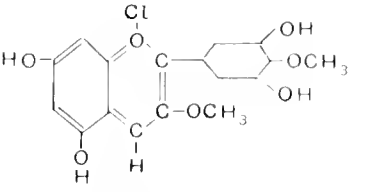


Delphinidin chloride.

Myrtillidin chloride has one, Oenidin chloride two methoxy groups in place of hydroxy groups in Delphinidin chloride, but the position of these groups is, as yet, uncertain; Willstätter tentatively gives them as:

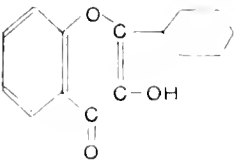


Myrtillidin chloride.

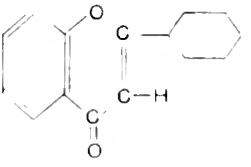


Oenidin chloride.

It will be noticed that all these are derivatives of flavonol VI and thus far no anthocyans corresponding to flavone itself VII have been



VI.



VII.

obtained from natural sources. As the present author has already pointed out in previous publications, in that flavones occur naturally and on reduction yield red pigments, doubtless anthocyans related to flavones will be found as the result of further investigation of the naturally occurring pigments.

The anthocyan pigments thus far isolated in a chemically pure and crystalline condition, and whose structures have now been definitely established, are:

(1) Cyanin, obtained from the corn-flower, and identical with that obtained from the rose (*gallica*). A glucoside which on hydrolysis yields the non-glucoside pigment cyanidin and two molecules of glucose.

(2) Pigment of the cranberry, a mono-saccharide of cyanidin, which on hydrolysis yields cyanidin and one molecule of galactose.

(3) Pelargonin, obtained from *Pelargonium zonale*, yields on hydrolysis pelargonidin and two molecules of glucose.

(4) Oenin, from deep-coloured wine grapes, gives oenidin and one molecule of glucose.

(5) Delphinin, from larkspur (purple), yields delphinidin, two molecules of glucose and two molecules of *p*-oxybenzoic acid.

(6) Myrtillin, from bilberry, gives myrtillidin and one molecule of glucose.

(7) Pigments from two types of hollyhock, one of which yields one molecule, the other two molecules of glucose and the non-glucoside pigment myrtillidin.

The chemical investigations above mentioned having established the relationship which the anthocyans bear to the flavone derivatives, the fact that the distribution of oxidases and peroxidases in the plant in many cases coincides with the distribution of the anthocyan pigments

can no longer be accepted as favouring the suggestion that the anthocyans are oxidation products of the flavones; its true interpretation has yet to be sought.

Chemical evidence has now established the facts:

(1) That the anthocyans always occur as glucosides (anthocyanins).

(2) That the same pigment may be capable of showing a blue, purple or red colour, according as it exists as alkali salt, free pigment or oxonium salt of some acid. All anthocyans do not, however, form blue alkali salts.

(3) That the anthocyans may be obtained from flavonols by reduction followed by spontaneous dehydration as shown above.

(4) That glucosides of flavonols can pass, by reduction, to glucoside anthocyans (anthocyanins) without intermediate hydrolysis.

The points (3) and (4) doubtless apply also to flavones, but as no natural anthocyans related to these have, as yet, been isolated, proof of this is naturally unavailable.

(5) All analytical evidence points to the molecular weights of the anthocyanidins being of the order of those of the flavonols.

The author does not propose to go deeply into the Mendelian significance of the chemical results discussed above, but would suggest that they appear to show that the factors *R* and *B* so frequently used to represent the power to produce red and blue anthocyan pigment respectively, are really complex factors representing power to produce different conditions of acidity and alkalinity in the cell sap, together with the power to produce anthocyan pigment independent of whether it is in the red, purple or blue form. It ought also to be noted that the factors *R* and *B* if looked upon thus must affect the production of ivory and yellow, for it is well known that in many ivory flowers the pigment of the flavone series is present, and it is only necessary to make the cell sap alkaline in order to produce a fine yellow flower. These are however matters that are better left for those researching upon Mendelian problems to deal with, but now that chemical investigation has thus far cleared the ground, the problems involved in the production of these pigments become somewhat more clear, and research in this field of botanical work should be stimulated and helped thereby.

OUR PRESENT KNOWLEDGE OF THE CHEMISTRY OF THE MENDELIAN FACTORS FOR FLOWER-COLOUR.

PART II.

By M. WHELDALE,

Fellow of Newnham College, Cambridge, and formerly Research Student of the John Innes Horticultural Institution, Merton, Surrey.

Since the appearance of Part I of the author's paper on this subject[1], further work on anthocyanin has been published by Willstätter, in conjunction with Bolton, Nolan, Mallison, Martin, Mieg and Zollinger. The present paper is concerned with the bearing of these later results on the genetics of flower-colour.

In Part I of the author's paper reference was made to Willstätter's first publication on anthocyanin[2] and the views contained therein, namely, that anthocyanin is an oxonium compound, which is purple in

[1] Wheldale, M., "Our Present Knowledge of the Chemistry of the Mendelian Factors for Flower-colour." *Journal of Genetics*, Cambridge, 1914, Vol. iv. (No. 2), p. 109. This communication was delayed six months in the press, and at the time of writing, Willstätter's second communication was not available and his third communication was not yet published.

The question as to the identity of the red produets, formed artificially by reduetion from flavones, with natural anthocyanins is considered in a separate paper in this journal:—Wheldale, M., and Bassett, H. Ll., "On a supposed Synthesis of Anthocyanin." *Journal of Genetics*, Cambridge, 1914, Vol. iv. (No. 1), p. 103. Owing to the above-mentioned delay, the latter paper appeared first and as regards this particular question of identity expresses the author's more recent views.

[2] Willstätter's commnnications referred to are:—(1) Willstätter, R., und Everest, A. E., "Ueber den Farbstoff der Kornblume." *Liebigs Ann. Chem.*, Leipzig, 1913, Bd. 401, p. 189. (2) Willstätter, R., "Ueber die Farbstoffe der Blüten und Früchte." *SitzBer. Ak. Wiss.*, Berlin, Mareh 1914, p. 402. (3) Willstätter, R., und Mallison, H., "Ueber die Verwandtschaft der Anthocyane und Flavone." *SitzBer. Ak. Wiss.*, Berlin, July 1914, p. 769.

its neutral state and forms red oxonium salts with acids and blue salts with alkalies.

Willstätter's second and third papers deal with the preparation and constitution of a number of anthocyanins in addition to the pigment of the Cornflower. The main facts of importance in both communications will be set out first as follows.

The analyses, published in Willstätter's first paper, of the cyanidin of the Cornflower in the form of the chloride have been found by him to be incorrect owing to imperfect drying and a new formula from analyses (by combustion) is given as:

Cyanidin chloride, $C_{15}H_{10}O_6HCl$ (original formula $C_{16}H_{12}O_7HCl$).

The anthocyanin of the Rose (*Rosa gallica*) was found to be a diglucoside of cyanidin and also the pigment, idäin, from the Cranberry to be a galactoside of cyanidin, giving two molecules of galactose on hydrolysis.

The additional anthocyanins[1] isolated are:

Delphinin (from *Delphinium* flowers), which gives on hydrolysis 2 mols. of glucose, 2 mols. of *p*-oxybenzoic acid and 1 mol. of delphinidin, $C_{15}H_{10}O_7HCl$.

Pelargonin (from *Pelargonium* flowers), which gives on hydrolysis 2 mols. of glucose and 1 mol. pelargonidin, $C_{15}H_{10}O_5HCl$.

Önin (from Grapes), which gives on hydrolysis 1 mol. glucose and 1 mol. önidin, $C_{17}H_{14}O_7HCl$.

Myrtillin (from Bilberry fruits and flowers of Hollyhock, *Althaea rosea*), which gives on hydrolysis myrtillidin, $C_{16}H_{12}O_7HCl$ and glucose.

Malvin (from Mallow flowers), which gives on hydrolysis malvidin, $C_{17}H_{14}O_7HCl$.

All the above substances were prepared in the crystalline form as hydrochloric acid salts and apparently analyses were made from such salts only.

The formulae for the pigments free from hydrochloric acid may be compared with the flavones as follows:

Cyanidin, $C_{15}H_{10}O_6$, isomeric with luteolin, kampherol and fisetin.

Pelargonidin, $C_{15}H_{10}O_5$, isomeric with apigenin.

Delphinidin, $C_{15}H_{10}O_7$, isomeric with quercetin and morin.

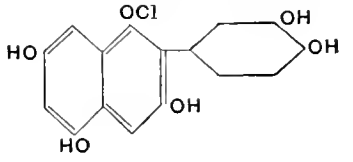
Önidin, $C_{15}H_{10}O_7(CH_3)_2$
Myrtillidin, $C_{15}H_{10}O_7(CH_3)$
Malvidin $C_{15}H_{10}O_7(CH_3)_2$
} are methylated derivatives of delphinidin.

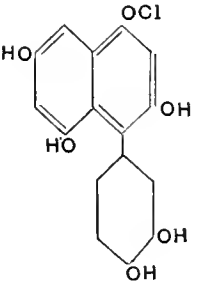
[1] In the present paper the distinction made by Willstätter between anthocyanin (glucosidal product) and anthocyanidin (non-glucosidal product) is not employed.

When heated with alkali, the various pigments split up, as in the case of flavones, into a phenol and an oxybenzoic acid, which were identified in each case:

Cyanidin gave phloroglucin and protocatechuic acid.
Pelargonidin „ „ „ *p*-oxybenzoic „
Delphinidin „ „ „ gallic acid (not isolated in pure state).

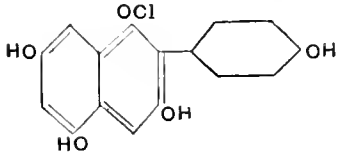
On the basis of the above decompositions and analyses (by combustion) Willstätter first suggests the following alternative constitutional formulae for three of the pigment salts:

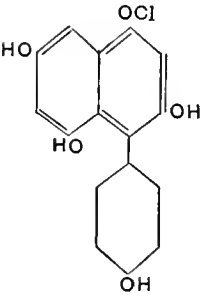
I. 

or 

II.

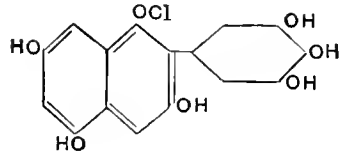
Cyanidin chloride.

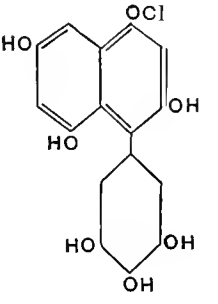
I. 

or 

II.

Pelargonidin chloride.

I. 

or 

II.

Delphinidin chloride.

In water and alcohol solution both the anthocyanins and their salts tend to become colourless with the formation of a colourless iso-form. Willstätter states that the analysis of the colourless iso-form in the case of delphinidin has shown that the change from the quinonoid to the non-quinonoid combination takes place with the addition of a molecule of water, though no more light is thrown on the reaction than is given in the following equation:

$$C_{15}H_{10}O_7HCl + H_2O = HCl + C_{15}H_{12}O_8.$$

In acid solution, on the other hand, and in the solutions of some neutral salts[1], the anthocyanins are stable owing to the formation of the oxonium salts, and the stability is maintained in the absence of excess of water.

Formulae of the type II, as shown above, are discarded by Willstätter since, for example, if such a formula were correct for cyanidin, it should be possible to obtain maclurin as an intermediate product of decomposition and this he did not achieve.

Adopting therefore formulae of type I, Willstätter then points out that the relationship between anthocyanins and flavones is such that it should be possible to obtain the red pigments from the yellow by reduction and, conversely, the yellow from the red by oxidation.

The question as to the formation of anthocyanins from flavones on reduction by means of nascent hydrogen has been considered in the author's previous paper. The phenomenon was first investigated by Combes[2] who maintains that he obtained a crystalline red pigment identical with natural anthocyanin by reduction of a naturally occurring flavone: these statements, however, are not supported by analyses. The same view of the identity of products of flavones with natural anthocyanins has been held by Everest[3].

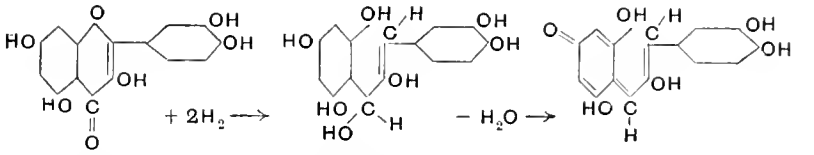
Willstätter, also, has investigated the reduction products of flavones in view of his hypothesis as to the origin of anthocyanins and he claims to have shown that in the process two products are formed, of which one is a true anthocyanin, the other not. In his experiments, an alcoholic solution of quercetin acidified with hydrochloric acid is reduced with sodium amalgam or magnesium. The bulk of the purplish-red

[1] See Willstätter's first paper.

[2] Combes, R., *C. R. Acad. Sci.*, Paris, 1913, T. CLVII. p. 1002 and p. 1454, and T. CLVIII. p. 272.

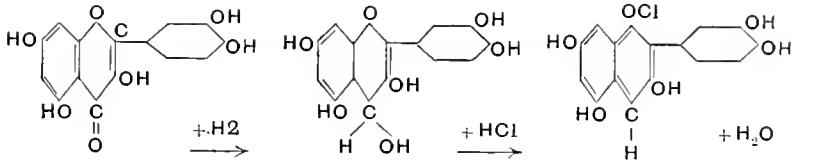
[3] Everest, A. E., "The Production of Anthocyanins and Anthocyanidins." *Proc. Roy. Soc.*, London, 1914, B, Vol. LXXXVII. p. 444. *Ibid.* Pt II. 1915, B, Vol. LXXXVIII. p. 326.

product formed he terms allocyanidin and he suggests that the reaction proceeds as follows with the opening of the pyrone ring:

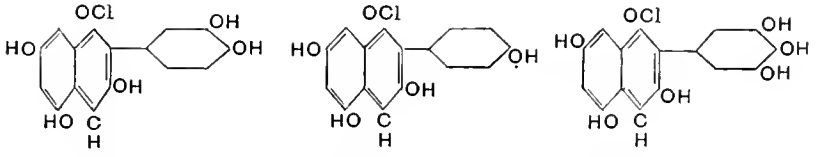


Allocyanidin forms a crystalline compound with hydrochloric acid and it is apparently on the analysis (by combustion) of this compound that Willstätter bases the above constitution of allocyanidin.

Allocyanidin chloride is unstable on heating in dilute hydrochloric acid but when the crude solution from reduction of quercetin with nascent hydrogen is diluted and heated in order to decompose allocyanidin chloride the solution does not entirely lose colour owing to the presence, according to Willstätter, of a small amount of a second substance. The latter formed with hydrochloric acid a crystalline product which on isolation and analysis proved to be identical with cyanidin chloride. From 33 gms. of quercetin, 0·165 gm. only of the product was obtained. The identity was established on the results of analysis (by combustion), appearance, solubilities and properties in general. It is suggested by Willstätter that the reaction proceeds as follows:

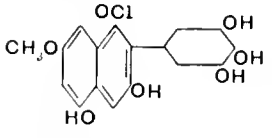


Since the constitution of quercetin is known, Willstätter considers the formula for cyanidin chloride to be established by the above reaction and very probably also the formulae for delphinidin and pelargonidin chlorides:

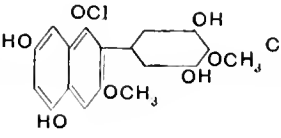


Cyanidin chloride. Pelargonidin chloride. Delphinidin chloride.

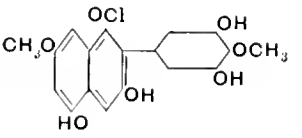
Other anthocyanins are represented as methyl derivatives of delphinidin:



Myrtillidin chloride.



Önidin chloride.



Malvidin chloride.

He points out moreover that the anthocyanidins in the acid-free form may be regarded as forming a series of reduced flavones:

Luteolin, kampherol, and
fisetin $C_{15}H_{10}O_6 \longrightarrow C_{15}H_{10}O_5$ pelargonidin.
Quercetin $C_{15}H_{10}O_7 \longrightarrow C_{15}H_{10}O_6$ cyanidin.
Myricetin $C_{15}H_{10}O_8 \longrightarrow C_{15}H_{10}O_7$ delphinidin.

Bringing Willstätter's views to bear upon the Mendelian factors for flower-colour, we necessarily come to the following interpretations. The chromogens of the pigments are flavones and the factor for colour is the power to bring about a simple reduction of the flavone accompanied by a change from divalent to tetravalent oxygen and a pyrone to a quinonoid structure. If the cell-sap is neutral the anthocyanin has the structure of an inner oxonium salt and is purple. A reddening factor must be interpreted as one which produces an acid cell-sap whereby the anthocyanin is enabled to form a red acid oxonium salt. Similarly a bluing factor is one which produces an alkaline cell-sap, blue anthocyanin being an alkaline salt of the purple neutral compound.

With the exception of the view that the anthocyanins are derived from flavones which was first suggested in 1909[1], the author's results are not in agreement with those of Willstätter. In the case of *Antirrhinum* investigated by the author in conjunction with Bassett, there is no doubt that the chromogen is the flavone, apigenin. On Willstätter's hypothesis that the anthocyanins are reduced flavones, the formula for *Antirrhinum* anthocyanin should be $C_{15}H_{10}O_4$ (apigenin being $C_{15}H_{10}O_5$), giving the percentage composition:

C ... 70·86 %,
H ... 3·94 %,
O ... 25·20 %,

[1] Wheldale, M., "On the Nature of Anthocyanin." *Proc. Phil. Soc.*, Cambridge, 1909, Vol. xv. p. 137.

whereas the percentage compositions actually found for the two forms of *Antirrhinum* anthocyanin are:

Red		Magenta	
C	... 51·81 %	C	... 50·50 %
H	... 5·01 %	H	... 5·11 %
O	... 43·18 %	O	... 44·39 %

Moreover the artificial red product prepared from apigenin by reduction with sodium amalgam does not give the qualitative reactions nor has it the percentage composition of the natural anthocyanin.

The red and magenta anthocyanins from *Antirrhinum* were not obtained in crystalline form, nevertheless their purity was guaranteed by the concordance of analysis results when the pigments were prepared independently from entirely different varieties. Also the products analysed were the pigments themselves and not the hydrochloric acid salts.

The analyses show that both red and magenta contain more oxygen than apigenin. Moreover the red pigment is not an acid salt of the purple or magenta: both are precipitated from acid solutions. Nor is the magenta an alkaline salt of the red. They are different substances, and cannot be converted the one into the other by such means.

In the work on *Antirrhinum*, the problem which has been before the author may be expressed thus:—What are the actual processes which lead to anthocyanin formation *in the living cell*? Only the answer to this question will enable us to interpret the Mendelian factors correctly.

Willstätter's percentage formulae for several anthocyanins coupled with the production, from quercetin by reduction, of a very small quantity of a substance claimed to be identical with cyanidin are not convincing reasons for regarding the natural process of pigment formation as one of reduction. In this respect the important point is whether cyanidin is formed from quercetin, delphinidin from myricetin, etc., *in the living plant.* Willstätter makes no mention of the flavones accompanying the anthocyanins he has isolated. Myricetin which should be the chromogen of delphinidin is at present known in *Myrica*, *Rhus*, *Haematoxylon* and *Arctostaphylos*. From *Delphinium consolida* kampherol has been isolated but it is quite possible that other flavones may be present in addition or different flavones in other species.

Further evidence is still needed of the connection between anthocyanin and flavone in the same plant, and between the natural

anthocyanin and the artificial pigment prepared from the same flavone. Only in *Antirrhinum* are such relationships known and they are not so far in accordance with the reduction hypothesis.

Willstätter dismisses as disproved the hypothesis suggested by the author[1] some years ago, with a view to explaining some of the phenomena, both physiological and chemical, of anthocyanin production. The hypothesis supposes several of the hydroxyls of the flavones *in the living cell* to be replaced by sugar and that only after hydrolysis of certain hydroxyls can changes take place at these points with the production of pigment. The hypothesis is more within the province of plant physiology than chemistry and was the outcome of observations upon the distribution of pigment in the tissues and the effect of factors such as light, temperature, drought, injury, sugar feeding, etc., on anthocyanin formation. The hypothesis has been said to be rendered valueless by the fact that in the formation of artificial anthocyanin, mono- or even di-glucosides of flavones can be employed and the pigment is formed on reduction in the cold without hydrolysis.

The existence of stable di-glucosides of quercetin which can be isolated and which, when treated in hot acidified alcoholic solution with nascent hydrogen, give a red product is no criterion of the conditions in which the quercetin exists *in the living cell*, nor of the reactions which convert it into anthocyanin.

Note. I should like to take this opportunity of correcting two errors made by me in a previous paper written jointly with Mr Bassett, i.e. "On a supposed Synthesis of Anthocyanin" and published in this Journal, Vol. IV. No. 1, p. 103.

On page 104, line 3, of the above paper, read:

"glucoside (of flavone) + water $\rightleftharpoons$ chromogen (flavone)
+ sugar x (flavone) + oxygen = anthocyanin."

On page 105, line 9, read:

"then reducing without removal of sugars."

[1] Wheldale, M., "On the Formation of Anthocyanin." *Journal of Genetics*, Cambridge, 1911, Vol. I. p. 133.

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Owing to an error in alignment it is requested that this slip be substituted for the Note on p. 376 of Vol. IV.

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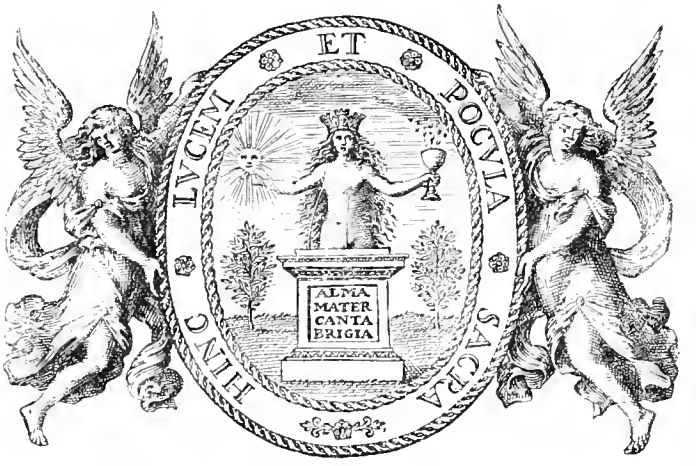
THE
CAMBRIDGE BULLETIN

No. XXXII



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THE CAMBRIDGE BULLETIN

No. XXXII May 1914

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Fig. 29. Elevation of part of a sanatorium pavilion of Doecker construction for 20 beds. The administration buildings are not shown

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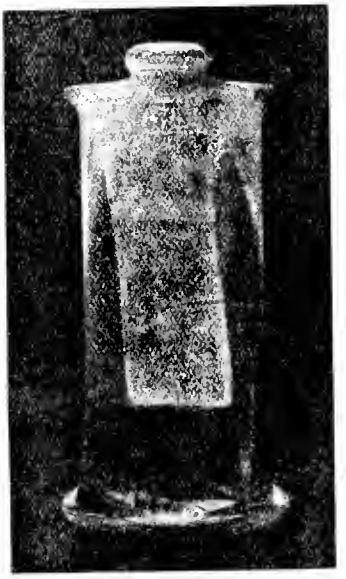

Fig. 10. *B. enteritidis sporogenes* enumeration jar

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Fig 133.- View of Matterhorn from the Capanna Margherita at sunset

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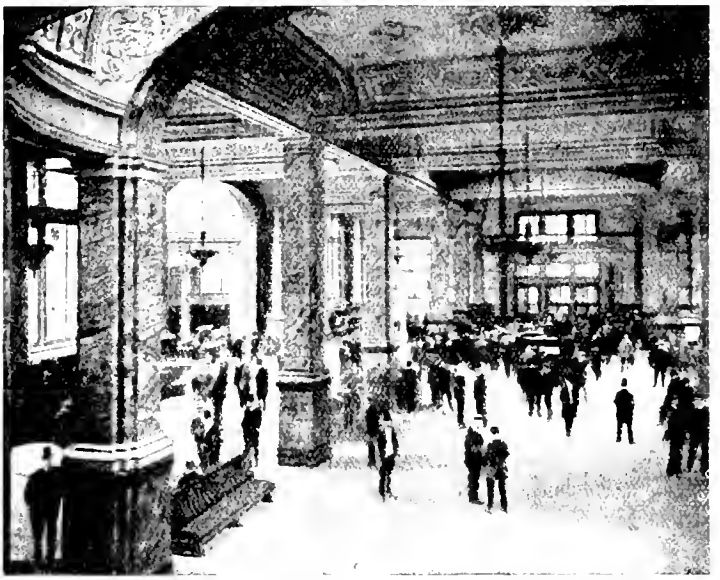

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The Elementary School Teacher. *Monthly, except July and August. Annual subscription 8s. net*

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Books published since 29 January 1914

THEOLOGY

Joshua: The Hebrew and Greek Texts. *By S. Holmes, M.A., Lecturer in Theology, Jesus College, Oxford, and formerly Senior Kennicott Scholar. Demy 8vo. pp. viii + 80. Price 7s. net.*

The result of Hollenberg's enquiry into the texts of the Book of Joshua in 1876 was in many passages favourable to the LXX; he strongly denies deliberate alteration but on the whole seems to uphold the general superiority of the M.T.

Ten years later a far less favourable attitude was adopted by the great scholar Dillmann; he affirms that the value of LXX, in this book as well as in others, has been much overestimated. Other scholars have given their support to this view, and Holzinger (1901) explicitly affirms that the statement of Dillmann—that LXX does not offer a more original text, but represents in many cases a deliberate endeavour to avoid difficulties—still holds good.

The present thesis dissents from this position and offers some fresh reasons in favour of the superiority of the LXX:

(1) The phenomenon of double and sometimes more frequent omission of the same word or expression in LXX in a large number of passages.

(2) The circumstance that in several cases where the two texts vary from one another, each text is consistent with itself; thus suggesting the hypothesis of a deliberate and systematic revision.

(3) The fact that the confused LXX passage, ch. v. *vv.* 4 f., when turned back into Hebrew requires only a slight emendation to give an intelligible text manifestly earlier than M.T.

Evolution and the Need of Atonement. *By Stewart A. McDowall, M.A., Trinity College, Cambridge, Assistant Master at Winchester College. Crown 8vo. pp. xx + 184. Price 4s. 6d. net.*

A second edition, revised and enlarged. The author addresses his work to those who are oppressed with the difficulties of their faith not as an apologetic, but as a re-statement of certain fundamental doctrines of Christianity from that point of view which the spirit of the age forces upon us. In the new edition a considerable section has been added to the discussion of original sin, and the treatment of the Atonement itself has been amplified by a new chapter linking the two portions of the book more closely.

Nestorius and his place in the History of Christian Doctrine. *By Friedrich Loofs, D.D.* See p. 4.

The Canticles of the Christian Church. *By James Mearns, M.A.* See p. 4.

The Poem of Job. *Translated in the metre of the Original by E. G. King, D.D.* See p. 5.

The Fourfold Gospel. Section II. The Beginning. *By Edwin A. Abbott. Diatessarica, Part X, Section II.* See p. 4.

The Bible of To-Day. *By A. Blakiston, M.A.* See p. 5.

Parerga Coptica. *Conscripsit Stephanus Gaselee, A.M., Collegii sanctae Mariae Magdalenae apud Cantabrigienses socius et bibliothecarius, et hoc anno alter e Procuratoribus Academiae.* II. De Abraha et Melchisedec. III. Hymnvs de Sinvthio. *Royal 8vo. pp. iv + 24. Paper covers. Price 1s. 6d. net.*

The Offices of Baptism and Confirmation. *By T. Thompson, M.A., of Saint Anselm's House, Cambridge. Small crown 8vo. pp. x + 254. Price 6s. net.*

One of the *Cambridge Handbooks of Liturgical Study*, under the general editorship of Dr H. B. Swete and Dr J. H. Srawley. Its aim is to trace the developments of the services in general outline, and to indicate the relations of various rites to each other; and to help those who desire to study the services in the ancient liturgical books.

The Second Book of Kings. *Edited by the Rev. G. H. Box, M.A. Fcap. 8vo. pp. xvi + 158. Price 1s. 6d. net.*

Isaiah xl–lxvi. *Edited by the Rev. W. A. L. Elmslie, M.A., and the Rev. John Skinner, D.D. Fcap. 8vo. pp. xxxiv + 138. Price 1s. 6d. net.*

Two new volumes in the *Revised Version for Schools* series, under the general editorship of Dr A. H. McNeile; the aim of the series is to explain the Revised Version to young students and at the same time to present, in a simple form, the main results of the best scholarship of the day.

A Commemoration Sermon *preached 9 December 1913, in the Chapel of Trinity College, Cambridge, by Henry Jackson, a Fellow of the House. Large crown 8vo. pp. 12. Paper covers. Price 6d. net.*

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Terence: Phormio. *Edited by John Sargeaunt, M.A., Assistant Master at Westminster. Extra fcap. 8vo. pp. xxiv + 130. With or without vocabulary. Price 3s.*

This edition, in the *Pitt Press Series*, should prove of general use in schools, especially for those students who are making their first acquaintance with this author. The editor has been concerned less with matters of textual criticism than with a presentation of the author's style in its modern equivalent and his general literary value.

Livy: The Revolt and Fall of Capua. *Edited by T. C. Weatherhead, M.A., sometime Headmaster of King's College Choir School, Cambridge; formerly Bell University Scholar. Extra fcap. 8vo. pp. xxvi + 166. With 3 maps. Price 2s.*

This book, which consists of Selections from Livy XXIII–XXVI, is one of the *Cambridge Elementary Classics*. The text of Livy has not been simplified, but the editor's object has been to give to those who are beginning this author such assistance as will serve to bridge over the undoubtedly serious transition from Caesar to Livy.

Caesar: Gallic War. Book VI. *Edited, with introduction, notes, and vocabulary, by E. S. Shuckburgh, Litt.D. Fcap. 8vo. pp. xxii + 116. With illustrations and a map. Price 1s. 6d.*

In the new *Cambridge Elementary Classics* edition, in which, to aid pronunciation, vowels long by nature are marked throughout the text.

ENGLISH

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The first volume of the *Cambridge Modern French Series* (see p. 20) contains the following stories : *Le Horla—Le Trou—Les Prisonniers—Qui Sait?—Menuet—L'Aventure de Walter Schnaffs*, with exercises and a vocabulary of less known words.

GERMAN

Julius Stinde. Die Familie Buchholz. *Edited by G. H. Clarke, M.A. Large crown 8vo. pp. xii* + 76. *Price* 2*s.* 6*d.*

In the *Cambridge Modern German Series* (see p. 21).

Formal introduction to Julius Stinde, says the editor, is rendered unnecessary by his European fame, Part 1 of *The Buchholz Family* having gone through 89 editions.

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History of Roman Private Law. Part II. Jurisprudence. *By E. C. Clark, LL.D.* See p. 7.

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The Bacteriological Examination of Food and Water. *By W. G. Savage.* See p. 6.

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An Atlas of Commercial Geography. *Edited by Fawcett Allen, Assistant Map-Curator to the Royal Geographical Society. With an Introduction by D. A. Jones, Librarian to the Royal Geographical Society.*

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The Annals of the Bolus Herbarium. *(South African College.) Edited by H. H. W. Pearson, Sc.D., F.L.S. Vol. I, Part 1. Price 5s. net.*

The first number of the first South African journal entirely devoted to botanical work. Two parts will probably be published each year and four parts will constitute a volume. The subscription price per volume is 15s. net.

Announcements

THE ROYAL SOCIETY

Vol. XIII of the Royal Society's Catalogue of Scientific Papers is the first of the fourth series, which will comprise the titles of papers published or read during the period 1884—1900, and will conclude the work undertaken by the Royal Society, namely a complete collection of the titles of papers for the whole of the 19th century. The present volume, which will shortly be published, covers the letters A and B.

Vol. III, Part II of the Subject Index, arranged under the superintendence of Dr Herbert McLeod, Director of the Catalogue, will also be ready immediately. It deals with Electricity and Magnetism and contains 23,300 entries, thus making in all 56,644 entries for the subject Physics for the years 1800 to 1900 inclusive.

THE CAMBRIDGE MODERN FRENCH SERIES

The aim of this series, which is under the general editorship of Mr A. Wilson-Green, is to provide Modern French texts equipped with exercises on the lines of the direct method. The volumes are divided into three groups and comprise:—

1. A short biography in French of the author.
2. A series of exercises, each containing passages for translation into French, and questions in French on (*a*) the narrative, (*b*) the words and idioms, (*c*) the grammar.

3. A French-English vocabulary for those who desire translation into English, instead of reading all in French.

Mr Sloman's edition of *Six Contes par Guy de Maupassant* is now ready (see p. 17), and the following volumes are in the press:

Senior Group. *Ce que disent les livres,* par Emile Faguet. Edited by H. N. Adair, M.A., Senior French Master, Strand School, London.

Middle Group. *Causeries du Lundi (Franklin et Chesterfield),* par C. A. Sainte-Beuve. Edited by A. Wilson-Green, M.A., Senior French Master, Radley College.

Junior Group. *La Maison aux Panonceaux,* par Mrs J. G. Frazer. (Exercises by A. Wilson-Green.)

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This series, which is under the general editorship of Mr G. T. Ungoed, is primarily intended for use on the direct method by pupils who have completed at least their first course in German. The texts are short and suitable for schools without being trivial in subject matter. In addition to a short sketch of the career and works of the author, each volume contains questions on the narrative, grammatical exercises, and subjects and outlines for free composition. In the most elementary texts appear phonetic transcriptions of short passages for reading and dictation.

The exercises are based entirely on the corresponding sections of the text. They consist of:

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   (*b*) on the use of words and phrases.
2. Exercises on Accidence, Syntax and Word-formation.
3. A subject for free composition suggested by an incident in the text, the main outline being also given for beginners.

A German-English vocabulary is supplied with each volume for those who desire it. Words which a pupil may be expected to know are not included.

The following two volumes have already been published:

Hackländer: Der Zuave. Adapted and edited by G. T. Ungoed, M.A. (Without vocabulary.) 2*s.*

Stinde: Die Familie Buchholz. Edited by G. H. Clarke, M.A. (See p. 17.)

Der tolle Invalide auf dem Fort Ratonneau, edited by A. E. Wilson, is in the press.

ENGLISH LITERATURE FOR SCHOOLS

Several volumes in this series have recently been reduced in price. A special prospectus will be forwarded on application.

FORTHCOMING BOOKS

Volume XI of *The Cambridge History of English Literature* will be ready shortly. It will deal with the writers of the closing years of the eighteenth century, and the early years of the nineteenth, notably the poets and politicians who were influenced by the French Revolution—Wordsworth, Coleridge, Burke. It will also contain sections devoted to Burns, Cowper, Crabbe, Blake, Bentham, Southey.

The Life, Letters and Labours of Francis Galton has been compiled by Professor Karl Pearson, Director of the Galton Laboratory, and the former friend and colleague of the great scientist. The work will be complete in 2 volumes, of which the first will be ready shortly.

Clio Enthroned, by Mr W. R. M. Lamb, deals with Thucydides—his style and the literary influences under which his work was composed. The writer emphasises the view that the aim of Thucydides was not merely to produce a truthful document, but to set the Muse of history upon her rightful throne.

The Philosophy of Biology, by Dr James Johnstone, is described by the author as "an attempt to understand the descriptions of the Science in the light of its later investigations"; the point of view and the methods of treatment adopted by him are those suggested by Driesch and Bergson.

Professor E. J. Rapson's work on *Ancient India* is written in a manner intelligible to all who take an interest in Modern India, and contains an outline of the history of the national, religious, and social systems of India which flourished between 1200 B.C. and the first century A.D. Two maps are included, together with several photographs of ancient monuments and inscriptions.

In Professor E. G. Browne's new book on *The Press and Poetry of Modern Persia,* the first part will contain a complete list of Persian newspapers, with particulars concerning each, whilst the second part will consist of specimens of the political and patriotic poetry of Modern Persia.

The six hundredth anniversary of the Battle of *Bannockburn* will be celebrated in June of this year, and the Press will shortly publish a work by Dr J. E. Morris in which the story of the battle is re-told in the light of recent investigations into the site of the struggle, the number of men engaged, and so on. The book will be illustrated by plans and photographs. The same author has written a *Modern History of Europe from 1558* for school use, which will also be ready shortly.

Dr J. R. Clark Hall, author of a prose translation of *Beowulf,* has now made a metrical translation of the same poem into modern English, in which he attempts to get as close as possible to the rhythm of the

original and at the same time to make the rendering acceptable to those who are unfamiliar with the peculiar structure of Old English verse.

A book on *English Folk-Song and Dance,* by Mr Frank Kidson and Miss Mary Neal, should be of special interest in view of the recent efforts which have been just in time to rescue a valuable, but rapidly disappearing, national inheritance, and which are now giving it a renewed vigour in the life of the people.

Perception, Physics, and Reality, by Mr C. D. Broad, Fellow of Trinity College, is an enquiry into the information that Physical Science can supply about the Real.

Philosophy: What is it? by Dr F. B. Jevons, consists of five lectures delivered before a branch of the Workers' Educational Association which had expressed a desire to know what Philosophy is; the truth, implied in this request, that Philosophy is the concern of the average man and of practical life is one which this book seeks to emphasise.

The British Revolution, by Dr R. A. P. Hill, deals with the political problems of the moment from the philosophical standpoint. The author seeks to show that Theory, so commonly the suspect of practical men, "is a draught-horse and will drag a load."

Pragmatism and French Voluntarism, by Miss L. S. Stebbing, is an essay written with especial reference to the notion of Truth in the development of French Philosophy from Maine de Biran to Prof. Bergson.

Dr F. R. Montgomery Hitchcock has written a study of the teaching of *Irenaeus of Lugdunum.*

A book on *Elementary Logic* by Mr Alfred Sidgwick gives some account, for beginners, of both the old system and the new in the study of this subject: i.e. it treats Logic (1) as a carefully limited subject to get up for an examination, and (2) as a free study of some of the chief risks of error in reasoning.

Dr A. R. Forsyth's lectures delivered before the University of Calcutta during January and February 1913, will be pnblished under the title of *Lectures introductory to the Theory of Functions of Two Complex Variables.*

A second edition of Volume I of Dr A. Russell's *Theory of Alternating Currents* will shortly be published in the *Cambridge Physical Series.* Besides many additions to the earlier chapters, four new chapters have been added to the book, including one on High Frequency Currents.

Dr Hobson's lecture on *John Napier, and the Invention of Logarithms, 1614,* commemorates the tercentenary of the publication of John Napier's work which embodied this great discovery.

Complex Integration and Cauchy's Theorem, by Mr G. N. Watson, Fellow of Trinity, is an addition to the series of *Cambridge Mathematical Tracts.*

Two volumes in the *Cambridge Technical Series* will be ready in the autumn: Mr Dales's book on *Mechanical Drawing* and Mr Hale's on *Household Science.*

The English Borough in the Twelfth Century consists of two lectures delivered in the Examination Schools at Oxford in October, 1913, by Mr Adolphus Ballard, Town Clerk of Woodstock, and author of a larger volume on *British Borough Charters.*

To the *Cambridge Archaeological and Ethnological Series* will be added a volume on *The Place-Names of Sussex* by Mr R. G. Roberts.

Mr David Hannay has written a work on *Naval Courts Martial,* of which the purpose is to make from the reports of Courts Martial some picture of what the old Navy was down to the end of the Napoleonic wars. The book will include reproductions of old engravings.

A work entitled *Outlines of Ancient History,* by Mr H. Mattingly of the British Museum, is a survey of history from the earliest times down to the fall of the Roman Empire in the West in 476 A.D.

In the autumn will be published new school histories of Greece and of Rome. The *History of Greece* is by Mr C. D. Edmonds, of the Royal Naval College, Osborne, and the *History of Rome* by Mr E. E. Bryant, of Charterhouse School. Each will contain a large number of illustrations and maps.

In the *Cambridge Bible for Schools* series, the volume on *Genesis,* by the Dean of Westminster, will shortly be ready. In addition to full notes and introduction it will contain a number of illustrations and maps.

An edition of *Lucan, Book VIII,* by Prof. J. P. Postgate, will be added to the Latin volumes in the *Pitt Press Series.*

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A Children's Book of Verse, compiled by Miss Alys Rodgers, has the double aim of supplying pieces suitable for recitation and general reading in both secondary and elementary schools, and of providing a collection of verses for out-of-school enjoyment.

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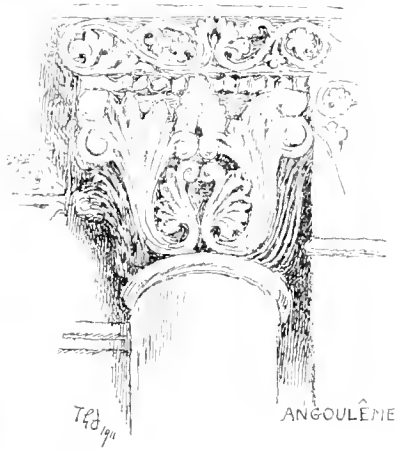

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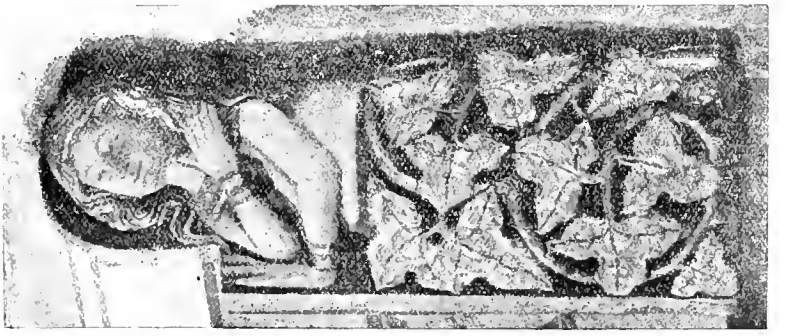

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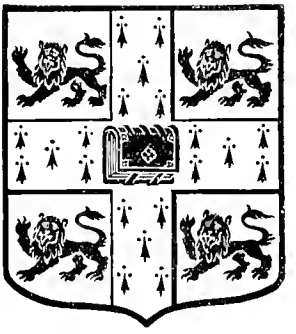

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CONTENTS

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