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THE EFFECTS OF INBREEDING AND SELECTION
ON THE FERTILITY, VIGOR, AND SEX-RATIO
OF DROSOPHILA AMPELOPHILA

By
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THE EFFECTS OF INBREEDING AND SELECTION
ON THE FERTILITY, VIGOR AND SEX RATIO
OF DROSOPHILA AMPELOPHILA

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INTRODUCTORY

The present report includes the results of two series of experiments on the fruit fly—*Drosophila ampelophila*. One set concerns itself primarily with the effects of inbreeding and the other with sex-ratios. The experiments on inbreeding grew out of work I had been carrying on on hybridization. In these hybridization experiments the effects on the developmental processes of hybrids between species too remotely related were especially emphasized. The converse of these experiments was, naturally, to study the effect upon the young between individuals too closely related. Fishes, upon which all my experiments in hybridization were made, do not lend themselves for purposes of inbreeding without elaborate breeding facilities. Mice seemed suitable for this purpose but, both at the outset of these experiments and since, these creatures have proven miserable failures in my hands. Among the insects, I tried the common willow beetle but this proved to throw only one generation annually in this latitude. It was desirable to have an animal with a brief life history, whose food could be easily obtained at all seasons and in which the sexes could be readily distinguished. In these respects the fruit fly is almost ideal. The facts herein considered confine themselves to this species.

The experiments on sex-ratio suggested themselves in connection with the inbreeding experiments and so were carried out along with the latter and after they were completed.

MATERIAL AND METHODS

The strain which is mostly under discussion in my inbreeding experiments came from a well-filled female that was taken from the window of my residence in Bloomington. Other strains were started at the onset. Some of these came from the banana bunches at the various groceries and others came from fruit which I had laid out for this purpose. None of these were carried further than two or three generations excepting two, called 6 and 7 in my records. The latter was discontinued after the tenth generation

since it had been from the beginning apparently less prolific. The strain 6 was carried for over seventy-five generations and is the one on which the experiments in inbreeding of this report are based.

For vivaria, tall stender dishes, tumblers, quinine bottles and lamp chimneys were given a trial. They were discarded in favor of 8-dram shell vials. These were compact, so that a large number of matings could be kept in a small space, and they were most convenient in manipulating the pairs during the frequent changes to new cages that was necessary all along. The open end of the shell vial was closed with a plug of absorbent cotton, not too compact, so as to afford some ventilation. The flies are strongly positive to light, so that the vials could be laid with their bottom toward the light and the cotton plug removed with safety for the introduction of food etc. Small trays holding fifteen of these vials were used and in this way the experiments could be readily and compactly stored in the incubator, or they could be packed into a valise to be taken along wherever I went. The food was exclusively well-ripened bananas. To prevent the larvae from pupating in the food, narrow strips of blotter or filter paper were introduced in which they seemed to be especially fond of pupating. It is, of course, apparent that the greatest care had to be taken to avoid contamination from flies without. The stock food had to be scrupulously watched and the instruments kept clean to avoid the introduction of eggs laid on them by extraneous females. The bananas, especially, as they come from the stores, are likely to be infected with eggs and larvae if the skin be in any way bruised or split.

The brothers and sisters were paired off, always within the first ten or twelve hours of their life as imagos. Up to this time mating has not occurred. In fact I have never found a pair that copulated during the first twenty-four hours or, if so, that produced fertile eggs.

INBREEDING AND SELECTION ON FERTILITY AND VIGOR

1. Introductory

That continued inbreeding acts deleteriously on the fertility and vitality of a race is a belief so firmly and generally established that it is seldom questioned. This has its origin largely in the common experience of breeders whose observations, unfortunately, are too often unreliable. There are not wanting experiments such as those of Van Guaita ('98) and Bos ('94) and others, scientifically conducted, which bear out this conclusion.

On the other hand, it is refreshing to encounter in the literature such reports as that of Gentry ('05) who believes not only that inbreeding is not necessarily harmful, but also that it may be beneficial to conserve and intensify the good points in his breed. Gentry's experiments were made on Berkshires. The most prolonged tests of close inbreeding that have been recorded were made by Castle ('06) on the same species with which the present paper deals. He inbred (brothers with sisters) for fifty-nine generations. He concludes that such close inbreeding does not necessarily result in a loss of productiveness and of vigor; at least that inbreeding cannot be regarded as a causal factor. Some of his results so nearly parallel those of the present writer that further reference to his results will be made in the body of the paper.

During the early part of October, 1903, a number of pairs were started breeding. These came from various sources in Bloomington. These different pairs were reared for the most part only a few generations, excepting pair No. 6 which was continued for about four and one-half years. During this time over seventy-five generations were produced. Toward the close of this period no exact count was kept of the generations so that only an approximate figure can be given. Five pairs of brothers and sisters were mated in each generation to insure against accidents that might terminate the strain if but one mating were made.

Along at the fifth and sixth generation it became more and more difficult to keep the strain alive with the five pairs of brothers and sisters that were mated each generation. The failure of an

occasional pair to produce young had hitherto been attributed to accidental conditions of food, etc., but this no longer seemed a satisfactory explanation of all the failures to produce young. This condition, was, therefore looked into more thoroughly. This was done by laying out instead of five pairs a much larger number from the offspring of a given productive pair. The greatest care was taken with the food, temperature etc. and it soon developed that a variable per cent of the pairs were sterile. These sterile pairs were to all appearances normal. It was clear now that, while inbreeding had not reduced the general vitality of the strain thus far, there had appeared a high degree of sterility.

2. Sterility

a. Character of the sterility. Examination of all the matings brought out the fact that in all cases eggs were present in large numbers. This seemed to suggest that the difficulty lay in the larvae either failing to emerge from the egg envelope or, succeeding in this, failing to carry themselves through the feeding stage or the transformation.

By a careful search of the food of the sterile pairs, after sufficient time for the larvae to mature had been allowed, it became evident that the difficulty lay at a time earlier than the pupal stage for none of the latter could ever be found. The food supplied these sterile pairs was the same as that of the fertile ones since it could not be foretold which pairs were going to prove infertile. Furthermore, the infertile pairs were usually kept for from twenty to thirty days, the best of food being supplied them from time to time. The same search showed that no larvae were present, at least so far as direct inspection of the food under a dissecting microscope could be depended upon.

It was always possible, of course, that the larvae failed to carry their development very far, and, thus, being small when they first emerge from the egg, might have been overlooked. It became necessary, consequently, to take the eggs as they were laid from time to time and keep them under observation to see whether the larvae ever emerged. This was done by placing a piece of banana

in the vial with a sterile pair and from time to time removing the eggs one by one with the point of a needle and placing them on a piece of moist filter paper in a separate vial. Usually twenty were placed in each vial and some food added for the larvae, should they emerge. Inspection of the eggs after twenty-four, forty-eight and seventy-two hours would readily reveal the number of eggs that had produced larvae. I have laid out thus at a great expense of time literally thousands of eggs from many infertile pairs, in many cases all the eggs that a given pair produced during the first twenty-five days of its life, but I have never seen a single egg that had hatched. Eggs of fertile pairs thus laid out will readily hatch so that all the larvae will have taken to the food twenty-four hours after the eggs are deposited.

Such infertile pairs copulate frequently and it would seem that impregnation should follow. I have never sectioned the eggs to see whether spermatozoa enter the eggs or whether they contain partially developed larvae which fail to hatch. I have, however, been able to determine in this strain which of the sexes is at fault. This was done in the following manner. After a pair by sufficient trial had proven itself infertile, the male was mated to a virgin female of a fresh strain that had not been inbred and possessed a high degree of fertility, and the female was similarly mated with a male, usually one whose fertility had been established. Sixty-four such cases were tried and in no case did the females fail to produce young and in no case did the males produce any although repeated copulations took place. It is evident from the foregoing, that, in this strain, the sterility lies exclusively in the male and that the female has lost, apparently, nothing in fertility. Castle (p. 735) reports, on the other hand, that either sex may be sterile. However, Castle took no account of the eggs and larvae but merely the production of pupae, so that his sterility cannot be with certainty compared to mine. It would seem, however, that in some strains infertility may be strictly confined to the males and in others to both sexes. That sterility is complete for all males, when it occurs, is shown by both our results.

b. Degrees of sterility. The foregoing experiments concerned themselves with such pairs as were completely sterile. Other pairs

of brothers and sisters from the same parents, however, were fertile. Judging from the productiveness of these, there was often a wide divergence. It would seem that, as a result of inbreeding, we had a condition of fertility ranging from absolute infertility to comparatively high fertility among the different pairs of brothers and sisters from any given pair of parents. To test this the following experiment was carried out: About two-hundred eggs from each of fifteen pairs of flies were laid out after the fashion indicated above. Ten of these pairs had been inbred for seventeen generations while five belonged to fresh stock that had not been inbred. Of the ten pairs of the inbred strain, five belonged to a strain which had arrived at a very low degree of fertility, namely only 36 per cent of the forty-two pairs tested were fertile (table 3, seventeenth generation, strain, A). These five pairs were brothers and sisters to many of the sterile pairs considered in the preceding section.

The other five pairs (of the ten inbred) were from a strain which had been held by selection to a high degree of fertility, namely 97 per cent of the thirty-four pairs tested were fertile. Both of these strains were descended from common great grandparents (table 3, seventeenth generation, strain B).

We have, thus, for comparison three conditions, namely, (1) eggs from a highly infertile inbred strain; (2) eggs from a highly fertile inbred strain; and (3) eggs from a presumably normal strain that had not been inbred. It should be added that the five pairs were taken at random and were not selected. Approximately the first two-hundred eggs of each pair were laid out in batches of about twenty to twenty-five to the vial. The number of eggs that hatched was noted in each case and also the number that emerged as imagos. Table 1 gives the summary of results.

From this table it appears that from the eggs which were taken from the inbred pairs with low fertility practically as large a per cent (97.27) hatched as from the eggs that came from the inbred pairs that showed a high fertility (98.2). The same is true in regard to the number that produced imagoes, 86.8 per cent and 85.1 per cent respectively. The fact clearly brought out here is that when infertility arises in this strain it arises suddenly and

does not present all intergradations. In other words, one does not find that among a large number of brothers and sisters some pairs whose eggs only partially hatch and other pairs that range in this respect, on the one hand, to perfect fertility and, on the other, to complete sterility. The fertility is either completely lost or it is of a high degree. Furthermore, when we compare the inbreds with the normals (not inbred) in regard to the percentage of eggs hatched no essential difference is observable. It would seem, therefore,

TABLE 1

Inbred (low fertility)

PAIRS	NUMBER OF EGGS PLACED	NUMBER OF EGGS HATCHED	NUMBER OF IMAGOS EMERGED	PER CENT OF EGGS HATCHED	PER CENT OF IMAGOS EMERGED
A.....	193	184	160	95.3	82.9
B.....	200	188	169	94.0	84.5
C.....	201	197	182	98.0	90.5
D.....	198	198	180	100.0	90.9
E.....	123	123	104	100.0	84.5
Total.....	915	890	795	97.27	86.8

Inbred (high fertility)

A.....	201	198	182	98.5	90.5
B.....	173	172	156	99.4	90.1
C.....	204	200	161	98.0	78.9
D.....	197	193	165	97.9	83.7
E.....	175	169	145	96.5	82.8
Total.....	950	932	809	98.2	85.1

Normals (not inbred)

A.....	215	211	193	98.1	89.7
B.....	70	70	48	100.0	68.5
C.....	153	152	132	99.9	86.2
D.....	224	218	144	97.3	64.2
E.....	158	155	144	98.1	91.1
F.....	146	127	109	87.7	74.6
G.....	223	222	205	99.9	91.9
Total.....	1189	1155	975	97.2	82.0

that the pairs that had not completely lost their fertility, in so far as hatching their eggs is concerned, had suffered no deterioration whatever as a result of seventeen generations of closest inbreeding.

A fact of further importance brought out by table 1 is that of the percentage of eggs that successfully produced imagos. This does not differ essentially in the two groups of inbreds nor do these differ essentially from the normals. Castle used as his measure 'productiveness,' meaning thereby the number of pupae that were successfully produced. Making allowance for some pupae which do not emerge, the imagos produced in my experiments were an approximation to his 'productiveness.' Inbreeding, consequently, does not affect adversely the productiveness of pairs that show any fertility at all.

Castle found that his strains showed an annual fluctuation in productiveness, the period of least productiveness falling in the late autumn and early winter. My own experiments extended over about four and one half years and, although I have been on the lookout for this, I have never observed it. As Castle himself suggests, this fluctuation was probably a function of the temperature of the room. My flies were kept in a room which varied from 60 to 80 degrees and, when this was not possible, they were placed in an incubator kept at about the same range of temperature. It may also be that the productiveness of his strain ran low at this time of the year because they were placed in new hands at the opening of the college year. My observation has been that it takes some time for a new man to learn all the conditions that make for a favorable rearing of these creatures so that Castle's low productive periods may be merely a measure of the training period of the experimenter.

3. Inbreeding and vigor

At the outset of the experiments it was the expectation of the writer that such rigorous inbreeding would early and violently show itself in the vigor and fertility of the animals. In this, however, he was largely disappointed. In the strain that is here under consideration no untoward results could be detected during the

first five or six generations. As previously stated, up to this time the method consisted in placing pairs of brothers and sisters in each of five vials to insure against mishaps. These mishaps consisted of drying up of the food, attacks of fungus and in some cases the escape of the flies themselves during the process of feeding etc. Those pairs that produced young were regarded as having escaped these various possible mishaps and were taken as indications of the vitality and productiveness of the strain. The expectation at that time was that any deleterious effect of the inbreeding would show itself in the offspring of any of the pairs. Consequently, when a given pair would produce offspring that was numerous, all well formed, vigorous, and in no apparent way differing from normal offspring, to see whether some slight influence might not be present that could not be detected by ordinary observation a definite measure was taken of (1) their rate of reaction to light and gravity, (2) the total number of eggs produced and (3) the percentage of eggs which hatched and emerged. An attempt was made to determine their length of life but this proved too prolonged to allow one to carry it out together with all the other incidents of the already too laborious experiments.

The reaction of this animal toward light and against gravity is well known. To get a measure of the rate of reaction the animals were made to travel through a glass tube that had been blackened for 16 cm. on the inside. This tube had a light placed at one end and was inclined about twenty-five degrees. From a glass vial the flies were admitted, one at a time, into the tube and the time from the moment of entrance into the blackened portion of the tube to their emergence was recorded. It was found essential that the two batches of flies (inbreds and normals) should be of the same age, be reared under the same conditions and that the temperature of the room be the same for the two batches. The results are as follows: at a temperature of 27.2° C. 133 normals took 16 seconds, average, to travel the distance, and 140 inbreds took 15.4 seconds. The two sexes in these two groups were about equal in number. In both groups the males travel the distance on an average in three seconds less time. It is clear from this that the normals and inbreds are equally responsive to these two

agents and that the latter have not suffered in this regard as a result of inbreeding.

In order to determine the total number of eggs produced it was necessary to isolate the pairs and twice each day pick off all the eggs that had been deposited in and around the food provided. This proved to be a most laborious process, for the eggs are too small to be followed safely with the naked eye and had to be removed individually with the point of a needle. Too much value must not be attached to this measure for the reason that the rate and, therefore, probably the number of eggs deposited seems to depend somewhat, at least, on the condition of the food present, and for the

TABLE 2

Strain 6

Number of generations inbred.....	2	3	5	6	8	9
Number of days eggs were counted.....	27	30	34	34	23	32
Total number of eggs laid.....	433	617	480	724	455	516

Strain 7

Number of generations inbred.....	2	3	5	6	9	10
Number of days eggs were counted.....	26	33	29	23	33	28
Total number of eggs laid.....	654	662	539	498	907	429

reason that only the eggs deposited during the first twenty-five or thirty days were counted. These creatures live to be very much older. We have kept females alive 153 days, but after the first twenty-five or thirty days the eggs come only in small numbers. Table 2 gives the actual counts of several females of both strains 6 and 7.

We see from the above counts that no material reduction has occurred in egg production during nine and ten generations of inbreeding. Such variations as occur may, of course, represent individual differences in the females.

The data given in table 1 of the relative hatching and emerging qualities of the young of normals and of pairs inbred for seventeen generations shows that there is no difference in this respect.

In so far as the above determination may be taken as a measure of the vitality of this species we are justified in concluding that from six to seventeen generations of inbreeding no appreciable deterioration has resulted. No such exact determinations were made in later generations, and it is possible that eventually the effects of inbreeding would manifest themselves, but my observations during seventy-five or more generations does not lead me to believe this.

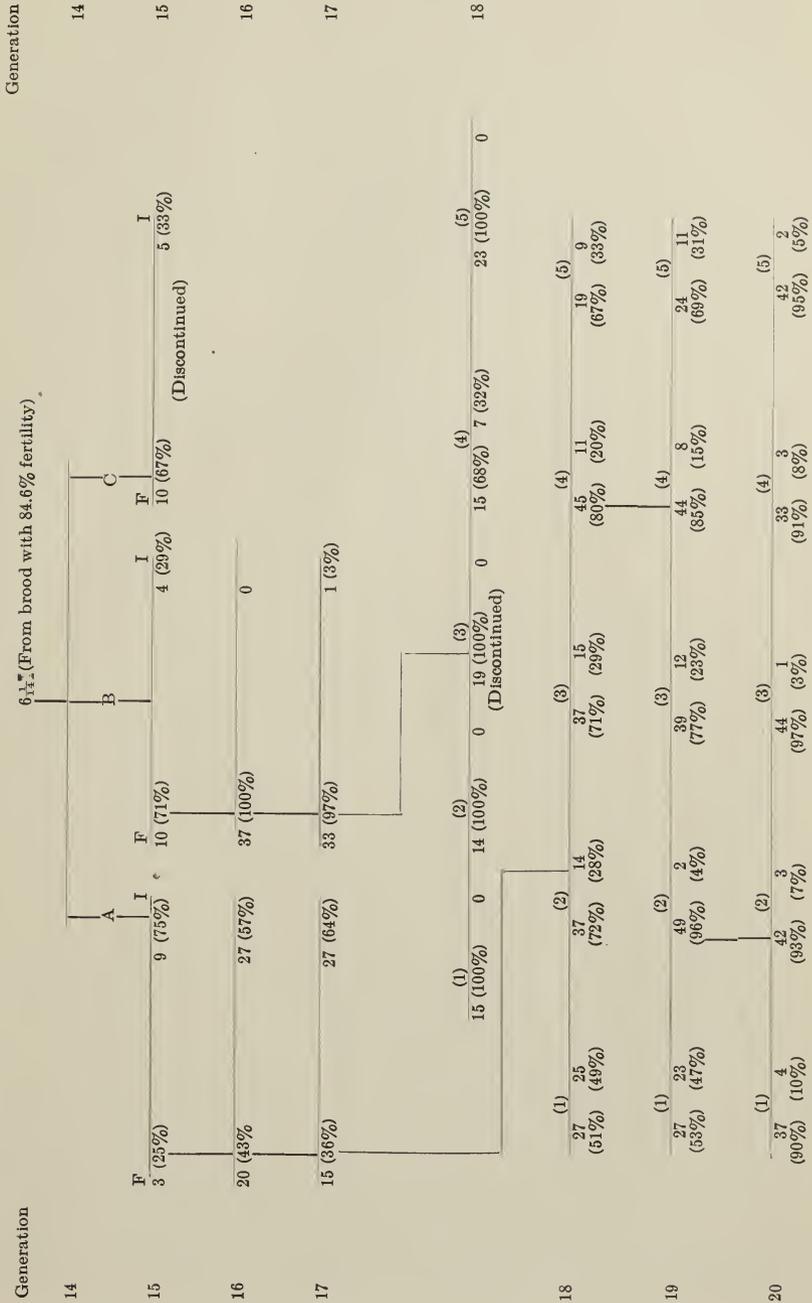
4. *Sterility and selection*

Along at the thirteenth and fourteenth generations the sterility had become very pronounced. Of the offspring of some of the pairs, more than 50 per cent of the males were sterile. On the other hand, while practically all pairs showed at least some degree of sterility this varied very much in the different brothers and sisters of the same brood. That this sterility was a direct physiological result of the inbreeding seemed to me very doubtful. To find the effects of inbreeding showing itself in such a specific way upon the males only, did not, to say the least, meet expectations. Furthermore, sterility was not wholly wanting in forms that had not been inbred.

It was highly desirable to continue the experiments on inbreeding, and yet to keep the strain alive, it was necessary to find some way to eliminate this high degree of sterility. The process that was most effective was selection. By continuing the strain of those pairs whose offspring showed the highest degree of fertility but at the same time continuing the rigorous inbreeding, it was possible almost completely to eliminate the sterility. This at the same time gave one of the severest tests as to whether inbreeding was the responsible factor, for if the sterility could be eliminated by continuing the very process of inbreeding the latter could not well be held to be the cause of it.

This was done as follows: In the fourteenth generation three fertile pairs of brothers and sisters from the same brood were isolated and mated. The offspring of each of these were mated in pairs to determine the degree of sterility. By reference to table

TABLE 3
History of Strains A, B and C



3, it will be seen that the pair marked *A* produced offspring out of which nine of twelve pairs tested were infertile; pair *B* produced offspring of which four pairs out of fourteen tested were infertile and pair *C* threw offspring with five pairs out of fifteen infertile. We have here, then, three pairs showing a wide variation in the degree of fertility of their offspring. Pair *A* showed 75 per cent of the pairs infertile and pairs *B* and *C* approximately the reverse ratio. In the further progress of the experiment pair *C* was dis-

TABLE 4

Strain A

	NUMBER PAIRS TESTED	NUMBER PAIRS FERTILE	NUMBER PAIRS INFERTILE	PER CENT PAIRS FERTILE	PER CENT PAIRS INFERTILE
18 (1).....	52	27	25	51	49
18 (2).....	51	37	14	72	28
18 (3).....	52	37	15	71	29
18 (4).....	56	45	11	80	20
18 (5).....	28	19	9	69	31

Average for 238 pairs 69 per cent.

Strain B

18 (1).....	15	15	0	100	0
18 (2).....	14	14	0	100	0
18 (3).....	19	19	0	100	0
18 (4).....	22	15	7	68	32
8 (5).....	23	23	0	100	0

Average for 93 pairs 92.5 per cent.

continued so that only pairs *A* and *B* were used. I shall in the further description of the experiment refer to the descendants of *A* as strain *A* and of *B* as strain *B*.

Before entering upon the experiment of selection it was necessary to ascertain whether, without selection, the descendants of pairs *A* and *B* continued to show a low and high fertility respectively. Accordingly, a single one of the fertile pairs of the 15th inbred generation of strain *A* and *B* was tested. Reference to the table shows that in strain *A* 27 pairs or 57 per cent of the forty seven pairs tested were infertile, while in strain *B* none of the thirty-

seven pairs tested were infertile. The same process was repeated with a pair of the sixteenth generation of the two strains. Strain *A* showed twenty-seven or sixty-four per cent of the forty-two pairs tested infertile and strain *B* one or three per cent of the thirty-six pairs tested.

Up to this point in the experiment only a single pair in each generation was tested as to the fertility of its offspring. It might well be that by chance in each case a pair of low fertility was taken in strain *A* and a pair of high fertility in strain *B*. To eliminate this possible error five pairs were taken in each strain and the fertility of their offspring determined. It was further desirable to obtain an estimate of the variability in the fertility of the pairs in the two strains as well as to get a more correct estimate of the average fertility of both. In the diagram these five pairs are designated as 18 (1), 18 (2), etc. Table 4 shows the number of pairs of offspring tested for each pair and the number and percentage of pairs fertile and infertile.

The fertility thus varied in strain *A* from 51 per cent in 18 (1) to 80 per cent in 18 (4), with an average fertility of 69 per cent. In strain *B* the fertility was much less variable in the different pairs, the only exceptions being 18 (4), the average fertility being 92.5 per cent.

We now have definitely established two strains, one of low and another of high fertility. The important part to be emphasized here is that this was produced by the process of selection from among the variable offspring of generation fourteen of the inbred strain. To make the experiment more complete it was now necessary to obtain a highly fertile strain out of the one with low fertility. Accordingly strain *B* was discontinued at this point and attention restricted to strain *A*. Five pairs, 19 (1), 19 (2), 19 (3), etc., were taken from among the offspring of 18 (4) because this showed the highest percentage of fertility. These were tested in the same way as in the preceding generation. Table 5 gives the details.

By selection it will be seen that the average fertility was raised from 69 per cent in the 18th generation to 75 per cent in the 19th generation. Among the five pairs used one 19 (2) showed an unusually high fertility (96 per cent). This pair was accordingly

TABLE 5

	NUMBER PAIRS TESTED	NUMBER PAIRS FERTILE	NUMBER PAIRS INFERTILE	PER CENT PAIRS FERTILE	PER CENT PAIRS INFERTILE
19 (1).....	50	27	23	53	47
19 (2).....	51	49	2	96	4
19 (3).....	51	39	12	77	23
19 (4).....	52	44	8	85	15
19 (5).....	35	24	11	69	31

Average fertility of 239 pairs 75 per cent.

taken to select from. Five pairs were again taken as before. The results appear in table 6.

Thus it will be seen that all five pairs showed a uniformly high degree of fertility. The average fertility of all the pairs was raised to 93.8 per cent.

5. Discussion

From the above series of experiments a number of important facts are brought out. 1. Sterility, as it appeared in the strain under consideration, is strongly transmissible through inheritance. 2. It is readily controlled by selection. 3. Inbreeding is probably not the physiological cause of it.

That this sterility is transmissible cannot be doubted. The faithfulness with which this occurs appears in the strains *A* and *B*. Both were derived from a common pair that showed a variability with respect to this character in the three pairs of its offspring

TABLE 6

	NUMBER PAIRS TESTED	NUMBER PAIRS FERTILE	NUMBER PAIRS INFERTILE	PER CENT PAIRS FERTILE	PER CENT PAIRS INFERTILE
20 (1).....	41	37	4	90	10
20 (2).....	45	42	3	93	7
20 (3).....	45	44	1	97	3
20 (4).....	36	33	3	91	9
20 (5).....	44	42	2	95	5

Average for 211 pairs 93.8 per cent.

tested. One of these possessed a high degree of sterility, while the two other pairs showed a low degree. The descendants of the latter constituting strain *B*, retained this low degree of infertility throughout. Similarly the descendants of the former, constituting strain *A*, retained their high degree of infertility up to the time when selection away from this condition was introduced. In the latter process the transmissibility of the character is again emphatically revealed. In the eighteenth generation, pair 4 showed a lower degree of sterility than any of the remaining four pairs of brothers and sisters. Breeding from this pair at once showed offspring with a decided decrease in sterility, compared with the eighteenth generation, the average of the nineteenth generation being 75 per cent of the pairs fertile as compared to 69 per cent of the latter. Again, in the nineteenth generation, pair 19 (2) showed a much lower degree of infertility than the other pairs. Continuing the strain from this pair, this character is faithfully reproduced in the offspring in that they average fertility of the latter is raised to 93.8 per cent.

It is important to note in this connection that Castle, in his experiments upon *Drosophila*, found that productiveness (which as previously noted is quite a different thing from the sterility here considered) was similarly transmissible and amenable to selection. Furthermore, Castle's experiments would seem to indicate that this character of productiveness behaves, in inheritance, after the Mendelian fashion, low productiveness acting as the recessive character. We have evidently to do here, both in the productiveness in Castle's experiments and in the sterility in my own, with characters that are germinal for they behave as such. In the strain upon which my experiments were made we have the further remarkable condition that the infertility is inherited only by the males.

It is clear that whatever the causal factor or factors to which the sterility may be attributed, it is relatively insignificant compared to the effect of selection upon it. Furthermore, the modification is a germinal one. That inbreeding may be responsible for its prevalence in the strain seems probable, but that it is responsible

for its origin is not believed. We have seen that the general vitality of the strain, as measured by its productiveness and its reaction to light and gravity, did not suffer as a result of seventeen generations of closest inbreeding. Failing in this, it is not probable that its effect would show itself in so specific a way as the sudden and complete sterility in certain males of the strain. The improbability is further supported by the fact that the inbreeding may be continued unabated if only care be exercised in the selection of the brothers and sisters to be mated, thereby even eliminating practically what sterility may have existed.

It is much more probable that the sterility arose spontaneously in this strain or that it is present to a varying degree in this species. With the character present and highly transmissible and subject to selection it is only necessary to carry on indiscriminate breeding to have the character appear in varying intensities depending upon the chance combinations. The rule of inbreeding would be only to intensify the chance combination of the character and to insure the more or less continued presence in the successive generations.

That this character of sterility is not unique to this inbred strain is evident from its rather frequent presence in pairs not inbred. In my own experience this sterility nearly always showed itself in the males. In one instance I found among a brood, besides a sterile male, two females that failed to deposit eggs although eggs were evidently present in the oviducts. Similarly Castle found in his strain a considerable amount of sterility, and this in some cases among the females. We see, therefore, that sterility is not altogether rare even in broods that were not inbred.

The same facts doubtless hold for the character of productiveness. Castle has shown this to be transmissible and amenable to selection. Inbreeding does not produce it but is instrumental, with indiscriminate mating, in intensifying it, or, if the strain be not eliminated thereby, of preserving it in the strain.

SEX-RATIO AND SELECTION

1. *Introductory*

The once rather generally accepted notion that nutrition was an influential factor in the control of sex, based on the experiments of Yung ('85), Born ('81), and others, has given place to the now as commonly accepted idea that sex is determined prior to or at the time of fertilization and is independent of the food. The experimental work of Cuénot ('99) King ('07) and others, and the splendid cytological researches of Wilson and his students are largely responsible for this change of view and have been so frequently reviewed in the various recent discussions of the problem of sex that they need not be further detailed here.

The writer tried some starvation experiments on *Drosophila* in 1904. During the past year more extensive experiments were carried on under his direction by Mr. Claude D. Holmes, on the effects of starvation during successive generations upon the sex-ratio. These are published under a separate title ('10). It will suffice in this connection, to state that the results coincide with those of recent workers, namely that nutrition does not affect the sex-ratio.

2. *The normal sex-ratio*

One fact was very apparent in these earlier tests and in all subsequent experiments, that, under the varying conditions in these creatures were reared, there was the same persistence of the predominance of females over males. Below (table 7) is given the

TABLE 7

FOOD	TOTAL NUMBER REARED	NUMBER OF MALES	NUMBER OF FEMALES	RATIO
Bananas.....	10506	4972	5534	1:1.113
Grapes.....	2161	995	1166	1:1.171
Tomatoes and grapes.....	4048	1943	2105	1:1.083
Bananas.....	10218	4757	5461	1:1.14
Total.....	26933	12667	14266	1:1.126

summary of four determinations on a large scale to obtain the normal sex-ratio. The flies were reared in the following manner. Mason jars containing a large quantity of food were exposed to flies in nature. The jars were left open until the larvae began to pupate when all flies were excluded by tying a guaze over the top. As the imagos emerged from time to time they were preserved and the sex-ratios determined. For 26933 individuals, the ratio was one male to 1.126 females.

In regard to these determinations only one question, so far as I can see, can be raised. This is the academic one of the greater mortality of the males during development or, to push the matter back a little further and to make it applicable to recent developments in our idea of sex, the greater mortality of the male determining sex cells. In reference to this it may be pointed out that the developmental conditions were as nearly normal as one can imagine. There was an abundance of food, air, light and moisture, and the larvae pupated in the remnants of the food in much the same manner as one finds them doing in nature. In this connection the experiments of Miss King ('07) on the influence of food on the sex ratio of *Bufo* are of importance. In this she finds that the mortality among the males is not greater than among the females. From these facts and from the knowledge that has come to me from the extensive rearing of *Drosophilas* for six years I am convinced that the sex-ratio in this species is not one of equality.

3. Control of sex-ratio by selection

If the sex-ratio of this species, then, is that of 1 male to every 1.126 females, this should be regarded as specific just as any other of the specific characters of the species. It should, therefore, be subject to fluctuations and to control like other specific characters.

Starting with this conception of sex-ratio, I wished to see whether it were possible to control this, within limits, of course, by the process of selection. The results of these experiments I propose to detail below.

To apply the selective process on the sex-ratio, the following simple method was employed. Two pairs were selected from

nature, the one showing a high, the other a low female ratio. These were selected as the parents of the two strains to be developed. From among the offspring of each of these two pairs a number of single matings were made. From among these the pair that showed the most favorable ratio in the desired direction was selected to continue the strain. The same process was repeated as often as desired.

From a number of pairs taken from a banana bunch in Bloomington June 12, 1907, two such pairs were obtained. These two pairs go by the numbers 206 and 207, showing the following ratio:

$$\begin{aligned} 206-52 \sigma : 135 \text{ } \varphi \text{ or } 1:2.59 \\ 207-84 \sigma : 75 \text{ } \varphi \text{ or } 1:0.89 \end{aligned}$$

A. *Strain 206 (high female ratio)*. The 206 strain will, for convenience, be called the female strain and the 207 strain the male strain, although, as will appear, the latter never developed into a predominantly male strain. In tables 8 and 9 are given in diagrammatic form the results of selection for five generations in the former and six generations in the latter. At the margin the generations are numbered 1, 2, 3 etc., and the sex-ratios are indicated.

The sex-ratio of the eleven pairs of brothers and sisters mated from the first generation of the female strain (206) varied from 1:93 (76 σ ; 71 φ) to 1:7.00 (8 σ : 56 φ).

The unusually high female ratio in the latter is probably attributable to the small number of individuals obtained from this pair. Two of the pairs threw a predominance of males (table 8 nos. 4 and 8). With the exception of no. 5, all the remaining pairs threw a high female ratio. The ratio for all the pairs was 1:1.67 (578 σ : 969 φ). We have here a female ratio very much higher than that characteristic of the species (1:1.14) and yet considerably below that of the parent pair (1:2.59). This may be regarded as a regression toward the normal ratio. It should be pointed out here that too much emphasis should not be placed upon the exact figures representing the ratios in the different pairs, since the number of individuals at best are rather small. In most cases, however, when the number of offspring obtained is fairly large, the ratio approximates the true one, so that in any given

TABLE 8
History of Strain 206

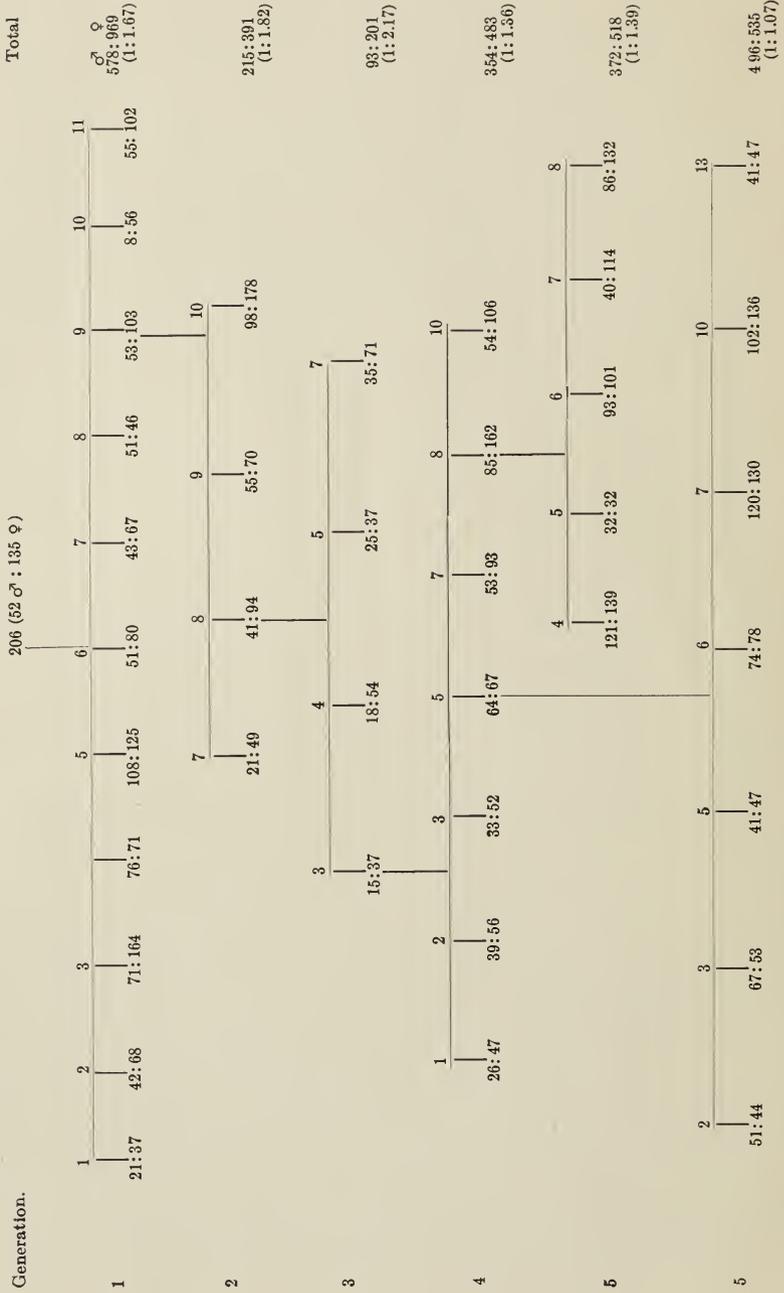
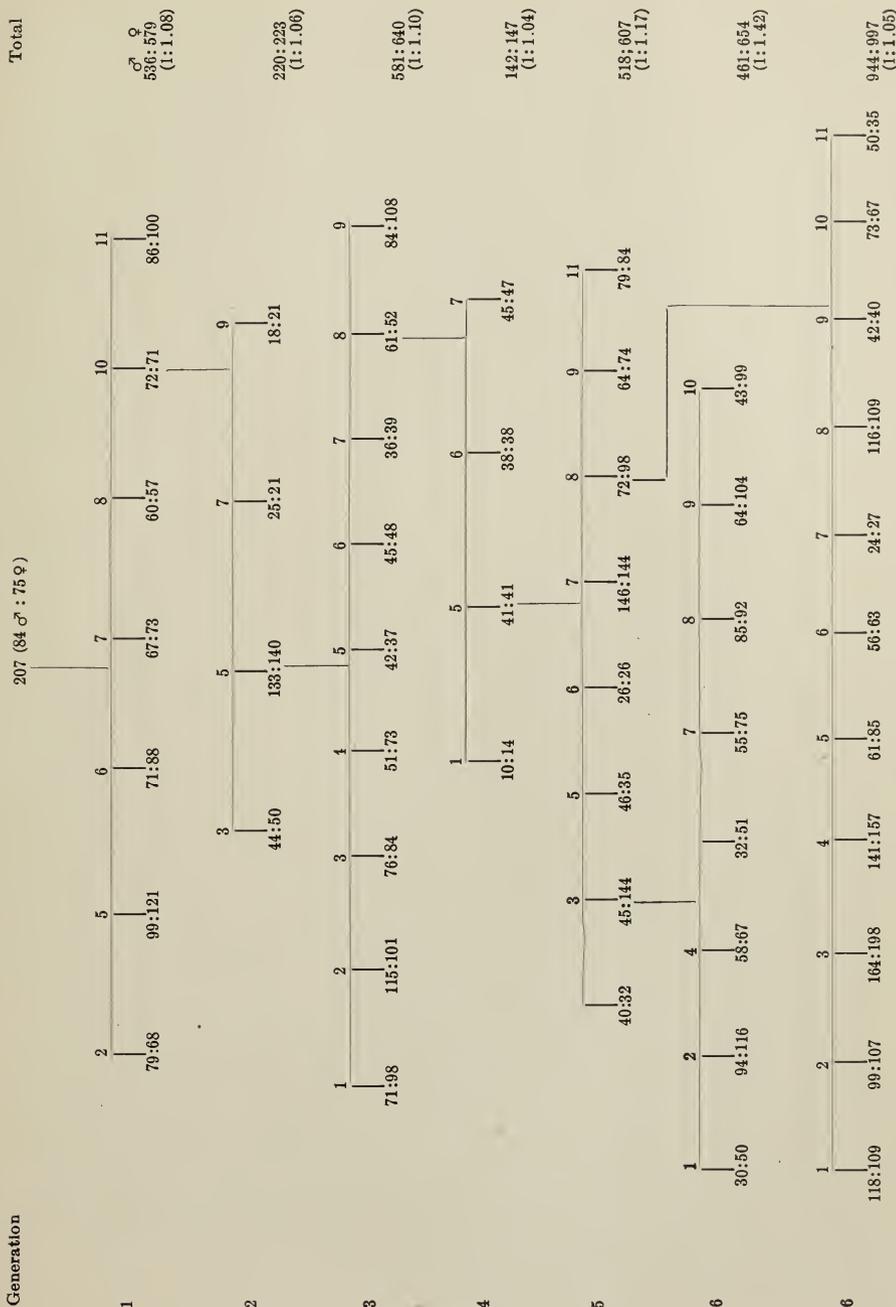


TABLE 9
History of Strain 207



pair from which a fairly large number of offspring has been obtained shows a high female ratio for instance, this may be taken as a pretty safe indication that the female ratio would be high if all or a much larger number had been obtained.

For the next generation ten pairs were taken from brood 9 with a ratio of 1:1.94. Brood 3, with a ratio of 1:2.31, would have been a more favorable one to select from, but this is not always possible since the matings must be made before all the offspring have emerged and therefore all the data for the complete ratio is obtained. Only four pairs of this series of matings came through safely, due purely to the lack of time to give them the attention they should have had. The four pairs threw the following sex-ratios: 1: 2.33; 1:2.29; 1:1.27; 1:1.81. The ratio for the entire brood was 1:1.82 (215 ♂: 391 ♀). This ratio was somewhat more predominantly female.

Pairs were now selected from the brood 8 with a ratio of 1:2.29. Of the seven pairs mated the offspring of only four was obtained and the number of young in each case was quite small. The ratio for all the offspring of the generation was 1:2.17 (93 ♂ to 201 ♀). The total number here involved is so small that not too much importance should be attached to the increased female ratio.

For the matings of the next generation there is little doubt that an unfortunate selection was made. The brood from which the matings were taken showed a ratio of 1:2.46 but this ratio was based on numbers so small (52) that it probably did not represent the true ratio of the pair. This may account for the drop in the ratio for all the broods of the 4th generation to 1:1.36 (354 ♂ 483 ♀).

Two sets of matings were now made from as many broods of the fourth generation. One of these series was again taken from the brood showing the most favorable female ratio 1:1.90 (85 ♂ 162 ♀), but the other series was taken from a brood showing a relatively low female ratio, 1:1.04 (64 ♂ 67 ♀). From the former the ratio of five pairs was obtained showing a ratio of 1:1.39 (372 ♂—518 ♀) and from the latter the ratio of 7 pairs, showing a ratio of 1:1.07 (496 ♂: 535 ♀).

b. Strain 207 (low female ratio). From pair 207 with a ratio of 1:0.89 (84 ♂ : 75 ♀) it was hoped to develop by selection a strain showing a low female ratio. Seven matings from the first generation produced 536 ♂ and 579 ♀, or a ratio of 1:1.08. The range of ratios of the individual pairs was from 1:1.22 (99 ♂ : 121 ♀) to 1:0.86 (79 ♂ : 68 ♀). This selection was continued for four generations, the matings being made from broods with a low female ratio. The ratios of all the offspring in the successive generations were 1:1.06 (220 ♂ : 223 ♀) 1:1.10 (581 ♂ : 640 ♀); 1: 1.04 (142 ♂ : 147 ♀); 1:1.17 (518 ♂ : 607 ♀) for the second, third, fourth and fifth generations respectively (See Table 9). This low female ratio showed itself rather uniformly in all the individual matings, a notable exception occurring in the fifth generation (see Table 9, pair 3.) with a ratio of 1:2.53 (45 ♂ : 144 ♀). On the other hand no pairs threw a great preponderance of males, the most notable among those from which a large number of progeny was obtained being pair 2 in the third generation in which the ratio was 1:0.87 (115 ♂ : 101 ♀). For the sixth generation two sets of matings were made as in the fifth generation of the strain 206. One of these was made from a brood with a ratio of 1:2.53 (45 ♂ : 144 ♀) and the other from a brood with a relatively low female ratio, 1:1.36 (72 ♂ : 98 ♀). From the former the total progeny of eight matings gave a ratio of 1:1.42 (461 ♂ : 654 ♀) and from the latter the ratio of eleven matings was 1.1.05 (944 ♂ : 997 ♀).

c. Discussion. It seems from the above experiment that the sex-ratio in this creature is a strongly transmissible character. Starting with a pair that throws an offspring showing either high or a low female ratio it was possible to maintain, by selection, a strain maintaining the respective ratios. The offspring from a given pair, when mated in pairs, show a considerable variation in the sex-ratio of their children. It is thus possible to develop a strain with a low female ratio from one with a high female ratio, or the reverse, as is shown in the fifth and sixth generation of experiment 206 and 207 respectively (tables 8 and 9). The sex-ratio is clearly amenable to selection like any other character.

It is an interesting fact that it is possible to develop a strain with a high female ratio much more easily and pronouncedly than a male strain. I have repeatedly tried to hold the sex-ratio to or below that of unity but without success. Not infrequently pairs will throw a predominance of males but it has not been possible to hold them there. The best I have ever been able to do is to hold it considerably below that of the normal, but never as low as unity. On the other hand, it is relatively easy to select in the direction of females even to the extent of 1 to 2.

It should be observed that in the breeding of these strains the most rigorous inbreeding was practiced. It might, therefore, be that the difficulty of selecting for a low female ratio results from the possibility that inbreeding tends toward the elimination of the males. My extensive experience in inbreeding these creatures, however, does not bear out this explanation. Furthermore, in the sixth generation of the high female strain it was possible in two generations to reduce this ratio to near unity notwithstanding that the same rigorous inbreeding was continued.

4. Relative influence of male and female in determining the sex-ratio

Having thus produced two strains showing a decided difference in the sex-ratio of their offspring I wished to determine two further points. First, whether the maternal or the paternal elements had an equal share in the control of this ratio, and second, whether this ratio was determined in the process of fertilization. To this end reciprocal crosses were made between the two strains and the proportion of the sexes in the offspring ascertained. Three experiments were performed in the following manner. From among a brood of each of the two strains a large number of individuals were taken. Before sexual maturity a number of males and females were isolated, while the remainder were allowed to reproduce. The latter gave a control for each of the strains. The isolated virgin females of one strain were mated with the males of the other. Each experiment thus consisted of four multiple matings. (1) A number of brothers and sisters belonging to the male strain. This furnished a control for the male strain. (2) A number of brothers and sisters belonging to the female strain. This furnished a control

for the female strain. (3) Females from the male strain mated with males from the male strain, and (4) the reciprocal of '(3)'.

In crossing two strains as in the above experiment three possibilities might obtain. First, that the two sexes have an equal influence in determining the sex-ratio; second, that either sex have a predominant influence and third, that a ratio result unlike that obtaining in either of the parental strains. While the first is probably the expected result, the experiments show in a most decided way that the male has little or no influence in determining the sex-ratio in this species (tables 10, 11 and 12). In most of the cases the ratio of the offspring falls pretty closely around that of the strain from which the females were taken. In two instances the ratios exceeded 100 per cent influence. The remaining ones, with the exception of strain 244 in which the male influence amounted to 35 per cent show the female influence almost near enough to 100 per cent to justify one in regarding the differences merely as fluctuations incident to the small number of individuals involved. The unusually great influence of the male in strain 244 might be accounted for in two ways. First the number of individuals involved in this experiment are relatively small so that the ratios of both the control and the crossed broods are not as reliable as in the other experiments. Secondly, the flies used for this experiment were taken from the earlier generations of the two strains, before, we may believe, any considerable selection had been applied to fix the character of the respective strains. Indeed, this seems to be borne out in the other experiments.

The materials of the three experiments were not all taken from the same generation but were taken from different generations in the development of the strain. Thus, in experiment 1 the broods were taken from the first generation of strain 206 and 207. In experiment 2 the broods came from the second generation of strain 206 and the third of 207. The third experiment was made from the fourth and fifth generations of strains 206 and 207 respectively. Arranging these experiments in a series, based on the length of time that selection had been practiced on the broods used, we see that the male influence decreases as the selective time increases.

TABLE 10

Experiment 1

No. of strain mated.....	No. 242 212 ₂ × 212 ₂		No. 245 212 ₂ ♀ × 214 ₀ ♂		No. 243 214 ₀ × 214 ₀		No. 244 214 ₀ ♀ × 212 ₂ ♂	
	♂	♀	♂	♀	♂	♀	♂	♀
Number of individuals.....	208	194	463	475	171	273	225	311
Sex-ratio (actual).....	1.00	0.98	1.00	1.03	1.00	1.60	1.00	1.38
Theoretical ratio.....			1.00	1.288			1.00	1.288
Influence of male parents....	7.3 per cent				35 per cent			
Influence of female parents..	92.7 per cent				65 per cent			

TABLE 11

Experiment 2

No. of the strains mated..	No. 271 252 ₁₀ × 252 ₁₀		No. 274 252 ₁₀ ♀ × 255 ₈ ♂		No. 272 255 ₈ × 255 ₈		No. 273 255 ₈ ♀ × 252 ₁₀ ♂	
	♂	♀	♂	♀	♂	♀	♂	♀
Number of individuals.....	332	545	589	919	739	818	680	698
Sex-ratio (actual).....	1.00	1.69	1.00	1.56	1.00	1.106	1.00	1.026
Theoretical sex-ratio.....			1.00	1.365			1.00	1.365
Influence of male parents....	22 per cent				0 per cent (-13)			
Influence of female parents..	78 per cent				100 per cent (1.13)			

TABLE 12

Experiment 3

No. of strain mated.....	No. 279 275 ₈ × 275 ₈		No. 281 275 ₈ ♀ × 278 ₇ ♂		No. 280 278 ₇ × 278 ₇		No. 28 278 ₇ ♀ × 275 ₈ ♂	
	♂	♀	♂	♀	♂	♀	♂	♀
Number of individuals.....	289	427	382	551	1022	1044	752	825
Sex-ratio (actual).....	1.00	1.477	1.00	1.50	1.00	1.021	1.00	1.083
Theoretical ratio.....			1.00	1.249			1.00	1.249
Influence of male parents....	0 per cent				13 per cent			
Influence of female parents..	100 per cent				87 per cent			

TABLE 13

		PER CENT OF FEMALE INFLUENCE	PER CENT OF MALE INFLUENCE
Experiment 1-from broods selected for one generation.....	212 214	92.7	7.3
	214 212	65	35
Experiment 2-from broods selected for 2 and 3 generations.....	252 255	78	22
	255 252	100	0
Experiment 3-from broods selected for 4 and 5 generations.....	275 278	100	0
	278 275	87	13

This fact of the prevailing or exclusive influence of the female in determining the sex-ratio occurs in some other species of animals. Phylloxerans (Morgan '09) and *Dinophilus apatris* (Korschelt '82). On the other hand, Whitney ('09) seems to have shown that in rotifers certain eggs which will produce males if unfertilized are changed to females, if impregnated. In the case of *Drosophila*, we can not be certain that the sex-ratio is established before fertilization since the experiments do not with certainty entirely exclude the male influence.

5. Discussion of sex-ratio

It is not the intention to enter into an elaborate discussion of the problem of sex control. The literature is certainly already sufficiently burdened with such. The writer wishes merely to point out briefly a few conclusions about sex in this species which his results seem to warrant.

The property of sexuality possessed by this species expresses itself not in the equal production, numerically, of its two states, male and female, but in an unequal production. Studies in normal sex-ratios involving a sufficiently large number of individuals are not numerous. The unequal production of the two sexes in the human species is well established. Montgomery ('08) has given the data of a large number of individuals of *Theridium* and finds a marked inequality in the sexes. The general assumption seems to be that an equal sex-ratio is the rule. It is not improbable,

however, that, as careful determinations upon different species multiply, the condition of unequal ratios will be found increasingly common. Any theory of sex must take into consideration this normal inequality in the sex-ratios.

Sex-ratio like color, size etc., is a character belonging to a species. Sexuality of course is not, for it is common to all species reproducing by the sexual method. The particular form of sexuality, however, the proportion of the two sexual persons to which it gives expression in the process of differentiation, this is specific. For *Drosophila ampelophila*, the ratio of one male to 1.126 females is a specific character. This is not a ratio of merely the present generations but has been transmitted from generations remote. It is inherited. It is the expression of the physiological condition to which the species has been developed by its environmental demands.

Like other specific characters this ratio should be subject to modification, but this should not be more easily done or by other methods, in general, than those used in the modification of other characters. From this view point it should not be expected that the sex-ratio in an animal could be materially changed by such agents as food, temperature, etc. A change in the proportion of the sexes involves a much more fundamental modification than simple starvation or the reverse is likely to induce. In regard to other characters, we have long ago ceased to regard them as modifiable by such methods, but in the case of sex, it is only recently that their futility is being entertained. The most potent factor and the one most generally used to modify a character is selection. If the experiments herein recorded prove what they are held to prove, this process of selection is a potent factor in the modification of the sex-ratio also. It would be interesting to try to line this fact up with the chromosomal conception of sex. However, the writer regards this as the task of those who are engaged in these interesting and important investigations.

SUMMARY

1. *Drosophila ampelophila* may be inbred (brothers and sisters) for seventy-five or more generations.

2. Inbreeding in itself is not deleterious to the fertility or vigor of this species.

3. Infertility normally occurs to a varying degree among the offspring of any pair. Promiscuous inbreeding among such offspring may perpetuate and even intensify this character. When sterility appeared in the strain experimented with, it was always complete, appeared suddenly and was confined to the male.

4. By the judicious selection of the brothers and sisters to be mated from a brood that shows a high degree of infertility, this infertility can be eliminated by selection although continuing the inbreeding in the closest possible way.

5. There is a wide divergence in the fertility and productiveness among the different pairs taken in nature, but by the proper selection and closest inbreeding these may be readily brought to either a high or low state with respect to these characters.

6. Many generations of closest inbreeding does not necessarily cause any loss in size, perfection of form, rate of reaction to light and gravity, egg production or length of life and sex-ratio.

7. The normal sex-ratio of this species in nature when reared under diverse conditions of food is one male to 1.126 females.

8. Different pairs in nature show a wide divergence in the sex-ratio of their offspring.

9. When the offspring from a pair with a given ratio are mated in pairs their offspring will show a wide range in the sex-ratio but in the aggregate will tend to reproduce the ratio of the brood to which they belong.

10. Sex-ratio is therefore a character that is strongly transmissible. By the proper selection of pairs tending to throw a high female ratio on the one hand or a low female ratio on the other it is possible to develop strains characterized by high or low female ratios.

11. In this species it is comparatively easy to develop a strain with a female ratio considerably higher than the normal but very

difficult to develop a strain with a female ratio much lower than the normal or even one in which the sexes are equal in number.

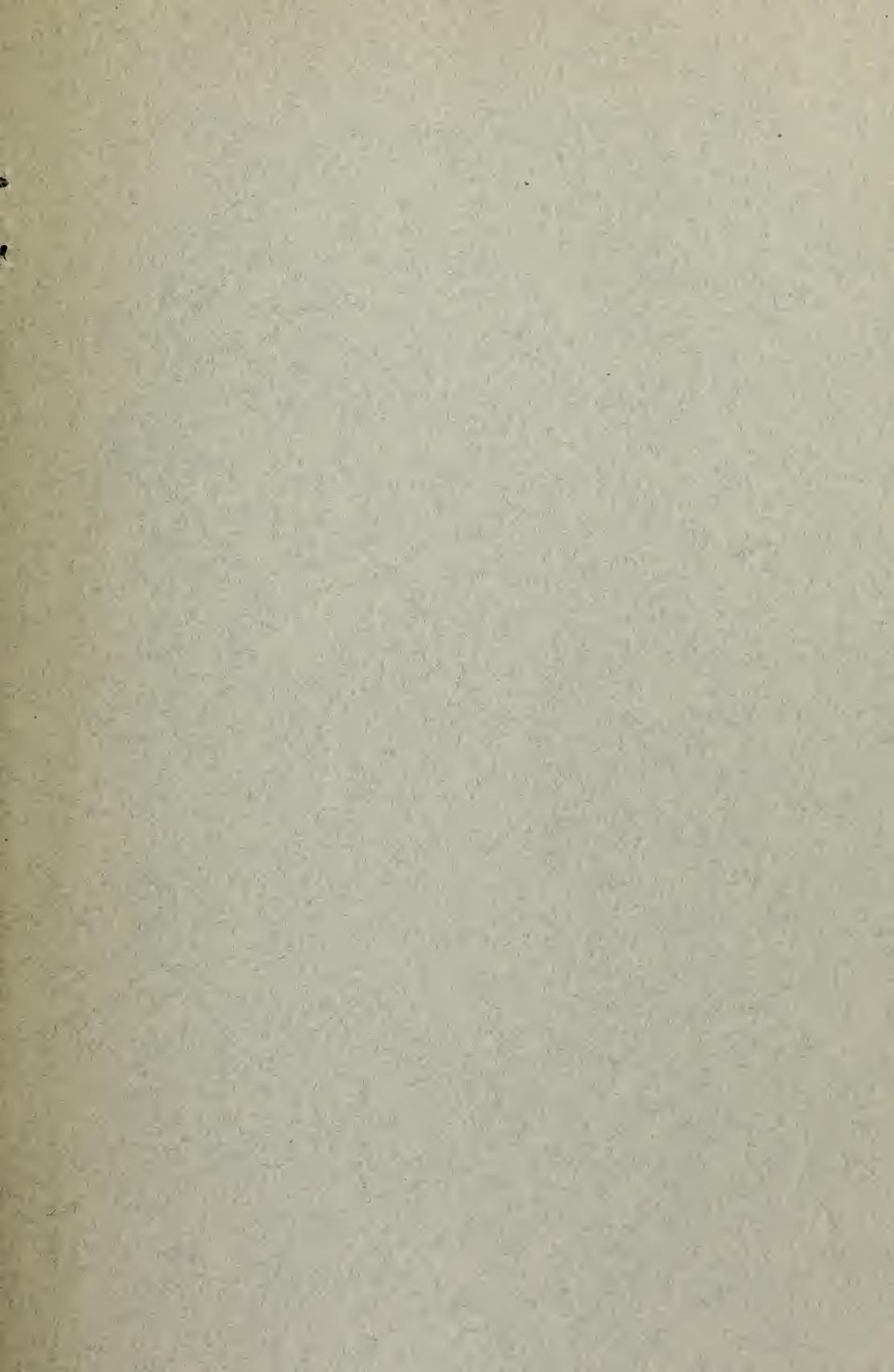
12. Sex-ratio is one of the qualities that is, like color, an inherent characteristic of this creature, strongly transmissible and amenable to the process of selection.

13. The female is almost wholly responsible in the transmission of the sex-ratio. For, if females from a strain possessing a high female ratio be mated with males from a strain possessing a low female ratio or vice versa, the offspring will show a sex-ratio which is wholly or very near that of the strains from which the females were taken.

14. Sex is probably very little, if at all, influenced at fertilization in this species, but is probably determined much earlier and by the female, but there seems no reason why this may not be influenced by various factors and in some species at fertilization.

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