JOURNAL OF GENETICS

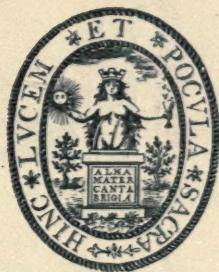# JOURNAL OF GENETICS

EDITED BY

W. BATESON, M.A., F.R.S.

DIRECTOR OF THE JOHN INNES HORTICULTURAL INSTITUTION

AND

R. C. PUNNETT, M.A., F.R.S.

ARTHUR BALFOUR PROFESSOR OF GENETICS IN THE UNIVERSITY OF CAMBRIDGE

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THE HISTORY OF *PRIMULA OBCONICA*, HANCE, UNDER CULTIVATION, WITH SOME REMARKS ON THE HISTORY OF *PRIMULA SINENSIS*, SAB.

By ARTHUR W. HILL,

Assistant Director, Royal Botanic Gardens, Kew.

Primula obconica, Hance[1], was introduced to England from China by Maries, one of Messrs Veitch's collectors who in 1879 sent home seeds from the Ichang gorges, where the great river Yangtse rushes out of the mountains. The plants raised from these seeds flowered in September, 1880[2].

In the *Botanical Magazine*, t. 6582 (Sept. 1881), the plant is figured and described under the name *P. poculiformis*, Hook, f. and in this figure the petals are shown with a simple notch or indentation similar to that of the common Primrose, *P. acaulis*. The wild specimens collected in China and preserved in the Kew Herbarium all show this character of the simply toothed perianth segments though they exhibit a considerable range of foliar variability. In the description the plant is said to have the habit and foliage of *P. cortusoides* (see t. 399 and t. 5528) and the calyx of the Himalayan species *P. filipes*—a native of rocks at Chuka in Bhotan at an elevation of 6500 feet.

P. obconica as introduced appears to have been a well-defined plant showing on the whole but little variation and, except for slight divergences in the colour of the flowers and character of the leaf margin, to have remained fairly true to type for about the first ten years after its introduction.

The finding of the wild plant in China by Maries is best described in his own words:—"When I was travelling in Central China, I was

[1] *Journ. Bot.* 1880, p. 234.
[2] *Hortus Veitchii*, pp. 82 and 292.

much puzzled how to bring out living plants 1100 miles to the coast at Shanghai. I, of course, took plants of the things I thought were best for garden purposes, but Ferns and herbaceous plants were altogether out of the question. I thought, however, that many seeds would germinate if they were kept in soil, so I collected surface soil from Ferns and Primulas, and other plants. This was kept in an old wine box and eventually taken to Hong-Kong. I took this home twelve months afterwards and the soil was 'sown' in a glass house. The first thing that came up was *Primula obconica* in large quantities, several shrubs, and a lot of ferns[1]."

Sir Joseph Hooker, writing in the *Botanical Magazine*[2], states that the plant is probably very variable and that the earliest flowering specimens sent by Mr Veitch were less hairy and had rounder and nearly entire leaves and very much smaller flowers than that figured in the plate.

The flowers are of a pale lilac colour with a yellow eye, and the perianth segments, which are rather narrow, show a deep apical notch. Messrs Veitch[3] speak of the colour in the virgin species as an "undecided lilac," while according to another writer[4] the flowers are said to be pinkish-white and it is suggested that white forms might be raised by careful selection. From a notice in *The Garden*[5], we learn that "the flowers though somewhat small are of a pleasing mauve tint. The (almost entire) leaves are large and broad and they form a distinct tuft which lies almost flat on the soil." In the *Botanical Magazine* on the other hand the leaves are represented as upstanding with lobulate-dentate edges[6].

With these preliminary remarks as to the incidence of variation, the detailed history of our modern forms may now be examined.

In attempting to trace the history of *Primula obconica* under cultivation it will be convenient to arrange the facts under different headings such as colour of the flowers, size, shape, fimbriation of the corolla lobes and doubling and lastly questions connected with hybridisation.

[1] *The Garden*, November 22, 1890, p. 479.
[2] *Bot. Mag.* 1881, t. 6582.
[3] *Hort. Veitchii*, p. 282.
[4] *Gardeners' Chronicle*, October 28, 1882, p. 565.
[5] *The Garden*, June 25, 1881, p. 655.
[6] See also *The Garden* for 1884, September 6, p. 236, the perianth segments are drawn with an apical notch.

Flower Colour.

For the first few years after its introduction, as has been shown, the flower colour is always recorded as pale or undecided lilac (Plate I, figs. 1, 2) or pinkish-white but references to the variable character of the plant are frequent. In 1886 there is a record of plants from the Royal Botanic Garden, Edinburgh, with blooms "ranging from mauve to lilac and frequently pure white[1]." "N. G." in the following year refers to the variability in flower colour and speaks of a few nearly white forms[2]. In the same note, it is mentioned that "we have observed in two or three of the plants this year a much deeper shade of rose round the eye than previously." This appears to be the earliest record of the appearance of the dark eye which was only found in a few cases among a batch of ordinary forms. The next record of the dark eye is in 1889 when "R. D." (Richard Dean) writes "The variety I have is of a very delicate mauve colour, with a slight purple ring round the eye[3]." In 1893 mention is again made of a distinct circle of a dark colour surrounding a lemon eye and the lemon eye itself is recorded as a novelty[4]. A "dark carmine shaded eye" is also mentioned in a note in 1895[5] and a "distinct eye" in the following year[6].

The "dark eye" is now a very common feature in many of the present day forms and the depth of the colour of the eye tends to increase as the flowers remain open (cf. Plate I, figs. 8 and 17, Plate II, figs. 27, 30, 32, 34).

White variety. The white variety to which references have already been given does not receive further mention in horticultural journals until the year 1896. In that year Messrs Vilmorin Andrieux et Cie[7] of Paris exhibited "*P. obconica* à grande fleur blanche" at the meeting of the French Horticultural Society, on Feb. 27th. This form had fimbriated petals. A pure white form was shown again, together with coloured varieties before the same Society by Messrs Vilmorin on May 2nd 1899[8], and was catalogued by Messrs Vilmorin in that year under the name "*P. o.* grande fleur blanche pur."

[1] T. W. Sanders in *Journ. Hort.* May 6, 1886, pp. 358, 359, with fig. 66.
[2] "N. G." in *Journ. Hort.* May 26, 1887.
[3] "R. D." in *Gard. Chron.* November 2, 1889, p. 504.
[4] "A. D." in *The Garden*, October 7, 1893, p. 327.
[5] J. C. Tallack in *The Garden*, April 6, 1895, p. 240.
[6] *The Garden*, December 12, 1896, p. 481.
[7] *Rev. Hort.* 1896, p. 238.
[8] *Rev. Hort.* 1899, p. 169, "A côté des coloris rose et blanc pur...".

I am informed by Mr A. W. Sutton that the pure white variety first appeared with his firm in 1899. The next reference to the white-flowered plant is in 1904, when a plant figured in the *Gardeners' Chronicle*[1] is described as having "white flowers or as nearly white as possible." Mr Gumbleton also in this year sent "almost white" flowers to the editor of *The Garden*[2] raised from seed obtained from Messrs Haage and Schmidt. Further a group of *P. obconica alba* was shown by Messrs Veitch at the Temple Show that same year, and it was remarked that ..."never before had been seen so near an approach to pure white[3]."

P. obconica has been largely grown by Her Grace Adeline, Duchess of Bedford, and has been the subject of numerous experiments by the head gardener, Mr Dickson. I am indebted to Her Grace for affording me every facility for obtaining information about the cultivation of the plant at Chenies. The first white *P. obconica* arose there in 1903, and its origin would appear to be independent from the white forms previously mentioned since it is alleged that no admixture from outside sources has taken place. In present day collections the white-flowering varieties can usually be easily distinguished by the darker tint of green in their leaves, and by their more delicate and paler stems. Messrs Veitch have a fine strain of this plant, very similar to those raised by Mr Dickson and others. The variety includes both toothed and fimbriated flowers and is found to come true from seed (Plate I, figs. 15, 16, 19, 20).

Rose variety. A very distinct break in colour and one of the earliest to arise was started with the development of the rose colour from the original pale lilac and this yielded the remarkable rose-carmine series which includes some of the most striking of our modern varieties.

Mr Sutton informs me that the first break from the original lilac was a good rose-pink, seed of which was offered by their house as *P. obconica rosea*[4] in 1895, and a variety under this name was exhibited at a meeting of the Royal Horticultural Society early in 1896 and was said to be an "undoubtedly most decided break in point of colour." Other references are to be found in *The Garden* about this time[5] (cf. Plate I, figs. 3—5). In 1897 Mr T. S. Ware[6] also showed a variety

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[1] *Gard. Chron.* 1904, p. 244, Fig. 103, p. 245.
[2] *The Garden*, 1904, January 9, p. 18, see also *l.c.* April 7, p. 304.
[3] "T." in *The Garden*, July 2, 1904, p. 3.
[4] *The Garden*, February 20, 1897, p. 143 "E. J."
[5] *The Garden*, November 7, 1896, p. 383, see also August 7, 1897, p. 110.
[6] *The Garden*, March 13, 1897, p. 197, March 20, 1897, p. 216.

with flowers of a "warm rose tint" and the managing director of Ware's Nurseries has sent me the following account of the origin and development of their rose-coloured forms:

"The first noticeable tendency (with us) of *Primula obconica* to produce other than the pale lilac flowers occurred about twenty years ago, when amongst a batch of many hundreds of seedlings (from seed obtained from a Continental source), five or six plants showed a deeper coloration beyond anything we had ever previously noticed. These plants were isolated, cross fertilized, and the seed saved separately from each plant. The resulting seedlings produced a fair amount of rose colours of varying shades. The best of these as regards depth of colouring, size of bloom, and good habit were retained, the remainder being destroyed. These were again cross fertilized and the selection carried on as before. Year after year seedlings were raised by this means (the colour becoming more intense each generation) until at last we reached the climax, that is, a deep self-rose of good habit and large flowers. In each successive batch of seedlings we always found one or two plants with extra deep and a greater number of serrations and of good colour. These we made the seed-bearing parent, as after repeated trials we found the serration was more pronounced in the offspring than when these plants were used as pollen bearers only, and so together with the development of the desired rose colour, the fimbriation or excess of serrations was proceeding at the same time in each successive generation" (cf. Plate I, fig. 3).

Messrs Vilmorin of Paris had also at this time produced a rose form of *P. obconica*[1], but it is not possible to determine whether the rose break originated in one place only or in several nurseries at about the same time, though the latter suggestion seems the more probable[2].

A form apparently very similar to these early rose or pink varieties is still to be seen planted out in the beds of the Temperate House at Kew where it has been grown undisturbed for many years (Plate I, figs. 3—5).

A rose variety[3] formed the starting point of the experiments carried out in the gardens of the Duchess of Bedford.

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[1] *Rev. Hort.* 1897, p. 141, announced in their catalogue for 1898 as "*P. obconica* à grande fleur rose vif."

[2] See also *l. c.* 1899, p. 548, with coloured plate. The variety "Rose Chamoise" was catalogued by Messrs Vilmorin in 1900.

[3] *P. obconica rosea*, seed purchased from D. W. Thomson, Edinburgh, who obtained seeds from Stewart and Co., Covent Garden. The origin of the seed was very possibly continental.

A further improvement in the production of a red *obconica* "Vesuve," was due to Messrs Rivoire, père et fils[1], and the variety "Vesuve" produced in 1903 was said to surpass in colour all other reds and carmines. Messrs Rivoire, like Messrs Vilmorin, attribute all their improvements simply to selection.

In the next year Messrs Barr and Sons exhibited their "Crimson King[2]," described as a "rich deep lilac-crimson, a decided advance in coloured obconicas," followed in 1905 by their "Crimson Queen[3]," a "deep crimson-rose." In the note on this plant it is remarked that the first pink and rose forms were thought to have been sent from a large Fifeshire garden ten years before. Rover[4] had also an assortment of reds from "Zartesten blassen Rosa bis zum dünkelsten Karmin." Messrs Sutton and Sons chronicle the production of a crimson form in 1906, and on Nov. 23, 1909 they exhibited a remarkable variety[5], "Sutton's Fire King," having terra-cotta crimson flowers with a yellow throat surrounded by a darker ring. The origin of this form is also attributed entirely to selection. Herr Georg Arends of Ronsdorf, who has kindly supplied information about his experiments with *P. obconica*, obtained varieties which he has named "rosea" and "kermesiana" (Plate II, fig. 35). In a letter dated Nov. 24, 1909, he writes "The last quite new colour I gained was the var. 'Feuer Königin' (Fire queen), that came out of the Kermesiana in four or five years' work. There is a kind of salmon-orange in the crimson of this variety and I think it will be possible to have a pure salmon-pink shade from it in a few years." So much interchange has gone on in recent years between nurserymen that it is highly probable that many of the varieties recorded by different houses have a common origin and are really the same plant. A dark claret form to which the name "Chenies excelsior[6]" has been given has the darkest coloured flower of the red series so far seen (Plate II, figs. 37, 38).

Violet-blue varieties. One of the most recent and striking colour shades which have been evolved in *P. obconica* under cultivation is a decided violet-blue shade which is now a well-marked and good colour. The improvement in this direction in England appears to be due very largely to the efforts of Mr Dickson. I am informed that a small

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[1] *Rev. Hort.* 1903, p. 442, see also *Rev. Hort.* 1906, p. 487.

[2] *Garden*, 1904, March 24, p. 261.

[3] *Garden*, 1905, February 9, p. 116.

[4] *Gartenflora*, 1905, 54, p. 82 ; see also *idem* 1903, p. 204.

[5] *Garden*, 1910, April 9, p. 179, with plate.

[6] *Gard. Chron.* 1911, April 29, p. 268.

flowered blue variety was raised a Chenies in the autumn of 1904, and this was followed by the production of a large-flowered blue form in 1906 (Plate I, figs. 10—12). A variety named *coerulea*, remarkable for the blue colour of the flowers, was shown by M. Ferard[1] at the French Horticultural Society, October 1907, and the production of a similar variety in France is of interest as it seems almost certain that the blue colour has been developed quite independently in two different places. Herr Arends has also produced a blue form and he says in his letter " out of the *white* I raised the blue, beginning with plants which only showed a slight bluish hue in the bud. It took about ten years to bring this colour out clearly." Unfortunately, Herr Arends has not supplied exact dates of the origin of his varieties, but it seems clear that his blue has had no connection with the similar English variety and may possibly have been independent of Ferard's var. *coerulea*. Magenta and deep purple forms (Plate II, figs. 22—26) have also been produced and should perhaps be more properly regarded as belonging to the red than to the blue series.

Size of flowers.

The increase in the size of the flowers was one of the first of the changes noticed in *P. obconica* after it had been under cultivation for a few years. In 1887[2] a few plants were reported to have produced flowers nearly double the size of the original plants. In France the tendency to variation in this plant does not appear to have received much notice until 1892[3]. In this year M. Lille of Lyons brought out a variety *grandiflora*, and in the following year a variety with flowers larger than the type was produced and fixed by Messrs Vilmorin and sent out by them in the year 1894 with the designation " à grande fleur améliorée."

In England in this same year plants grown at Gunnersbury Park are reported to have had flowers which approached very closely in size to those of the ordinary Chinese Primula[4]. In 1895, however, Mr Tallack, writing in *The Garden*[5], expressed the view that there had been little or no advance on the best flowers of former years, and that

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[1] *Rev. Hort.* 1907, p. 531.

[2] *Journ. Hort.* 1887, May 26, p. 417 (N. G.). See also *The Garden*, 1886, March 13, p. 241, and *Gard. Chron.* 1890, February 8, p. 175 (D.).

[3] *Rev. Hort.* 1897, p. 374, 1899, p. 548, *ibid.* 1893, p. 123.

[4] *The Garden*, 1893, March 25, p. 242; see also *ibid.* p. 327.

[5] *The Garden*, 1895, April 6, p. 240.

it was not likely that further advance would take place owing to the difficulty in obtaining seed from the better varieties.

Herr Arends who commenced experimenting with *P. obconica* in 1888, says that by careful selection and inter-crossing of the best strains he first succeeded in obtaining his var. *grandiflora* with larger pale-lilac flowers and that this was followed by various colour breaks in later years.

At the present day the increase in size of the flowers in comparison with those of the plants originally introduced is very marked. The largest flowers have been noticed principally in pink and lilac-purple shades and have measured as much as 4—5 cm. in diameter[1] (Plate I, figs. 13, 15; Plate II, fig. 30).

Fimbriation.

The commencement of the fimbriation of the corolla segments in *P. obconica* is a matter of some interest since this form of variation appears to occur, sooner or later, in most species of Primula under cultivation. Such variation of course is particularly noticeable in the cultivated Chinese Primula, and as far as can be ascertained from the earliest records this tendency to fimbriation had already been initiated when the plant was under cultivation in China[2]. In wild species of the genus, except in a few cases, the corolla segments show a simple notch at the apex[3] but under cultivation fimbriation has developed in *P. Forbesii*, *P. verticillata*, *P. kewensis*, *P. Sieboldii*, *P. japonica* and also in the varieties of the common Primrose *P. vulgaris*. In the allied genus *Cyclamen*, fimbriation of the petals has also been developed as a result of cultivation[4].

Another point of interest in connection with the fimbriation of the corolla is that this variation has, without doubt, been developed quite independently in different places, and at different times. The earliest case of which a record has been traced occurred in the garden of Mr J.

[1] See also *Gartenflora*, 1904, 53, p. 139, *ibid.* 1905, 54, p. 82, and *Rev. Hort.* 1906, p. 487.

[2] The *Botanical Register*, 1821, tom. 539.

[3] *Primula Winteri* (*Gard. Chron.* 1911, March 4, p. 130, *Gard. Mag.* 1911, March 4, p. 163, with figs.), is of interest in this connection as the corolla segments are strikingly fringed. *P. Stuartii*, *P. petioloris* and some other species also have fringed corolla segments in the wild condition.

[4] See Thiselton-Dyer, " The cultural Evolution of *Cyclamen latifolium*," in *Proc. Roy. Soc.* LXI. (1897), p. 143.

Crook of Forde Abbey[1], blooms being said to show "distinct evidence of fringing of the edges." It should be added that these plants were supposed to be the result of crosses with *P. sinensis* but it will be seen later that the evidence in support of this view is very slight.

The next record comes from France, about two and a half years later, a plant with fimbriated white flowers being exhibited by Messrs Vilmorin in February 1896[2], before the French Horticultural Society. M. Mottet writes in reply to queries addressed to M. Ph. L. de Vilmorin about this var. *fimbriata*: "The variety first appeared here in 1896 in a large-flowering white strain, in the form of slight denticulations on the edge of the petals. This was selected carefully and the variety announced in our catalogue in the spring of 1897," he adds that its origin had nothing to do with any seed or plants outside their own stock. In the early autumn of 1897 a var. *fimbriata* was being grown at Kew[3] from seed obtained from Messrs Heinemann of Erfurt (cf. Plate I, fig. 3).

The fimbriated variety was also produced independently at Kew and the fringed character was attributed to the results of high cultivation. As the variety was not considered an improvement its cultivation and selection at Kew were discontinued. In 1899 Messrs Vilmorin[4] were exhibiting this variety with rose-coloured flowers, and from thence onwards a var. *fimbriata* was offered in most seedsmen's catalogues (cf. Plate I, figs. 15, 17; Plate II, fig. 30). Mr A. W. Sutton writes that the fimbriated form appeared at Reading in 1901 and he adds " we do not, however, catalogue this, as the fimbriation has the effect of making the flowers look smaller."

Double Flowers.

One other striking floral variation in *Primula obconica* has yet to be described, namely the occurrence of double flowers (Plate I, fig. 21). This appears to have originated with Messrs Vilmorin and as far as can be ascertained nowhere else. M. Mottet writing in *Le Jardin*[5] early in 1901 mentions that the double variety was put in commerce in that

[1] *The Garden*, 1893, Oct. 7, p. 327.

[2] *Rev. Hort.* 1896, p. 238 ; *ibid.* 1897, p. 141.

[3] *The Garden*, 1897, September 18, p. 227 ; *Gartenflora*, 1897, 46, p. 143, text fig. 23.

[4] *Rev. Hort.* 1899, p. 169 ; see also *The Garden*, 1904, April 7, p. 304; *Gard. Chron.* 1904, April 16, p. 244.

[5] *Le Jardin*, 1901, p. 89, fig. 52, see also *Rev. Hort.* 1901, p. 238, figs. 100, 101 ; *Journ. Hort.* 1901, p. 14. In 1902 Sir Trevor Lawrence received an award of merit, R.H.S., for a' mauve-purple coloured semi-double var. of *P. obconica*, see *Journ. Hort.* 1902, p. 548.

year, and in a recent letter he informs me that the double variety may have appeared some years before it was sent out, Messrs Vilmorin do not remember exactly, but they state that at first the flowers were rather small and only half double but that since then the variety has been greatly improved in size and duplication of the flowers and that it now reproduces as well as any other variety. The colour is stated to have been pale rose like the type. Herr Arends informs me that he obtained seeds of the double form from Messrs Vilmorin and attempted to improve the variety by crossing with his own plants. In the course of several years he has obtained " larger flowers, stronger growth and rose-coloured flowers as well as the old lilac ones." Reference to modern catalogues shows that there are now several double varieties offered by nurserymen.

Doubling of the flowers in the genus *Primula* as is probably well known, may take place in one of two ways. In the case of *P. sinensis* the modern varieties such as Crimson King,. etc., double the flowers by corona-like outgrowths from the corolla lobes at the back of each anther and the coloured surface of the outgrowth faces that of its corolla lobe or in other words the added lobes show the colours reversed.

In the old double white variety of *P. sinensis*, known as *P. sinensis* var. *flore pleno*, which appears to have been produced about the year 1839[1] and which can only be propagated by cuttings, the doubling is of a "hose-in-hose" character, the colours of the added lobes not being reversed but corresponding in arrangement to those of the original corolla. In *P. obconica* the doubling is also "hose-in-hose" and there is no reversal of arrangement and colour as in the modern *P. sinensis* doubles. *P. obconica*, however, sometimes shows a tendency to doubling in yet another manner owing to the splitting of upgrowths of the corolla between adjoining corolla lobes, these ridges split towards the centre and so tend to produce a kind of corona with the colours reversed, but with this difference that the "corona lobes" alternate with the corolla lobes while in *P. sinensis* they are opposite to them.

This method can at present be hardly considered as more than a tendency towards doubling, for it may only be shown by some of the flowers in an inflorescence; it is however of considerable interest and it seems possible that by careful selection a new type of double *P. obconica* might be developed (Plate II, figs. 27, 29).

[1] See *Paxton's Magazine of Botany*, vi. 1839, p. 262. This sport appears to have arisen at Henderson's Nursery, Pine Apple Place, London.

Variation or Hybridisation.

That *Primula obconica* as cultivated to-day is a totally different plant from the wild species introduced from China in 1879–80 is a perfectly obvious fact, but as to the cause or causes which may underlie the changes which have taken place there is a considerable difference of opinion. Some of those who have worked for many years on this plant and to whom several of our modern improvements are due contend that the amelioration of this species is due in the main to hybridisation with other species of the genus, while others who have been equally successful in raising new varieties protest against this view and claim that the improvements are entirely due to selection and cross-fertilization of the best forms within the species. It is unfortunate that experiments with this plant have been undertaken from the horticultural rather than from the purely scientific point of view and that the results which tend to be matters of conjecture and assumption have been accepted in many cases as proved fact. For there has been a tendency in some quarters to assume that because a given variation appeared to fit in with a preconceived idea or with expectation, therefore such a variation was due to a definite experiment or series of experiments.

In order to arrive at a conclusion as to the right explanation of the course of events displayed by the history of *P. obconica* it will be necessary to examine the evidence somewhat in detail.

In the foregoing pages it has been shown that for some ten to fifteen years after its introduction *P. obconica* displayed but little tendency to vary. A slight increase in the size and slight changes in the colour of the flowers were recorded such as would be expected under artificial cultivation, but the bulk of the seedlings tended to come more or less true to the original form. According to Messrs Vilmorin[1] the first variations in *P. obconica* were noticed in 1892. In England variation both in size and colour is recorded on several occasions between 1886 and 1893[2], but compared with present-day forms the improvement of the plant does not appear to have been very striking until about fifteen years after its introduction. Messrs Ware name about 1890 as their starting point, while Messrs Veitch consider the year 1898, in which their Feltham nurseries were opened, as the date from

[1] S. Mottet in *Le Jardin*, 1901, p. 8.
[2] See especially *The Garden*, 1888, p. 550, 1890, p. 354, and 1893, p. 242; *Journ. Hort.* 1887, p. 417; *Gard. Chron.* 1890, p. 175.

which their improvements should be reckoned. Even as late as 1897 "R. D." (R. Dean) writes in the *Gardeners' Chronicle* (1897, p. 65) that "so far comparatively little variation has appeared among seedlings. The blossoms of some are larger, rounder and stouter than others and there is a tendency to deepen the tints of some individuals to something approaching mauve."

As with all new introductions gardeners very soon attempted to "improve" *P. obconica* by crossing with other species of the genus, and *P. sinensis* appears to have been tried in most cases as the pollen parent. As early as 1887 it was suggested[1] that the variations noticed might be due to attempts to cross *P. obconica* with Alpine auriculas and Primroses though it was considered as unlikely. That *P. sinensis* pollen was answerable for the improvement in the flowers is put forward in the case of the first occurrence of fimbriation recorded in 1893[2], and several references to the action of *P. sinensis* are to be found from that date onwards. In 1896[3] Dr Masters exhibited a "hybrid" at the scientific committee of the Royal Horticultural Society supposed to be the result of crossing *P. obconica* with the wild form of *P. sinensis*, but there was apparently very little to distinguish it from the female parent, and in the spring of 1898[4] Mr Shea exhibited the result of a similar cross with (?) cultivated *P. sinensis* before that committee in which the influence of the Chinese Primula appears to have been accepted, though the predominance of the female is recorded. The possibility of hybridising *P. obconica* with *P. sinensis* was accepted definitely in Germany and Herr Arends informs me that a fine batch of hybrids was raised at Fürsten Walde near Berlin in 1893 "with the growth and leaves of *obconica* and size and colour of *sinensis* flowers." He adds in a further letter that the plants had the "large brilliant flowers of *sinensis*. They represented in perfection that which we had tried to get for so many years." These plants all died without having been described or figured and it is not now possible to say whether they may or may not have been hybrids, but in the light of our present knowledge it would appear to be a matter of considerable doubt. Herr Arends states that he has made this cross again and again but without result and Messrs Sutton, Veitch and Vilmorin[5] all express the

[1] *Journ. Hort.* 1887, p. 417.   [2] *The Garden*, 1893, p. 327.

[3] *Gard. Chron.* 1896, pp. 600, 790.

[4] *Gard. Chron.* 1898, p. 119.

[5] See *Le Jardin*, 1901, also *The Garden*, 1897, pp. 193, 197, 213, 216, 227, 394; 1899, pp. 144, 366; 1910, LXXIV. p. 179: *Rev. Hort.* 1899, p. 548; 1906, p. 487.

opinion that no hybridisation has ever taken place between *P. obconica* and *P. sinensis*.

Mr Valentine, managing director of Messrs Ware's Nurseries, also says that all their improvements are regarded as being due to selection and cultivation.

M. Mottet points out that the failure to produce artificial hybrids in the genus *Primula* is all the more curious since many natural hybrids in the genus are known as for instance among Alpine species and with *P. officinalis*, *P. acaulis*, and *P. elatior*.

It has been suggested once or twice that *P. obconica* and *P. sinensis* may have hybridised naturally in the same way that *P. kewensis* arose in the first instance[1], but this view is opposed by M. Mottet[2], who even goes so far as to say that even *P. kewensis* cannot be considered to be a hybrid. This latter case has, however, been proved more than once by artificial crosses carefully made at Kew[3].

Besides *P. sinensis* various other species of *Primula* have been used in the attempt to produce hybrids such as Alpine auriculas and primroses[4]; *P. floribunda*, *P. verticillata*, *P. japonica*, *P. farinosa*, *P. cortusoides*, *P. sikkimensis*[5], etc., but with regard to all these it is stated that though seedlings were often obtained there was no evidence of hybridisation.

In an account of an attempt to cross *P. obconica* with a well-coloured form of *P. Sieboldii cortusoides* "J. H. W." writes that the latter plant was used as the seed parent and every care was taken to prevent self-fertilisation. Seed was duly formed but the seedlings were nothing but *P. Sieboldii cortusoides*[6].

Several interesting varieties have been exhibited in recent years by the Duchess of Bedford; Mr Dickson, the head gardener, started the experiments in 1901 with a fimbriated variety of *P. obconica* and pollen of Polyanthi, Primroses and *P. Sieboldii* in varieties was used, later the pollen of *P. cortusoides*, *P. sinensis* and *P. rosea*. Mr Dickson claims that his results are due to the use of *P. sinensis* pollen and that one plant shows distinct evidences of the effect of *P. rosea*. In April, 1911, Mr Dickson showed a plant at a meeting of the Royal Horticultural Society[7] under the name of "Chenies excelsior" (Plate II, figs. 37, 38)

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[1] See *Rev. Hort.* 1906, pp. 448, 449, fig. 176, where M. Grignan puts forward this suggestion to explain the origin of *P. obconica superba* raised by M. Nonin.

[2] *Rev. Hort.* 1906, pp. 498, 499. [3] See *Kew Bulletin*, 1910, p. 325.

[4] *Journ. Hort.* 1887, p. 417.

[5] *The Garden*, 1897, p. 193. [6] *Gard. Chron.* 1897, p. 128.

[7] *Gard. Chron.* April 29, 1911, p. 268.

and he informs me that the pollen of *P. japonica* was used to fertilise the deep red-flowered plant of *obconica* mentioned above which was thought to show evidence of the influence of *P. rosea*. In general habit however the plant shows no trace of *P. japonica* either in leaves or flowers. The flowers are of a dark claret-magenta colour not more intense but not unlike that which has been produced by other growers. The inflorescences tend to become whorled as in *P. japonica* but this occurs not uncommonly with robust plants of *P. obconica* towards the end of their flowering season. The results achieved in the short space of about eight years are certainly very remarkable, but except for colour changes in the flowers there appears to be no evidence to support the view that the plants should be considered as hybrids especially as similar series of forms are known to have been produced elsewhere by selective methods alone without any recent attempts at hybridisation.

Yet another species of *Primula*, namely *P. megasaefolia* from the Caucasus, is claimed to have been successfully hybridised with *P. obconica*. Herr Arends of Ronsdorf writes that he started working with *P. megasaefolia*, then recently introduced, about the year 1902 and produced in course of time the strain to which he gave the name *P. obconica giguntea*. Professor Pax to whom some of the plants were sent accepted them as hybrids between *P. obconica* and *P. megasaefolia* and in his account of the genus in the *Pflanzenreich*[1] has given the name *P. Arendsii*, to this supposed hybrid.

In habit this *gigantea* strain undoubtedly shows some differences from the ordinary *grandiflora* type in its stouter leaves and pedicels, but the flowers both as to calyx and corolla are those of *P. obconica* and the plants do not appear to show any character which can be definitely attributed to the influence of the pollen of *P. megasaefolia*.

Forms of *P. obconica* closely resembling the *gigantea* of Arends appear to have been raised in different nurseries and by other means about the same time. *P. obconica robusta* raised at Lyon, for instance, by M. Choulet, is stated to be the same thing as *P. obconica gigantea*, but to have been produced entirely as a result of selection, Messrs Rivoire of Lyon write as follows on this subject:

"Vous avez raison de mettre en doute l'origine supposée de cette Primevère et de vous refuser à croire à une hybridation; c'est d'ailleurs, là aussi, l'avis de l'obtenteur. Nous ne pouvons que vous confirmer

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[1] *Das Pflanzenreich*, IV. 237, *Primulaceae*, p. 346. There is also a note in *Gartenflora*, 1908, 57, p. 632 on *P. obconica gigantea rubra*, "the first true dark red hybrid of the new *gigantea* race."

dans votre opinion en vous disant que nous avons, depuis l'apparition du *Primula obconica*, tenté des hybridations avec un grand nombre de Primevères. Nous n'avons jamais réussi, et les variétés que nous avons mises au commerce, telles que Vésuve (à fleurs rouge carmin) et *robusta*, ont été obtenues uniquement par voie de sélection.

" Nous connaissons d'autres horticulteurs qui ont tenté de leur côté des hybridations, mais également sans succès. Aussi ne croyons-nous nullement à l'origine hybride signalée par l'horticulteur allemand qui annonce le *Primula obconica gigantea*.

"A ce propos, nous vous serions obligés de rappeler que le *Primula obconica robusta*, que nous avons annoncé l'an dernier et qui a été obtenu par M. Choulet, chef des cultures florales du Parc de la Tête-d'Or, présente absolument les mêmes caractères que ceux qui sont signalés pour la variété *gigantea*, c'est-à-dire feuilles de consistance ferme, fleurs de dimensions très grandes (les plus grandes connues 5 centimètres de diamètre) de couleur blanc lilacé, ombelles énormes et surtout *tiges rigides*, qui lui ont fait donner ce nom de *robusta*[1]."

Whatever may be the explanation of some of the forms of *P. obconica* which have been obtained, it is evident that numerous attempts have been made to effect hybridisation with other species and that a great deal of work has also been done on the lines of selection and cross fertilisation of the best varieties. In the cases of the assumed hybrids it is remarkable that the results, whatever species may have been the pollen parent, are all strikingly similar and only a better form of undoubted *P. obconica* has been obtained. Further the forms alleged to have been produced by hybridisation can hardly be distinguished from those produced by selection. In this connection also it is worthy of note that in the only case on record where the pollen of *P. obconica* was used the seedlings raised were purely of the type of the female parent[2] (*P. Sieboldii cortusoides*). The experiments in hybridisation appear to have been made with proper care in many cases and the conclusion seems to be suggested that the pollen may in some way stimulate the development of the ovule without effecting hybridisation. The case of the orchid *Zygopetalum Mackayi*[3] crossed with the pollen of other genera but always yielding seedlings closely resembling the female parent may perhaps be considered as a somewhat parallel case.

It is true that Arend's *gigantea* strain shows a stoutness in the leaves which is more marked than in the ordinary forms and there is

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[1] *Rev. Hort.* 1906, p. 487. [2] See p. 15, and *Gard. Chron.* 1897, p. 128.
[3] See *Journ. R. Hort. Soc.* xxi. 1897, pp. 476, 477; *Orchid Review*, vi. 1898, p. 19.

also the case of a peculiar claret-coloured, small-flowered form produced by Mr Dickson, which is unlike any other variety I have met with. This latter plant was the only one of its kind produced by a cross alleged to have been made by *P. obconica* and *P. rosea splendens*. The leaf of this plant also differs somewhat from the normal though it is quite like that of some of the wild specimens preserved in the Herbarium at Kew. The whorled character of the inflorescence, also, which has been developed in the variety "Chenies excelsior," cannot be accepted as an indication of hybridisation with *P. japonica* since it may occur in uncrossed plants.

The evidence for hybridisation in *P. obconica* cannot therefore be regarded as convincing. A careful series of experiments have been conducted at the John Innes Horticultural Institution at Merton in which the pollen of eight species of Primula has been tried. The results so far obtained tend to show that good seed has been produced only with the pollen of *P. obconica* itself all other crosses being failures, and this corresponds with the results of similar experiments made at Kew. It is just possible however in view of the conflicting evidence that further careful experiment might demonstrate some form of hybridisation for it may be, as Doncaster[1] suggests in dealing with the question of crossing between species, that the multiplicity of characters concerned makes analysis very difficult and thus the evidence of hybridisation may not be apparent.

It has been pointed out above that the difficulty of producing artificial hybrids in the genus *Primula* is somewhat remarkable in view of the fact that natural hybrids are not uncommon between certain species. Whether any natural hybrid between *P. obconica* and any other species exists is not certainly known but a specimen preserved in the Kew Herbarium collected by Wilson (no. 4052) in Western China suggests such a possibility. Mr J. F. Duthie who has kindly examined the plant is of the opinion that it may be a natural hybrid between *P. obconica* and *P. cortusoides*; and in this connection it is of considerable interest to find that a plant (or plants?) of *P. cortusoides* came up with the seed of *P. obconica* collected by Maries in Ichang, the specimen being preserved at Kew. Mr Duthie says of Wilson's plant—"It agrees with the former (*P. cortusoides*) in the shape, texture and pubescence of the leaves, but the calyx is that of *P. obconica.*"

In its native home *P. obconica* appears to show a considerable range

[1] L. Doncaster, *Heredity*, Cambridge University Press, 1910, Chap. VIII. p. 109.

of variation, though the seed sent to England would appear to have belonged to a fairly uniform type. The plants lately collected by Forrest and referred by him to *P. Listeri*[1], King, are now considered to be the variety *glabrescens* of Franchet. Many of the specimens, however, show marked differences from the typical *P. obconica* and are also no doubt quite distinct from *P. Listeri*, but it seems open to doubt whether all of Forrest's specimens from Yang pi in Western Yunnan are rightly included under *P. obconica* and whether some should not rather be considered as belonging to a distinct though only slightly differing species, intermediate perhaps between *P. obconica* and *P. Listeri*.

P. obconica and P. sinensis.

The history of *P. obconica* and of the changes in form and colour which it has undergone in the comparatively few years of cultivation suggests that it may afford a parallel to the case of the long-cultivated Chinese Primula whose origin is still a matter of dispute and controversy. *Primula sinensis* unlike *P. obconica* was not introduced to this country as a wild plant but as a species which it is believed had long been cultivated in Chinese gardens[2]. *P. sinensis*, Lindl., as described and figured in the *Botanical Register*, 1821, t. 539, as *P. praenitens*, Ker.-Gawl., and figured in the *Botanical Magazine*, 1825, t. 2564, is not a wild form but a domesticated plant. The first figure published depicts a flower with the corolla segments fimbriated and it is of interest to notice that the later illustration in the *Botanical Magazine* shows the corolla segments with the notched apex and the plant is in general characters very similar to *P. sinensis stellata* of to-day. The two pictures are of interest in connection with the history of *P. obconica*, *P. Forbesii*, *P. japonica*, *P. cortusoides*, *P. Sieboldii*, *P. kewensis*, etc. The amelioration of *P. sinensis* both as to flower colour and shape

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[1] See *Gard. Chron.* 1909, November 20, p. 544, with figure.

[2] The following account of *Primula praenitens* is given in the *Bot. Reg.* VII. 1821, t. 539.

"It had been brought by Captain Rawes from the gardens at Canton, where it had probably found its way from some far more northern quarter of the Chinese Empire. Samples in a dried state had been previously submitted by Mr Reeves, a gentleman in the employment of the East Indian Co. at Canton."

In the figure the corolla segments are fimbriated and the calyx has many lobes; the account continues—"The plant not having been known in its wild state, can we be sure that the multiplication of the segments of the calyx does not arise from luxuriance induced by exotic cultivation?...."

and the remarkable leaf development have proceeded steadily since its introduction so that now many of the cultivated races are very distinct from the plants introduced about 1821. What we may ask was the earlier history of the plant under the hands of the Chinese? Is it too great a step to take to consider that the plant found by Henry in the limestone gorges of Ichang is really the original wild type of this species? I for one, in the light of the history of *P. obconica,* am inclined to think that it is not too great, and that we have in this little plant with its lilac flowers the true wild type of the species. Some corroboration seems to me to be given to this view by the variety *flore pleno* of *P. sinensis.* This plant somewhat closely resembles Henry's wild type in foliage and may be considered as offering a parallel to the old-fashioned double white and double lilac primroses which in the dim past must have been derived from *P. acaulis.*

P. sinensis also offers another interesting parallel to *P. obconica* in respect of the old double white variety since it appears that this arose as a sport about the year 1839 after *P. sinensis* had been in cultivation about eighteen years. *P. obconica* has also yielded a similarly constituted double variety as a result of cultivation about twenty years after its introduction.

Conclusion.

The conclusion to which one is led from the investigation of the history of *P. obconica* under cultivation would therefore appear to be that the amelioration and development in form and colour of the flowers, etc. which have taken place during the past thirty years must be attributed to selective processes. The evidence which has been adduced in support of theories of hybridisation with other species is not sufficiently confirmed by facts to justify its acceptance.

In view, however, of certain doubtful points and of some interesting questions as to the influence of foreign pollen in effecting fertilisation it would seem desirable to suspend full judgment until the results of further careful experiments in the fertilisation of *P. obconica* with foreign pollen have been obtained.

CHRONOLOGY OF *P. OBCONICA*.

DESCRIPTION OF PLATES.

PLATE I.

1. Pale lilac-flowered form with toothed corolla segments very near the type originally introduced from China. (3)
2. The same to show the calyx. (3)
3. Fimbriated pink variety similar to the first fimbriated forms produced. (1)
4. Side view of toothed pink variety. (1)
5. The same face view. (1)

Figs. 1—5 from plants grown undisturbed for many years in the Temperate House, Royal Botanic Gardens, Kew.

Figs. 6—12 illustrate the development of the blue strains of *P. obconica*.

6. Lilac-blue variety raised by selection, Messrs Veitch and Son, Feltham. (37)
7. Calyx of the same. (37)
8. Lilac-blue variety with red eye. (27)
9. Calyx of the same. (27)
10. Blue variety raised by Mr Dickson, Head Gardener to Her Grace Adeline, Duchess of Bedford. (38)
11. Calyx of the same. (38)
12. Violet-blue variety with darker stellate eye, raised by Mr Dickson. (28)

13. Large soft pink flowered variety, raised by Messrs Veitch. (35)
14. Calyx of the same. (35)
15. Large pure white fimbriated variety, raised by Messrs Veitch. (33)
16. Calyx of this variety showing tendency to fimbriation. (33)
17. Rose-pink variety with crimson eye, fimbriated, Messrs Veitch. (29)
18. Calyx of this variety. (29)
19. Toothed white variety with conspicuous yellow eye, Herr Arends. (20)
20. Side view of the same flower showing the conspicuously lobed calyx. (20)
21. Double pink variety, hose-in-hose type, raised by Messrs Vilmorin, Paris, drawn from a plant sent by Herr Arends. (24)

PLATE II.

22. Purple variety with conspicuous yellow eye, Herr Arends. (21)
23. Side view of the same flower. (21)
24. Calyx showing its hemispherical shape with unconspicuous teeth. (21)
25. Large-flowered deep purple variety with reddish eye, Messrs Veitch. (30)
26. Fimbriated calyx of the same. (30)
27. Deep rose-pink flowered variety with red eye, showing splitting of the corolla at the sutures between the corolla segments represented by colourless lines, cf. fig. 29, Herr Arends. (10).
28. Calyx of this variety with acuminate segments. (10)
29. Flower enlarged to show the splitting of the raised sutures between the corolla segments producing the commencement of a form of "reversed" doubling, cf. fig. 27. (9)
30. Very large pale pink fimbriated variety, raised by Messrs Veitch. (26)
31. Calyx of the same. (26)
32. Violet-pink variety with large crimson eye, Messrs Veitch. (28)
33. Calyx of the same. (28)
34. Deep rose-flowered variety with crimson eye, Herr Arends. (7)
35. Crimson variety with yellow eye, Herr Arends. (22).
36. Calyx of the same. (22)
37. "Chenies excelsior" (see *Gard. Chron.* April 29, 1911, p. 268), raised by Mr Dickson. (39)
38. Calyx of the same. (39)

Specimens of all these and other varieties are preserved in the Herbarium of the Royal Botanic Garden, Kew, and the numbers in brackets at the end of each description refer to the numbers in the Herbarium.

ACCOUNT OF A FAMILY SHOWING MINOR-BRACHYDACTYLY.

By H. DRINKWATER, M.D., F.R.S. (Edin.), F.L.S.

In the autumn of last year (1910) a medical friend resident in Liverpool informed me that a relative of his, whilst making his official medical inspection of school children, had seen a boy whose hands appeared to be of the same Brachydactylous type which I had described in a communication to the Royal Society of Edinburgh in November 1907. It naturally occurred to me that some family whom I had already examined had removed to Lancashire; but as soon as this boy's name was communicated to me I knew that he did not belong to any of the families already described.

In December I wrote to the Headmistress of the school which the boy had attended and received the following reply:—

<div style="text-align:right">Dec. 29. 10.</div>

Dear Sir,

   Your letter has just reached me having been forwarded from ———[1].
The boy, whom you refer to, has now left school.

 His parents live at ———. He is a rather peculiar boy, and dull by nature. At school we used to attribute his stupidity to the fact that his parents are related —(first cousins).

 His short fingers did not seem to hinder his manual work, but they are remarkably short. I remember being told that the grandfather, on the father's side, had also very short fingers. The boy is now 13 years of age and is apprenticed to a joiner. Dr ——— very kindly offered to have the boy's fingers examined at a Liverpool Hospital, but the parents refused their consent. The father is a rather intelligent man and by occupation a salesman at ———.

 If I can supply you with any further information I shall be very pleased to do so.

<div style="text-align:center">I am,
Yours faithfully,
W. ———.</div>

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[1] Names and addresses omitted for obvious reasons.

This letter contains three statements which are of interest from the biological standpoint.

(1)   As to the brachydactylous condition of the boy's hands.

(2)   The presence of the same condition in a grand-parent.

(3)   The blood-relationship of the parents.

Clearly it was a case for further inquiry. I endeavoured to get some more particulars by correspondence with some other people who knew the boy's family but without success. All the information obtainable was that the boy's parents and all his brothers and sisters were normal (Fig. 1), and the only known brachydactylous member, besides

Fig. 1.   Erroneous Chart.

himself, was his paternal grandfather[1]. Moreover the parents would not consent to have either a radiograph or photograph of the boy's hands taken.

I mention these facts in order to point out the un-reliability of second-hand information, for it will be seen in the sequel how erroneous the statements were from *all* sources. Accurate details can generally only be acquired by personal investigations. I have paid two visits to this boy's family and their relatives and though I have not succeeded in persuading them to do all I wished, I have been able to gather together sufficient particulars to make it possible to describe the essential feature of the abnormality, and so indicate its hereditary bearings.

I have interviewed most of the boy's relatives including the great-grandmother, grandmother, uncles, aunts, and cousins, and have made several measurements and obtained some radiographs and a couple of photographs. This is most satisfactory considering the great reluctance of these people to do anything which can possibly lead to identification.

I am greatly indebted to Mr Thurston Holland for the excellent radiographs which are amongst the best I have ever seen.

The abnormality can be traced through five generations. The oldest surviving members of the family amongst the abnormals are the man No. 5 in the chart (Fig. 2) and his sister No. 7. No information

[1] Three correspondents declared that the boy's *parents* were normal.

Pedigree of a Family showing MINOR BRACHYDACTYLY.
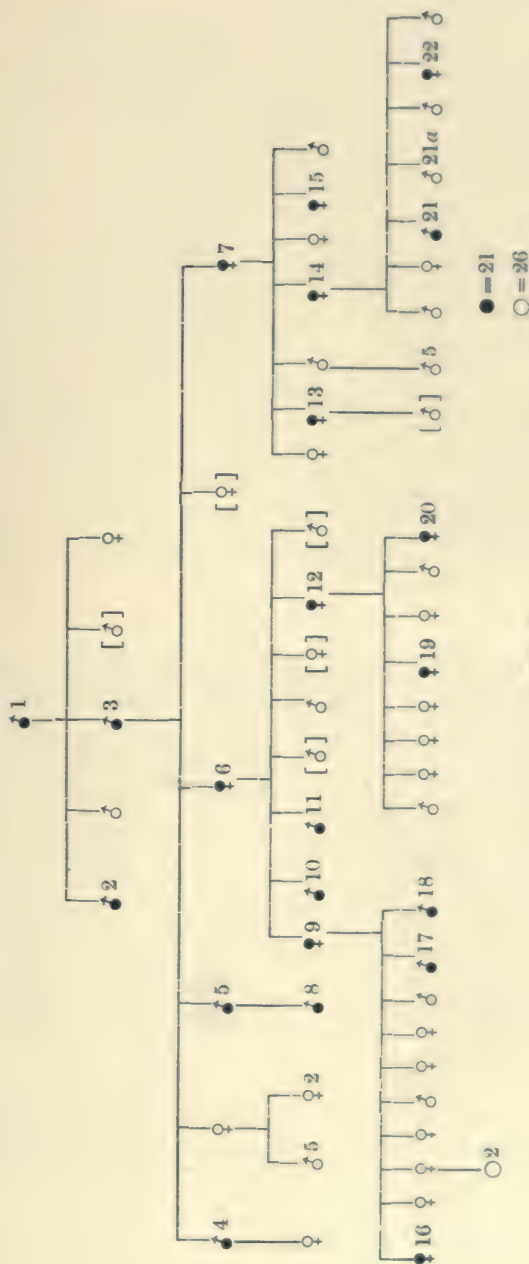
Fig. 2.

could be obtained from No. 5 as he is bordering on senile dementia. No. 7 was most reluctant to give me any information and *tried* to mislead me. (As an example, I may mention that on asking about her brother No. 5, and mentioning him by name, she said she had no such brother.)

The facts about No. 1 and his five children were however correctly given by her, and were confirmed by her mother, the widow of No. 3, and by other members of the family from tradition.

The abnormals are represented by black circles and the normals by white. Where there has been the least doubt as to the individual being normal or abnormal the circle is included in brackets; these must not be counted in reckoning the ratio of abnormals to normals. The abnormals only are numbered.

The information I first obtained to the effect that the schoolboy (No. 21) was the only affected member of his family was not correct, for it is seen that not only is a younger sister (No. 22) of the same type, but the mother also (No. 14): and it was not "the grandfather on the father's side" but the maternal grandmother (No. 7) who had transmitted the abnormality.

There were 16 abnormal members of the family alive at the time of my visits and I interviewed each of them, but I regret to say that in some cases I was not allowed to take any measurements.

What is the condition of the hands?

The abnormality resembles in many respects that described in my paper "An Account of a Brachydactylous Family[1]": whilst there are other features in which it differs. The fingers are not so short, and for this reason I propose to term the condition "Minor-Brachydactyly." The former family will be referred to as "No. 1 family."

Fig. 3 shows the hand of the boy already referred to (No. 21). The upper hand is that of a normal brother who is two years his junior (No. 21a).

The Brachydactylous condition is sufficiently evident, but that it is not so marked as in No. 1 family is shown by comparing Fig. 4 which is taken from my former paper.

As the bones of this boy's hands are not yet fully ossified, it will be well, before describing them from the radiograph, to point out the

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[1] *Proceedings of the Roy. Soc. of Edin.* Vol. xxviii. Part i.

Fig. 3. Hands of abnormal and normal brothers.

Fig. 4. Members of No. 1 Family. The lower hand is "Brachydactylous."

peculiarity as seen in the hands of an adult, and for this purpose I shall select the radiograph of a woman, No. 9 (Fig. 5).

What chiefly strikes one is the shortness of the middle phalanx in each finger. It is to this peculiarity that the shortening of the hand is principally due.

Fig. 5.   Hand of woman ($\times \frac{3}{4}$).   The middle phalanx is very short.

In the normal hand, the middle phalanx is intermediate in length between the first and the third. All the phalanges are seen to be distinct and separate: there is no union (ankylosis) of the second to the terminal phalanx, such as occurred in No. 1 family in every case

in the first and little fingers and frequently in the middle and ring
fingers also. (See Fig. 6.)

Fig. 6. Hand of adult woman (slightly reduced). Belonging to No. 1 Family.

The variations of the bones from the normal type are shown, in outline, in Fig. 7 where *A* represents those of a normal finger; *B*, those of a minor-brachydactylous finger, and *C*, a brachydactylous finger from No. 1 family. The phalanges are numbered 1, 2 and 3 in each case (3 is the terminal one which supports the finger nail).

In *C* it will be observed that the second phalanx (2) and the third (3) have become united into one bone: whilst in *B* the second phalanx is short, but remains as a separate bone.
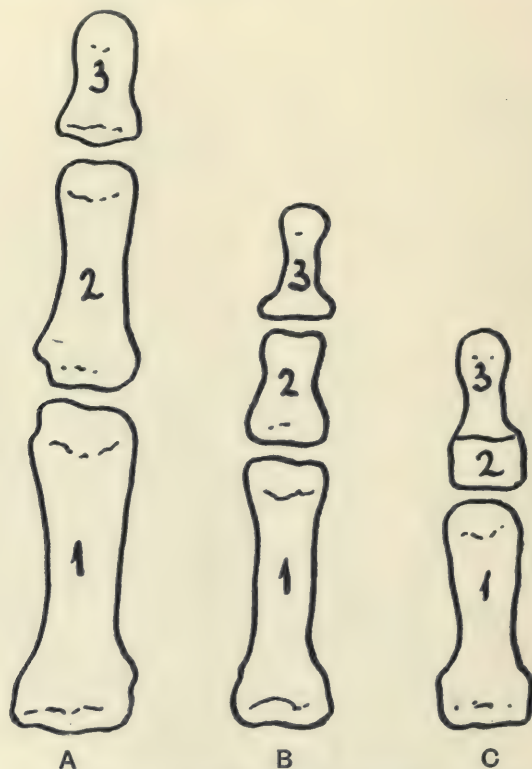
Fig. 7. Normal and Brachydactylous Phalanges. (Natural size.)

A. Normal.   B. Minor-Brachydactylous.   C. Brachydactylous.

What is the cause of this shortening?

Each phalanx during childhood shows, normally, a thin bony plate at its base. This plate is called the *epiphysis*; it is attached to the larger portion—the *shaft* of the bone—by an intervening layer of gristle, which, being transparent to the X rays, shows in a radiograph as a blank space. (Figs. 8, 9.)

In adult life this piece of gristle becomes ossified, and with the shaft and epiphysis forms one bone.

Fig. 9 shows the epiphysis at the base of the first phalanx and one at the base of the third but the second phalanx is seen to be without any epiphysis except in the case of the middle finger.

This absence of epiphysis accounts for a good deal of the shortening of the finger, but not for the whole of it; for it is obvious that the shaft of the bone is of less than normal length and the first and third phalanges are also slightly shorter than they should be.

Fig. 8. Outline of bones (not fully ossified) of brachydactylous finger of a youth.

1. First phalanx with epiphysis (ep.) at base.
2. Second phalanx without epiphysis.
3. Terminal phalanx with epiphysis (ep.).

There are three factors producing the shortening of the second phalanx:

(1)   The slight shortness of the shaft of the bone.

(2)   The absence of the epiphysis.

These have already been referred to, but

(3)   There is still another factor and to this is perhaps attributable the chief share in the production of the shortening.

I have already drawn attention to the cartilage or gristle between the shaft and the epiphysis. So long as this cartilage remains the bone can and does increase in length with the growth of the individual but when it has become ossified, then no further growth, in length, of the

Fig. 9.   Hand of boy showing absence of epiphysis at base of second phalanges except
in the middle finger and thumb (natural size).

bone can take place at this point. Now this layer of cartilage does not become ossified in the average individual until about the twentieth year, so that until that age the phalanx can and does increase in length. If however ossification occurs prematurely, then the growth of the bone will be arrested and a permanent shortening will be the result. This is exactly what has happened in this family especially in the first and little fingers. In Fig. 5 the second phalanx is seen to be short in all the four fingers. This is the hand of a woman (No. 9). Fig. 10 shows the hand of her son (aet. 14). Here there is no sign of the epiphysis of the second phalanx except in the middle finger where it has already united to the shaft. In the other fingers it has never been present or has united during infancy. The abnormality in this boy will therefore be an almost exact repetition of the mother's.

These cases represent the extreme type of deformity in this family.

Fig. 11 shows a modification of this type in the adult. It is the hand of No. 12.

The second phalanx is seen to be much more shortened in the index and little fingers than in the middle and ring fingers. Why is this?

I think the explanation is furnished by the radiograph (Fig. 12), which shows the hand of her daughter aged 8.

In this girl's hand there is an apparent absence of the epiphysis in the fourth finger, and in the index finger ankylosis has already occurred, whilst in the middle and ring fingers the epiphysis is still separated by cartilage.

When growth is complete this hand will be like the mother's with the second phalanx much shorter in the first and fourth fingers than in the second and third.

In another child of No. 12 aged 2 years the ossification of the cartilage has already occurred in the first finger (Fig. 13).

The third factor concerned in the production of shortening of the middle phalanx is, therefore, premature ossification of the cartilage intervening between the shaft of the bone and its epiphysis.

The essential feature of the abnormality in No. 1 family was stated to be "an absence of the epiphysis at the base of the second phalanx" with subsequent ankylosis of the second to the first phalanx, so that we have in the present family an abnormality which is essentially the same developmentally but stopping short of ankylosis.

The second phalanx in the middle finger is less affected than in the others and this was also a characteristic of No. 1 family.

The second phalanx of the thumb differs in the two families : in No. 1 the basal epiphysis was absent but in this family it is present.

The abnormality in the toes appears to be practically identical in the two families for in both there is an absence of the middle phalanx-

Fig. 10.   Hand of boy (× ¾).   No. 18 in chart.

epiphysis and in the adults there is ankylosis of the second and terminal phalanx in the small toes.   In each abnormal individual both hands and both feet are symmetrically affected, so that, in this respect, the peculiarity is as extensive as in No. 1 family.

Fig. 11. Hand of Woman (slightly reduced). No. 12 in chart.

Fig. 12.   Hand of girl, aged 8.   (Natural size.)

Fig. 13. Hands of girl aged 2 years, showing very early union of epiphysis in first and little fingers.

Fig. 14 is taken from an adult member of this minor-brachydactylous family.

The measurements so far as they have been made are given in the following table.

Measurements of Abnormals.

Number in Chart	Age	Middle Finger	Hand	Height
5	81	$2\frac{5}{8}$ inches	$6\frac{1}{2}$ inches	62 inches
7	—	$2\frac{3}{8}$,,	6 ,,	59 ,,
8	33	$2\frac{3}{8}$,,	$6\frac{1}{2}$	$60\frac{1}{2}$,,
9	54	—	—	—
10	51	$3\frac{1}{8}$,,	7 ,,	62 ,,
12	40	$2\frac{1}{2}$,,	$6\frac{1}{4}$,,	60 ,,
13	—	$2\frac{3}{8}$,,	$6\frac{1}{8}$,,	57 ,,
14	—	$2\frac{5}{8}$,,	$6\frac{3}{4}$,,	$58\frac{3}{4}$,,
16	33	—	—	—
17	17	—	—	$57\frac{1}{4}$,,
18	15	—	—	$54\frac{3}{4}$,,
19	8	—	—	—
20	2	—	—	—
21	14	$2\frac{1}{4}$,,	5 ,,	$54\frac{1}{2}$,,
22	5	$2\frac{1}{8}$,,	$4\frac{1}{4}$,,	$47\frac{1}{4}$,,

Scanty as the figures are they indicate very decidedly two characteristics, viz. :

(1) The shortness of the fingers.

(2) The shortness of stature.

(1) The average length of the middle finger of the seven adults (male and female) is 2·57 inches which is fully three-quarters of an inch less than the normal.

The shortness of the hand of the boy (No. 21) is shown not only by the photograph (Fig. 2) but by comparison with the hands of two of his younger brothers as shown in the following table :

	Age	Finger	Hand
No. 21 (abnormal)	14	$2\frac{1}{4}$ inches	5 inches
Brother (normal)	12	$2\frac{3}{4}$,,	6 ,,
,, ,,	7	$2\frac{3}{8}$,,	$5\frac{1}{4}$,,

(2) The shortness of stature is well shown by the photograph of No. 21 (aged 14) and his younger brother (aged 12) who is normal and the taller of the two.   (Fig. 15.)

The average stature of the four women Nos. 7, 12, 13 and 14 is 58·6 inches and of the three men Nos. 5, 8 and 10, 61·5 inches.

Fig. 14. Foot of adult showing abortive middle phalanx. (Natural size.)

Fig. 15. Photograph of two brothers. The shorter boy is aged 14, and is Brachydactylous. The taller is aged 12 and is normal.

Now these figures are very remarkable from their close approximation to the same measurements in No. 1 family where they were respectively 58¼ and 61 inches.

The women are therefore about 4¾ inches and the men about 8 inches below the normal height.

It is the general opinion that the abnormals have better health than their normal relatives. The abnormals are slightly more prolific than the normals though the numbers are too small to enable one to draw conclusions on this point. Increased fecundity was a marked feature of No. 1 family.

In both families a much larger proportion of normals have remained unmarried. The schoolmistress stated that the parents of the boy (No. 21) are cousins but such is not the case and I could not hear of any intermarrying in the family.

Mendelism.

This family illustrates certain Mendelian rules:

(1) There is perfect segregation. The abnormality is either not transmitted or it is transmitted fully: i.e. so as to involve the digits of both hands and both feet.

(2) The abnormality is transmitted only by the abnormals and never by the normals, so that all the descendants of normals are normal.

(3) The offspring of parents, *one* of whom is abnormal (= dominant) the other normal (= recessive), should theoretically show 50 °/$_\circ$ of each type. The descendants, in this family, counting only those whose type is known for certain, amount to 47, and of these 21 are abnormal: i.e. 21 abnormals to 26 normals giving 44·6 instead of the theoretical 50 °/$_\circ$.

But this percentage is not to be regarded as positively correct. It is certain that it is *as high* as this, but not at all certain that it does not more closely approximate to the theoretical number.

When I first interviewed the woman No. 9 in the chart she informed me that of her ten children only *one* had short fingers like her own, viz. her eldest daughter (No. 16) and a casual inspection would have confirmed her statement. The shortening is so inconspicuous that *in some of the children it is only detected by flexing the finger*, and then the shortened middle phalanx is noticeable but

not otherwise. By this means I was able to show her that her two youngest children's hands were brachydactylous and this is confirmed by the radiographs.

In adult life the shortness of the hand is conspicuous enough and cannot be overlooked, but this is not so during childhood, so that it is possible that of the few children whom I could not see and who were declared to be normal, one or two may be of the abnormal type, and if so would make the percentage of abnormals still more closely approximate to the theoretical figure.

I have been able to obtain radiographs of the hands of Nos. 9, 12, 14, 17, 18, 19, 20, 21 and 22 and of the feet of all the same except No. 9.

[Figure 4 is reproduced by kind permission of the Royal Society of Edinburgh.]

A CRITICAL EXAMINATION OF RECENT STUDIES ON COLOUR INHERITANCE IN HORSES.

By A. H. STURTEVANT,

Columbia University.

ABOUT a year ago I published a paper on the inheritance of colour in the American Harness Horse[1]. I concluded that the colour of these horses is, in general, determined by five factors: C (chestnut or yellow), hypostatic to the others and always present; H (Hurst's factor or black); B (bay), epistatic to H; R (roan), and G (gray). There were at that time two other papers on colour inheritance in horses with which I was not acquainted. The first, by Dr E. H. Harper[2], was not written from a Mendelian standpoint, but contained confirmatory evidence for my views about black and gray. The second, by Prof. James Wilson[3], had already covered most of the points which I brought out, and some others as well, although Wilson does not seem to have had a very clear idea of the factors concerned, as is shown by his attempt to represent the gametic constitution of his stallions by only two symbols each. There is one point on which we reached quite different conclusions—namely, the position of brown, which I shall discuss later. These three papers deal with five different breeds, the English Thoroughbreds and Shires and Scotch Clydesdales being treated by Wilson, the French Percherons by Harper, and the American Harness Horses by myself. It is my purpose in the present paper to compare and combine the contents of these three contributions.

Wilson and I agree that chestnut stands at the bottom of the scale, and neither of us found any horse lacking the factor for it.

[1] *Biol. Bull.* XIX. No. 3, August 1910, p. 204.

[2] *Biol. Bull.* IX. No. 5, October 1905, p. 265.

[3] *Scient. Proc. Royal Dublin Soc.* 12 (N. S.), No. 28, 1910.

The next factor, that for black, *H*, was discovered and correctly interpreted by Hurst[1]. Wilson's statistics show that this factor is carried by bays, browns, and blacks, though he gives no factorial interpretation of his results. The figures for matings of chestnuts, i.e., of *h h* individuals, are as follows:—

Breed		Colour of Foals				Authority
		Chestnut	Black	Brown	Bay	
Thoroughbred	...	1095	9 (bay or brown)			Hurst
Shire	... ...	44	1	1	5	Wilson
Trotter	... ...	69	0	0	0	Sturtevant
Total	...	1208	16 not chestnuts			

Hurst also gives five other authorities for the statement that chestnut breeds true, in various other breeds of horses.

Hurst, Wilson, and I all found a good many sires producing no chestnut foals, i.e., homozygous for *H*. These are blacks, bays, browns, grays, and roans. The total foals from heterozygous sires and chestnut mares is as follows:—

Breed		Chestnut	Not Chestnut	Authority
Thoroughbred	...	347	355	Hurst
Clydesdale	... ...	3	2	Wilson
Trotter	... ...	65	56	Sturtevant
Total	...	415	413	

My next factor was *B*, or bay, and I considered brown as being usually a heterozygous form (*CHBb*), although I also realised that horses with the above constitution are often bays. I found what I thought was a brown stallion with the formula *CHBB*, but this horse (Prodigal) as I have since found, is recorded in the later volumes of *Wallace's Year Book* (my principal authority) as a bay. The change was made after he had become famous as a sire, and is therefore probably correct. I was inclined to explain the four browns recorded as produced by two black parents by supposing that some *CHbb* horses are browns. I now believe these four cases to be errors in the record, this opinion being based on Wilson's and Harper's figures for matings of blacks to blacks (see below).

Wilson reached a quite different conclusion. He regards bay and brown as dominant to black. His idea as to the relation between bay and brown is shown by the following passage. "The relative positions

[1] *Proc. Royal Soc.* 77 B, 1906, p. 388.

Black × Black

Breed			Colour of Foals			Authority
		Black	Bay	Brown	Chestnut	
Percheron ...	...	49	(2 not black)			Harper
Shire ...	...	39	3	0	2	Wilson
Clydesdale ...	...	36	0	2	0	Wilson
Trotter ...	...	34	2	4	2	Sturtevant
Total ...	...	158	5	6	4	

Black × Bay

Thoroughbred	...	1	33	27	14	Wilson
Shire ...	...	39	125	43	19	Wilson
Trotter ...	...	16	48	31	7	Sturtevant
Clydesdale ...	...	40	104	67	7	Wilson
Total ...	...	96	310	168	47	

Black × Brown

Thoroughbred	...	8	12	20	0	Wilson
Shire ...	...	39	19	36	4	Wilson
Clydesdale ...	...	61	34	106	1	Wilson
Trotter ...	...	11	5	9	1	Sturtevant
Total ...	...	119	70	171	6	

Bay × Bay

Thoroughbred	...	1	1295	125	270	Wilson
Shire ...	...	13	287	18	28	Wilson
Clydesdale ...	...	6	243	59	5	Wilson
Trotter ...	...	1	46	3	9	Sturtevant
Total ...	...	21	1871	205	312	

Bay × Brown

Thoroughbred	...	10	744	365	123	Wilson
Shire ...	...	23	133	56	5	Wilson
Clydesdale ...	...	25	206-	254	5	Wilson
Trotter ...	...	9	81	31	8	Sturtevant
Total ...	...	67	1164	706	141	

Brown × Brown

Thoroughbred	...	6	78	114	11	Wilson
Shire ...	...	7	20	27	2	Wilson
Clydesdale ...	...	32	34	165	0	Wilson
Trotter ...	...	5	7	7	0	Sturtevant
Total ...	...	50	139	313	13	

of bay and brown remain to be settled; and although there is evidence
in favour of brown being dominant to bay, this conclusion is not clearly
established. It must be remembered these are the colours breeders
have the greatest difficulty in discriminating; and errors affect sires
and dams and foals. In regard to sires it has been possible to correct
the registered colours in several cases; and while every correction has
increased the evidence in favour of brown being dominant, it is still
possible that there may be other explanations, as, for instance, that bay
is a diluted brown."

I shall first give the figures bearing on this question, and then
discuss their significance in connection with the two views given above.

My hypothesis that brown is a heterozygous colour was based upon
two facts. In the first place, I am unable to see any very sharp line
between bay and brown. Wilson evidently thinks the two colours are
distinct, but I can find no definite statement as to what the difference
is, although he discusses the distinction between the various colours at
some length. Secondly, all the browns from which I could find any
fair number of foals produced some blacks, with the exception of the
stallion Prodigal, which, as I have explained above, now turns out to
be a bay. I have found 15 such brown sires producing black foals.
In Wilson's tables appear five brown and one doubtful bay or brown
Clydesdale sires, all of which have a fair number of black foals. There
are also five brown Thoroughbreds. Two of these sired no blacks
among 93 and 95 foals, respectively, though each has a foal recorded
as "brown or black." Ladas has one black and one black or brown
among 97 foals, Desmond three black and two brown or black among
48 foals, and Wolf's Crag seven black and one brown or black
among 95 foals. Here we meet an interesting fact—the extreme
scarcity of blacks among English Thoroughbreds. Wilson believes
that most, if not all, the recorded blacks are really browns, and was
not able to find a genuine black stallion. The Desmond mentioned
above is recorded as a black, but Wilson ascertained that he is really
a dark brown, and the same was found to be true of all the mares
recorded as black of which he could get definite and reliable informa-
tion. Only about 1 °/$_{o}$ of these Thoroughbreds are recorded as blacks.
Of course by my hypothesis it would be hard to explain how there
could be about 14 °/$_{o}$ browns and only 1 °/$_{o}$ blacks. However, I shall
not try to explain this, as the hypothesis is pretty well disproved by
the result of mating browns together. If it were correct such matings
should produce 25 °/$_{o}$ blacks, 25 °/$_{o}$ bays, and 50 °/$_{o}$ bays and browns.

As is shown above they do produce a trifle less than 10 °/₀ blacks. I am still convinced, however, that there is something in my original hypothesis. It could scarcely be a coincidence that twenty brown sires should be heterozygous and none homozygous, if we except the Thoroughbreds. Among the latter I believe that Wolf's Crag, Desmond, and perhaps Ladas are heterozygous, for it is evident that in a population containing only 1 °/₀ recessives there would not be a large proportion of heterozygous mares, and a heterozygous stallion would of course not produce recessives when mated to pure dominant mares. Again, it will be noticed in the tables above that, excluding chestnut foals, black mated to bay gives only 16 °/₀ black foals, while black mated to brown gives 33 °/₀, or twice as many. And while brown to brown gives only 10 °/₀ blacks, bay to bay gives still fewer—only about 1 °/₀, or 3 °/₀, if we omit the Thoroughbreds, of which the table illustrating this class has a relatively large proportion.

According to Wilson's hypothesis that brown is dominant to bay, bay to bay should produce no browns. This would require the further hypothesis that the 205 browns recorded from such matings are errors in description, which certainly does not seem to me to be probable. Again, there should be some brown sires producing no bay foals, but as a matter of fact all of the 25 brown sires found produce a large proportion of bays. I have, in fact, never yet found a sire which did not produce bays. Finally, as stated above, I am unable to agree with Wilson that bay and brown can be satisfactorily separated. I base this upon my own observation, upon the frequent changes from bay to brown and *vice versa* which he mentions finding in the Clydesdale records, and the similar changes which I have observed among Harness Horse records, and upon the frequent recording of English Thoroughbreds as "bay or brown." My conclusions, then, are that brown and bay are not distinct, brown being merely a dark bay, and that brown is more often *CHBb* than *CHBB*, and never *CHbb*. It would be interesting to know whether or not the heterozygous bays are darker than the homozygous ones.

In regard to gray there is no great difficulty. I thought when I published my first contribution that I had a non-conformable case, where two brothers not gray were siring about 50 °/₀ gray foals. One of these, Dispute, I now know to have been wrongly recorded, as his owner, Mr John Taylor, and another breeder, Mr W. B. Gill, both write me that he is a gray. The supposition therefore is that his brother was also a gray, though I have been unable to verify this. But if he

was, then the whole family works out exactly according to expectation. The produce from one gray parent and one not gray is as follows:

Breed		Gray Foals	Not Gray Foals	Authority
Percheron	...	31	29 – ?	Harper
Thoroughbred	...	73	56	Wilson
Shire	...	146	186	Wilson
Clydesdale	...	9	15	Wilson
Trotter	...	141	142	Sturtevant
Total	...	400	428 – ?	

Of the 428 – foals neither gray nor chestnut 38, plus an unknown number of the Percherons, are blacks. This gives a clue as to whether or not the bay factor is necessary before *G* can cause gray. If it is, then these 38 + blacks should be eliminated from the table, and the result would be 400 grays to 390 – bays and browns, or, leaving out all Percherons, 369 grays to 361 bays and browns. Now gray is an unpopular colour, and it seems very unlikely that as large a proportion of the gray foals would be recorded as of the other colours, and I should therefore expect fewer grays than the calculation calls for, rather than more. For this reason, and because no black has yet been found to carry the *G* factor, I believe that *G* can cause gray in the absence of *B*, though this is by no means proven as yet.

Grays to grays give the expected 3 to 1 ratio.

	Colour of Foals				
Breed	Bay	Black	Brown	Gray	Authority
Shire	3	1	0	12	Wilson
Thoroughbred ...	0	0	0	1	Wilson
Percheron ...	10	not gray		31	Harper
Trotter... ...	1	0	0	1	Sturtevant
Total ...	15	not gray		45	

Just what this *G* factor is is a rather difficult question. Gray differs from the colours hypostatic to it both in the possession of white hairs, and in the mottled or dappled pattern. Now this dappled pattern, or one very similar to it, is to be seen also on chestnuts, bays, browns, duns, and sometimes even blacks. In the case of browns, the spot inside may be lighter than the ring around it, that is it may have more brown hairs, or this condition may be just reversed. If there is a separate dappling factor, then gray would not act like an ordinary colour due to a single factor, but would be produced by non-gray parents, unless the dappling factor is present in most horses. Many

horses seen on the streets do not show dappling, but it is possible that it would show on most of them at the time they are shedding their coats. This whole question is one which can be settled only by further investigation.

In my former paper I suggested (p. 215) that perhaps all horses carrying the roan factor are roan, the type of roan, or the ground colour, depending upon the colour the horse would have been if it had not had that factor. Wilson had already made the same suggestion, as I now find, but he has given little more evidence on the subject than I did. I have written to a good many breeders of Trotters, in order to try and get information which would help out on this point, but have not much to offer as yet. What little I have, however, is not encouraging. In my tables of sires homozygous for the bay factor (see former paper), and therefore producing no black foals, were two roans, Jay Bird and Margrave. I am informed that both these stallions were bay roans, and that Jay Bird's roan foals are also bay or red roans. Margrave likewise has some bay roan foals and a full brother of the same colour. This is according to expectation, but I am also informed that Margrave has at least one blue roan colt. Moreover, his former owner, who now owns the full brother mentioned above, writes me that their dam, Spanish Maiden, was a blue roan. Not only did this mare produce a supposed *BB* colt, but she was also the daughter of a *BB* stallion, Happy Medium. Margrave has 65 non-black foals and Happy Medium has 69, neither being credited with a single black, though eleven of Happy Medium's bays and browns are from black mares, so that it is very improbable that both should in reality be *Bb* animals. Either Spanish Maiden and probably the Margrave colt are not blue roans, or blue roans can carry the *B* factor.

Wilson's tables give some information not brought out elsewhere, so I shall take the liberty of reproducing them, in part. He considers iron gray as a kind of roan. Of course his records may use that term in a different sense from that in which American horsemen do, but unless that is true iron gray is not a roan at all, but merely a young gray. We, in America, mean by that term a very dark gray, which the casual observer might mistake for a roan, but which later develops into the ordinary dapple gray, and, still later, into white with black hairs in the mane and tail, and on the feet. It is, moreover, the invariable colour of a young gray, so far as I know. Wilson classifies these iron grays separately but has only a few of them, and I shall omit them because of my doubt as to their real position—a doubt which his data

do not help to clear up. The tables below were drawn entirely from the Shire records. Just what roan means is not made clear. Does it include only red or bay and chestnut roans, or may it include some blue roans whose colour is not more definitely stated in the records?

One Parent Roan

Colour of other Parent	Colour of Foals						
	Chestnut	Black	Bay	Brown	Gray	Blue Roan	Roan
Chestnut ...	7	2	7	2	1	0	8
Black ...	0	5	0	2	0	3	9
Bay ...	4	2	30	11	1	1	39
Brown ...	1	2	14	7	1	1	19
Gray ...	0	0	0	1	5	1	6
Blue Roan...	0	0	2	0	2	0	2
Roan ...	0	0	1	0	0	0	5

One Parent Blue Roan

Colour of other Parent	Colour of Foals						
	Chestnut	Black	Bay	Brown	Gray	Blue Roan	Roan
Chestnut ...	0	0	1	0	0	1	0
Black ...	0	1	0	0	0	0	0
Bay ...	1	1	4	1	0	1	0
Brown ...	0	2	1	2	0	5	2
Gray ...	0	0	0	2	0	0	0
Blue Roan...	0	0	0	0	0	0	1
Roan ...	0	0	0.	0	0	0	1

There is no evidence in this table against the hypothesis reached independently by Wilson and myself, but neither is there any of very much significance in favour of it. That black to blue roan gives one black, and blue roan to blue roan gives one roan is all very well, provided the one roan is not a red roan, but it does not take us very far. The table is of value, however, as showing that roan to chestnut black, brown, or bay, gives 80 roans to 118 not roans. Adding my figures for the same cross gives 225 roans to 284 not roans. I believe that this deficiency in roans is at least partly due to the fact that roans vary in the amount of white they show, and the breeders are apt to avoid calling a roan such if they can help doing so, as the colour is unpopular. Still, if this practice were very general it should lead to many roans being produced with neither parent recorded as roan, which is not the case, such records being rather rare. It is quite possible that there are other complications in this case.

Neither Wilson nor I had enough evidence to enable us to make even a safe guess as to the relation between gray and roan. I have

since obtained more information however. Below are the results of mating grays to roans.

Breed	Colour of Foals				Authority
	Black	Brown	Gray	Roan	
Shire ...	0	3	5	7	Wilson
Trotter ...	1	0	2	4	Sturtevant
Total	1	3	7	11	

The expectation is $50\,\%$ of the epistatic colour, $25\,\%$ of the other, and $25\,\%$ of colours hypostatic to both, i.e. brown, bay, etc. There are $50\,\%$ roans, but the numbers are small, and the grays are dangerously close. Wilson's tables show five grays produced among Shires by mating roans to chestnuts, browns, bays, or roans, but he says a similar number of cases of gray apparently carrying roan could be found. I have found four cases where gray has seemed to carry roan, two of which, however, are from doubtful sources, and the other two, of course, might easily be due to mistakes somewhere. I have found no case of roan carrying gray. Of the above four roans from gray by roan, two were reported as gray roans, one was a bay roan, and one either bay or chestnut roan. Of Wilson's, one is a blue roan, and the others are roans, the type not being made clear. The most probable view of the matter seems to me to be that the $G\,R$ horses are blue or gray roans, which may perhaps be sometimes mistaken for grays and recorded as such.

I did not treat dun in my paper, but Wilson has worked with it. He assumes, though without saying so, that all duns are due to the same factor. This may be correct, but there are certainly at least two types of duns, differing from each other much more than do the bay and brown he is so careful to separate. One type has black " points," and
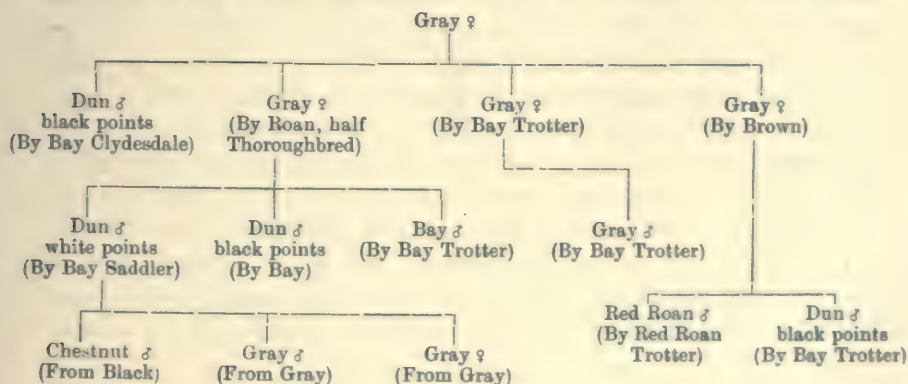
the other has very light, almost white, mane and tail, and no black on the feet. The light type is sometimes described as cream-coloured. That the two are related the pedigree given here indicates, though just what the relation is does not appear.

Lumping all duns together and adding my figures to Wilson's give the following as the result of mating duns to various colours.

		Colour of Foals						
Colour of other Parent		Chestnut	Black	Brown	Bay	Gray	Roan	Dun
Chestnut	...	0	0	0	1	0	0	1
Black	...	1	1	0	1	0	0	2
Brown	...	0	0	0	1	0	0	1
Bay	...	1	1	0	2	0	1	2
Gray	...	0	0	2	0	7	0	5
Dun	...	1	0	0	1	0	0	2

Wilson gives a case of gray to gray producing dun, and the above pedigree shows three gray mares which carried a dun factor. Therefore, assuming that there is a single dun factor and making no attempt to explain the differences among the duns, we should agree with Wilson in placing the colour between bay and brown on the one hand and gray on the other. But such an assumption is rather hazardous as the case stands now, and I should not like to make any generalizations about dun until more evidence is at hand.

Mr W. P. Newell has supplied me with information about an interesting family of white horses. The ordinary white horse is of course merely an old faded-out gray, but this is a family of real whites. Mr Newell gave Professor W. E. Castle some information about these horses, on the basis of which Professor Castle considered the colour to be an extreme spotted condition dominant to the ordinary colours[1]. I have now some further information, which makes the case an interesting one. These horses are said to be somewhat variable in colour. To use my informant's words: " The colour of skin is white or so-called pink, usually with a few small dark specks in skin. Some have a great many dark spots in skin. These latter usually have a few dark stripes in hoofs; otherwise the hoofs are almost invariably white. Those that do not have dark specks in skin usually have glass or watch eyes, otherwise dark eyes....I have one colt coming one year old that is pure white, not a coloured speck on him, not a coloured hair on him, and with glass eyes." The term "glass eye" means a white eye. Therefore the colt described above is almost an albino in appearance.

[1] *Breeder's Gazette*, LIX. 15, 1911, p. 948.

However, his sire is one of the dark-eyed somewhat spotted whites, his dam being a brown Trotter. Since "glass" eyes occur not infrequently in pigmented horses it seems probable that this white-eyed albino (?) is really an extreme case of spotting, plus an entirely independent "glass" eye. Mr Newell writes that white mated to white gives about 50 °/₀ white to 50 °/₀ pigmented. He reports only three matings of white to white. The results of these were, one white, one roan, and one gray. Apparently, then, the white factor stands at the very top of the series. However, I am not sure that this is the whole story, as it would be a peculiar coincidence if it were a mere accident that the only two non-whites produced from white by white are representatives of the only two other colours having white in the coat, and these both such uncommon colours. On the other hand, it does not appear that white by pigmented gives a large percentage of grays and roans.

In addition to those already mentioned I wish to extend my thanks to the following horsemen, who have supplied me with information used in this paper: Messrs S. J. Fleming, W. B. Wallace, D. W. Northrop, T. Sterneman, and G. M. Garth.

Summary.

It seems probable that chestnut always breeds true. Therefore the placing of C (chestnut or yellow) at the bottom of the scale probably represents the condition of nearly all breeds of horses. Epistatic to it is H (black). Next comes, in the breeds studied, B (bay or brown), epistatic to both the preceding. G (gray) is next higher. Next is R (roan), which is probably always evident when present[1] and which probably merely causes a sprinkling of white hairs, without otherwise affecting the colour. Finally, we have W (white).

[1] Unless suppressed by the next factor, W.

A FURTHER CONTRIBUTION TO THE STUDY OF RIGHT- AND LEFT-HANDEDNESS.

By R. H. COMPTON.

In a paper presented to the Cambridge Philosophical Society in June 1910[1] I discussed the phenomena connected with the occurrence of two stereo-isomeric forms of seedling in two-rowed Barley (*Hordeum distichum*); the difference between them being shown in the mode of folding of the first leaf above the coleoptile (Diagram 1). The two
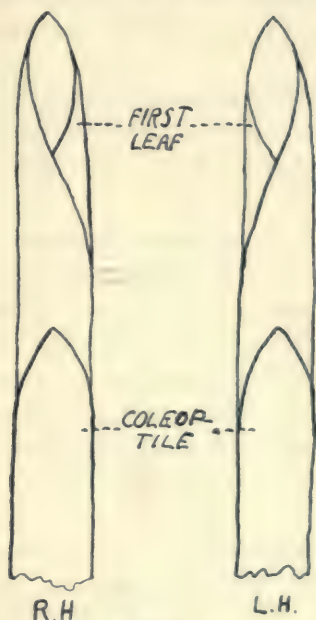
Diagram 1.

kinds of fold were called respectively right- and left-handed, the convention adopted being explained in the introduction to the paper.

[1] R. H. Compton, "On Right- and Left-Handedness in Barley," *Proc. Cambridge Phil. Soc.* xv. p. 495, 1910.

The difference between the two kinds of seedling may appear at first sight to be of an insignificant character, and not worthy of serious attention. That this is not so is amply evinced by the detailed account of the morphology of the plant given by Schoute[1] in his fine work on the tillerage of cereals. It is there shown that the direction of folding of the first foliage leaf has a direct connection with the side towards which (in Barley, but not in other cereals) the axillary bud is displaced from the median line, and also with the side of the plant on which the first foliage leaf (after the prophyll) of every lateral axillary branch is produced. The successive leaves, arranged distichously on the stem, normally alternate in their mode of folding[2]: thus all the leaf edges on one side of the plant lie beneath those on the other. Since the first foliage leaf of the axillary bud is always produced in the plane at right angles to that of its subtending leaf and on the side towards its underlying edge, it follows that all the first foliage leaves of lateral axes of the first order are towards the same side of the main axis. In these and other ways it is demonstrated by Schoute that the mode of folding of the first leaf is closely bound up with the symmetry and morphology of the whole plant.

Unfortunately reverse conventions are used by Schoute and myself in distinguishing right- and left-handed plants. In what follows, however, I propose to adhere to the convention adopted in my earlier paper.

Two-Rowed Barley.

In my previous paper it was concluded that there is no hereditary nexus between successive generations of two-rowed Barley in respect of right- and left-handedness. The statistics advanced were somewhat restricted, and did not attain the same degree of accuracy as those advanced in reply to the other questions proposed. Further results have since been acquired and are given below in order to place my previous conclusion on a firmer basis: for the conclusion itself is unaltered.

[1] J. C. Schoute, "Die Bestockung des Getreides," *Verh. d. K. Akad. v. Wetensch. t. Amsterdam* (Tweede Sectie); Deel xv. No. 2, pp. 8—24, Feb. 1910.

[2] Failure of this regular alternation of *LH* and *RH* leaves is mentioned by Schoute (*loc. cit.* p. 20), and also by Stratton and Compton, "On Accident in Heredity, with Special Reference to Right- and Left-Handedness," *Proc. Cambridge Phil. Soc.* xv. p. 508, 1910. I made observations on twenty Maize plants, following them as far as 6—10 leaves, and noting the fold of each leaf as it appeared : of these thirteen showed the normal alternation of *RH* and *LH* throughout; the other seven showed a disturbance of the regular sequence.

In 1910 a number of ears of "Kinver Chevalier" Barley were sown separately in the ground[1] and the plants produced were grown to maturity. Four of these families were selected for further study by reason of the eccentricity of the ratios of *LH* to *RH* which they exhibited[2]: it being thought that such ears might give the clearest indications whether the characters or the ratios were hereditary. Ear I gave an unusual excess of *LH* seedlings; ears II, III and IV an excess of *RH*.

Table I summarises the results obtained. In the first column is given the reference numeral to the ear in question together with the fold of the first leaf of the plant which bore it (in 1909). Columns 2 and 3 show the numbers of *LH* and *RH* seedlings produced from the ear in 1910. The ears yielded by the 1910 plants were harvested separately and were sown on wet string-canvas. The numbers of *LH* and *RH* seedlings so produced were counted and the results are displayed in the rest of Table I. The data are classified according as the

<p align="center">TABLE I.</p>

Parent Ear, 1910			Offspring, 1911								
			From *LH* plants			From *RH* plants			Total		
No. and twist 1909	LH	RH	LH	RH	LH/RH	LH	RH	LH/RH	LH	RH	LH/RH
I R	15	3	845	622	1·36	123	69	1·78	968	691	1·401
II R	6	8	88	69	1·28	197	131	1·50	285	200	1·425
III L	5	9	477	332	1·44	794	547	1·45	1271	879	1·446
IV L	7	12	550	378	1·46	495	409	1·21	1045	787	1·328
Totals			1960	1401	1·397	1609	1156	1·392	3569	2557	1·398
			58·28 °/₀ LH			58·19 °/₀ LH			58·26 °/₀ LH		

seedlings were the offspring of left-handed (cols. 4 and 5) or right-handed (cols. 7 and 8) parents in 1910. In columns 10 and 11 are given the totals from *LH* and *RH* parents added together. The ratio *LH/RH* is calculated in each case. In the lowest line of the table are given the sum of each column and the ratios for all four ears taken together.

The bottom line of the table shows at once the extreme closeness of the ratios found among the offspring of *LH* parents on the one hand and *RH* parents on the other. This appears to be conclusive evidence

[1] The results of these sowings were given in my earlier paper, p. 504.
[2] The average ratio for this variety of Barley was found to be 1·390 *LH* : 1 *RH* (58·18 °/₀ *LH*).

that the direction of folding of the first leaf is not inherited. The same conclusion is to be reached from a consideration of the offspring of each individual ear I—IV. Ear I, which produced a great excess of *LH* plants in 1910, gave in 1911 a ratio almost identical with the average: ears II—IV, which gave an excess of *RH* plants in 1910, also approximated to the same ratio in 1911.

Thus the conclusion of my earlier paper with respect to the non-inheritance of right- and left-handedness in the fold of the first leaves of two-rowed Barley is amply confirmed. This must be clearly distinguished from the fact that the ratio between rights and lefts is approximately the same in successive generations, as shown in Table II for Kinver Chevalier Barley.

TABLE II.

Year	LH	RH	LH/RH	Per cent. LH
1909[1]	730	546	1·337	57·21
1910[2]	379	259	1·463	59·41
1911	3569	2557	1·396	58·26
Total	4678	3362	1·390	58·18

We may say that the ratio *LH/RH* is hereditary though right- and left-handedness themselves are not.

Further confirmation was obtained at the same time of another conclusion previously stated: viz. that " The same ratio subsists among the seedlings whether produced from the odd or the even rows of seed on the parent ear" (p. 505). In the present experiments the offspring were as follows :—

TABLE III.

Rows	LH	RH	LH/RH	Per cent. LH
Odd ...	867	615	1·410	58·43
Even ...	2702	1942	1·391	58·19
Total	3569	2557	1·396	58·26

The totals obtained for all the varieties of two-rowed Barley studied up to the present are as follows:—11,185 *LH*, 7980 *RH*. Ratio *LH/RH* = 1·4016. Percentage *LH* = 58·362.

[1] Compton, 1910, p. 497.
[2] *Ibid.* p. 499.

SIX-ROWED BARLEY.

A few sowings were made with *Hordeum hexastichum*, var. *pyramidatum*. In this species all three flowers produced at each notch of the spike are hermaphrodite and set seed. Thus there are three odd and three even rows on each ear. The three rows in each case are called "left," "middle," and "right": that row being called "left" which is towards the left hand when the ear is held upright with the middle row towards the observer. Diagram 2, which is partly ground-plan, partly elevation, will make this convention clear.
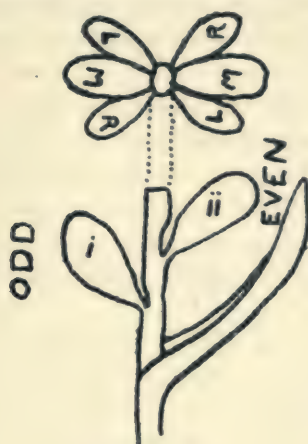
Diagram 2.

The three odd rows in several ears gave in all 55 *LH* and 45 *RH* seedlings: the ratio *LH/RH* being 1·22 (°/₀ *LH* = 55). Exactly the same ratio was given by the offspring of the three even rows; the numbers being 60 *LH*, and 49 *RH*. In Table IV, therefore, the two

TABLE IV.

Rows		LH	RH	LH/RH	Per cent. LH
Left	...	60	46	1·304	56·6
Middle	...	87	76	1·145	53·4
Right	...	71	48	1·479	59·7
Total		218	170	1·282	55·98

"left" rows—odd and even—are added together, and the same is done for the "middle" and "right" rows respectively.

Thus, as in the two-rowed varieties, there is an excess of left-handed offspring from all positions on the spike. The numbers studied

are rather restricted, so that the ratios cannot be considered exact; and the discrepancies from the average given by individual rows are well within the limits of probable error. We may conclude with a certain degree of safety that the position of the seed on the ear has no effect upon the method of folding of the first leaf of the seedling.

<div align="center">OATS.</div>

The mature plant of many species of the Gramineae exhibits a more or less pronounced tendency on the part of the leaves to twist loosely into the form of a screw[1]. The direction of the screw is constant, so far as known, in the case of the common cereals, and it is sometimes used as a diagnostic character for distinguishing different crops in their early stages[2].

The leaf-blades of Barley, Wheat and Rye are rolled to the right (i.e. like an ordinary right-handed screw), while Oats show leaf-blades rolled to the left. This twist is quite independent of the mode of folding of the leaves in the young state (and of the leaf-bases when mature); all the leaves of the same plant showing the same twist, although the direction of folding is normally reversed at successive nodes.

We have seen that in Barley, where the leaves exhibit a right-handed torsion, there is a definite and constant excess of seedlings whose first leaf shows the mode of folding which I have called left-handed. The question thus arises whether Oats, in which the leaf-blades show the reverse or left-handed torsion, will give a different ratio between seedlings showing the *LH* and *RH* modes of folding of the first leaf.

A sowing was made of a random sample of "Thousand Dollar" Oats, and the seedlings were counted for rights and lefts. There were found 469 *LH* and 576 *RH*. That is to say, there exists in Oats a considerable excess of *right-handed* seedlings. The ratio *LH/RH* is 0·814 (44·88 °/₀ *LH*); whereas in Barley (adding together all the varieties studied) it is 1·4016 (58·362 °/₀ *LH*) (above, p. 56).

We thus find that, in the cases of Barley and Oats, the reversed torsion of the mature leaves is accompanied by a reversal of the ratio between left- and right-handed seedlings in respect of the fold of the first leaf. What is the nature of this association, or whether it is merely fortuitous, it is impossible at present to decide.

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[1] Hackel, "Gramineae," in Engler and Prantl, *Nat. Pflfam.* II. 2, p. 4.

[2] Percival, *Agricultural Botany*, p. 511.

ITALIAN MILLET.

The seedling of *Setaria italica* exhibits a long internode between the scutellum and the base of the plumular sheath (cf. Maize). A number of seedlings were counted, and there were found 258 with the first leaf folded in the left-handed, 217 in the right-handed fashion. Ratio $LH/RH = 1·19$ ($54·1 °/_\circ LH$). A marked excess of left-handed seedlings occurs here also, though not so great as in Barley.

RYE.

Secale cereale is an unfavourable plant for this purpose, owing to the narrowness of the leaves and the frequency with which both margins are curled inwards in the upper portion[1]. Out of 30 seedlings, 16 were *LH* and 14 *RH*: showing that both conditions occur here also, though the numbers are insufficient to allow a ratio to be calculated.

MAIZE.

As is well known, the infructescence or cob of *Zea Mais* is comparable with an annular fasciation: its construction is such as might be obtained by the fusion in a cylinder of a number of pairs of rows of fruits[2]. The number of such double rows varies from 2 to 11 according to the variety and the strength of the plant. In every case it is easy to distinguish the rows of a single pair from the adjacent rows of contiguous pairs. There is a shallow furrow on the cob between adjoining double-rows which becomes evident if the cob be cut or broken across, though it is usually impossible to see it in the ordinary condition owing to distortion of the rows at the ends of the cob. It is said that the Maize cob invariably possesses an even number of rows of grain: this being of course the result of its construction from pairs of orthostichies.

In speaking of the Maize cob I propose to distinguish odd and even rows of seeds, in the same way as in Barley: for it may be considered as in a sense equivalent to a fused ring of ears of two-rowed Barley[3]. Holding a Maize cob upright, and considering a single pair of rows of grain towards the observer, that row which is towards his left hand will be called odd, and that row even which is towards his right hand.

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[1] Cf. Compton, 1910, p. 496, first foot-note.

[2] Another case of an hereditary ring-fasciation is described in my paper on "The Anatomy of the Mummy Pea," *New Phytologist*, x. p. 249, 1911.

[3] Hackel (*loc. cit.* p. 20) remarks that "die einzelnen Doppelzeilen je Einer Aehre von Euchlaena entsprechen."

The accompanying ground-plan (Diagram 3) will make this convention clear.

  The first leaf of a Maize seedling is folded in the same way as in the other species of Gramineae here considered : both right- and left-handed seedlings occurring. The question whether there is any connection between the direction of folding and the position of the seed on the cob was answered in the affirmative by Macloskie[1], who stated that " The grains arising on adjoining rows in the ear of corn are of different castes, and produce antidromic plants[2] " : and again, " In the particular ear examined the grains of the dextral row were all with dextral embryos, and those of the sinistral row had sinistral embryos[3]." In 1910 I sowed the seeds of rather an old cob of Maize, keeping the
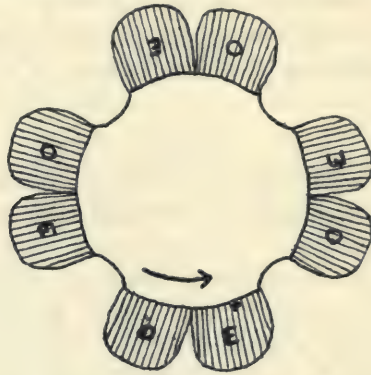
Diagram 3.

rows separate : comparatively few of the seeds germinated, but these were quite sufficient to show that both left- and right-handed seedlings were produced from the same orthostichy. I announced my failure to confirm Macloskie's statement in my earlier paper (p. 503). Later, Professor Macloskie sent me a copy of a further paper[4], of whose existence I was unaware, in which he corrects his previous statements as follows :—" I now find that two-thirds of the grains in the row opposite my right hand have the left margin of their leaves external, and the other third have their right margin external, these proportions being reversed for the row opposite my left hand."

[1] "Dimorphism and Rhizomycete of Maize," *Princeton Coll. Bulletin*, v. p. 84, Nov. 1893.

[2] "Antidromy in Plants," *Amer. Naturalist*, xxix. p. 973, Oct. 1895.

[3] "Antidromy of Plants," *Bull. Torrey Bot. Club*, xxii. p. 379, Sept. 1895.

[4] "Antidromy in Plants," *Princeton Coll. Bulletin*, vii. p. 107, Nov. 1895.

It was considered desirable to pursue the enquiry further, as statistics on the subject had not been published, and as both Macloskie's statements and my own were based (as now appears) on insufficient data. With this object a number of Maize cobs of different varieties[1] were procured, and sowings were made of the seeds from each row separately[2]. The seedlings were counted for rights and lefts as in previous experiments, and the results are summarised in Table V.

If we consider the total number of seedlings of Maize from all sources, given in the last line of the table (columns 12—15), we find that right- and left-handed plants occur in almost exactly equal numbers: the ratio LH/RH for 6189 seedlings being 1·008[3].

This is a striking difference from what was found in Barley, Oats, etc., where there is always a considerable excess of one kind, LH in Barley, RH in Oats.

Not only is the ratio for the total number of Maize seedlings very near unity, but the offspring of individual cobs, as recorded in columns 12—15 of Table V, also show a reasonably close approximation to the same ratio. In some cases it may appear that the ratio diverges from unity so far as to be outside the limits of fluctuating variability: this is the case in cobs I, II, V, VI, X, and XIV. In cobs I and VI this is clearly explicable as the result of the smallness of the numbers of seedlings; in cob X it appears to be the result of an accidental excess of seedlings from odd rows—an occurrence to be explained below. But in the other three aberrant cobs the ratios may be significant.

If we now consider the results given by odd and even rows of seeds taken separately (cols. 4—11 in Table V) a marked discrepancy from this ratio of equality becomes evident. On the whole, as shown in the last line of the table, *the offspring of the odd rows contain an excess of individuals with the first leaf folded in the right-handed direction: the even rows produce a corresponding excess of left-handed seedlings*[4]. In the case of 2966 seedlings from the odd rows of 16 ears of Maize

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[1] I am much indebted to Mr A. O. D. Mogg, of Caius College, Cambridge, for kindly naming many of the varieties used.

[2] The irregularly arranged seeds from the "butts and tips" of the cobs were discarded.

[3] It should be observed that there was an excess of 85 seedlings from odd over those from even rows; and that, for reasons to be discussed later, this renders the ratio 1·008 slightly too small: after applying the necessary correction the ratio was found to be 1·010.

[4] A somewhat fanciful comparison may be made with the Cyprinodont fish *Anableps anableps*, in which according to S. Garman (*Amer. Naturalist*, p. 1012, 1895), the males are 3/5 *RH*, 2/5 *LH*; while the females are 2/5 *RH*, 3/5 *LH*: the dimorphism being shown by the external genital organs.

TABLE V.

Ref. No. of Cob	No. of Rows	Variety of Maize	Odd Rows				Even Rows				Total			
			LH	RH	L/R	Per cent. LH	LH	RH	L/R	Per cent. LH	LH	RH	L/R	Per cent. LH
I	4	Yellow Flint	17	30	0·56	36·2	30	27	1·11	52·6	47	57	0·82	45·2
II	8	—	76	84	0·91	47·5	95	55	1·73	63·3	171	139	1·23	55·2
III	8	—	45	52	0·87	46·4	49	39	1·26	55·7	94	91	1·03	50·8
IV	8	—	74	93	0·80	44·3	81	68	1·19	54·4	155	161	0·96	49·1
V	8	—	51	117	0·44	30·4	102	67	1·52	60·4	153	184	0·83	45·4
VI	8	—	10	23	0·43	30·3	18	14	1·29	56·3	28	37	0·76	43·1
VII	10	Hickory Horsetooth	71	70	1·01	50·4	68	69	0·99	49·6	139	139	1·00	50·0
VIII	10	?	51	68	0·75	42·9	58	43	1·35	57·4	109	111	0·98	49·5
IX	12	Compton's Early	76	113	0·67	40·2	103	77	1·34	57·2	179	190	0·94	48·5
X	12	Early Star Leaming	96	124	0·77	43·6	99	98	1·01	50·3	195	222	0·88	46·8
XI	14	Austin's Colossal	120	106	1·13	53·1	96	113	0·85	45·9	216	219	0·99	49·7
XII	14	Compton's Early	50	60	0·83	45·4	59	51	1·16	53·6	109	111	0·98	49·5
XIII	16	?	95	139	0·68	40·6	137	99	1·38	59·1	232	238	0·98	49·4
XIV	18	Eureka	225	182	1·24	55·3	221	185	1·20	54·4	446	367	1·22	54·9
XV	20	Austin's Colossal?	100	109	0·92	47·9	111	111	1·00	50·0	211	220	0·96	49·0
XVI	22	Wisconsin	212	227	0·93	48·3	235	203	1·16	53·7	447	430	1·04	51·0
XVII	8	Yellow Flint	—	—	—	—	—	—	—	—	111	109	1·02	50·5
		[Miscellaneous]	—	—	—	—	—	—	—	—	65	57	1·14	53·3
		Total	1369	1697	0·857	46·16	1562	1319	1·184	54·22	3107	3082	1·008	50·20

there were 228 more *RH* than *LH* individuals: and among 2881 seedlings from the even rows there were 243 more *LH* than *RH* individuals. There can be no doubt that so large a divergence from the mean ratio unity is significant: especially as in the majority of the Maize cobs considered separately a similar result was obtained.

Further, individual rows of seed gave the same excess of *LH* or *RH* offspring according as they were even or odd. There is no need to give the data for all the cobs: but one set of results will be quoted in full as an example of the phenomena encountered. The numbers shown in Table VI were yielded by cob XIII, and may be taken as typical of those given by the others[1].

TABLE VI.

	Number of Offspring			
Number of Row	Odd Rows		Even Rows	
	LH	*RH*	*LH*	*RH*
1	14	19	—	—
2	—	—	14	16
3	7	25	—	—
4	—	—	18	14
5	16	16	—	—
6	—	—	20	11
7	9	22	—	—
8	—	—	20	12
9	13	14	—	—
10	—	—	19	9
11	15	13	—	—
12	—	—	15	11
13	13	9	—	—
14	—	—	15	13
15	8	21	—	—
16	—	—	16	13
Total	95	139	137	99

The reference numbers of the rows in the first column are arbitrary: No. 1 was a casually selected odd row, No. 2 the even row of the same pair, and so on all round the cob in the direction of the arrow in Diagram 3. The results for even and odd rows are displayed in different columns for the sake of clearness. It will be seen that five of the eight odd rows gave an excess of *RH* seedlings, and that seven of

[1] In this particular cob the seeds of each row were sown in order of position, with the object of determining whether there was any further regularity in the distribution of rights and lefts over its surface: no such pattern, however, could be detected.

the eight even rows gave an excess of *LH* seedlings. There are altogether three exceptional rows in which the proportion of rights and lefts are reversed, and one in which $LH/RH = 1$. Such exceptions occur in all the cobs studied, both in even and in odd rows: they are probably to be attributed to fluctuating variability of the same kind as was found in the ratios for ears of two-rowed Barley, and represented by normal curves[1].

It must be noticed that exceptions to the general rule are not lacking in individual cobs, as will be seen on reference to Table V. Cob VII shows a ratio of very near equality for both odd and even rows. Cob XIV shows a ratio of about 1·2, not only for the even rows (where it is normal), but for the odd rows as well: a result which may be compared with that obtained in Barley. Cob XI is the most striking exception, for here the odd rows gave a definite excess of lefts and the even rows a similar excess of rights: this being the exact reverse of the usual results. Another cob, doubtfully of the same variety as cob XI— viz. cob XV—also gave somewhat abnormal results, so that it is possible that different varieties of Maize may behave in different ways[2]. No further cobs of the same varieties as VII and XIV could be procured.

But despite exceptions, both in the offspring of individual rows and of single cobs, the general conclusion appears to be justified that odd rows on the Maize cob give an excess of right-handed, and even rows an excess of left-handed, offspring. It remains to find a reason for this behaviour.

It was thought possible that the position of the ovule with respect to its neighbours, and the consequent differences in pressure which would be experienced by ovules according as they were produced in odd or even rows, might cause differences in the shape of the early environment of the embryo which would be to some extent reflected in the mode of folding of its first leaf. This hypothesis was tested by the following experiment. A cob (XVII) was chosen whose rows were considerably distorted, and whose seeds consequently showed much variety of shape. The seeds were divided into three lots according to the relative thickness of the two lateral edges: looking at the outer end of the seed with the embryo uppermost it was placed in class (*a*) if the *LH* edge was narrower than the *RH*, class (*c*) if the reverse was the case, or class (*b*) if it could not be definitely included among (*a*)

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[1] Compton, 1910, p. 501.

[2] Further experiments are being made with this variety of Maize.

or (c). (See Diagram 4 in which one is supposed to be looking at the distal end of the seed *in situ*, the shaded semilunar area representing the position of the embryo.) The three classes were sown separately and the offspring counted, the results being as follows:—

TABLE VII.

Class	Offspring	
	LH	RH
a	38	37
b	40	39
c	33	33
Total	111	109

Thus the three classes of seeds gave precisely similar results, the ratio being equality in each case—i.e. the same ratio as given by the whole number of Maize seeds studied.
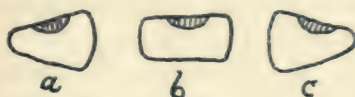
Diagram 4.

Though this experiment failed to give a positive result, it cannot be considered as conclusive evidence against the theory it was designed to test. It seems probable *a priori* that a variation in symmetry of the seedling should be produced by asymmetry of the space within which the embryo develops, and that this asymmetry should be produced by the pressure of neighbouring seeds: in fact that asymmetry in the parent should directly produce asymmetry in the offspring[1]. Several examples are indeed given by Macloskie[2] showing that the seeds from opposite sides of a bilateral fruit (silicle or legume) are "antidromic"— a word which he uses very broadly to cover many different kinds of asymmetry.

[1] An essentially similar hypothesis has been suggested by van Biervliet to account for the apparent inheritance of human right- and left-handedness (see footnote to p. 68). He remarks, "Nous savons que la femme droitière a la hanche droite plus développée, la gauchère la hanche plus forte à gauche. La structure du bassin ne pourrait-elle influer sur la position de l'embryon? ou du moins *favoriser* le développement preponderant du côté gauche?" ("L'homme droite et l'homme gauche," *Revue philosophique de la France et de l'Etranger*, XLVII. p. 385, 1899). The existence of left-handedness among the children of a *LH* father and a *RH* mother, as recorded by Jordan, tends to cast doubt on this hypothesis but does not destroy it altogether. The collection of a greater number of pedigrees is the most obvious method of solving the problem.

[2] *Bull. Torrey Bot. Club*, XXII. p. 379, 1895.

An alternative explanation would be possible on the basis of a hypothesis of a somatic segregation of characters[1] if it be assumed (i) that the characters of right- and left-handedness are represented in the gametes, and (ii) that odd rows tend to produce an excess of female gametes bearing right-handedness, even rows an excess of those bearing the character for left-handedness. (The male gametes may be left out of account when considering large numbers, for pollination is anemophilous and promiscuous.)

The alternative explanations are (i) that the influence determining the fold of the first leaf is of a feeble nature and acts upon the gamete or embryo, which itself has no special inclination to become right- or left-handed, but must choose one or the other: or (ii) that the female gametes are definitely right- or left-handed, and that a certain amount of somatic segregation occurs.

In order to maintain this latter alternative explanation it would be necessary to show that right- and left-handedness in Maize are in some way hereditary characters. The statistics give strong evidence that this is not the case. Almost all the 17 Maize cobs studied gave ratios between lefts and rights very nearly alike (p. 62): but it is almost inconceivable that in every case the parent plant should have had the same direction of fold in its first leaf.

It is evident that in different cereals there are tendencies to produce different ratios; Barley for instance gave a very different result from Oats: and it is possible that the inverse ratios given by Maize cob XI point to some similar divergencies between different varieties of Maize. But the fact that different plants give different ratios and that (in the case of Barley at least) the ratio is constant for successive generations does not imply that the characters of right- and left-handedness themselves are hereditary.

We may therefore conclude that there is no evidence for the inheritance of the direction of fold in the first leaf of Maize: and this conclusion harmonises with the similar one previously reached in the case of Barley. A further argument against the second alternative explanation is the fact that, though much sought, up to the present no direct proof of somatic segregation affecting gametes of the same sex has been obtained, however probable the existence of such a phenomenon may appear.

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[1] See W. Bateson and R. C. Punnett, "On the Inter-relations of Genetic Factors." *Proc. Roy. Soc.* B, Vol. LXXXIV. p. 6, 1911. "On Gametic Series involving Reduplication of certain Terms," *Verh. d. naturf. Ver. in Brünn*, Bd. XLIX. 1911 and *Journ. Genet.* Vol. I. 1911.

It seems therefore almost impossible to maintain the hypothesis of somatic segregation of right- and left-handedness in Maize : and despite the negative result of the single experiment on the subject, I incline to embrace the first explanation proposed, viz., that the difference in offspring between odd and even rows is due to the direct influence of spatial relationships on the developing embryo.

In two-rowed Barley no such dependence upon position was found : it may be suggested that the difference in the case of Maize is due to the close packing together of adjacent rows of seeds ; for in two-rowed Barley the individual seeds develop without lateral pressure from neighbouring rows such as they experience in Maize.

GENETIC SPIRALS.

It is not proposed at present to enter fully into the question of genetic spirals from the point of view of ratios and heredity : experiments are in progress, and it is hoped to publish a paper on this subject at some future date. Meanwhile it is interesting to remark that the ratio LH/RH, for genetic spirals also, diverges from equality in a more or less marked degree in the cases investigated. Bonnet[1] found 43 RH and 30 LH stems among a collection of 73 plants of Chicory. Out of 458 plants of *Lepidium sativum* I found 241 LH and 217 RH.

Not only is there a divergence from equality in the case of different plants, but on one and the same plant there may be a considerable excess of branches showing one spiral over those showing the reverse one. Valuable data were obtained[2] by Dr A. H. Church and Mr E. G. Broome with respect to the genetic spiral of certain individuals of different species of Pines : the following numbers were recorded :—

TABLE VIII.

Tree		Year	Number of cones	Per cent. LH	Per cent. RH
P. austriaca	...	1900	100	41	59
,,	...	1901	1000	46·4	53·6
,,	...	1902	500	46·0	54·0
,,	Average	1900—02	1600	45·94	54·06
P. pumilio	...	1900	100	47	53
P. laricio	...	1900	100	68	32
,,	...	1901	400	71	39
,,	...	1902	600	69·16	50·83
,,	Average	1900—02	1100	69·82	30·18

[1] *Recherches sur l'usage des feuilles*, p. 179, 1754.

[2] A. H. Church, *The Relation of Phyllotaxis to Mechanical Laws*, pp. 92, 351, London, 1904.

These results are very striking, and as Church remarks, "The element of chance appears quite out of the question" in the case of *P. laricio.*

It may be suggested that perhaps differences in conditions (sunlight, prevailing winds, etc.) between opposite sides of the tree may have caused an excess of cones to arise towards one side: and that, since the genetic spiral of a lateral branch depends on its mode of insertion, this might be the cause of the excess of one kind of spiral among the cones. At present, however, it is impossible to decide whether this accounts for the phenomena, or whether there is a definite tendency in the apical meristem to produce a genetic spiral of one direction rather than the other.

Conclusions.

The present paper deals with the dimorphism found in certain Gramineae in respect of the mode of folding of the first leaf of the young plant. It is in part a continuation of a previous paper (cited above) to which reference should be made for an explanation of the conventions used in describing the phenomena, and for a summary of the literature of the genetics of right- and left-handedness in general[1].

[1] Further references to works on the genetics of right- and left-handedness may be inserted here.

H. E. Jordan ("The Inheritance of Left-Handedness," *American Breeders' Magazine*, Vol. II. pp. 19, 113: 1911) gives a number of human pedigrees which show that functional left-handedness is hereditary, in certain cases apparently in conformity with a simple Mendelian scheme. The most remarkable human pedigree on record is perhaps that given by Aimé Péré (*Les courbures latérales normales du rachis humain*, Toulouse, 1900, p. 71: quoted by D. J. Cunningham, "Right-Handedness and Left-Brainedness," *Journ. Anthropol. Inst.* XXXII. p. 273, 1902). In this family no fewer than twenty-six left-handed individuals are recorded: the marriage of a *LH* ♀ × *RH* ♂ gave eight sons and six daughters, all left-handed,—a fact in strong contrast to Jordan's hypothesis of the dominance of right-handedness, and suggesting the reverse assumption. A number of other instances of inheritance of left-handedness in man are given by F. Lueddeckens (*Rechts- und Links-händigkeit*, Leipzic, 1900). A general summary, with a full bibliography, of our present knowledge of human asymmetry is given by K. von Bardeleben ("Ueber bilaterale Asymmetrie beim Menschen und bei höheren Tieren," *Anat. Anz. Ergänzungsh.* z. Bd. XXXIV. p. 2, 1909).

H. de Vries, *The Mutation Theory* (Engl. trans.), II. p. 561, London, 1911, finds that the peculiar torsion of the stem in certain races of *Dipsacus sylvestris* is partly hereditary, but that the *direction* of the twist is not transmitted. About equal numbers of *RH* and *LH* individuals are produced normally: and in an experiment in which only *RH* plants were allowed to flower in two successive years the offspring comprised 245 *RH* and 239 *LH* plants.

In this paper it was shown that in two-rowed Barley there is no inheritance of the right- and left-handed characters, nor is there any regularity in the distribution of right- and left-handed seedlings on the ear.

(1) The previous conclusions in the case of two-rowed Barley are confirmed by new experimental results: in particular it is amply shown that, while the ratio of lefts to rights is maintained through three successive generations, the kind of asymmetry itself is not inherited.

(2) In Maize it also seems clear that there is no inheritance of right- and left-handedness as such.

(3) In six-rowed Barley an excess of LH seedlings was also found: and there was no conspicuous variation in the ratio LH/RH as between different rows of grain on the ear: but the numbers examined were too small to be decisive.

(4) In Maize the ratio LH/RH is very near unity (1·010). *Setaria italica* shows, like Barley, an excess of left-handed seedlings, LH/RH being 1·19 (54·1 °/₀ LH). Both stereo-isomeric forms are also present in Rye.

(5) Oats show a considerable excess of right-handed seedlings, the ratio LH/RH being 0·814 (44·88 °/₀ LH): it thus gives a result the inverse of that found for Barley. This may be in some way connected with the fact that in Barley the mature leaf-blades are generally slightly twisted into a right-handed screw, while in Oats the torsion is in the reverse direction.

(6) The seeds on a Maize-cob give different ratios of left-handed seedlings according to their position: the seeds on odd orthostichies giving an excess of right-handed, those on even rows an excess of left-handed, offspring. This result is obtained in the majority of cobs, but there are exceptions. On the average, deduced from 5847 seedlings from 16 cobs of several varieties and sizes, the ratio LH/RH for even rows is 1·184, for odd rows 0·857 (54·22 and 46·16 °/₀ LH respectively).

(7) An attempt to discover whether there was any connection between the shape of the seed of Maize and the direction of fold of the first leaf gave a negative result.

(8)   Nevertheless, it appears probable that the diverse ratios given by odd and even rows in Maize result from a difference in shape of the environment of the developing embryo, this being connected with the different spatial relationship of the seeds on the cob.   An alternative hypothesis involving somatic segregation of symmetry characters represented in the gametes is dismissed as highly improbable.

BOTANY SCHOOL, CAMBRIDGE,

November 1911.

SPECIES HYBRIDS OF *DIGITALIS*.

By W. NEILSON JONES, M.A.,
University College, Reading.

Historical.

Hybrids between the various species of *Digitalis* have been recorded from time to time and in many cases can be produced without difficulty or even occur in nature. A general summary of the literature up to 1881 is given by Focke in *Die Pflanzen-Mischlinge.* The hybrids most frequently recorded are those between *D. purpurea* and *D. lutea*, and between *D. purpurea* and *D. grandiflora*[1], the accounts being somewhat contradictory.

The cross between *D. purpurea* and *D. lutea* has been re-investigated by J. H. Wilson and described in the Report of the 3rd International Conference on Genetics (1906). Briefly, the facts concerning the hybridising of these two species are as follows:

It was found much easier to effect the cross when *D. purpurea* was used as pollen parent. The reciprocal crosses differed from one another as to their flowers, in each case more closely resembling the seed-parent. The hybrid having *D. purpurea* as seed-parent had larger and wider flowers of a rose colour although the *D. purpurea* used was a white flowered variety without coloured spots (from which it was concluded, incidentally, that colour may be latent in a white foxglove). In the cross in which *D. lutea* was used as seed-parent the flowers were narrower and in colour creamy-yellow or almost white with a pale rose flush, even when a purple *D. purpurea* was used. The flowers of both hybrids had purple spots inside the corolla tube.

In a subsequent series of experiments Wilson found that the F_1 plants of both hybrids varied considerably among themselves as to flower colour. This was possibly due to the use of an impure strain.

The reciprocals were indistinguishable until they flowered.

No fertile seed was obtained from either hybrid.

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[1] *D. grandiflora* L. is synonymous with *D. ambigua* Mur.

The two species *D. purpurea* and *D. grandiflora* have recently been hybridised by me at University College, Reading. The results of these experiments form the subject of the present paper, before describing which a short summary of previous references to the cross may be of interest. For convenience *D. purpurea* and *D. grandiflora* will be, in what follows, referred to as *D.P.* and *D.G.*

Kölreuter failed to obtain viable seed as the result of crossing *D.P.* and *D.G.*, whichever species was used as seed-parent.

Gärtner obtained the hybrid (*D.P.* × *D.G.*) in quantity, but only a single example of the reciprocal cross: he found both hybrids infertile[1] and (*D.P.* × *D.G.*) the more like *D.P.*[2] In another passage, however, he says the latter hybrid resembles more the pollen-parent[3].

Of the hybrid (*D.P.* × *D.G.*) he writes: "flowers somewhat shorter than *D.P.*, about 4 cm. long and 2·2 cm. broad, wider at the mouth. Colour: pale red with a yellow tinge and with very pale, hardly noticeable, irregular, confluent markings, especially in the under part of the corolla." (*D.G.* × *D.P.*) is recorded as more slender in growth and showing a greater resemblance to *D.P.* Flowers 5 cm. long and 1 cm. broad, of a pale purple colour, with externally a yellowish, internally a pale purplish sheen. Numerous small dark purple spots were present.

Godron also succeeded in obtaining the hybrid (*D.G.* × *D.P.*), which he found fertile when pollinated by *D.P.* The resulting F_2 plants had smallish, bright red flowers, while their leaves closely resembled those of *D.P.*

Focke found no difficulty in obtaining (*D.P.* × *D.G.*), but apparently was unsuccessful with the reciprocal cross. In describing these hybrids he says that in contrast to the case of (*D.P.* × *D. lutea*), the leaves here show a considerable resemblance to those of *D.P.* The young leaves of the hybrid are broad and downy but have no well-marked petiole. The flowers are intermediate in size between those of *D.P.* and *D.G.* The colour of the flowers is: "outside, a dull purple, paler on lower side; inside, paler purple with washed-out yellow reticulate markings and having dark purple spots on lower side, each surrounded by a yellowish-white halo."

This description will be found to agree essentially with that given below of this hybrid grown at Reading, where I have obtained plenty of plants of (*D.P.* × *D.G.*), but only one of (*D.G.* × *D.P.*). There seems no doubt therefore that it is much easier to effect this cross when *D.P.*

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[1] Flor. (*B.Z.*) 1833, p. 365.　　　　　[2] *Loc. cit.* pp. 288, 401.
[3] *Loc. cit.* pp. 225, 226.

is used as seed-parent. In this connection it may be noted that the hybrid (*D.P.* × *D.G.*) has been found in many places growing wild, e.g. in Dresden, Hanover, &c., and is referred to as *D. fulva* Lindl. A plant, evidently of the reciprocal (*D.G.* × *D.P.*), was recorded by G. F. W. Meyer in the Harz in 1827 and referred to as *D. fucata* Ehrh.

Another species hybrid mentioned by Focke is that between *D. lutea* and *D. obscura*.

The reciprocals were much alike in habit and leaf characters, but very different as to flowers, (*D. lutea* × *D. obscura*) having a much longer, narrower, and straighter corolla than the reciprocal.

In the reciprocal crosses between *D. grandiflora* and *D. lanata* Gärtner's description indicates that each of the hybrids is more like the seed-parent (p. 225). On another page, however (p. 404), he classes this cross among those that follow the paternal parent more closely.

These contradictory statements may have resulted from the then prevalent belief that the characters of the male were the more potent in heredity[1], a belief no doubt fostered by Schleiden's interpretation of the fertilisation process.

The general conclusions to be drawn from the above records are :

(1)   The reciprocal crosses between *Digitalis* species are unlike.

(2)   In the crosses between *D. purpurea* and *D. lutea*, between *D. lutea* and *D. obscura*, and between *D. grandiflora* and *D. lanata* the reciprocals show a greater resemblance to the seed-parent than to the pollen-parent.

(3)   Regarding the cross between *D. purpurea* and *D. grandiflora* the evidence is somewhat contradictory; but on the whole points to the reciprocals resembling the seed-parent as in the other cases[2].

The result of the present investigation supports this view.

The present investigation.

In the crosses made at Reading between *D. purpurea* (*D.P.*) and *D. grandiflora* (*D.G.*) both reciprocals were obtained : they differ from one another and from the parents. Speaking generally, the characters of both hybrids are intermediate between those of the parent species, but each hybrid resembles the seed-parent to a greater degree. An attempt has been made to analyse these differences with the object of determining whether the greater general resemblance to the seed-parent was due to the fact that although the reciprocals possessed similar

[1] See *Genetics Report*, III. p. 213.
[2] See Focke, p. 470.

characters each of these characters was developed rather differently according to which species was used as female parent, or whether the one hybrid possessed characters derived from its female parent which were entirely absent from the reciprocal and *vice versa.*

The *D. grandiflora* used in these experiments was raised from seed obtained from Haage and Schmidt of Germany and has given offspring true to type for several generations.

The *D. purpurea* bore flowers of a deep purple colour. Unfortunately the same plant was not used for both crosses : that used in the case of (*D.P.* × *D.G.*) subsequent breeding proved to be a pure strain. There is no reason to believe that the plant used in the reciprocal cross was otherwise, although this was not proved by selfing. Below is a detailed comparison of these four plants—two parents and reciprocals.

1. *Stature.* *D.P.* = 4 feet or more. *D.G.* = about 2 feet. (*D.P.* × *D.G.*) = about 3 feet. (*D.G.* × *D.P.*) = about $2\frac{1}{2}$ feet.

Thus the stature of the hybrids is intermediate between the parents ; that having the taller female parent being of greater size than the reciprocal.

Of course these numbers are only to be taken in quite a general sense, as a well-grown plant of (*D.G.* × *D.P.*) would no doubt be taller than a poorly grown plant of (*D.P.* × *D.G.*). Still, under similar cultural conditions there seems no doubt that (*D.P.* × *D.G.*) is distinctly taller than (*D.G.* × *D.P.*). The season was a very dry one, so that all the plants were rather below the normal height.

2. *Inflorescence.* The form of the inflorescence in the two species is quite distinct ; the raceme of *D.P.* is straight (Plate III, fig. 1), while that of *D.G.* is strongly curved at the apex (Plate III, fig. 2). In (*D.P.* × *D.G.*) the end of the raceme is curved, but not through more than a right angle—the plants having in consequence quite a characteristic appearance (Plate III, fig. 3). In (*D.G.* × *D.P.*) the form of the inflorescence is indistinguishable from that of *D.G.*

3 and 4. *Flower.* The corolla of *D.P.* is dark magenta-purple and the lower side of the tube marked on the inside with dark purple spots of varying size (Plate IV, fig. 4). That of *D.G.* is a rather dark yellow (the shade varying somewhat in different plants) and in place of the spots occurring in *D.P.* is a brown network-like pattern (Plate IV, fig. 5). This network marks the position of the veins of the corolla and, as can be seen from the figure, the markings are thicker and more pronounced transversely. Comparing now the hybrids : (*D.P.* × *D.G.*) is a salmon-purple colour with dark red spots (Plate IV, fig. 6), while

Character	D.P.	(D.P. × D.G.)	(D.G. × D.P.)	D.G.
1. Stature	4 feet	3 feet	2½ feet	2 feet
2. Inflorescence. (Plate III, figs. 1—3)	Straight	Half curved	Curved right over	Curved right over
3. Flower colour. (Plate IV, figs. 4—7)	Purple	Red with yellowish tinge	Yellow with pink flush	Yellow
4. Markings on Corolla. (Plate IV, figs. 4—7)	Many large red spots	Few small red spots	Few small red spots	No spots, brown reticulate markings
5. Shape of Corolla.	See Plate IV, fig. 4	Intermediate, most like D.P. (Plate IV, fig. 6)	Intermediate, most like D.G. (Plate IV, fig. 7)	See Plate IV, fig. 5
6. Spots on anthers	Spotted	Slightly spotted	Slightly spotted	No spots
7. Sepals. (Text-figs. 5—8)	Large, broad and veined	Intermediate, rather more like D.P.	Intermediate, rather more like D.G.	Small, narrow and not veined
8. Calyx. (Text-figs. 1—4)	Sepals overlap	Sepals overlap	Sepals not overlapping	Sepals not overlapping
9. Hairs on sepals. (Text-figs. 21—25)	Clubbed and pointed	Clubbed only	Clubbed only	Clubbed only
10. Fruits. (Text-figs. 9—16)	Curved. Two furrowed	? slightly curved ? 2 furrowed	? Straight ? 4 furrowed	Straight 4 furrowed
11. Nectaries. (Text-figs. 9—12)	Small and inconspicuous	Somewhat small and inconspicuous	Rather large and conspicuous	Large and conspicuous
12. Leaf-shape	Broad, obtuse, petiolate	Intermediate, rather nearer D.P.	Intermediate, rather nearer D.P.	Narrow, pointed, not conspicuously petiolate
13. Leaf Venation. (Plate V, figs. 8—11)	Conspicuous network of large veins raised above general surface	Network of veins raised above general surface	Some veins slightly raised above general surface, hardly forming a network	A few veins raised slightly above general surface, not forming network
(Text-figs. 17—20)	Large, transversely running veins	Some large, transversely running veins	Very few large, transversely running veins	No large, transversely running veins
14. Leaf margin. (Text-figs. 34—37)	Crinkled, coarsely crenate	Not crinkled, coarsely crenate-serrate	Not crinkled, coarsely dentate, teeth prolonged into points	Not crinkled, serrate
15. Epidermal cells. (Text-figs. 26—33)	Smaller than D.G.	Intermediate	Intermediate	Larger than D.P.
16. Thickness of lamina. (Text-figs. 42—45)	·2 mm.	·2 mm.	·2 mm.	·33 mm.

($D.G. \times D.P.$) is a greenish-cream (very much lighter than in $D.G.$) with pale rose flush above, also with spots (Plate IV, fig. 7). In neither hybrid are the brown markings characteristic of $D.G.$ apparent.

5.   The shapes of the corolla tubes in the hybrids also show a preponderant influence of the female parent (see Plate IV).

6.   The anthers of all except $D.G.$ have dark purple spots; the spots in the hybrids, however, are smaller and less numerous than in $D.P.$

Calyx.   In all the four forms of *Digitalis* under consideration the sepals are of three sizes; the upper being the smallest, the two lower laterals largest, and the two upper laterals intermediate in size.

7.   Comparing now corresponding sepals of these plants: those of $D.P.$ are large, broad and ovate and have numerous, conspicuous veins raised above the surface (text-fig. 5): those of the other species $D.G.$ are small and narrow with few, very inconspicuous veins not raised above the surface, lateral branchings being quite invisible in the fresh green sepals (text-fig. 6).

The sepals of the two hybrids are intermediate in every particular, but differ from one another in that ($D.P. \times D.G.$) (text-fig. 7) is nearer the $D.P.$ type as regards shape and veining than is ($D.G. \times D.P.$) (text-fig. 8). In this latter, the sepals tend to be almost the same width throughout their length, instead of narrowing gradually towards their bases.

8.   The way in which the sepals of any one flower are united is also characteristic: in $D.P.$ and ($D.P. \times D.G.$) the members of the calyx overlap one another in the fully opened flower (text-figs. 1 and 3), while in $D.G.$ and ($D.G. \times D.P.$) gaps occur between them (text-figs. 2 and 4).

9.   The nature of the multicellular hairs on the calyces is also worth notice.   In $D.P.$ the hairs are long, slender and of two kinds, viz. with pointed end-cells and rounded end-cells (text-figs. 17 and 18).

In $D.G.$ the hairs are all of one kind—short, stout and with rounded end-cells (text-fig. 19).

In the hybrids the hairs are intermediate in size and all have rounded end-cells (text-figs. 20 and 21), there being no very noticeable difference between the reciprocals.

This distinction in size and structure of the hairs was not found to hold in the case of the ordinary foliage leaves, on which the hairs are of different character and common to all four types of plants.

Figs. 1—16.

DP 17   18   DG 19   DP × DG 20   DG × DP 21

$\frac{1}{10}$ *mm.*

Figs. 17—21.
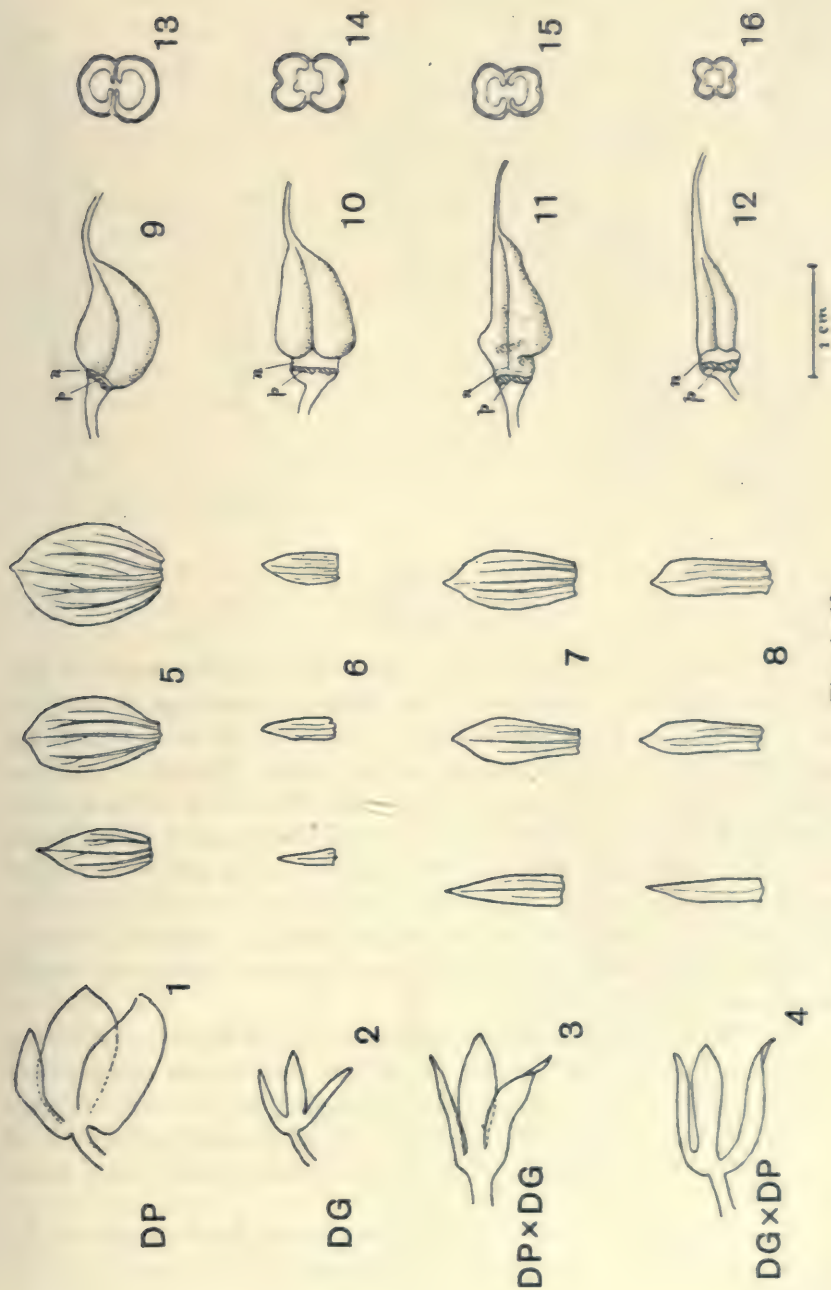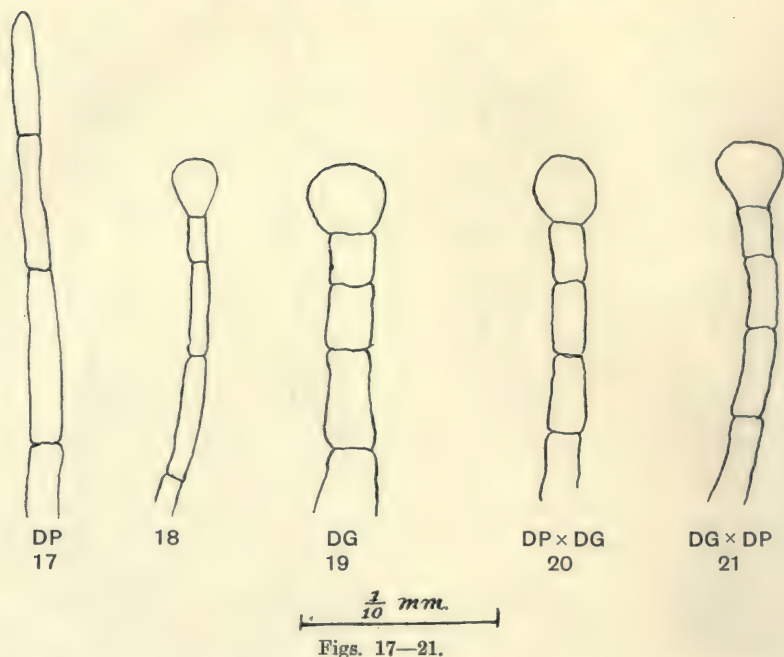
10. *Fruits.* The fruits of *D.P.* are markedly curved longitudinally and have furrows running along the sides *only* (text-figs. 9 and 13): those of *D.G.* are almost cylindrical and have furrows running not only along the sides but also top and bottom, as is evident in the cross-section of the ovary (text-figs. 10 and 14). The fruits of the hybrids were too imperfect to allow any very definite conclusions to be drawn as to their shapes had they swollen properly; but in (*D.P.* × *D.G.*) the upper and lower furrows are practically invisible on the outside, but are indicated on the inside wall of the capsule (text-figs. 11 and 15); while in (*D.G.* × *D.P.*) these furrows are also more or less observable on the outside (text-figs. 12 and 16).

11. Although these general characters of the hybrid capsules are admittedly unsatisfactory for the reason given above, the nectaries are more completely developed and easy of comparison. Thus the nectary in *D.P.* is a rather inconspicuous, narrow band of tissue at the base of the capsule and forms part of the general curve of this latter (text-fig. 9 *n*).

The nectary of *D.G.*, on the other hand, is rendered conspicuous by its breadth and by not forming part of the general curve of the capsule (text-fig. 10 *n*).

The nectaries of the hybrids are intermediate in character between those of the two species, but differ from one another in inclining markedly towards the respective female parents as to size and shape in relation to the capsule (text-figs. 11 and 12).

The differences between the four plants is not confined to the flower parts, as the leaf characters alone are sufficiently distinct to enable one easily to assign any plant to one of the four classes.

12. *Leaves.* The leaves of *D.P.* are large and broad (the radicle ones being as much as 30 cm. long and 10 cm. broad), ovate in shape and, with the exception of those some way up the flowering axis, with a well-marked petiole.

The leaves of *D.G.* are never as large as the above (the larger ones are about 18 cm. long and 3 cm. broad) and much more linear in shape. They taper gradually to a rather sharp apex instead of being obtuse as in *D.P.*, and there is no demarcation between lamina and petiole, the leaf gradually narrowing towards its base which is much broader than in *D.P.*

The leaves of the hybrids are intermediate in size and shape : those of (*D.P.* × *D.G.*), however, resemble more closely the leaves of *D.P.*, while the leaves of (*D.G.* × *D.P.*) are almost indistinguishable from *D.G.* as regards shape.

The above differences in leaf-form are most noticeable in the radicle leaves.

13. Much more definite than the differences in shape are the differences in texture and surface of the leaves of the four types. Photographs of the under sides of the radicle leaves of *D.P.*, *D.G.*, (*D.P.* × *D.G.*), and (*D.G.* × *D.P.*) are shown in Plate V.

In *D.G.* (Plate V, fig. 9) it will be seen, that while the midrib and some of the larger longitudinally running veins are raised somewhat above the general surface of the leaf, the smaller veins are quite invisible.

In *D.P.* (Plate V, fig. 8), however, all but the very smallest veins stand up prominently above the surface in the form of a network covering the entire under surface of the leaf.

There is a corresponding difference in the upper surfaces of these leaves ; *D.G.* being quite smooth except for slight depressions along the midrib and some of the larger veins, while the whole upper surface of *D.P.* is covered with a conspicuous network of furrows.

As will be seen from figs. 10 and 11 of Plate V, the hybrids are intermediate between these two conditions, the reciprocals differing in

that each follows the maternal type. The upper surfaces of the leaves show similar peculiarities.

That the above differences are due not merely to degree of prominence of the veins but also to a dissimilar system of venation may be seen from text-figs. 22—25.
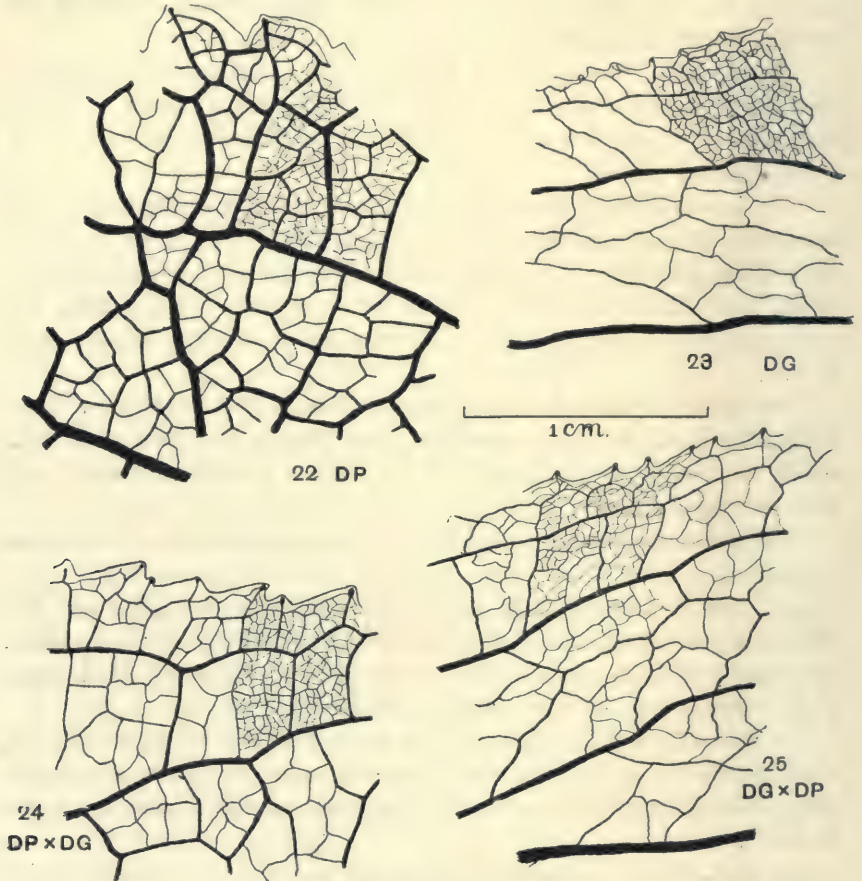
Figs. 22—25.

These are camera lucida drawings of leaves bleached in Mayer's solution, stained in safranin and afterwards cleared and mounted in Canada balsam. In *D.P.* there is a network system of large veins, whilst very fine veins fill up the meshes of this network. In *D.G.*, on the other hand, the only large veins are the midrib and a few longitudinally running laterals (which are much smaller than the large veins of *D.P.*)—the rest of the system consisting entirely of veins all of much

the same size and considerably broader than the finest veins of *D.P.*
The reciprocals (text-figs. 24 and 25) differ from one another in the
greater resemblance to the seed-parent in each case.

14. The margins of the leaves also show well-marked characters.
That of *D.P.* is coarsely crenate and (presumably by its more rapid
growth relatively to the rest of the leaf) crinkled so that it cannot be
made to lie flat (text-fig. 34).

The margin of *D.G.* is serrate, the coarseness of the serrations
varying greatly in different leaves of the same plant, but always much
smaller than the crenations of *D.P.* (text-figs. 35 *a* and 35 *b*). In
(*D.P.* × *D.G.*) there is an intermediate condition of affairs both as to
size and shape of the marginal projections (text-fig. 36): while in the
reciprocal these projections are rather larger and run out into long
points, the furrows between always forming a smooth concave curve
(text-fig. 37) instead of a sharp angle.

15. Text-figs. 26, 27 and 28, 29 are camera lucida drawings of
the upper and lower epidermis of leaves of *D.P.* and *D.G.*

Comparison shows that the cells of *D.G.* are larger and have more
irregular walls than *D.P.* The stomata of *D.G.* are larger though less
numerous than in *D.P.* Since *D.P.* has the larger leaf this is the
reverse of what might have been expected.

The epidermal cells from the leaves of the hybrids are intermediate
in character with a slight leaning towards the female parent (text-
figs. 30, 31 and 32, 33).

The epidermal cells differ so much in different leaves and different
parts of the same leaf that this character is somewhat indefinite, although
the drawings were taken as representing the average condition.

16. Transverse sections show that the leaves of *D.G.* (text-fig. 43)
are thicker than *D.P.* (text-fig. 42), the average thickness in the two
cases being ·2 mm. for *D.P.* and ·33 mm. for *D.G.* Both reciprocals
resemble *D.P.* as regards thickness of leaf (text-figs. 44 and 45). The
greater thickness of the leaves of *D.G.* seems due both to the larger size
of individual cells and also to the greater number present.

Discussion of results.

It must be concluded from the above analysis of the reciprocal
crosses between *D. purpurea* and *D. grandiflora* that, in general, the
expression of any character in the hybrids is intermediate between its
expression in the two parents, the reciprocals differing from one another
in that each shows a greater resemblance to its seed-parent.

Species Hybrids of Digitalis

Figs. 26—33.

34   DP

35 a   DG

35 b   DG

36   DP × DG

37   DG × DP

1 cm.

Figs. 34—37.

38

42

39

43

40

44

41

45

1 mm.
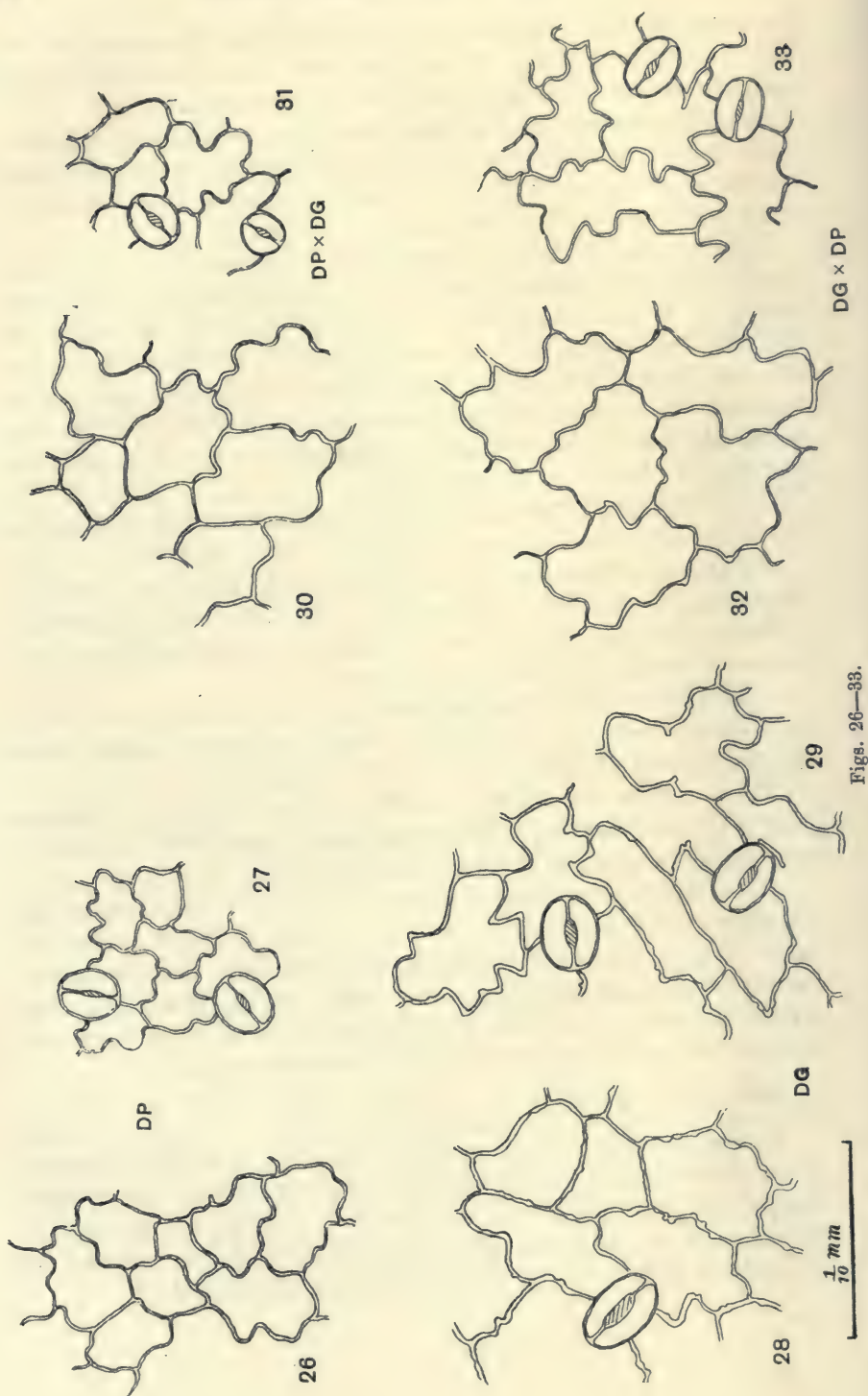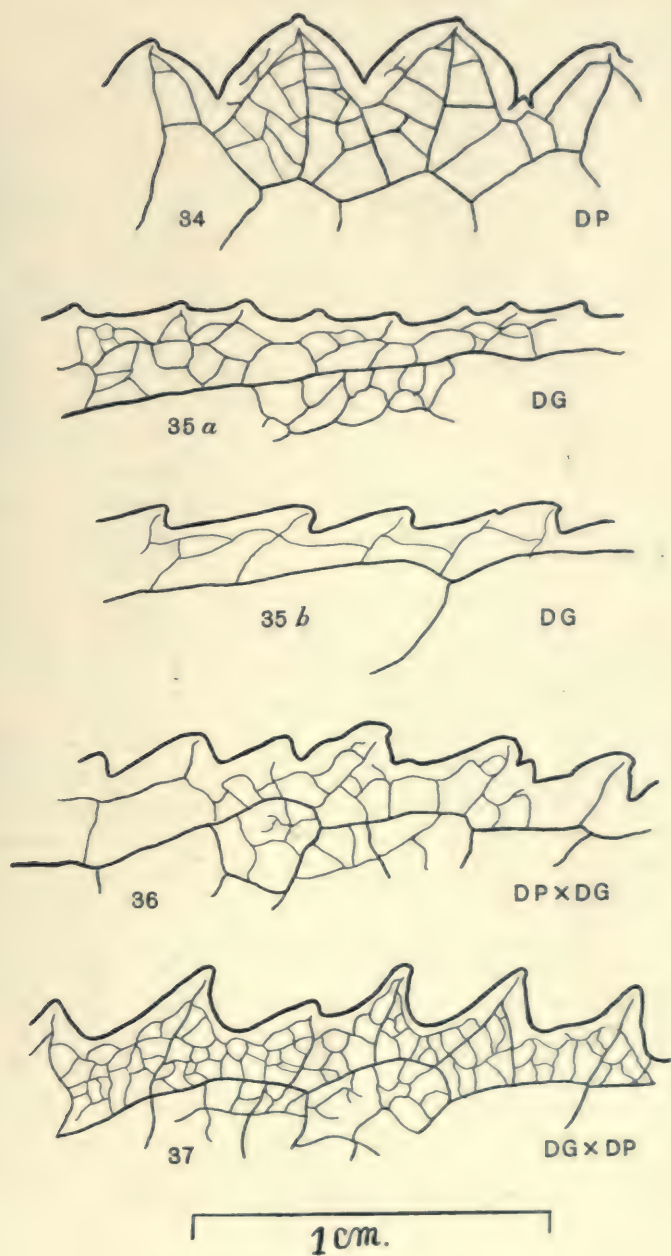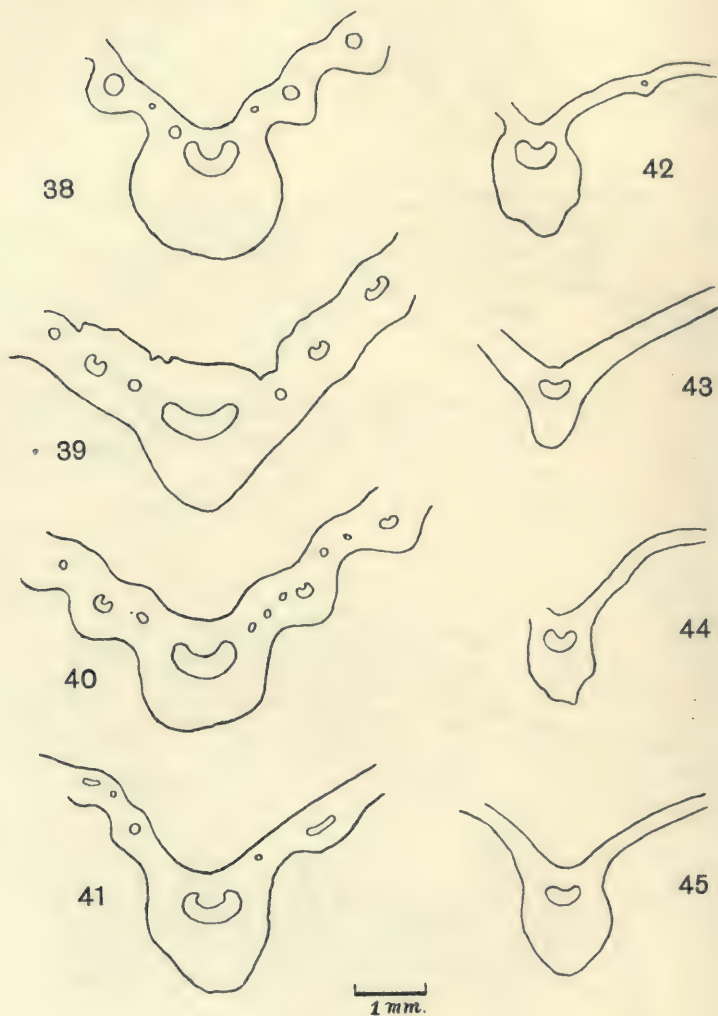
Figs. 38—41 are sections through leaf bases.

Figs. 42—45 are sections halfway up leaf laminas.

In the case of certain characters, however, there is complete dominance in the hybrids irrespective of whether these characters are derived from the maternal or paternal sides (e.g. the thickness of the leaf, the possession of a swollen end-cell by the calyx hairs and the occurrence of purple spots on the inside of the corolla tube).

It would seem probable that cases where a character is completely dominant in the hybrid, and where it is exactly intermediate between its expression in the parents, are simply the end terms of a continuous series. Also, if there is a tendency for the reciprocals to differ, one might expect it to be most evident in cases where the dominance is not very definite.

In fact the degree of dominance of any character (i.e. the degree to which any character is expressed in an individual heterozygous for that character), and the difference occurring between the reciprocals in the expression of a character, are possibly two quite distinct phenomena resulting from entirely different causes. Varying degrees of dominance are found in varietal crosses and the phenomenon does not appear to be of much significance. It is in the latter peculiarity—the difference between the reciprocals—that most interest lies.

The first explanation that suggests itself is that the cytoplasm of the egg-cell has an influence on the subsequent development of the embryo. This influence may be due to hereditary determinants being carried by the cytoplasm. For example, if the cytoplasm of the egg-cell is provided with a certain kind of plastid, the seedling resulting from fertilisation of this egg-cell will contain these plastids. If however the cytoplasm of the egg-cell does not contain the plastids, then the resulting offspring will be devoid of them whatever the nature of the individual from which the male gamete is derived.

Sap colours might be inherited similarly by means of vacuoles, if there is any truth in the tonoplast theory of Went and de Vries.

The hereditary behaviour of "Albomaculata," varieties of *Mirabilis Jalapa, Antirrhinum*, &c., is commonly accounted for on some such basis as the above[1].

But much more subtle determinants, such for example as specific enzymes, might be carried by the cytoplasm of the egg-cell. These would ensure that the course of metabolism followed would be that of the female parent: and such cytoplasm might provide an environment

[1] See Baur, *Einführung in die experimentelle Vererbungslehre*, 1911, p. 163, &c.; cf. however Baur's hypothesis regarding white-leafed Pelargonium, p. 167.

more favourable to the expression of the characters of its own nucleus (the female nucleus) than of those brought in by the male nucleus.

But although such an explanation would fit the present case of *Digitalis*, it is difficult to apply to the case of *Œnothera* recently investigated by de Vries[1], in which the reciprocals follow the male parent[2].

Even in *Œnothera*, however, although the reciprocals resemble the male parent in respect to size and shape of leaves and flowers, &c., they take after the female parent in some features: e.g. (*Œ. biennis* × *Œ. muricata*) has the green leaves of *biennis* and not the blue-green of *muricata*.

De Vries found that both reciprocals between the above two species bred true for an indefinite number of generations. He has found also that the result of crossing the hybrids together is different according to which is used as seed-parent.

Thus, using *B* and *M* to stand for *Œ. biennis* and *Œ. muricata*, the cross $(B \times M) \times (M \times B)$ gave plants resembling pure *Œ. biennis* with no trace of *muricata*: while $(M \times B) \times (B \times M)$ gave as offspring pure *muricata*.

The hypothesis adopted by de Vries to explain the above results is that pollen grains and ovules carry different sets of characters. If the characters carried by *Œ. biennis* ovules and pollen grains are represented by $B\,♀$ and $B\,♂$, and those by *Œ. muricata* by $M\,♀$ and $M\,♂$, then $(B \times M)$ has the constitution $B\,♀\ M\,♂$, and is an entirely different cross from $(M \times B)$ which has a constitution $M\,♀\ B\,♂$.

It is further supposed that there is selective sterility of half the pollen grains and ovules in such a way that the ovules of the hybrid $(B \times M)$ are of one constitution only, namely $B\,♀$—those of the constitution $M\,♂$ being abortive. Similarly, the pollen grains are of one kind only—namely those carrying $M\,♂$.

The same scheme is applied *mutatis mutandis* to the reciprocal cross $(M \times B)$.

Thus it will be seen that on selfing $(B \times M)$ there will be a meeting of male and female gametes carrying respectively $M\,♂$ and $B\,♀$, giving a zygote $B\,♀\ M\,♂$. In other words the hybrid breeds true.

[1] " Ueber doppeltreziproke Bastarde von *Œ. biennis* und *Œ. muricata*," *Biol. Centralbl.* **xxxi.** p. 97, Feb. 1911.

[2] For other cases in which reciprocals differ in the direction of resembling male parents see Focke.

Papaver somniferum L. × *P. caucasicum* M.B. as regards general size, colour of flowers, &c. (Godron, *Rev. d. Sc. natur.* 1878, N. 2).

Probably flower colour in *Petunia* (Gärtner).

Also when $(B \times M)$ is pollinated by $(M \times B)$, the factors involved will be $B \,♀$ from the $(B \times M)$ ovules and $B \,♂$ from the $(M \times B)$ pollen grains. The resulting zygote having the constitution $B \,♀\; B \,♂$ resembles pure *biennis*. Thus the hypothesis suggested by de Vries fits the facts observed in *Œnothera* completely. The only criticism to be brought against it is, that although sterility of half the ovules and pollen grains is known to occur in many *Œnothera* species[1], there is at present no proof that the sterility is selective.

The behaviour of other species of *Œnothera* when hybridised would appear to be explicable in the same way.

A similar explanation, depending on a difference in constitution between ovules and pollen grains, might be advanced to account for the behaviour of the *Digitalis* hybrids; only in this case it would be the ovules which carried characters most like the parent plant instead of the pollen grains as in *Œnothera*. What the result of crossing the *Digitalis* hybrids together might be, and whether they would behave similarly to the *Œnothera* hybrids, it is impossible to say, since the crosses proved infertile with me.

Seed however was obtained as the result of pollinating the hybrids by their parents, and the resulting seedlings may throw some light on this problem, although they are as yet too young to show any characteristic features.

The inheritance of doubleness, &c., in Stocks has also been explained on the basis of a difference between pollen grains and ovules[2].

The phenomena exhibited by *Œnothera* and *Digitalis* are not necessarily, of course, of the same kind: and it is possible that the resemblance of the reciprocal crosses to the pollen parent as seen in the former is due to a difference in constitution of pollen grains and ovules, while their resemblance to the seed-parents in the latter is due to the influence of the cytoplasm of the egg-cell (either direct or indirect).

[1] J. M. Geerts, "Beiträge zur Kenntniss der Cytologie und der partiellen Sterilität von Œ. Lamarckiana," *Recueil Trav. Bot. Néerlandais*, v. p. 93, 1909.

[2] E. R. Saunders, *Journal of Genetics*, I. 4, 1911.

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EXPLANATION OF FIGURES.

D.P. and *D.G.* are used throughout as abbreviations for *D. purpurea* and *D. grandiflora.* All illustrations are either photographs or drawn to scale with camera lucida.

PLATE III.

1. Inflorescence of *D.P.*
2.   ,,   *D.G.*
3.   ,,   (*D.P.* × *D.G.*).

PLATE IV.

4. Flower of *D.P.*
5.   ,,   *D.G.*
6.   ,,   (*D.P.* × *D.G.*).
7.   ,,   (*D.G.* × *D.P.*).

PLATE V.

8. Underside of leaf of *D.P.*
9.   ,,   ,,   *D.G.*
10.   ,,   ,,   (*D.P.* × *D.G.*).
11.   ,,   ,,   (*D.G.* × *D.P.*).

The scale by fig. 11 marks centimetres.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.　D. P.

Fig. 5.　D. G.

Fig. 6.　D. P. × D. G.

Fig. 7.　D. G. × D. P.

Fig. 8.

Fig. 9.

Fig. 10.
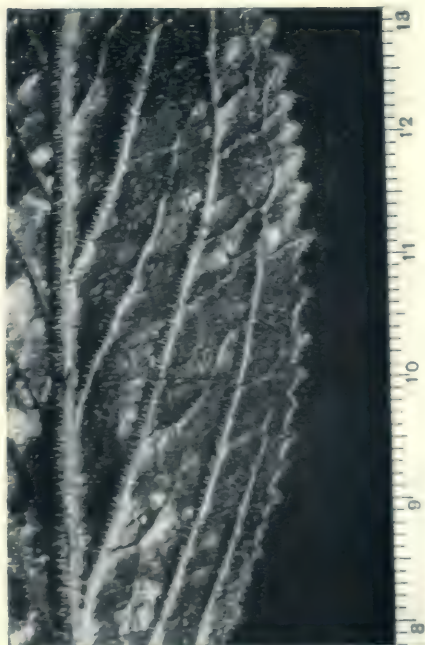
Fig. 11.

NOTES ON INHERITANCE OF COLOUR AND OTHER CHARACTERS IN PIGEONS.

By L. DONCASTER, M.A.
Fellow of King's College, Cambridge.

FOR some years I have carried on experiments on the heredity of certain characters in pigeons, and since circumstances prevent me from continuing the work further, I publish the results so far as they have gone, although they are in many respects incomplete. The space at my disposal was not sufficient to do the work on a large scale, so that although it has been in progress for almost six years, the results are not very extensive. In beginning the work, three separate series of experiments were planned of which the first was concerned with the inheritance of leg-feathering.

Series A. Leg-feathering.

Since the colours were very complicated and difficult to describe, in this series no attempt will be made to deal with them. The first
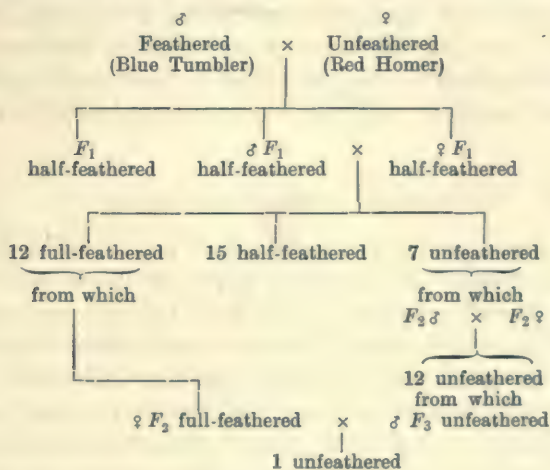
Fig. 1. (Series *A*.)

mating consisted of a 'Red' Homer ♀ with no feathers on the legs crossed with a Blue Tumbler ♂ with fully-feathered legs. The legs and toes were completely feathered, the feathers on the outer and middle toes being of considerable size. These two birds produced three young, all with feathers on the legs and the outer and middle toes, but not on the inner toe. The feathered cock then died, and two of the F_1 young were paired together and produced 34 young in F_2. Not all of these lived till maturity, but they were classified as 12 fully-feathered like the feathered grandparent (4 being doubtful as to whether they were 'fully' or 'half' feathered), 15 half-feathered like the F_1 birds, and 7 with no trace of feathering. There was thus evident segregation, but with an apparent excess of fully-feathereds. Two of the unfeathered F_2 birds were paired together and gave 12 young with no feathers on the legs or feet; the extracted recessives thus bred true. I failed to get any young from F_2 fully-feathereds paired together, but one of these paired with an unfeathered bird produced by the F_2 unfeathereds gave one young one with unfeathered legs. Although therefore the feathered parent was apparently fully-feathered, this experiment proves it to have been heterozygous. The excess of fully-feathered in F_2 is thus explained, and it may be concluded that leg-feathering and its absence behave as an allelomorphic pair, but that the character is incompletely and somewhat irregularly dominant.

It should be mentioned that in another series of experiments described below (mating *Da*), two Fantails paired together neither of which had feathered legs, produced among four young, one in which the legs and toes were distinctly though slightly feathered. No other similar case occurred among the birds of this series, and the occurrence of feathering in this one bird must probably be considered as a 'sport.'

Series B. Tail feathers, oil gland, and colour.

In the second series of experiments, a Red Tumbler ♀ was crossed with a White Fantail ♂, in order to follow the inheritance of the colour and of the 'fantail' character. The Red Tumbler was completely red except that the outer webs of the outer tail feathers were white; the legs were red, claws pale, bill pink, and iris light pink. The White Fantail had all its feathers completely white, the legs red with pale claws, bill pink, iris very dark brown. It had 23 tail feathers. These two birds produced ten young which lived long enough to be capable of description. They were all very similar in character. The general

colour of the feathers was smoky black, not the full black of a true
black pigeon, but a very deep smoky brown. The contour feathers
were darker than the quill-feathers; the primaries were distinctly
tinged with red, and in the young the contour feathers had reddish
tips, but these, as is usual in young pigeons, disappeared at maturity.
The red in the wing quill-feathers remained. The tail was long, most
of the feathers dark grey with a black band at the tip, and the outer
webs of the outer feathers whitish. In all the birds there were white
feathers, but the distribution of these varied somewhat. The rump
was generally grey, mottled with white, the upper and lower tail
coverts were partly or wholly white, and there were always some
white feathers in either tail or wings, and generally in both. The
distribution of the white feathers was not necessarily symmetrical on
the two sides of the body, and sometimes there were scattered white
contour feathers on the breast and under parts. The legs were dark
red, some of the claws generally dark, and the bill dark brown. The
tail feathers varied from 13 to 16 in number.
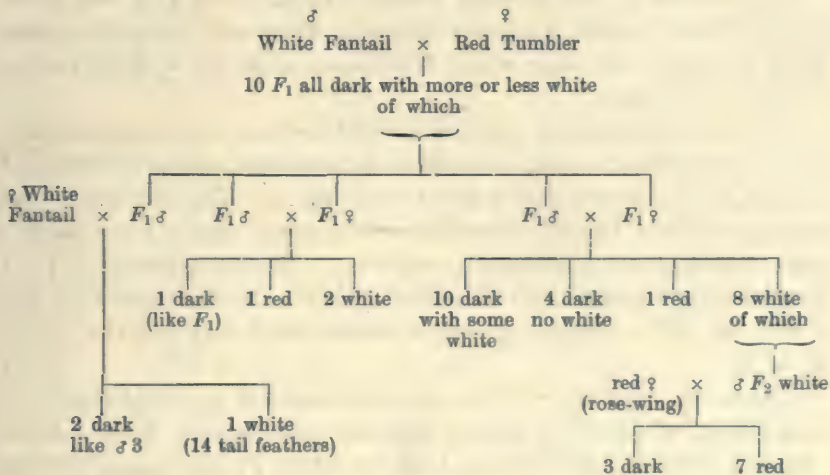
Fig. 2. (Series *B*.)

Two pairings were made between these F_1 young. From the first,
only four young were produced, all of which died before they were fully
fledged. Two were quite white, one red with one tail feather nearly
white and the primaries speckled with white, and the fourth closely
like the parents (dark smoky, with red in the webs and some white
feathers). From the second pairing between F_1 young 23 young were

produced, 8 white, 1 red, and 14 smoky. The red had some primaries and tail feathers white. The dark F_2 were not so uniform as those of F_1; 4 of them had no white feathers, the remaining 10 had the white distributed much as in F_1. But the ground colour of these birds varied considerably from an almost uniform smoky brown, with a tinge of red in many of the feathers, to a dark smoky blue-grey, with quite distinct bars across the wings and at the tip of the tail. Only two of the 14 dark birds had conspicuous wing-bars, but they were indicated in several others. The tail feathers varied in number from 12 to 16; none of the F_2 young showed anything that could be called a fantail. The presence or absence of the oil-gland at the base of the tail was unfortunately not always recorded; it is absent in the Fantail, but was present in all the F_1 birds which were examined. In F_2 it was recorded as absent in 4, present in 10; of the latter one had an extremely small oil-gland and another had a double one. The fact that the oil-gland was found in all the F_1 birds examined, and in 10 out of 14 in F_2, suggests that it behaves as a dominant Mendelian character.

In addition to the pairings between F_1 birds, an F_1 ♂ was paired with a White Fantail ♀, giving one pure white and two smoky young. Both the latter had some white, distributed as in the F_1 birds, and one had dark wing-bars.

In order to determine whether a white bird can bear the determinant for one or another colour, as happens in mammals, one of the white F_2 young (♂) was paired with a Red (rose-wing) ♀. The rose-wing is like the original Red Tumbler described above, except that it has a patch of white feathers near the base of each wing. The extracted white (in F_2 from White Fantail × Red Tumbler) paired with the rose-wing gave 7 reds, all with a varying amount of white, and 3 dark like the original F_1 birds.

Since the original cross between white and red gave in F_1 uniformly dark birds, which when paired together gave among their coloured offspring both red and dark, and since a white F_2 bird crossed with red gave both red and dark, it may be assumed that the original white fantail bore one kind of colour determinant, which on meeting red gave smoky (F_1), but that the F_2 white was heterozygous for colour determinants, bearing red as well as another. Since the dark birds in F_2 were not uniform, but in some cases were bluish with wing-bars, it seems probable that the determinant borne by the original fantail was for 'blue' (slate-grey with dark wing-bars), and that this meeting red gives the smoky heterozygote described.

C ♀ blk.   ×   ♂ wh.

3 blk. wh.

10 wh. blk.

Ca II wh. blk. ♀ × wh. blk. ♂

6 wh.   4 wh. blk.   5 blk. wh.

3 blue and wh.

blk. wh.
ex *C*

C Ca III
blk. wh. ex
Ca II

3 wh. blk.   ×   1 blk. wh.

C Ca I
wh. blk. ex
Ca I

Ca I wh. blk. ♀ × wh. blk. ♂

7 wh. blk.   1 blk. wb.   1 blk. with   1 blue
6 wh. feathers   and wh.

wh. blk.
ex *C*

C Ca II
♂ blk. with wh.
feathers ex *Ca I*

♀ blk. wh.
ex *C*

1 blk. wh.   ×   2 blk.

1 blue

C b
Blue and wh.
ex *Ca I*

Blue ex
C Ca II

1 blue   ×   2 probably blue,
but died before
it was certain they
were not blk.

3 wh.   ×   2 blk. wh.

2 wh. blk

C Ca D
♀ wh. blk. ex
C Ca I

♂ wh. blk.
ex *D*

4 wh.   4 wh. blk.   1 blk. wh.

C Ca IV
♂ blk. with wh.
feathers ex *Ca I*

♀ blk. ex
C Ca II

1 wh. blk.   2 blk. wh.   1 blk. with   3 blk.
5 wh. feathers

N
blk. wh. *C Ca IV* × blk. wh. ex *Ca II*
1 wh. blk.

Fig. 3. (Series C. Black and White Fantails.)

Series C and D.   Black, blue, and white.

The third series of experiments was concerned with the inheritance of colour only. In it two original pairings were made, *C* and *D*, a Black Fantail hen by White Fantail cock and the reciprocal cross white hen by black cock. The Black Fantails used were guaranteed of pure stock by the breeder who supplied them, all their feathers were full black except the webs of the tail feathers and primaries, which were very finely mottled with whitish, especially at the base, giving the web a greyish appearance. After a number of young had been obtained from these birds paired with White Fantails, the black cock and hen were paired together and produced two young, one of which was full black with practically no grey on the webs of the quill-feathers, the other black with as much grey as the parents, and four white feathers, two primaries in the left wing and two covert feathers in the right. It is possible therefore that one or both the original black parents was not pure bred. Control experiments were made with pedigree birds as described below.

Fig. 4. (Sèries *D*.)

The results of pairing these Black Fantails with Whites were as follows. In Series *C* (black ♀ × white ♂, p. 93) 13 young were produced, all pied black and white, but of these 10 had a preponderance of white, and three of black. The birds varied considerably, but those with excess of white may be described as white fantails, generally with most of the tail feathers black with grey along the shafts, and black patches of varying size and distribution on the body and wing coverts. The three with excess of black were black birds, with scattered white

feathers on the body, wings and tail, and in two out of the three with the rump almost or quite white.

In Series D (white ♀ × black ♂) there were 7 young resembling on the whole the F_1 from Series C, but with the white and black more evenly distributed. These birds might be described as mottled or chequered with black and white, or as white thickly scattered with black feathers, with most of the primaries and rump feathers and some of the tail feathers white. After 7 young had been hatched the white hen died, and another was paired with the cock (mating D_2), and produced 12 young[1]. These on the whole had more white than those of the first family, but it varied in the amount from one case in which the bird was quite white, with the exception of a small black patch on the right side of the rump, to white birds sprinkled with black feathers on the head, body and wings, and with a number of the middle tail feathers black. It was impossible to classify these birds sharply into classes with more or less white.

Pairings were made between F_1 birds from both matings C and D. From C two such pairings were made both between birds with preponderance of white. In one ($Ca\ I$) 13 F_2 young were produced, 3 white, 7 white with black patches, 2 black with white feathers (of which one had only 6 white feathers) and one 'blue' with a good deal of white. Many of the blue (slate-grey) feathers had a trace of rusty colour, and in the left primaries and tail the feathers were white speckled with grey; the secondaries and wing coverts of both wings and the tail feathers had dark grey tips, giving a distinct tail-bar and a suggestion of a wing-bar.

In the second pairing ($Ca\ II$) between F_1 birds from Series C 18 young were produced, 6 white, 4 white with black patches, 5 black with white feathers, and 3 blue and white. The blue feathers were in excess of the white, but as in the previous case the blue was not clean, but was dark and tinged with brown or rusty.

In Series D three pairings were made between F_1 birds. In the first only 4 young were produced, 3 white with black patches, and one white with patches of blue. The F_1 cock then died, and was replaced by another, from which pairing 15 young were raised, 7 white, 4 black with white, 4 blue and white, the white preponderating in three of the latter, and the fourth being mottled.

In the third pairing between F_1 from Series D 10 young were reared, 5 white, 3 white and black, 1 black and white, 1 blue and white.

[1] These two matings are included together in Fig. 4, p. 24.

In all the blues from Series D, as from Series C, the blue feathers were tinged with rusty brown.

In all then from F_1 of Series D 29 F_2 birds were reared, of which 12 were white, 6 white and black (white preponderating), 1 white and blue, 5 black and white (black preponderating) and 5 blue and white. From Series C, 31 F_2 birds were produced, 9 white, 11 white and black, 7 black and white, 4 blue and white, or adding the two series together, out of a total of 60 F_2 young, 21 white, 17 white and black, 1 white and blue, 12 black and white, 9 blue and white.

There are several points connected with these results which call for comment. First, the factor for blue is evidently introduced by the whites, but being recessive to black does not appear in F_1. In F_2 almost exactly one-fourth of the coloured young are blue (9 out of 38). My results in this respect confirm those of Staples-Browne[1] obtained with other breeds. It is interesting that the blue of the wild rock pigeon should be recessive (hypostatic) to the more recently acquired black of the domestic breeds.

Another point is the absence of extracted blacks. No fully black bird was produced in F_2, and only one with as few as 6 white feathers. The fact that the original blacks when paired together gave a black with some (4) white feathers may indicate that pure blackness is not a stable character. A third point is the excess of whites over expectation (21 out of 60 where 15 would be expected). This is not a great difference in itself, but a similar excess was found in Series B above (10 out of 27), and again in some of the matings described below.

It has been seen that F_1 birds with more white than black in the plumage when paired together give among their coloured offspring not only young like themselves, but also blacks with a relatively small amount of white. With the object of discovering whether this difference corresponds with a difference of constitution several pairings between such 'black with white' birds were made. Since birds of the same generation were not available, F_1 birds were crossed with F_2 which were nearly similar in distribution of colour. In the first of these crosses ($C\,Ca\,II$) an F_1 from C, black, with nearly white rump and a few scattered white feathers, was crossed with an F_2 from $Ca\,I$, black with only six white feathers. Four young were produced, one black with white, two wholly black, and one blue with no white feather. In this case therefore there were three self-coloured to one black with a few white feathers. But in a second pairing of the same kind ($C\,Ca\,III$) of

$F_1 \times F_2$ both with excess of black, but with more white than in $C\,Ca\,II$, of four young, three were white-with-black and one black-with-white. In two other matings also between black-with-white birds ($C\,Ca\,II$ and N) white-with-black was produced. In one case where a similar mating between white-with-black birds was made ($C\,Ca\,I$, F_1 from $C \times F_2$ from $Ca\,I$) 2 white-with-black and 2 black-with-white appeared. It appears then that birds with excess of black can be produced from those with excess of white, and *vice versa*. But the only matings from which pure blacks were produced were those in which two black-with-white birds were paired together ($C\,Ca\,II$ and $C\,Ca\,IV$). The latter of these pairings consisted of an F_1 cock from mating C, paired with his own black daughter produced in the mating $C\,Ca\,II$; these two produced 1 white-with-black, 3 black-with-white and 2 black.

Since these results seemed somewhat confusing, and might perhaps be partly due to the impurity of the original stock of black fantails, I obtained two well-bred black fantails from fanciers which had no white speckling on the webs and paired them with whites (K and L). In mating K (white ♀ × black ♂) two young were produced, one of which died before it was fully fledged, but it appeared to be completely black. The other was patched black and white, the patches of colour being larger than in Series C and D. The white hen then died, and on pairing the black cock with another (mating K_2), one black and white mottled young one was produced. In the converse cross (L) one young one only was produced which was black-with-scattered white feathers, and most of the tail white. The pied F_1 bird from K mated with the F_1 from L gave 9 young, 4 white, 3 black-with-white, 1 black with one white tail feather and 1 blue and white, with black wing and tail-bars. The Series K and L thus differ from C and D in producing one full black F_1 bird, and in producing no white-with-blacks. They differed also in that most of the black-with-white birds had the central tail feathers white, while those in the Series C and D were almost always black.

The results appeared at first very perplexing, especially the absence of blacks in F_2, and the fact that black-with-whites and white-with-blacks when paired together can each produce the other. These facts indicate that two or more pairs of allelomorphs must be present, and an explanation may possibly be sought on lines similar to those suggested by Mudge with regard to the inheritance of piebaldness in Rats. If we assume that pattern depends on two pairs of characters, P for piebaldness and S for full (self) colour, each allelomorphic with its absence (p and s),

and that colour requires a factor **C**, allelomorphic with its absence **c**, and further that **S** and **P** are neither of them completely dominant (epistatic) over the other, we get the following zygotic types, opposite each of which I put the pattern which it may be provisionally assumed to represent :

SS pp CC,	**SS pp Cc**	Black
SS Pp CC,	**SS Pp Cc**	Black with grey webs
SS PP CC, **SS PP Cc** **Ss Pp CC**		Black with some white feathers
Ss PP CC,	**Ss Pp Cc**	Black with white
Ss PP Cc and all combinations containing **P** and **C** but not **S**		White with black

That is to say, birds homozygous for **S** are black, with or without grey webs or very few white feathers according to whether **P** is present or absent. Birds heterozygous for **S** but containing **P** are in general black with white, but if homozygous for **P** and heterozygous for **S** (**Ss PP Cc**) they may have preponderance of white. Birds without **S** but containing **P** and **C** are white with black.

If this scheme at all approaches the truth it explains (1) the absence of whites in five matings ($C\,Ca\,II$, $C\,Ca\,III$, $C\,Ca\,IV$, N, and Cb) made between birds with excess of colour over white (18 coloured, no white). (2) The excess of whites in families where they occur is also explained if it is assumed that a bird is white which contains **C** but not **S** nor **P** (**ss pp CC, ss pp Cc**). If this is so, such a bird crossed with a white containing **S** or **P** but not **C**, should give coloured offspring. I have not been able to test this suggestion, but Staples-Browne in crossing a White Fantail with a White Tumbler got a coloured F_1. His suggestion is that the Fantail was a dominant white, but the explanation here suggested would lead to the same result.

In conclusion, it should be mentioned that there was no evidence in my experiments that the two young hatched from the same pair of eggs are more often alike than young from the same parents out of different nests.

ON HETEROCHROMIA IRIDIS IN MAN AND ANIMALS FROM THE GENETIC POINT OF VIEW.

By C. J. BOND, F.R.C.S.

CONTENTS.

PART I.

Irregularity of Eye Colour Pattern in Man and Animals.

It has been known for a long time that in a certain proportion of the population the eyes of one and the same individual are of a different colour sufficiently marked to attract attention. In earlier references to heterochromia attention was chiefly directed to the frequent association of this condition of unequal pigmentation of the iris with diseased conditions, such as glaucoma, cataract, cyclitis, and corneal opacities, generally in the lighter coloured eye.

Thus Sir J. Hutchinson (1) in 1869 reported three cases with imperfect vision in the blue eye. Marcus Gunn (2) and Sym (2a) in 1889, and Malgat (3) note the association with cataract in the lighter eye.

Fuchs (4) in 1906 reported 38 cases, of which a large proportion suffered from cataract and a considerable number from opacities of cornea or vitreous.

Anton Lutz (5) after an exhaustive study of the subject of heterochromia also corroborates the frequent recurrence of diseased conditions in the lighter eye, and comes to the conclusion that hereditary influence as a causative factor in heterochromia has not been clearly established.

From these observations, and from the experience of other ophthalmic surgeons, there can be no doubt that intra ocular disease, especially when associated with increase of intra ocular tension, may bring about, under certain conditions, anterior depigmentation, partial or complete, of the iris of the diseased eye.

But beyond this limited group of cases, in which irregular iris pigmentation is associated with disease (frequently antecedent) of the affected eye or eyes, there is a larger group (growing in size in proportion to the care which is exercised in searching for and examining the cases) in which the irregular pigmentation of the iris is congenital and unassociated with any evidence of disease.

Thus Thorpe (2b) and Allan (2c) record such cases. J. Ross (2d) found 11 cases of heterochromia in a series of 5000 patients in which the irregular eye colour was not associated with any symptoms.

In these congenital and healthy cases the demarcation between the pigmented and the less pigmented, or the unpigmented portions of the iris in the same eye, is often sharply defined with clear tangential margins quite unlike the merging of pigmented into unpigmented areas in disease.

Further, in a certain number of these congenital heterochromia cases a familial incidence strongly suggests a genetic origin. When we find the heterochromic condition closely associated with certain varieties of domesticated animals, and in individuals in which there is no evidence of ocular disease, when further we find that it makes its appearance in man and in animals during the inter-breeding of individuals and varieties of different eye colour and pattern, then we are, I think, fully justified in considering that the whole subject of heterochromia of the iris should be re-investigated from the genetic point of view.

It is the object of this paper to record some observations which bear on this hereditary aspect of the problem.

Coincidentally with the important discovery announced by Hurst (6) in 1907 (and independently by Davenport later) that the inheritance of eye colour in man followed Mendelian lines, a further attempt was

made by Hurst to subdivide his duplex type into a *self-coloured pattern* in which the pigment was spread over the whole anterior surface of the iris, a *ring pattern* in which it was deposited in a circle round the margin of the pupil, and a *spotted pattern* in which it was collected into discrete spots or patches. Owing to lack of material and to the fact that deposition of pigment tends to increase during childhood, Hurst was unable to establish fully the genetic relations between these types, beyond showing that the self-coloured behaved as a dominant towards the ringed pattern.

From Hurst's description it would appear that the genetic factor or factors which control eye-colour in the human species (although they may be classified into two or more sub-types) operate under normal conditions in both eyes equally, that is to say they are bi-lateral or bi-iridial in action.

It is the object of this paper to draw attention to some examples of irregular iris pigmentation in man and in some varieties of domesticated animals and birds in which this symmetrical arrangement is departed from.

It has been known for a long time that in a certain proportion of cases in the human species, perhaps one or two per 1000, the two eyes of the same individual are of different colours. When this condition of heterochromia does occur, according to my own experience, the darker or coloured eye presents some shade of the duplex type, either arranged on the self-colour pattern, or more frequently affecting a larger or smaller portion of the iris either in a continuous sheet or in discrete sections, the remainder of the iris being blue or some lighter shade of the coloured pattern. The other or lighter eye is either simplex or more generally a lighter shade of the duplex pattern of the coloured eye.

Different degrees of this condition of asymmetrical colouration are met with, varying from complete heterochromia to one small sector of darker colour in an otherwise duplex eye.

In the course of the examination of the pigment distribution in a considerable number of individuals an irregular type of the duplex pattern will be found which does not conform to the self-coloured or the ringed or the spotted pattern described by Hurst.

In an otherwise blue or grey, i.e. a simplex eye, a portion of the iris, generally embracing the whole diameter from periphery to pupillary margin, and more or less triangular in shape, with its base at the periphery, will show in different cases different degrees of pigmentation from light yellow to dark brown.

In some individuals this duplex ray takes the form of a darker pigmented sector or patch on a ground colour of lighter duplex instead of a blue background.

This radial sector of duplex pattern in an eye of simplex or lighter duplex ground colour, I propose to call the *ray pattern* of the duplex type.

Iris Structure in Man.

Although as we shall see later cases of irregular pattern of iris pigmentation also occur in animals and birds, yet the pigmentary deposit is less sharply defined in outline, less sectorial or ray-shaped in them than in man. It is probable that this difference in pigmentary pattern may depend on the very definite radial arrangement of the connective tissue stroma of the iris in the human subject. This framework tissue and the muscular fibres which dilate the pupil form a network of radiating fibres with lozenge-shaped interstices which generally converge to form a ring with knot-like intersections at a little distance from the pupillary margin, and it is in the situation of this ring of intersections that the greatest deposit of pigment occurs in the so-called duplex ring pattern.

This ray-like formation of supporting tissue is well seen in the blue human iris in which there is no obscuring anterior pigment, and even more clearly still in the albinotic iris.

In the iris of birds and of many animals on the other hand the concentric zone-like disposition of the fibres is more marked and obscures the radial pattern.

Prevalence of the Ray Pattern in Man.

Out of 200 consecutive eye-cases attending Dr Henry's clinic there were three examples of this ray type, that is 1½ per cent. of eye cases (not of the general population). Of these *one* was a brown or duplex ray on a blue or simplex background. *Two* were dark brown rays on a ground colour of lighter brown and in one of these a few dark pigmentary spots were present in addition to the pigmented ray.

Through the kindness of Mr Ridley and other medical men and nurses I have examined 40 other cases of the ray pattern in the last six months.

In 21 of these a well-defined duplex triangular ray or rays existed on a simplex background, the other eye in the same individual being of the simplex type.

In 19 a dark duplex ray was present on a lighter shade of brown or yellow as a background, the other eye being also light duplex.

In two of the 40 cases there was more than one ray. In five cases the pigment covered more than half the surface of the iris in a continuous sheet with sharply defined ray-like margins.

In nine cases the duplex ray in the one eye was associated with the ring duplex pattern in the other eye.

In one case both eyes were of the duplex type, but in the left eye the anterior pigment was absent from two small triangular areas, one on either side of the iris in the equatorial line, thus giving the appearance of two pale blue rays in these situations. In one case only was the irregular ray pattern bilateral (E. Frake, P_1).

The Hereditary Transmission of the Ray Pattern.

In 19 cases in which the eye colour of the parents could be verified by personal examination, in 15 one parent was duplex and one simplex, in four cases both parents were duplex.

Four families (Baines, Frake, Masters and Whitby) are of especial interest in which more than one example of the ray pattern occurred in the same family, and their pedigrees are shown as far as the facts could be ascertained in the accompanying tables.

In the "A" family (Masters), Fig. I, two sisters D. M. and I. M. showed narrow but distinct dark brown rays on a yellow and blue background respectively in different eyes and on different sides of the iris in each case. In one the other eye was faintly duplex, in one simplex. The father of these children was simplex and the mother dark duplex. Three other children were regular simplex and one regular duplex in the same family.

In the "B" family (Baines), Fig. II, the father G. B. had a well-marked duplex ray covering the inner half of the left iris, the remainder being blue and the right iris yellow duplex in the ring form.

One daughter F. B. had a corresponding yellow brown ray covering the outer side of the left iris, the inner half being blue and the right eye yellow brown ring duplex like the father.

One son W. B. the lower half and inner and outer quadrants of the right iris were brown leaving a ray of blue in the middle of the upper half of the iris. The left eye was ring duplex like the father and sister.

The mother of this second family of ten children, of which two were of the ray pattern and the remainder all simplex with the exception

of one boy faintly duplex, was herself simplex with a duplex mother and a simplex father.

The father of the male parent was simplex and the mother unknown.

The father G. B. had had a previous family (Family I) of eleven children by his first dark duplex wife; of these eleven children eight were dark brown and three simplex, and no cases of ray or irregular pattern occurred in this first family.

In the "*C*" family (Frake), Fig. III, the father (of unknown parental eye-colour) is a ray-duplex of self-type, that is to say the iris is dark brown leaving some ray or lozenge-shaped blue spaces round the pupil in both eyes.

The mother is a self-coloured duplex of yellow tint.

Of the nine children one son E. F. has a dark brown, almost black ray in the outer side of the left iris, the remainder of the iris and the other eye being light brown.

One daughter L. F. has multiple dark brown rays covering the greater part of the inner half and part of the outer half of the left iris, the remainder being blue and the right eye dark brown.

One son A. F. has a dark patch in the upper and outer part of the right iris, the remainder of this and the left iris being light brown ring-duplex.

Four Families (Masters, Baines, Frake, Whitby) showing Heredity of Ray Pattern.

Bracketed circles represent the two eyes of *one* individual, the lower circle represents the right eye.

"*A*" Family.   Masters.
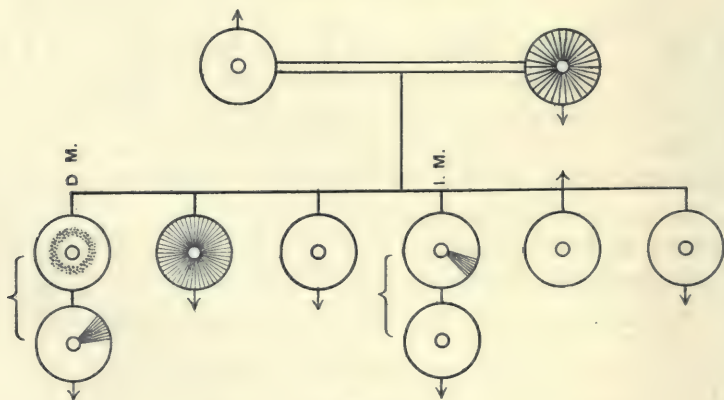
Fig. I.

"*B*" *Family. Baines.*
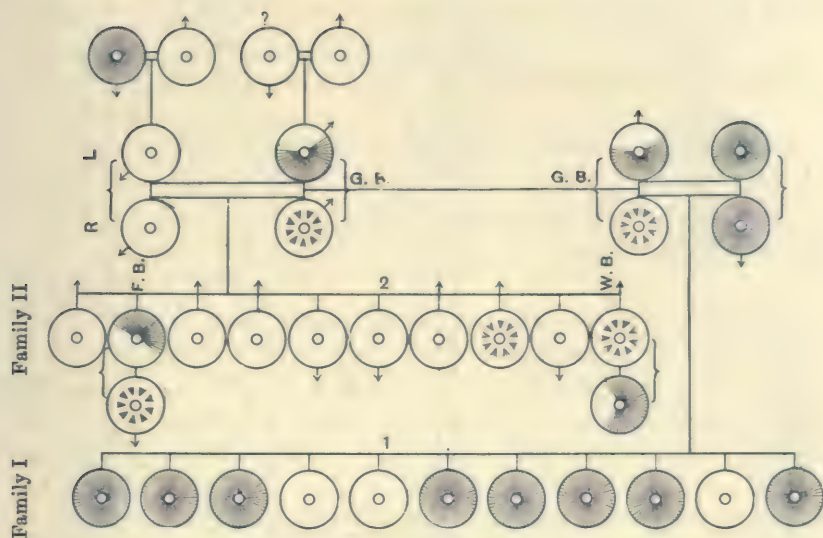
Fig. II.

"*C*" *Family. Frake.*
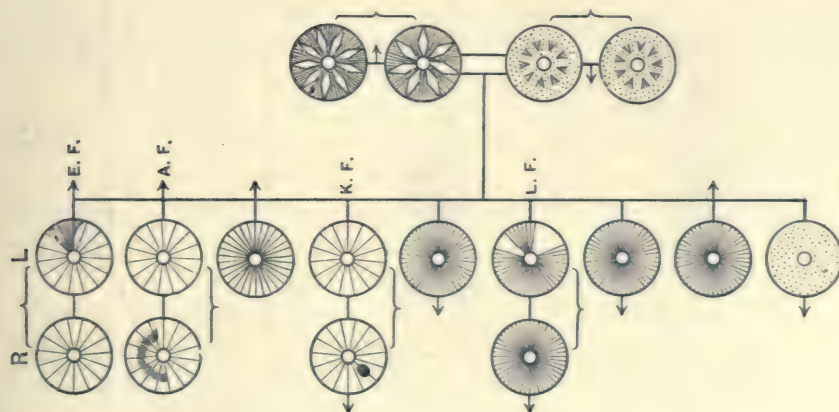
Fig. III.

"D" Family. Whitby.

Florence
Light Brown Hair

Ethel
Brown Hair

Fred
Light Brown
Hair

Walter
Straw Coloured
Hair

Kate
Brown Hair

Edward
Straw Coloured
Hair

Albert
Straw Coloured
Hair

Evelyn
Light Brown
Hair, Myopia

Thomas
Brown Hair

Annie
Reddish Yellow
Hair

Twins

Fig. IV.

One daughter K. F. has a black spot on a lighter duplex background on the lower half of the right iris, the left also being brown duplex.

Four children W. F., G. F., I. F. and Al. F. are dark brown self-duplex, and one B. F. is a green self-duplex.

The father I. F. has two sisters also with irregular iris pigmentation of the ray-type.

In the "D" family (Whitby), Fig. IV, the father is a heterozygous ring-duplex of irregular pattern with excess of pigment in the upper part of the ring; the mother is a heterozygous ring-duplex, and three cases (Florence, Ethel, Kate) of ring-pattern heterochromia occur in a family of ten children.

In two other families (Martin and Cavanagh) familial facts occurred. In one (Martin) a duplex ray in the child was associated with an irregular dark patch in a ring-duplex in the father (Case 9, Fig. V and Case 9, Fig. VI). In one (Cavanagh) a duplex ray in the mother was associated with a dark patch in a ring-duplex in the son (Cases 22 and 23, Fig. V).

The following diagrams will serve to illustrate the form the ray pattern assumed, and the position the coloured sector occupied on the iris in 24 cases, in which, with the exception of cases 9, 22 and 23, no familial incidence was recorded.

Irregular Type of the Ring Pattern.

One case, a woman *aet.* 34, Mrs A. B. (Case 8, Fig. VI), was seen of abnormal structure pattern apart from pigmentary abnormality in a simplex eye. In both blue eyes an inner ring of dark blue immediately surrounding the pupil was itself surrounded by a wider ring of light blue reaching nearly to the periphery of the iris. This outer ring had the dog-toothed margin and the radial striated appearance of the simplex iris, the peculiar appearance of two concentric rings being due to the outer zone of more opaque radial fibres round the inner ring of circular transparent fibres.

In Case 1 L. C. a broad brown ring surrounded the pupil in the right eye and a narrow yellow ring the pupil of the left eye, giving an appearance of heterochromia in the two eyes.

In Case 5 (shown also as Case 12 in the ray-table) is a duplex ray in an otherwise simplex right eye. This ray was associated with two dark pigment patches on the lower half of the sclerotic.

In one Case 6 N. H. the difference in area and degree of

Ray Pattern
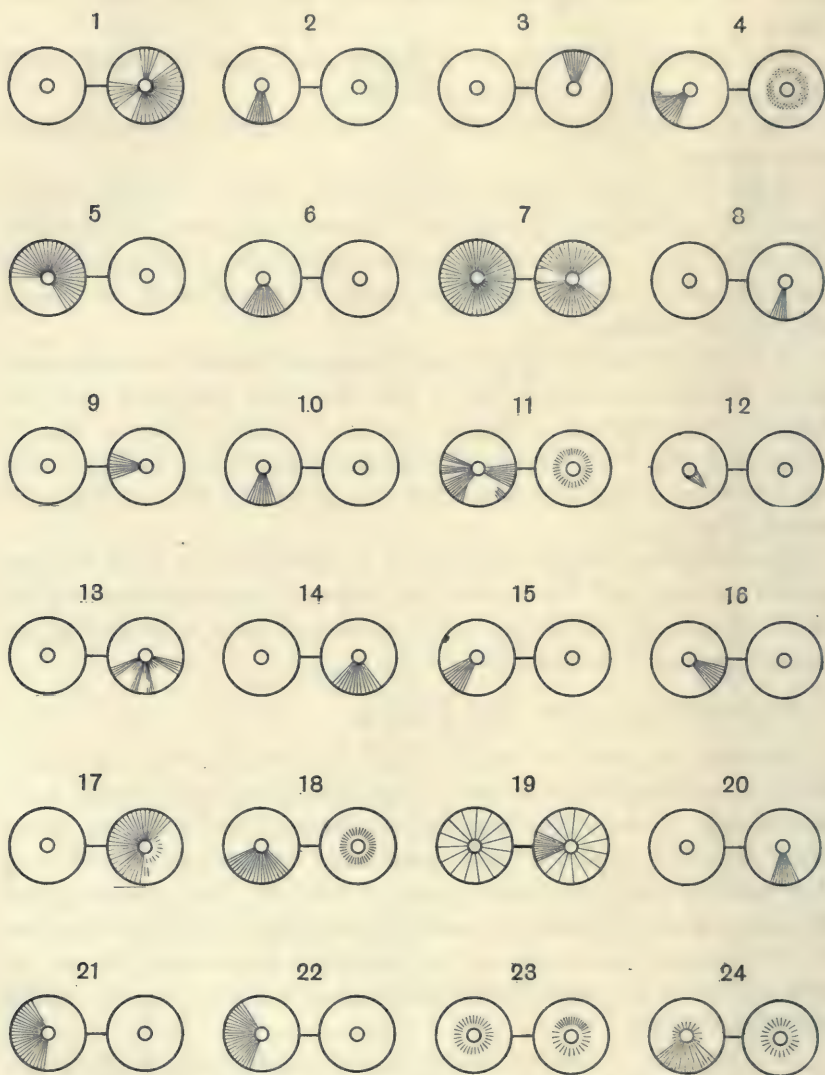
1. Higginson.   2. Lilley.   3. Tate.   4. Tompkins.   5. P. B.   6. Mrs H.
7. Scott.   8. S. H.   9. Martin.   10. Turner.   11. A. G.   12. N. R.
13. Randall.   14. Stevens.   15. Aston.   16. Thomson.   17. Mrs Day
18. Nunn.   19. Jackson.   20. Taylor.   21. Bodycote.   22. Cavanagh ♀.
23. Cavanagh ♂.   24. Grundy.

Fig. V.

pigmentation in each ring was so marked as to make the right eye appear dark brown and the left eye light yellow.

In one Case 7 E. E. a yellow brown ring was associated with black pigment in each eye most marked in the lower half of each iris.

In Case 9 the spots were limited to the right eye. This man was the father of the ray-duplex (Case 9).

Ring and Spotted Patterns.
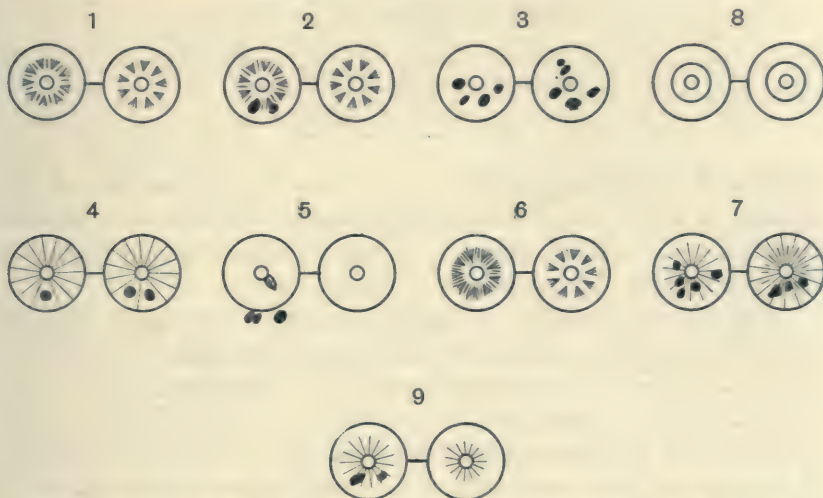
1. Colton.   2. Martin.   3. Hives.   4. N. Jones.   5. N. Richards.
6. N. Harvey.   7. Evans.   8. Mrs A. Ball.   9. Martin.

Fig. VI.

In one case B. C. No. 23 ray pattern, a duplex ring with irregular black spots in the upper half of the left iris in the son, was associated with a brown duplex ray in the outer half of the right iris. In the mother (Case 22) the rest of the iris and the left eye being blue.

Prevalence of the ring pattern.

There were 14 ring pattern cases in 88 duplex individuals occurring in the consecutive series of 200 cases of Dr Henry's clinic.

Prevalence of the spotted pattern.

In 26 individuals out of the 200 cases spots or patches of darker pigment were present on a lighter duplex background and in a few cases on a simplex background.

Thus we see that out of 22 examples of a single ray only two were *above* the horizontal pupillary equator, two were *on* the equator and

Distribution of the Spot and Ray Patterns on the Iris.

Spotted Pattern:

In both eyes   7

In one eye... 19

- Above the horizontal equator   5
- On the horizontal equator ...   4
- Above and below equator   ...   4
- *Below* equator...   18

Distribution of the Ray:

Single Ray above equator ...	2
,, on ,, ...	2
,, *below* ,, ...	18
Multiple rays above and below	11
Total	33

18 *below* it in various positions in the lower half of the iris. In the 11 cases in which a large portion of iris surface was covered by multiple duplex rays the greater number and wider and more deeply pigmented rays occurred in the lower half of the iris.

This inferior situation of the duplex ray is suggestive when we recall the fact that congenital coloboma of the iris, which is often bi-lateral and which is probably dependent on imperfect closure of the choroidal fissure, is nearly always found in the lower half of the iris and usually in the downward vertical direction. The occurrence of coloboma of the iris has been recorded in the upper portions of the iris, and an attempt to explain this fact has been made by supposing that in these cases some rotation of the developing optic cup has carried the choroidal fissure out of its usual inferior position. It is also interesting to note that in some of the amphibia (frog and toad and some newts) a colour coloboma of the margin of the iris in the vertically downward direction normally exists in the adult animal. Plate VII, fig. 3.

The fact that while coloboma is nearly always vertically downwards the pigmented ray is rarely in the exact vertical midline but usually displaced to one or other side of the lower half of the iris, would at first suggest a different origin for the abnormality in the two cases.

But it is necessary to remember that coloboma is due to non-closure of a gap which nearly always forms in the same position, while the ray of pigment is the result of the in-growth of mesoblastic tissue which eventually becomes pigmented through this choroidal fissure, and the spreading out round the globe of this tissue in a radial or fan-shaped manner, to form the iris etioma. The habitual presence of the pigmented rays in the lower half of the iris may be due therefore to the

failure of this pigmentary tissue to spread from its point of entrance further round the globe in these abnormal cases.

Irregular Iris Pigmentation in Animals.

My attention was drawn to this subject by finding that out of 100 wild rabbits shot on a small uninhabited island in the Orkneys four animals exhibited unequal pigmentation of the iris in one or both eyes.

In three animals the upper portion of the iris in both eyes above the horizontal pupillary equator was blue, the animal being partly wall-eyed, while the lower or lower anterior portion was deeply pigmented (duplex type). In one animal this irregularity was limited to the left eye. Plate VI, figs. 1 and 2.

It is well known that "wall" eye occurs in many species of domesticated animals. I am indebted to Dr Sydney Turner for some interesting information about "wall" eye in Great Danes.

The wall-eyed condition, that is the simplex type of iris, may be present in both eyes in the same animal or it may affect one eye only.

In the latter case it may affect the whole or only a portion of the same iris, the remainder being of the normal brown colour or duplex pattern.

While "wall" eye is most common in the Harlequin variety of the Great Dane, that is in an individual of a bi- or tri-coloured patchwork pattern or piebald coat colour, it is also found in other breeds of dogs such as the English Collie, especially the "marled" variety, and in the bobtailed Old English Sheep Dog and I have also seen it in the Dalmatian.

It is also noteworthy that in the individual dogs that I have examined the unpigmented areas frequently occupy some portion or the whole of the lower half of the iris, the opposite of the condition found in man.

"Wall" eye has also been observed in piebald and in so-called "skewbald" horses but not exclusively in this breed.

This association between irregular iris pigmentation and patchy Harlequin coat is important.

Thus in the series of the 100 Orkney rabbits there were 81 of the wild grey colour, the remaining 19 showed some irregularity of coat pattern, five had white patches on or in the neighbourhood of the right shoulder, five on the left shoulder, three white patches on one or more limbs, and six had white colouration of the forepart of the body, neck

and head, roughly corresponding to the Dutch pattern. (See Illustration.)

Distribution of Coat Colour in Orkney Rabbits.

	Wild Grey ...	81			
100				White patches on Right Shoulder	5
	Patched ...	19		,, ,, Left ,,	5
				,, ,, Limbs ...	3
				Dutch Pattern	6

Irregular Dutch Pattern in Orkney Rabbits associated with "wall" eye.

Now it was only in these rabbits which showed the Dutch pattern of coat colour that the "wall" eyes were observed. Plate VI, figs. 1 and 2.

I understand from Prof. Punnett that "wall" eye occurs in the Dutch breed and it is known that five or six years ago some tame white, or brown and white rabbits were turned down by a farmer on this uninhabited Orkney island, it is possible therefore that some of these were of the Dutch type.

Heterochromia in pigeons.

I cannot find any systematic record of eye colour in pigeons, but I have observed one or two points in the course of my own breeding

experiments in pigeons during the last seven years which are of interest in this connection[1].

It is well known that whiteness of plumage is associated in pigeons with a dark brown iris or "bull" eye. Bull eye is an expression used by fanciers to denote a dark brown eye which looks black at a distance and in which it is difficult to see the outline of the pupil without close examination. If however the bull eye be examined at an angle with a lens, an outer zone of pinkish red colouration can be observed near the periphery of the iris which at first suggests red pigment in this situation. After removal of the eye however and the emptying of the blood vessels the pink colour largely disappears and microscopical sections of the iris show that the pink appearance is due not to the deposition of pigment but to exposed blood vessels on the anterior surface of the iris. Thus the bull eye is a simplex eye in which the posterior uveal pigment is seen through the delicate iris tissue.

A homozygous white fantail cock with "bull" eyes was mated to a black fish-tailed short billed tumbler hen with pink eyes[2].

The F_1 offspring of this cross were black and white, and brown (with blue) and white hybrids, all with dominant orange red or duplex iris.

Two of these, a black and white cock and a brown blue and white hen (brother and sister) were mated together and produced in the F_2 generation:

Chocolate Brown	Black	Black and White	Brown, Blue, and White	White
2	4	21	11	13
Red eye	Red eye	Red eye	Red eye	"Bull" eye

Of these F_2 heterozygotes a black and white cock with orange red eyes was mated to a brown, blue and white hen, with orange red eyes, producing in the F_3 generation:

Black and White	Brown, Blue and White	Blue	Blue and White	Almond	Almond and White	White
4	5	2	3	1	1	2

Of these the black and white, brown and white, almonds, and almonds and whites, have the dominant red eye, the white the recessive

[1] Heterochromia in pigeons is apparently according to fanciers more common in Tumblers and chiefly in the Pied varieties, it also occurs in Homers.

[2] It should be stated that in the pigeon experiments the pairs, when once mated, were not confined, but were allowed to mingle with other birds in the same loft. If care be taken to keep all the adult birds properly mated, I have not found any difficulty arise from non-isolation.

bull eye, and only the blues, and blues and whites show irregular iris pigmentation.

One of the blue and white birds of the F_3 generation is especially interesting, having a full orange red eye on the right side and a " bull " eye on the left.   Plate VII, figs. 1 and 2.

On careful examination with a lens however the red anterior pigment in the right eye is found to be partly wanting in a segment of the lower half of the iris downwards and a little forwards from the pupil, leaving the underlying brown or " bull " ground colour exposed in this situation.

This individual bird thus shows a duplex pattern with coloboma of the *colour pattern* in the right eye and the bull eye which corresponds to the human simplex type in the left eye.   This bird also showed some irregular white patches on the head indicating further irregular pattern of feather colour.

The details of the breeding of these birds are as follows:

```
                     ♂                          ♀
              White Fantail    ×    Black Tumbler
                Bull Eyes             Pink Eyes

              Black and White   ×   Brown, Blue and White
                Pink Eyes              Pink Eyes
                   44                      26

              Brown, Blue          Black
      Brown    and White   Black   and White    White
        2         11         4         21         13

   Almond                Blue and  Black and  Brown, Blue
   Almond  and White Blue  White     White    and White    White
     1        1       2      3         4          5           2
   Pink     Pink                     Pink       Pink         Bull
            Irregular iris pigmentation
```
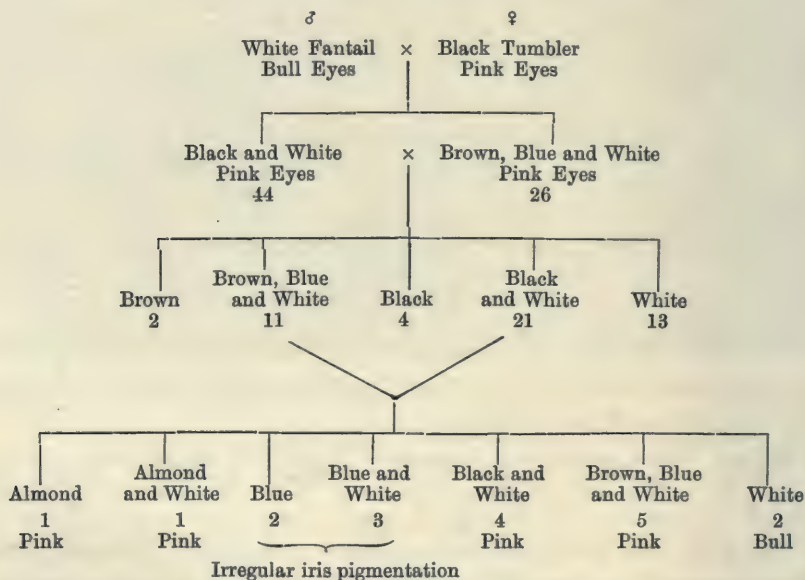
In this case then the heterochromia and the irregular duplex pattern in the right eye have appeared in the F_3 generation during the repeated interbreeding of the black and white, and brown, blue and white heterozygous offspring of a bull eyed white fantail, and a pink eyed black tumbler.

This heterochromic blue and white tumbler-fan hybrid was mated to a full sister, almond in colour with pink eyes, with the following result:

Blue and White × Almond
Heterochromia ♂ | Red ♀

Blue	Blue and White	Almond	Almond and White	White
12	14	2	4	6

and the reappearance of heterochromia in some number unrecorded of the blue and white birds.

Thus it would seem that the mating of a self-colour black with pink eye with a recessive white with "bull" eye is associated with the appearance of broken-up feather colour pattern and a broken-up iris anterior pigment pattern as in the Orkney rabbits.

Moreover the irregular iris pattern is so far only found in the blue and white birds, that is in birds of a dilute black and white colour pattern.

The gametic factor or factors, which in the self-coloured and self-white parents control the whole feather pattern, and in the self-coloured iris and the "bull" iris control the whole iris pattern, have undergone some change by which in the birds with parti-coloured eye and feather pattern they now control independently different feather areas and separate eyes and different portions of the iris in the same individual, and the question arises, What is the nature of the change in the constitution of these gametic factors which is responsible for this altered behaviour of unit characters?

Harlequin feather colour pattern in pigeons.

The result of mating two self black fantail cocks of prize strain with orange red eyes from the same loft with two pure bred white fantail hens with "bull" eyes in 1905 was to produce F_1 hybrids of different type in each case. In the "A" mating most of the F_1 hybrids were black and white piebalds with bull eyes, in the "B" mating all the F_1 hybrids were blacks with (in most cases) a few white feathers on the rump and one or two white primaries in one wing. These black hybrids had the orange red eye. [The details of these two matings are given in the tables on pp. 118, 119.]

But the point of interest is that the self mating of the F_1 heterozygotes of the "A" and "B" cross respectively resulted in a different proportion of extracted types in the F_2 generation in each case, although the proportion of selfs to pied, two to one, was about equal

in both. The self mating of a pair of the "harlequin" hybrids of the "A" cross produced in six years:

Blacks	Blues	Black and White Harlequin	Whites	
11	4	17	22	(Total = 54)

The self mating of a pair of the "black" hybrids of the "B" cross produced in the same time:

Blacks	Blues	Black and White Harlequin	Whites	
26	14	25	16	(Total = 81)

see Chart, pp. 118, 119.

It is of interest to compare this result in pigeons with the two extracted types of the "Irish" variety in hooded rats obtained by Doncaster and Mudge by crossing the wild grey with the albino rat (Bateson (9), p. 84).

On re-mating the original black cock of the "A" mating to another white fantail hen of a different strain ("C" mating) all the offspring F_1 were blacks with white rump feathers and white wing primaries like the F_1 black offspring of the "B" cross.

This result of the second mating shows that the difference in parental gametic constitution which led to the difference in colour pattern in the F_1 hybrids of the "A" and "B" crosses was introduced by the white recessive female parent of the "A" cross, and it is this difference of gametic constitution in this parent that led also to the unequal distribution of extracted types and the excess of recessive whites in the F_2 generation of the "A" cross over that in the F_2 generation of the "B" cross[1]. The association between eye colour and feather colour was preserved in these parallel matings, the harlequin F_1 offspring of the "A" cross retaining the "bull" eye and the black F_1 offspring of the "B" mating the orange eye. The bull eye of these F_1 and extracted F_2 harlequin birds, however, has a pinker appearance than the bull eye of the normal white fantail[2].

Heterochromia in cats.

In his experiments on heterochromia in Angora cats in 1907, Przibram (7) found that the mating of an asymmetrically coloured animal having one blue and one yellow eye with a symmetrically

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[1] The distribution of recessives, intermediates and dominants as revealed in the charts (pp. 118, 119) suggests a grouping of these three classes of offspring, especially in the case of the white recessive birds.

[2] In both matings the recessive whites showed the "bull" and the dominant blacks the orange eye.

coloured animal having two blue or two yellow eyes resulted in the production of both asymmetrically and symmetrically pigmented offspring.

Przibram concludes that asymmetric animals can be traced back to asymmetric ancestry. The asymmetry can also be altered during hereditary transmission, thus either eye colour of the asymmetric parent can appear in the symmetric form in the offspring.

The conditions (genetic) under which asymmetry of eye colour first appears are unknown.

Association between eye colour pattern and skin or coat colour pattern.

Recent researches by Pearson, Nettleship and Usher on albinism in man (8) show that the piebald, that is the black and white skin colour pattern, does occur though rarely in individuals belonging to the coloured races of mankind. In some of these cases of piebald negroes a familial association with complete albinism has been traced, in others with leucoderma and some other conditions having a pathological or somatic origin.

On the other hand a piebald skin colour, apart from pathological conditions, has not (so far I believe) been recorded among European races. This may be due to the fact that no systematic search has been made for this condition.

Some authorities, A. R. Gunn (13), are of opinion that partial albinism does occur among Scotchmen but in these, as in some cases of partial albinism, the dissociation is between eye colour and skin and hair colour, rather than between different areas of skin colour, though the important fact that albinos of different strains may carry different pattern factors is suggestive.

Thus, although a considerable number of cases of partial or complete heterochromia of the iris occur during the inter-breeding of duplex with duplex, and duplex with simplex types of eye colour, no cases of piebald skin colour have been recorded so far as arising during the inter-breeding of light complexioned and dark complexioned European varieties.

If some segregation of skin colour factors does occur in subsequent gametogenesis in the offspring of mulatto hybrids as stated by some observers, even in such cases the segregation process would seem to affect the self-colour patterns as a whole.

In this respect then the black and white human hybrid (mulatto) and the pied animal hybrid, e.g. the Dutch or Orkney rabbit, are dissimilar.

" *B* " MATING. BLACKS.

White Fantail □ P_1 ×   Black Fantail ■   ♂

F_1

1905 May July Aug. Sept. Dec.
1906 Jan. March April May June Aug. Oct. Nov. Dec.
1907 Feb. March May July Oct. Dec.
1908 Feb. March May June

" *A* " MATING. HARLEQUINS.

White Fantail □ P_1 ×   Black Fantail ■   ♂

F_1

1905 March May July Oct. Dec.
1906 Feb. May June July Aug. Sept. Oct. Nov.
1907 Jan. Feb. March April May June Aug. Sept.

Chart = result over 7 years of mating together one pair of Black and White F_1 young of White ♀ and Black ♂ Fantail P_1.

□ = White. ■ = Black and White. ⊠ = Blue. ■ = Black.

■ ▣ Represent relative proportion and distribution on wings and body of black and white in the "Blacks" of the "B" Mating.

Column labels: White 16 | Black and White 25 | Blue 14 | Black 26

Dates: July, Sept., Oct., Dec., 1909 Jan., Feb., March, May, June, July, Sept., Nov., 1910 Jan., Feb., April, June, Aug., Oct., Nov., Dec., 1911 Feb., March, April, June, Aug.

Chart = result during 7 years of mating together one pair of Black and White F_1 young of White ♀ and Black ♂ Fantail P_1.

⊠ = Blue. □ = White ▨ = Black and White. ■ = Black.

□ = White with black feathers in right wing.

◪ ▨ ◪ Represent relative proportion of black and white in the Harlequin of the "A" Mating.

Column labels: Black 11 | Blue 4 | Black and White 17 | White 22

Dates: Oct., Dec., 1908 Feb., March, June, Sept., Dec., 1909 Feb., March, April, June, Oct., 1910 Jan., April, May, June, Aug., Sept., Nov., 1911 Aug., Sept.

The parti-coloured iris occurs during inter-breeding fairly frequently, but the parti-coloured skin must be extremely rare in the human species, whereas in domesticated animals the parti-coloured or "wall" eye and the pied coat pattern both occur when self colour is mated with white.

This fact strongly suggests that the factors for self colour in the negro or the factor for self white in the European, or both of them, offer more resistance to disintegration than the factors which control the self-duplex and the self-simplex patterns in the human eye.

Some association no doubt does exist between skin-colour and eye-colour even in the human subject; the dark brown or black iris and the black skin of the negro, the blue eye, reddish hair and white skin of the northern races illustrate this point.

The same is certainly true in many wild species and domestic varieties of animals, and even in extracted hybrid varieties we have the association of the agouti and blue coat colour with black eyes in mice, and chocolate eye pigment with pink eyes in the Himalayan rabbit, the Siamese cat, and the Cinnamon canary (Bateson, p. 114) (9).

But owing possibly to the difficulty of breaking up the self-skin colour factor in man and the greater susceptibility of the self-colour iris factor to disintegration, the association which originally existed between these factors is partially dissolved when they are called upon to behave independently under the altered conditions of inter-breeding.

Thus in the native Irish race, dark complexion and dark hair have become associated with the violet or simplex eye. It is a suggestive fact that the factor for self skin colour in man which refuses to disintegrate when mated with absence of colour or white skin should apparently refuse also to segregate in gametogenesis.

Some anomalous facts about albinism in the human subject are also of interest in this connection.

A woman M. T. *aet.* 35, the daughter of a brown-haired, blue-eyed father, and a red-haired, blue-eyed mother, and one of four children (two daughters with red hair and blue eyes, and one son with dark brown hair and blue eyes) was herself born with white pigmentless hair, eyebrows, and eyelashes and pink pupils and pigmentless irides, in fact with all the characters of the true albino. At the age of puberty the hair of the head of this woman gradually assumed a red tinge and is now the ordinary fiery red colour, while the eyebrows and eye structures have remained free from pigment.

In this case the association which existed in childhood between the recessive characters, absence of skin and hair pigment, and absence of

iris and choroid pigment, has been broken up by the appearance in later years of a dominant character, e.g. hair pigment in the hair of the scalp though not in the hair of the face.

Bateson also refers to the existence of similar abnormal cases of albinism in man, pp. 226–7. See also Pearson, Nettleship and Usher, *On Albinism in Man.*

In one sense this unusual development of a later epistatic character in an albino is only what happens normally in the case of other characters in the young of animals and of human beings.

All children, with few exceptions, are born with simplex or blue eyes, the epistatic duplex character only begins to appear from 4 to 8 or 10 weeks after birth.

The same is true of kittens and the young of other animals, while in young pigeons hatched with " bull " eyes the red or yellow anterior pigment begins to be deposited 6 or 8 weeks after hatching.

In fact following the law of recapitulative ontological development with the addition of characters, epistatic characters seem usually to follow and not precede recessive characters in the process of the unfolding of unit characters in the zygote.

Hair Pattern in Man.

But although as we have seen the self-skin colour seems in the case of man at present to resist influences of a genetic kind calculated to break it up into subordinate factors (a state of things which may account for the fact that, when in the human species irregular or parti-coloured iris pigmentation occurs a like particulate distribution of skin colour pattern does not usually occur along with it, at any rate in European races), yet there are some facts which suggest that the factors which control hair structure and its distribution in the human subject are subject to similar disturbances to those which affect the factor for iris pigmentation.

As a general rule the corkscrew (ellipsoidal sectioned) black hair of the negro is dominant over the wavy (oval sectioned) brown hair of the European.

And in the majority of the F_1 hybrids of the negro and white cross this dominance extends over the whole of the scalp area to all the hair of the head. (Plate IX, fig. 2.)

In two children L. M. and B. M. out of a family of nine the F_1 offspring of an English woman with straight brown hair and a West African negro (Plate VIII) some segregation of the corkscrew and

straight hair pattern occurred. The curly tufted negro hair covered the sides of the scalp while the straighter hair formed a patch on the vertex in each case. (Plate IX, fig. 1.)

In these two children genetic factors which normally control the epistatic negroid type of hair structure over the whole scalp behaved differently in different scalp areas, and the true explanation of irregular or ray iris pattern and of harlequin coat colour pattern will probably apply to this abnormality in hair structure pattern also.

PART II.

IRREGULAR EYE COLOUR PATTERN AND THE CONSTITUTION OF GAMETIC FACTORS.

Irregular iris pigmentation of the ray type and indivisibility of gametic factors in heredity.

It has been customary to regard gametic factors as indivisible units like the chemist's atom.

Thus Punnett(10) (*Mendelism*, p. 39) says, " Unit characters (in the zygote) are represented by factors in the gametes which behave in the process of heredity as indivisible entities."

Now if in the " *B* " human family we regard the male parent G. B. as a heterozygous duplex of irregular or ray pattern, then, according to expectation, the mating of this individual with a simplex recessive should result in equal number of simplex and duplex offspring in the F_1 generation, whereas the actual numbers are seven simplex and three duplex of which two are of the irregular ray pattern like the father in a family of ten.

But a further difficulty arises with the manner of transmission of the irregular or ray pattern itself.

For although two of the three duplex children are partial or ray duplex like the father, the distribution of the irregular pattern is not the same in the parent and the children.

The pigmented ray appears in the right eye instead of the left (as in the father) in one child, and in the opposite half of the same iris in the other child.

How can we explain this different incidence of pattern in the passage from father to children ?

Either the complication arises in some alteration in the composition or arrangement of the duplex factors in the gametes of the male parent,

or it is brought about by some modifying factor introduced by the gametes of the recessive female parent.

It will be best to leave the final conclusion till all the evidence has been considered.

There are certain facts about these irregular forms of eye colour in man and pied coat colour in animals which suggest that *presence* and *absence* of gametic factors though essential are not the only conditions which determine the behaviour of unit characters.

These facts suggest that much depends on the manner in which any factor is present in the gamete which carries it, and on the way in which it incorporates itself with, or is incorporated by, the gamete which bears the alternative factor during the process of gametic union.

We already know that much depends on the volume of the factor present.

The difference in appearance between the homo- and heterozygous dominant depends on doubleness or singleness of dose of the dominant character.

In this connection I should also mention Davenport's (11) remarks on the imperfection of dominance in which he concludes that alongside of dominance we must place an important modifying factor, the factor of the strength or potency of the representative of the given character in the germ plasm.

The influence of reciprocal gametic contribution is well known in the case of sex controlled characters.

In other cases the appearance and behaviour of unit characters have been shown to depend on the intermediation of a second and in some cases of a third factor, introduced by the gamete which carries the recessive character. Thus black eye colour in certain varieties of mice is attributed to the interaction of two factors (Bateson, p. 112)(9).

The limitation of colour to certain skin areas in pied individuals, in which pied pattern is dominant, has been explained by the restraining influence of the factor for pied pattern on the factor for self colour (Bateson, p. 84)(9).

The investigation of irregular types of eye colour pattern throws some light on the relationship between these different factors for eye colour especially in the human subject, because in man the close association which exists in the black and white races between eye colour and skin colour has been partly dissolved in the European or mixed races, and one complicating element has been thereby removed.

It seems clear, from the familial distribution of these cases of irregular eye colouration, that the ray eye pattern in man, like the Dutch coat pattern in the rabbit, is controlled by a definite gametic factor or factors.

The duplex ray pattern like the duplex self-colour and ringed patterns seems to be dominant over the simplex character (see Baines, 2nd family). It would also appear to be dominant over self-colour duplex when the self-colour duplex is of lighter shade than the ray duplex (see Frake), but this dominance only occurred in two out of nine children and then only in one eye, the other being self-colour duplex. In two others the self pattern was associated with irregular patches of darker pigment as we shall see later. These facts suggest that ray pattern is only dominant over self-colour pattern when the original factor for self-colour has undergone some dilution or when disintegration and dilution are operating together.

Concerning this difference in genetic composition between self-colour and Dutch pattern, Bateson says (p. 142)(9):

" Physiologically we should I suppose refer it to differences in the distribution of one of the chromogenic factors, rather than to the presence or absence of an additional factor."

It is these differences in the distribution of one or both of the chromogenic factors that I wish to consider in the light of the evidence derived from cases of irregular iris pigmentation in man and animals.

It is true that there are albinos of different factorial composition in every species, just as there are dominant and recessive " whites " among fowls. There are some extracted albinos which carry the factor for self-colour, and others which carry the factor for pattern, though both lack the colour developer factor itself.

The different results obtained in mating the black fantail cock with the two white fantail hens of different gametic composition afford another example of the influence of the gamete which bears the recessive character on the determination of colour pattern.

But while by the assumption of a colour factor awaiting development in the albino and a developer factor in the self-coloured animal we can by the mutual interaction of these factors explain the restriction of the colour unit character to certain skin areas, and the dilution of colour in zygotes of different gametic composition, we have not thereby explained the original distribution of these different factors in either the albino or the pied animal.

Thus it appears that besides presence and absence, and besides

singleness and doubleness of dose, and besides reciprocal gametic introduction, and besides intermediary action of restraining factors, and besides colour basis and colour developer factors, there still remains some gametic cause for the abnormal behaviour of the unit characters in the individuals with irregular eye colour pattern. Now it seems possible that this unexplained irregularity of pattern may depend on some deficiency in integration of the dominant duplex factor, some abnormality in the way in which it is present in the gamete which carries it, or in the way it is incorporated by the gamete which carries the recessive or the alternative character.

Of the characters possessed by gametic factors two of the most important are TOTAL VOLUME, that is, size or quantitative presence, and DEGREE OF INTEGRATION, that is, qualitative presence.

The reduction of the total volume of gametic factors occurs probably during gameto-genesis and the important point is that this reduction of volume may apparently take place with or without a concomitant process of partial disintegration of the factor concerned into its subordinate units. That is to say reduction of volume may be quantitative or qualitative.

Thus the factor for self-eye colour has, in ordinary individuals, a definite total volume; it possesses a definite capacity to spread epistatically over the whole anterior surface of the iris and so to produce the self-coloured pattern in the duplex eye.

In certain less numerous cases (14 out of 88) the volume of this factor is reduced and its epistatic influence is restricted to a circular zone of iris round the pupil, and the RING PATTERN appears; in other individuals it is limited to certain patches on the iris and produces the SPOTTED PATTERN; in others again (3 cases in 200) it only operates over certain sectors of the iris and then forms the RAY PATTERN.

In all these cases the reduction in volume occurs in association with a qualitative and disintegrative change, a change in which the original factor for self-colour undergoes subdivision into subordinate factors independently controlling different areas (either rings, or patches, or rays) of the same iris.

In other cases again there is no subdivision or disintegration and the reduction in volume (or capacity of influence) of the factor for self-colour takes the form of quantitative dilution, of deficient pigment saturation over the whole of the anterior surface of the iris, and in this way the various tints of the duplex pattern, from yellow to dark brown, are brought about.

If we imagine the factor for self-eye colour to have been built up during animal evolution out of two primary factors, one for each eye, and each of these again out of a number of secondary factors controlling the deposit of pigment in different sectors of each iris, and that all these factors have become welded together into one whole, and now act as one integrated factor, then we shall associate the appearance of the irregular or ray pattern with some preceding disintegrative change in the factor for self-eye colour in the human species.

Some disintegration, partial or complete, is the first step in the origin of a new colour pattern. · It provides the opportunity for the re-arrangement of subordinate factors which takes place during gameto-genesis and gametic union.

From this standpoint we should regard the appearance of pied coat colour and pied eye colour, or "wall" eye, in the Orkney rabbits, and the appearance of the heterochromic eyes in the blue and white tumbler fantail hybrid of the third generation, as the outcome of a disintegration of the factors for self-coat colour or self-eye colour and this disintegration appears during the mating of individuals of different genetic composition.

The establishment of these new types on a basis of genetic stability (such as we see in the ring and ray iris pattern in man, and the Dutch coat pattern in rabbits) becomes then a matter of the re-welding of these secondary factors into one newly integrated factor, of a fresh type, and with a different arrangement of its component parts.

Thus regarded the only way to obtain the piebald variety in the human species is to disintegrate the factor for self-colour which at present controls, in different degrees of dilution, the whole skin area, and which at present ordinarily refuses to segregate in gameto-genesis.

The way to bring about irregular iris colour pattern is to break down (as we have done in the heterochromic pigeon) the self-colour factor for both eyes into two factors controlling its different eyes, and these again into subordinate factors controlling different areas in the same iris.

The inter-mating of dark duplex with light duplex and duplex with simplex types results in the appearance of partial or complete hetero-chromia in some cases in the human species, and the familial distribution of these cases of irregular iris pigmentation suggests the possibility of the establishment under certain conditions of a heterochromic variety of the human race.

Looked at from this point of view, the difference between individual

and racial, and between varietal and specific characters would seem to be a matter of integration of the gametic factors concerned in each case, dominance and integration being closely associated.

Thus the difference between the human mulatto hybrid and the spotted negro hybrid and the pied animal hybrid is this. In the mulatto the reduction of the volume of the colour factor is brought about by a quantitative process of equal dilution over the whole skin area, and not by a qualitative process of disintegration and segregation of component factors for different skin areas as in the spotted negro or the pied animal hybrid.

By assuming the occurrence of a process of disintegration we extend the principle of segregation into the constitution of gametic factors, we assume INTRA as well as INTER factorial segregation.

If *inter* factorial segregation can explain the behaviour of unit characters on Mendelian lines in the normal heterozygote, then *intra* factorial segregation can explain the irregular behaviour of unit characters in the abnormal heterozygote.

If the foregoing conception of gametic architecture be at all true, then it must stand the test of experience.

The establishment of the Dutch pattern of coat colour in the rabbit should show some evidence of the welding process by which two or more less integrated unit characters (or rather the subordinate factors which control them) have been integrated into one factor for Dutch pattern.

In the same way the resolution of the Dutch pattern into its component unit characters should show, as it does in the case of the Orkney rabbits (p. 112, Part I), the various steps in the disintegrative process.

The same process can be seen at work in the genesis of the hetero-chromic pigeon.

If true, this DISINTEGRATIVE THEORY should be applicable also to the problem of the comparative sterility of inter-special and the comparative fertility of inter-varietal hybrids.

Moreover it is quite independent of any pre-conceived notion as to the ultimate nature of genetic factors. It is equally applicable to the theory of gametic constitution which rests on an architectural or mechanical as to one which rests on a chemical basis in the organisation of the germ plasm.

Since writing this paper my attention has been called to H. H. Laughlin's(12) observations on the inheritance of colour in

shorthorn cattle, in which he puts forward a chemical theory of "Intra-zygotic inhibition and reaction in response to specific set (? genetic) conditions," p. 27, and alludes to the possibility that "unit characters may arise from a partial destruction of larger units, and that a determiner for a unit character behaving precisely in unit fashion may be a 'complex' capable of being shattered into a large number of independently behaving characters," p. 26.

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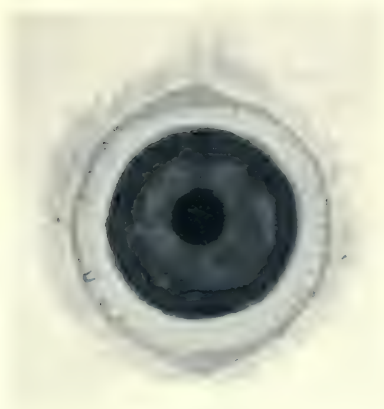
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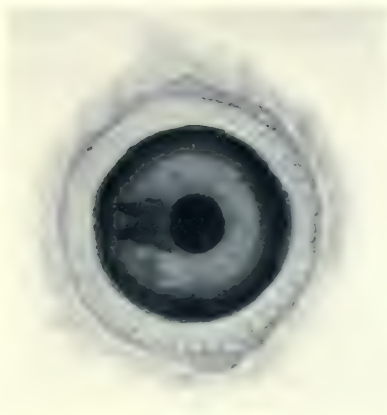
Fig. 1.

Fig. 2.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 1.

Fig. 1.

Fig. 2

EXPLANATION OF PLATES.

PLATE VI.

"Wall eye" in an Orkney rabbit of Dutch colour pattern. Note absence of anterior pigment in the upper half of the left iris and the upper and anterior portions of the right iris.

Fig. 1. Left eye.

Fig. 2. Right eye.

PLATE VII.

Heterochromic Pigeon. Fig. 1. Left bull eye.

Fig. 2. Right orange eye with a gap in the orange pigment (colour coloboma) in the lower half of the iris.

Fig. 3. Adult frog shows gap in anterior pigment of iris (colour coloboma) in downward vertical direction in the lower half of the iris.

PLATE VIII.

Fig. 1. Nine children, the offspring of an English woman (wavy hair) and a West African Negro (corkscrew hair). The two youngest (marked ×) show differentiation of hair pattern with wavy hair on the vertex.

PLATE IX.

Fig. 1. L. M. Boy aet. 4, shows wavy European hair pattern on vertex, corkscrew pattern on sides.

Fig. 2. Girl aet. 6, shows corkscrew negro hair pattern all over scalp.

SECOND REPORT ON THE INHERITANCE OF COLOUR IN PIGEONS, TOGETHER WITH AN ACCOUNT OF SOME EXPERIMENTS ON THE CROSSING OF CERTAIN RACES OF DOVES, WITH SPECIAL REFERENCE TO SEX-LIMITED INHERITANCE.

By RICHARD STAPLES-BROWNE, M.A.

CONTENTS.

Introduction.

In the *Proceedings* of the Zoological Society for 1908, p. 67, I published a report on the inheritance of colour in Domestic Pigeons with special reference to reversion. The colours chiefly dealt with there were black and the "reversionary blue" of Darwin, which may be considered as the type classed as "black-chequer" by the fanciers (Plate V of the report), and also the relations of these two forms to white.

In the present paper the colours considered are black, dun, blue and silver, their relations to each other, and the behaviour of the two latter when mated to white. The experiments on these colours are still in full progress, so that this report must be considered merely as a preliminary account of them. Enough has, however, been done to demonstrate a sex-limited inheritance in silver. The same may possibly be shown in dun also on further experiment[1]. It is found that, whereas a black ♂ mated to a silver ♀ gives all the offspring black, yet a silver ♂ mated to a black ♀ gives black ♂s and dun ♀s. This result is similar to that obtained from the reciprocal matings of Cinnamon and Green Canaries. Further evidence on the sex-limited inheritance of silver in pigeons is obtained from the experiment, alluded to below, on the matings of Silvers and Reds.

The possibility of a sex-limited inheritance of white in pigeons was suggested in my previous paper (p. 85) to account for the great excess of whites produced from the matings of heterozygous reversionary blues with whites in Exps. 16—23. This was particularly marked when the white was ♂, and Prof. Whitman's experiments with Doves were instanced in support of this proposition. The present series of crosses gives very little further evidence as regards white, but reference to experiment 58 of the present report would suggest that it is not sex-limited in these pigeons.

I have recently made a series of experiments on the crossing of coloured and white doves. These matings confirm Prof. Whitman's results, and an account of them is included in the present report. Specimens of the Turtle Dove (*T. turtur*) and the Barbary Cage Dove (*T. risorius* var. *domesticus*) have been mated respectively with the Java Dove, a white variety of the latter. These crosses show conclusively that the phenomenon of sex-limited inheritance of white does occur in these species.

Brief Statement of Results.

In the present report three sets of experiments with pigeons are included.

1. *Matings of blue and silver Rock Doves to each other and to whites.* The whites used being Fantails and whites extracted from the Barb-Fantail crosses already described.

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[1] The evidence from this year's matings, as far as it goes, supports the suggestion that the inheritance of dun is also sex-limited. (See p. 156.)

2. *Matings of Silver Owl and Black Fantail pigeons.*

3. *Matings of Blue Dragoon and Dun Carrier pigeons.*

In the case of the Rock Dove crosses the sex-limited inheritance of silver was not discovered, as in all cases the matings made were those of silver ♀ × blue ♂. In this case the offspring were all blue according to expectation. The reciprocal mating of blue ♀ × silver ♂ is being tried this year.

To test fully this question the following matings are necessary:

    1.   dun   ♀ × black ♂.
    2.   black ♀ × dun   ♂.
    3.   silver ♀ × blue   ♂.   *
    4.   blue   ♀ × silver ♂.
    5.   silver ♀ × black ♂.   *
    6.   black ♀ × silver ♂.   *
    7.   dun   ♀ × blue ♂.   *
    8.   blue   ♀ × dun   ♂.

Of the above, Nos. 3, 5, 6 and 7, marked with an asterisk, have been made; the remainder are being tried this year, as is also the relation of Silver to Dun.

The results obtained so far from these crosses are as follows:

    3.   silver ♀ × blue   ♂ gave all offspring blue.
    5.   silver ♀ × black ♂ gave all offspring black.
    6.   black ♀ × silver ♂ gave *black ♂ and dun ♀*.
    7.   dun   ♀ × blue   ♂ gave all offspring black.

Supplementing the evidence of sex-limited inheritance in silver the following matings may be given, but it must be understood that the shade and patterns of the offspring are for the present purpose neglected:

Silver ♀ × Red (containing yellow) ♂ gave 3 red and 2 yellow.

Red ♀ × Silver ♂ gave 5 red ♂s and 3 dun ♀s.

The inheritance of red and yellow will not be alluded to again in this paper. The question is complicated, and no statement can be made until much more work has been done. The experiments with the red birds derived from the Barb-Fantail crosses were continued, but the birds were ultimately lost by disease. A new series has been started, the reds used being Dragoons.

The following experiments confirm the statement in my previous paper that colour is dominant to white; they also show the dominance of blue to silver, and suggest that of black to dun, with the sex-limited provision.

Two pattern characters have also been studied in the Rock Dove × white matings, viz. Chequering and the colour of the rump, and it is shown that the chequered form is dominant to the non-chequered form in both blues and silvers. The matings of chequered birds to blacks and duns have not yet been undertaken other than the matings of the "reversionary blues" described in my previous paper.

Further it has been found that the white rump of the Rock Dove is dominant to the blue-rumped form. These two results have already been mentioned by Mr Bateson (*Mendel's Principles of Heredity*, p. 43, 1909).

A valuable paper has recently been published by Messrs Bonhote and Smalley (*P.Z.S.* 1911, p. 601), dealing with the relation of blue and silver, and also with chequering. I am able to confirm their results of the dominance of blue to silver, and chequered to non-chequered forms. They have, however, not made many matings between blue ♀ and silver ♂. Only four are recorded. In two of which (Nos. 154 and 158) the ♀ may have been heterozygous, and in the other two (Nos. 159 and 162) it was known to be so. The sex-limitation of the inheritance has consequently not been found. The remainder of the paper deals with Grizzles and Mealies, birds of which I have had but little experience as yet.

The numerical results of the Rock Dove matings, and also of the Rock Dove × white matings follow fairly closely the Mendelian ratios as the table on p. 135 shows. In all experiments where more than one type of offspring is to be expected allowances must be made for the small totals produced.

As regards the relations of the four colours black, blue, dun and silver, there is little doubt that all can be correctly represented as a series made by the combinations of the presences and absences of two pairs of factors. Silvers breed true and are therefore the double recessive. Blues can throw silvers or breed true. Duns have not yet been tried, but presumably can throw silvers. Blacks have thrown duns and blues, but I have not hitherto had a certain silver; this however would come only once in 16 birds. Moreover dun × blue gives black. Taking **B** as black, and **D** as a factor for density of colour, **BD** is black and **bd** silver. I had been disposed to regard **bD** as dun, and **Bd** as blue, thinking, that

Experiments on Colour.

Mating				Experiment Numbers	Expectation	Result
Blue × Blue	...	...	...	53, 70, 72	All blue	20 blue
,, ,,	...	...	...	47, 49	13·5 blue, 4·5 silver	15 B., 3 S.
,, ,,	...	...	...	51, 54, 55, 65, 66, 74	27 blue, 9 white	28 B., 8 W.
,, ,,	...	...	...	60	5·6 B., 1·9 S., 2·5 W.	6 B., 2 S., 2 W.
Silver × Silver	...	...	...	61, 68, 69	All silver	31 silver
Blue × Silver	...	...	...	48, 71	All blue	12 blue
,, ,,	...	...	...	59, 67	11 B., 11 S.	11 B., 11 S.
Blue × White	...	...	...	50, 52	All blue	10 blue
,, ,,	...	...	...	64	18·75 B., 6·25 S.	20 B., 5 S.
Silver × White	...	...	...	56, 57, 58, 62, 73	All blue	15 blue
,, ,,	...	...	...	63	9·5 B., 9·5 S.	13 B., 6 S.

Experiments on Chequering.

Mating		Experiment Numbers	Expectation	Result
Non-chequered × Non-chequered		47, 48, 49, 69, 70, 71, 72	All non-chequered	50 non-chequered
,, ,,		55, 74	10·5 non-C., 3·5 W.	9 non-C., 5 W.
Chequered × Chequered	...	67	All chequered	10 chequered
,, ,,	...	65, 66	6 C., 2 W.	4 C., 4 W.
,, ,,	...	61	12 C., 4 non-C.	11 C., 5 non-C.
,, ,,	...	54, 60	11·81 C., 3·94 non-C., 5·25 W.	10 C., 7 non-C., 4 W.
Chequered × Non-chequered	...	68	All chequered	8 chequered
,, ,,	...	53, 59	9·5 C., 9·5 non-C.	9 C., 10 non-C.

Experiments on Colour of Rump[1].

Mating		Experiment Numbers	Expectation	Result
White rump × White rump	...	47	5·25 W. rump, 1·75 B. rump	5 W. rump, 2 B. rump
Blue rump × Blue rump	...	49, 70	All blue rump	12 blue rump
White rump × Blue rump	...	72	All white rump	2 white rump

is to say, that blue is a dilute form of black, and silver a dilute form of
dun. Messrs Bonhote and Smalley, however, take the contrary view,
regarding silver as dilute blue. As there is no other colour on which
the two middle terms of the series can be tested (as they can for
example in mice), it is not possible absolutely to distinguish between
these two alternatives. In appearance some silvers may confidently be
said to contain no black (e.g. Silver Rock), but in others (e.g. Silver Owl
and Dragoon) the colour of the wing bars hardly differs at all from that
of blues, and probably contains true black pigment. Unfortunately
microscopical tests, though extensively tried, have not hitherto provided
any satisfactory criterion between the various pigments, and do not add
much to what can be seen on ordinary inspection.

[1] In these experiments silvers are not included, as it is impossible to be certain whether
the rump is coloured or not.

Account of the Experiments.

The numbering of the experiments herein described is continued from that of my previous experiments on colour contained in my first report. Thus the present series of crosses begins at Exp. 47 and ends at Exp. 91. The matings are divided as follows:

Exps. 47—49 (see Table I) consist of matings of Rock Doves.

Exps. 50—74 (see Tables II—V) consist of the matings of Rock Doves to Whites.

Exps. 75—78 (see Table VI) consist of the matings of Owls, Fantails, Carriers and Dragoons.

Exps. 79—91 (see Table VII) consist of experiments with Doves.

Rock Doves used in Experiments.

The specimens of *Columba livia* used in these crosses came from two sources: (1) three ♂s from a pair taken at the Isle of Achill, on the west coast of Ireland, and (2) one ♂ and one ♀ from Lincolnshire. Irish birds were used in Exps. 50, 71 and 72 of the matings with white, whilst Lincolnshire birds were used in all the other crosses with whites.

Irish Rock Doves.

These birds were kindly sent to me in 1906 by Mr J. L. Bonhote, who had bred them in his aviaries from a pair obtained by him from Achill. He informed me that the birds there were quite wild, and he saw no varieties in the flocks there. There were no tame pigeons within a radius of about thirty miles. Mr Bonhote has bred 19 birds in all from his Irish Rock Doves and their descendants, all true to type, with one exception. This bird was chequered, but since it was produced from a pair of birds that had their liberty, the possibility of a cross was not excluded[1]. From the descriptions of the crosses made with these birds it will be seen that no question arises as to their purity, and it was found that in F_2 from the mating of an Irish Rock Dove with a white the pattern factor segregated out quite cleanly (*v.* Exp. 51).

Lincolnshire Rock Doves.

These birds were obtained in 1903 from Lincolnshire through a dealer's advertisement. They were stated to be wild caught, but I could obtain no reliable information concerning them. Their appearance was identical in every respect with that of the Irish birds and other pure

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[1] *v.* Bonhote and Smalley, *P. Z. S.* 1911, p. 605.

specimens. On breeding them together, however, certain varieties were obtained, and, as will be seen, they were obviously heterozygous in at least two characters. It frequently happens that semi-domesticated birds join the Rock Doves in their breeding haunts, and no doubt often cross with them. It is stated in Yarrell's *British Birds*, 4th edition, Vol. III, p. 14, that "even in Yorkshire and Northumberland the birds found are open to the suspicion of not being pure wild birds." In 1906 I visited several caves near Flamborough Head which were frequented by these birds in large numbers, and, although no varieties were seen on the day of my visit, I was informed by the boatmen that lighter and darker birds and whites were frequently seen and shot there.

Test experiments with Lincolnshire Rock Doves.

When the Lincolnshire Rock Doves were bred together a mixed generation, consisting of three distinct types, was produced. To determine the relationship of these types to one another matings in the direct line were continued and two more generations were raised. The details of the experiments are given in Series A (Exps. 47—49), and the results tabulated in Table I.

Types of birds produced from the Lincolnshire Rock Doves.

(1) *Typical C. livia.* These birds were identical in every respect with their parents. This type behaves as a dominant to the other two types produced. One of these birds, mated to white, forms a starting point of some of the experiments described in Series B (*v.* Exp. 52).

(2) *Blue rumped Rock Dove.* The plumage of this type resembled that of the sub-species of Rock Dove known as *C. intermedia.* The colour of the rump was slightly lighter than that of the back. On examining the series of skins of *C. intermedia* in the British Museum I noticed that specimens varied slightly in the colour of the rump, some being darker than others. Mr Blyth informed Darwin that the rumps of *C. intermedia* were sometimes albescent (*Animals and Plants under domestication,* 2nd edition, Vol. I, p. 193). In the whole of these experiments thirty birds of this type have been produced, and although the colour of the rump varied slightly, the character was always quite distinct, and could be seen at a very early age. In the following descriptions this type is alluded to as Blue with no white feathers (Bl. no wh.).

(3) *Silver.* In this type the blue of the Rock Dove is replaced by a light silver, or, more strictly speaking, cream colour, whilst the wing and tail bars are dun. The head, neck, flight feathers, and tail are much lighter and browner than those of the blue, but are considerably darker than the plumage of the wing coverts, back, breast, and under parts. It is extremely difficult to determine with certainty whether the rump is a very light shade of silver or white, but after examining a large number I did not feel convinced that I had found a really white-rumped bird. In the figures given for the experiments on rump character, therefore, these birds are omitted. Silvers are recessive to both types of blues described above, and breed true when mated together. The silver ♀ produced by breeding together the two Lincolnshire Rock Doves formed the starting point of the experiments described in Series C.

TABLE I.

Experiments with Lincolnshire Rock Doves.

Exp. No.	Female	Origin from Exp.	Also used in Exp.	Male	Origin from Exp.	Also used in Exp.	Blue no white	Typical *Columba livia*	Silver
							Offspring		
47	Typical *C. livia* (no number)	—	—	Typical *C. livia* (no number)	—	—	2	5	1
48	Silver 43	47	55,56,57, 58, 68	Typical *C. livia* 44	47	50,52	4	6	—
49	Blue, no wh. 27	48	—	Blue, no wh. 39	48	—	8	—	2

Details of the matings of Lincolnshire Rock Doves.
Series A. (v. Table I.)

Exp. 47. Typical *C. livia* ♀ × Typical *C. livia* ♂.

These two birds, when mated together, produced eight offspring, of which five were typical *C. livia*, two were blue with no white feathers, the rump being a lighter shade of blue, and one was silver with dun wing and tail bars, the rump being apparently a very light silver. Now since subsequent experiments show that blue is dominant to silver and white rump dominant to blue rump, we may conclude that had this experiment been prolonged we should have had White-rumped blues, Blue-rumped blues and Silvers in the proportion of 9 : 3 : 4. The observed figures were 5 : 2 : 1.

Exp. 48.   Silver ♀ 43 × Typical *C. livia* ♂ 44.

Both birds were raised in Exp. 47.   Ten young were produced, all being blue, of which six had white rumps and four blue rumps.   The experiment shows the dominance of blue to silver.

Exp. 49.   Blue, no wh. ♀ 27 × Blue, no wh. ♂ 39.

These birds, both raised in Exp. 48, produced a family of ten, of which eight were blue and two silver.   The rumps of the offspring were of a lighter shade of blue and silver respectively, but no white feathers were seen.

On the crossing of Rock Doves with White Domestic Pigeons.

From the foregoing description of the Rock Doves obtained, and the varieties produced from them, it will be seen that four types were available for crossing with whites, viz.:

> Typical *C. livia* (Irish).
> Typical *C. livia* (Lincolnshire).
> Blue-rumped Rock Dove.
> Silver Rock Dove.

The Blue-rumped variety was not used as a starting point for crossing experiments, owing to the limited space at my disposal, but the other three types were mated to whites[1].   The results are given in detail.   The crossing of the Irish typical Rock Dove (Exps. 50 and 51) and of the Lincolnshire Rock Dove (Exps. 52—55) with whites are given in Series B (*v.* Table II).   The experiments with the Silver are given in Series C (*v.* Table III).   The remaining matings here described (Series D and E) were suggested by the preceding series of experiments.

No fresh colours appeared in these matings, the birds bred in the whole series of experiments consisting entirely of varieties of blue or silver, to be described in detail later, and, of course, extracted whites.

From the matings of the typical Rock Doves with whites no silvers resulted.   From the result of Exp. 48, already described, this was to be expected, and we had no evidence that the Irish Rock Dove was heterozygous in this or any other character.

From the matings of silver with white, however, I obtained some *blues in every case.*   It appears, therefore, that the factor Bl., which is present in the blue and absent in the silver, may be carried by the

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[1] For an account of the whites used see below, p. 141.

white and so, when meeting with colour in the silver, reconstitutes the original blue. In these experiments one case is met with (Exp. 63) where the white is heterozygous in Bl., and so both blues and silvers are produced from the mating of a silver with a white, and no doubt further search would have revealed a white from which the factor was absent.

In these experiments both blue and silver chequers occurred, the blues being chequered with black, and the silvers with dun. Chequering is dominant to its absence. As will be seen it was introduced by the whites used. Whites can be homozygous for the presence or absence of this factor, or heterozygous respecting it. The degree of chequering varied considerably, and, in the case of the blues, a few of the darker birds were with difficulty distinguished from the "reversionary blues" of the Barb-Fantail matings.

From these matings coloured birds having a certain amount of white in their plumage were also obtained. The amount of white varied very considerably, and the birds can be roughly classified as follows:

(*a*) *Coloured birds with very few white feathers*, i.e. considerably less than in a typical *C. livia*. These birds have not been bred from, and the significance of the few white feathers is not apparent. The presence of a very few white feathers does not necessarily imply that the bird is heterozygous, for I have already shown in my first report that a blue with very few white feathers (No. 19 raised in Exp. 5) when mated to a white (Exp. 13) did not produce any white offspring.

(*b*) *Typical C. livia.* These birds were obtained in F_2 from the cross between Irish Rock Dove and white.

(*c*) *Coloured birds with several white feathers.* When bred together these have produced whites, and they are comparable with the mottled birds produced in the Barb-Fantail experiments. White feathers were found both on chequered and non-chequered birds.

Lastly recessive whites were produced. No coloured feathers were seen on the plumage of any white, with the single exception of one bird raised in Exp. 66. A pair of whites, which were being kept for further use, were allowed to mate up in an aviary containing several other birds. These produced one white bird. Beyond this no pairing of whites together has been made. In the Barb-Fantail experiments it was shown that whites bred true, and there is no reason to believe that those raised in these crosses would behave otherwise.

White pigeons used in these crosses.

The whites used were either Fantails or extracted whites from the Barb-Fantail matings. In my first report the origin of the strain of Fantails used is given in detail, and a pedigree of the actual birds appended. The two white Fantails used in the present crosses (40 ♀ and 23 ♂) will be found in the pedigree. The extracted whites were bred as follows :

37 ♂ from Barb-Fantail, Exp. 16.

50 ♂ from a red in F_2 × White. (See Exp. *d.* in note on page 70 of *Barb-Fantail Report.*)

53 ♂ from Black w.f. × White. The black w.f. being bred from the mating of Red × White. (Exp. *e.* in note.)

All the other whites used in these matings were raised in the present series of experiments.

Types of birds produced in the Rock-Fantail Cross.

The offspring of these crosses fall naturally into three very distinct classes, viz., blues, silvers and whites. The coloured birds are further classed as chequered or non-chequered, and as having no white feathers or some white feathers, this latter type being further subdivisible, as stated above.

The classification of birds into chequered and non-chequered types is complicated by the occurrence of five birds which appear to be *intermediates.* These show a slight darkening of some of the lower wing coverts, which is not noticeable unless the bird is caught. They occurred in the experiments as follows :

<div style="text-align:center">

In Exp. 60 an intermediate silver.

„ 61 „ „ „

„ 64 „ „ blue.

„ 73 two „ blues.

</div>

These two latter birds were mated together in Exp. 74. Only two blue birds were produced from them, neither of which showed any trace of chequering. It is however quite possible that chequers would have been produced had more young been raised. No other matings of these birds have been made.

Pending further investigation, these birds are provisionally classed as non-chequers, but a note is appended to each experiment in which they occurred.

Descriptions of the several types follow :

Non-chequered blues.

Type I.   Blue-rumped Rock Dove.

A description of this type has already been given in the section on the mating together of Lincolnshire Rock Doves.

Type II.   Blue with very few white feathers.   (Blue, v.f. w.f.)

These birds resembled the preceding type, except for the addition of a very little white.   This was confined chiefly to the rump, vent and thighs.   The amount on the rump did not exceed 10 feathers, as far as could be judged.   In one specimen a very few white feathers appeared over the beak.   In another there was one white primary wing feather, and six white feathers on the rump.   These latter did not reappear after the moult, leaving a blue bird with only one white feather in the wing.   Another bird had one white primary and two white tertiaries in addition to a little white on the rump, vent and thighs.   In every case the amount of white feathers was considered to be less than that found on the rump of a typical *C. livia*.

Type III.   Typical C. livia.

The birds classed under this head resembled very closely the Rock Dove.   The amount of white on the rump did not appear in all cases to be quite so extensive, but no white feathers were seen elsewhere. In one specimen two blue feathers were seen on the white rump.

Type IV.   Blues with several white feathers.   (Blue much wh.)

In these birds the white was greatly in excess of that found in *C. livia*.   A mottled appearance was generally obtained.   In all cases white feathers were present on the rump, though not necessarily to the extent found in the Rock Dove, also on the vent and thighs.   In about half the specimens raised this white was continued on to the abdomen. With two exceptions, white feathers occurred on the head, varying in amount and exact situation, but usually on the occiput.   In most cases this white was continued down the neck, and occasionally even on to the breast.   With one exception, all these birds had some white flight feathers, and frequently all the flight feathers were white.   The bastard wings and carpal joints were often white.   Occasionally also some of the under tail coverts, and in one case three white tail feathers were noticed.   The wing coverts and back were generally free from white. The amount of white varied considerably, and the distinction between some of these specimens having less white and the birds described as belonging to Type II was not very sharp.

Chequered Blues.

Type V. Blue chequer, no white feathers. (B.C. no wh.)

The head and neck were of a slightly darker shade of blue than that found in *C. livia*, as was also the breast. Here a little dappling with a still darker shade was sometimes seen. The wing coverts and back were very definitely chequered with black. In some of the more lightly chequered feathers the outer webs only showed the black colour, but in the darker specimens both webs were similarly coloured. In nearly every case the extreme tip of the feather was of the ordinary blue colour. The black wing bars were very much wider than in *C. livia*, and appeared to be part of the general chequering. The flights and tail feathers were as in the Rock Dove. The black tail bar was present. Abdomen and under parts as in the Rock Dove.

Type VI. Blue chequer, with some white feathers. (B.C. some wh.)

Under this head are classed all Blue chequers in which any white feathers were seen. Chequered varieties of both Types II and IV were met with, but no example of a chequered bird having the true Rock Dove pattern as regards white occurred.

Non-chequered Silvers.

Type VII. Silver, no white feathers. (Silver, no wh.)

This type has already been described above. The rump was considered to be a very light silver in all cases.

Type VIII. Silver, with some white feathers. (Silver, some wh.)

Only three birds of this type were raised (*v.* Exps. 60 and 63). The amount and distribution of the white on these specimens were similar to that described under Type IV. As far as could be seen no typical white-rumped pattern occurred, but as the rump of the silvers was in all cases so extremely light, this type might very easily have been overlooked if it had occurred. The same remark applies to the occurrence of silvers with a few white feathers on the rump, vent and thighs, and these birds also would have probably been classed under Type VII. White feathers on the head and neck, as well as white flight feathers, on the other hand, were easily seen, as in the three specimens recorded as belonging to the present type.

<center>*Chequered Silvers.*</center>

Type IX.   Silver chequer, no white feathers.   (S.C. no wh.)

In these birds the ground colour was silver, and the chequering dun. The pattern and distribution of chequering being as in the blue chequers. The wing and tail bars also were dun. As in the blue chequers, the amount of chequering varied, so that light and dark chequers were obtained. In the darker specimens a little dappling was seen on the breast, the remainder of the plumage being similar to that of the non-chequered silvers, but slightly darker.

Type X.   Silver chequer, with some white feathers.   (S.C. some wh.)

As in the case of B.C. some wh., all silver chequers showing white feathers are classed together. The amount and distribution of the white corresponded with that of Types II and IV. No silver chequers showing the typical Rock Dove pattern occurred. As the general plumage of the chequered birds was darker than the non-chequered, white feathers were easily discernible.

<center>*Whites.*</center>

Type XI.   White.

No coloured feathers were seen in the plumage of these birds except on one specimen raised in Exp. 66, which had a small red tick under the right eye.

<center>*Details of the matings of Rock Doves with Whites.*
Series B.   (v. Table II.)</center>

Exp. 50.   White ♀ 4 × Typical *C. livia* ♂ (no number).

The Rock Dove ♂ used was one of the Irish birds sent to me by Mr Bonhote. The white ♀ was bred in Exp. 54 (*v. infra*), being a bird in F_2 from a Lincolnshire Rock Dove and a White Fantail. From this experiment only two birds were raised. They were blue, with black wing and tail bars, and had a few white feathers on the rump, vent and thighs, and also some white flight feathers and tertiaries. The amount of white on the *rump* was not so extensive as in *C. livia*, but, it will be noticed, white appeared in other situations in which it is absent in the Rock Dove. No chequering appeared on these birds, so the experiment is a great contrast to Exp. 53 (*v. infra*) in which a Lincolnshire Rock Dove mated to a White Fantail gave *all* chequered offspring. The

TABLE II.

Typical Rock Doves mated to Whites and subsequent matings in the direct line.

Exp. No.	Female	Origin from Exp.	Also used in Exp.	Male	Origin from Exp.	Also used in Exp.	Offspring					
							Blue no wh.	Blue few wh. fs.	Typical *Columba livia*	Blue much wh.	B. C. some wh. fs.	White
50	White 4 ...	54	—	Typical *C. livia*	—	—	—	—	—	2	—	—
51	Blue, w. f. 7 ...	50	—	Blue, w. f. 6	50	—	—	—	2	4	—	1
52	White Fantail 40	—	17	Typical *C. livia* 44	47	48, 53	—	—	—	—	8	—
53	B. C. some wh. 64	52	—	Typical *C. livia* 44	47	48, 52	1	1	—	4	1	—
54	B. C. some wh. 63	52	—	B. C. some wh. 67	52	—	1	1	—	3	4	2
55	Blue, much wh. 51	54	—	Blue, much wh. 3	54	—	—	1	—	6	—	4

explanation of this being that the F_1 birds raised in Exp. 52 were heterozygous in the factor for chequering, and produced in Exp. 54 birds homozygous for non-chequering, of which the white ♀ 4 used in this mating was one.

Exp. 51.   Blue, several w.f. ♀ 7 × Blue, several w.f. ♂ 6.

These were the two F_1 birds raised in Exp. 50. They produced seven young, of which six were raised to maturity. These consisted of four birds similar to the parents, and two almost identical with *C. livia*. The white feathers on the rump were not quite so extensive as in the Rock Dove, but there were no white feathers elsewhere, showing that the pattern-factor had segregated out fairly cleanly. It was remarkable that from the crosses of the Lincolnshire Rock Doves with Whites no typical *C. livias* were extracted ; this may possibly be accounted for by the fact that the Lincolnshire bird used was heterozygous in respect to rump-character, as has already been shown in Exps. 47 and 48. In addition to the six birds already described for the present mating, one young bird was produced which died on hatching. It had white down and white beak and claws. Since the blues have yellow down and some black on the beaks and claws, I have no hesitation in recording this as white.   The F_2 generation, here produced, is therefore, 2 Typical *C. livia*, 4 Blues with several white feathers, and 1 White, and is in very close agreement with the Mendelian ratio 1 : 2 : 1 which is the expectation for this mating.

Exp. 52.   White Fantail ♀ 40 × Typical *C. livia* ♂ 44.

This blue rock dove ♂, raised in Exp. 47, is the same bird that was used in Exp. 48, where it was shown to be heterozygous as regards the colour of the rump. A family of eight birds was raised from this mating. All were of the chequered type, with varying amounts of white feathers.

They may be roughly divided into (*a*) those with very few white feathers and (*b*) those with several white feathers. The first class, consisting of three birds, had a few white feathers on the head, rump, vent and thighs. One of them had in addition one white flight feather and one white tertiary. The second class (five birds) had white in the same situations but of greater extent, the white on the head often being continued on to the neck. They also had several white flight feathers and tertiaries, and one or both bastard wings were white. The occurrence of these two classes may possibly be connected with the heterozygosis of the ♂ parent respecting rump character.

Exp. 53.   Blue chequer, several w.f. ♀ 64 × Typical *C. livia* ♂ 44.

The ♀ was raised in the last experiment, and was mated with its own father. Seven young resulted. Of these only one was a chequered bird with a very few white feathers on the rump and one on the head. The remaining six were unchequered blues, of which one had a blue rump and showed no white feathers. Another bird had only a very few white feathers on the rump and none elsewhere, there being much less white than is found in a typical *C. livia*. The four other birds had more white than in *C. livia*. There were white feathers on the head rump, vent and thighs in all four, and in three of these also on the neck, abdomen, flight feathers, tertiaries and bastard wings. These birds presented a mottled appearance. The Mendelian expectation for this mating is chequered and non-chequered birds in equal numbers. The result obtained, 1 : 6, however falls far short of this. The only other mating of heterozygous chequer and homozygous non-chequer (Exp. 59) gave 8 chequered : 4 non-chequered. In this case the discrepancy is in the other direction, and the total of the two matings 9 : 10 gives practically equality. From the behaviour of the chequered birds in the subsequent matings there is no reason to believe that there is any disturbing or selective factor at work. It is to be regretted that no matings were made to test the gametic composition of the blue with very few white feathers. The number of birds of this type produced during the series of experiments was small, and the sexes of those living at the same time did not admit of their being mated together. It is probable however that they would have given blues with no white, but we cannot predict whether or not whites could have been produced from them. On the other hand the blue birds with much white would certainly have given whites, as the mating of two similar birds in Exp. 55 shows. As regards the colour of the rump, both parents of

this family were heterozygous in this character, and two blue rumped birds were to be expected in a family of seven. With such small numbers, however, the appearance of only one is not surprising.

Exp. 54. Blue chequer, few w.f. ♀ 63 × Blue chequer, several w.f. ♂ 67.

These two F_1 birds, raised in Exp. 52, were mated together and produced 11 young. Of these one was blue with no white feathers, another blue with two white feathers on the rump and a very few on the vent and thighs. The remarks on a similar bird produced in the last experiment are equally applicable to this specimen. The other coloured birds produced from this mating all had a considerable amount of white in their plumage. Of these three were non-chequered, whilst four were chequered. White feathers were present on the head, neck, rump, vent, thighs, abdomen, often on the breast; also there were white flight feathers, tertiaries, bastard wings, and sometimes one or more tail feathers. In addition to the coloured birds already enumerated, two whites were produced.

Exp. 55. Blue, much white ♀ 51 × Blue, much white ♂ 3.

These two birds, raised in Exp. 54, had white feathers on the head, neck, rump, vent, thighs, flights and tertiaries. The ♂ had white on abdomen and bastard wings in addition. The young produced were:

One blue with very few white feathers, viz. six white feathers on the rump, and one white flight feather tinged with blue, six of the blue with much white type, and four whites.

In this case we are dealing only with one pair of allelomorphs, the presence and absence of blue. The expectation is 3 blues : 1 white. Of the blues one would have no white whereas two would show white feathers. The fact that the blue with very few white feathers occurs where blue with no white is expected, and the contrast between this bird and the other coloured offspring is so marked, is very suggestive that this type is the homozygote which has not segregated quite cleanly. The expected ratio is 2·75 : 5·5 : 2·75, the observed figures 1 : 6 : 4. The discrepancy is not very great for so small a total. It is also seen here that the coloured type with much white is dominant to that with only a few white feathers. It is possible that this is connected with the dominance of the white rump.

TABLE III.

Silver mated to White, and subsequent matings in the direct line.

Exp. No.	Female	Origin	Also used in Exp.	Male	Origin	Also used in Exp.	Offspring									
							Blue no wh.	Blue few wh. fs.	Blue much wh.	B. C. no wh.	B. C. some wh.	Silver no wh.	Silver some wh.	S. C. no wh.	S. C. some wh.	White
56	Silver, no wh. 43	47	48, 57, 58, 59, 69	Wh. Fantail 23	—	2	—	—	—	—	2	2	—	—	—	—
57	Silver, no wh. 43	47	48, 56, 58, 59, 69	White 37	16	—	—	—	—	—	2	—	—	—	—	—
58	Silver, no wh. 43	47	48, 56, 57, 59, 69	White 50	See last report	19, 20, 22	—	—	—	—	7	—	—	—	—	—
59	Silver, no wh. 43	47	48, 56, 57, 58, 69	B. C. some wh. 116	57	—	1	1	—	1	—	2	—	6	1	—
60	B. C. some wh.115	56	—	B. C. some wh. 114	56	—	—	—	1	1	4	5	1	11	1	2
61	S. C. no wh. 31	59	62, 67	S. C. no wh. 2	59	—	1	—	—	—	2	—	—	—	—	—

TABLE IV.

F'_2s from Silver × White mated to Whites, and subsequent matings of offspring from these crosses.

Exp. No.	Female	Origin	Also used in Exp.	Male	Origin	Also used in Exp.	Offspring									
							Blue no wh.	Blue few wh. fs.	Blue much wh.	B. C. no wh.	B. C. some wh.	Silver no wh.	Silver some wh.	S. C. no wh.	S. C. some wh.	White
62	S. C. no wh. 31	59	61, 67	White 20	55	—	—	3	2	—	2	—	—	—	3	—
63	White 32	60	—	S. C. some wh. 14	59	—	—	—	—	—	8	—	—	1	5	—
64	B. C. no wh. 1	59	—	White 22	60	—	2	1	1	—	17	1	2	—	—	1
65	B. C. some wh. 35	64	—	B. C. some wh. 6	64	—	—	—	—	3	1	—	—	—	—	—
66	B. C. some wh. 32	64	—	B. C. some wh. 46	64	—	—	—	—	2	6	—	—	—	—	3¹
67	S. C. no wh. 31	59	61, 62	B. C. some wh. 10	63	—	—	—	—	—	—	1	1	1	1	—
68	S. C. some wh. 11	64	—	Silver, no wh. 28	61	69	—	—	—	—	—	6	2	6	2	—

¹ One of these birds had a small red tick.

Series C. (*v. Table III.*)

Exp. 56.   Silver, no white ♀ 43 × White Fantail ♂ 23.

Exp. 57.   „   „   „   × Extracted white ♂ 37.

Exp. 58.   „   „   „   × Extracted white ♂ 50.

In the above three matings the same silver ♀, raised in Exp. 47, was paired to three white birds, the origins of which have already been stated. In all 11 young were produced. This F_1 generation consisted entirely of blue birds chequered with black and having some white feathers. The birds were practically indistinguishable from those produced by the mating of a typical *C. livia* with a White Fantail in Exp. 52, and corresponded closely to them as regards amount, variation, and distribution of the white feathers.

In this experiment not only is the factor for chequering introduced by the white, but also the additional factor which when present with silver produces blue.

Special interest attaches itself to Exp. 58, owing to the fact that the white ♂ had given a very aberrant result when previously mated to blues carrying white. These matings have been described in my previous paper on the inheritance of colour in domestic pigeons[1]. If reference is made to Exps. 19, 20, and 22 it will be noticed that, instead of the expected equality of blue and white birds, young were obtained in proportion of 9 whites to 3 coloured. The suggestion was there made that the inheritance of white might in some cases be sex-limited, and comparable with cinnamon in Canaries and white in some species of Doves. Had this been the case with the pigeon in question we should have expected a certain number of white ♀s in the F_1 generation from such a mating as the present one. Here, however, the seven birds produced are all coloured. This experiment very strongly suggests that another explanation must be looked for regarding the unconformable numbers produced in the Barb-Fantail matings.

Exp. 59.   Silver, no white ♀ 43 × Blue chequer, some white ♂ 116.

The silver ♀ is the same bird that was used in the last three experiments. The blue chequer ♂, raised in Exp. 57, is of the F_1 generation. This mating is therefore mother and son. The ♂ had white feathers on the head, rump, vent and thighs, one flight feather

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[1] *P. Z. S.* 1908, pp. 81—84.

white tinged with blue, and four white tertiaries. Twelve young were produced as follows:

> Blue, chequered 1, non-chequered 2.
>
> Silver, chequered 7, non-chequered 2.

White feathers were noticed on two birds only, viz. one blue and one silver chequer. In both instances they consisted only of a very few white feathers at the vent. We should naturally, however, presume that about six birds were carrying white although they were not distinguishable by plumage. In this experiment equality was expected between both blue and silver, and also chequered and non-chequered forms. Although the numbers obtained deviate from the Mendelian figures, the totals for the whole series of matings, as has been already explained, approximate closely with the expected ratio.

Exp. 60.   Blue chequer, several w.f. ♀ 115 × Blue chequer, several w.f. ♂ 114.

These two F_1 birds were raised in Exp. 56. Both had several white feathers, the distribution of which has already been indicated. A family of 10 F_2 birds was produced, as follows:

Blue, non-chequered, much white, 1.
Blue, chequered, much white, 3
  „   „   very few w.f. on rump, vent and thighs, 1 ⎫ 5.
  „   „   no white feathers, 1 ⎭
Silver, non-chequered, much white, 1[1].
Silver, chequered, very few w.f. on rump and thighs, 1.
White 2.

The numerical results of this F_1 mating follow fairly closely the Mendelian ratios, the observed figures being:

> Coloured : White : : 8 : 2.

Of the coloured birds:

> Blue : Silver : : 6 : 2.
>
> Chequered : non-chequered : : 6 : 2.

White feathers in plumage.

> No w.f. : very few w.f. : several w.f. : : 1 : 2 : 5.

Exp. 61.   Silver chequer, no wh. ♀ 31 × Silver chequer, no wh. ♂ 2.

Both these birds were raised in Exp. 59. The mating was continued for two years, and 16 young were produced, all silver, of which 11 were

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[1] This bird was an intermediate and is counted as non-chequered.

chequered and 5 non-chequered[1]. The Mendelian expectation on this mating was chequered : non-chequered :: 12 : 4, with which ratio the figures obtained comply. No white feathers were produced; we may therefore presume that white was not being carried by either parent. In the nest a few of the birds were thought to have some white feathers, but these were seen to be light silver when the birds grew up. One bird also which had some feather deformity was counted as non-chequered in the nest; on maturing, however, when the feathers attained their normal development, it was seen to be a light chequer.

Series D. (v. Table IV.)

Exp. 62. Silver chequer, no white ♀ 31 × White ♂ 20.

This small experiment was made to further demonstrate that the factor for blueness can be carried by a white bird. Here the silver chequer ♀ is the same bird that was used in the last mating (Exp. 61), which there gave silver birds only when mated to a silver ♂. The white ♂, raised in Exp. 55, carries the blue factor, but not the chequering factor, which is introduced by the silver. Only two young were raised from this mating, both being blue chequered with black, having only a very few white feathers at the vent and thighs.

Exp. 63. White ♀ 32 × Silver chequer, very few w.f. ♂ 14.

The ♀ is an extracted F_2 white raised in Exp. 60, the silver chequer ♂ is from a $DR \times R$ mating in Exp. 59. The mating was continued for three years, and 19 young were produced, as follows:

Blue, very few wh. feathers ....3 ⎫ 5 ⎫
   „ many wh. feathers ....2 ⎭   ⎬ 13.
Blue Chequer, very few wh. feathers ...3 ⎫ 8 ⎭
   „   „ many wh. feathers.........5 ⎭

Silver, many wh. feathers 2   2 ⎫
Silver chequer, no wh. feathers............1 ⎫   ⎬ 6.
   „   „ very few wh. feathers ...1 ⎬ 4 ⎭
   „   „ many wh. feathers ......2 ⎭

Here the white ♀ is obviously heterozygous in the blue character. We should have therefore expected an equality of blues and silvers. There is however an excess of blues. As regards chequering we know the silver chequer ♀ to be heterozygous in this character, but the

[1] One of these birds was an intermediate.

result of 12 chequered to 7 non-chequered obtained from the mating is not sufficiently definite to state the composition of the white. As regards white the silver chequer ♀ is homozygous for colour, as no white birds are produced from this mating. It is worth noting, however, that, although all the young are heterozygous as regards white, still one of them shows apparently no white feathers, and seven have only a very few.

Exp. 64. Blue chequer, no white ♀ 1 × White ♂ 22.

The ♀ was raised in Exp. 59, the ♂ in Exp. 60. The mating was continued for three years, 25 young being raised. Of these two were blue without chequering and having no white feathers, one was blue showing a few ticks of darker shade which has been described as intermediate in chequering, but classed here as non-chequered, and also having several white feathers, 17 were blue chequers with some white feathers, and five were silver chequers with some white feathers. Of the blue chequers nine had very few white feathers, and eight had several white feathers. All the silver chequers had several white feathers. As regards the factor for blue and the factor for chequering both parents are obviously heterozygous, and we should therefore expect a 3 : 1 ratio for both characters. The homozygous non-chequered forms fall rather short, and it is curious that no unchequered silvers appeared, although only one or two at most could be expected in 25. The Mendelian expectation for the four forms would be 9 : 3 : 3 : 1, or with our figures 14·04 : 4·68 : 4·68 : 1·56 ; our actual results being 17 : 5 : 3 : 0.

Exp. 65. Blue chequer, very few w. f. ♀ 35 × Blue chequer, very few w. f. ♂ 6.

Exp. 66. Blue chequer, very few w. f. ♀ 32 × Blue chequer, very few w. f. ♂ 46.

These four birds, raised in Exp. 64, were all reckoned as having very few white feathers. From the two pairs eight young were raised, of which four were blue chequers and four whites. Here we have a very high figure for extracted recessives, as the proportion should have been six chequered to two whites. The totals here are, however, too small. As regards white feathers the chequer raised in Exp. 65 was reckoned as having several white feathers, the three raised in Exp. 66 had none. The occurrence of a white bird with a small red patch or tick under the right eye in Exp. 66 is remarkable. When the bird was first described three months after hatching this abnormality was not noticed. It was, however, seen after the moult when the bird was a year old. It will

be noticed, on reference to my previous paper (*P. Z. S.* 1908, p. 70), that similar birds appeared in the Barb-Fantail crosses, and it was suggested that this character had been introduced by the Fantails used. If this explanation is a correct one it holds equally good for this case, as the Fantails used in these crosses were related to those used in the Barb-Fantail matings.

Exp. 67. Silver chequer, no white ♀ 31 × Blue chequer, few w. f. ♂ 10.

The silver chequer ♀, raised in Exp. 59, is the same bird that was used in Exps. 61 and 62, giving all silvers when mated to silver, and blues when mated to white. The blue chequer ♂ was raised in Exp. 63, from the mating of a white heterozygous in the blue character with a silver chequer. This bird had a few white feathers on the rump, vent and thighs, and also three white flights. From this mating 10 young were raised, all chequered, showing that one of the parents is homozygous in this character. This must obviously be the blue ♂, as the silver ♀ has produced in Exp. 61 non-chequered birds. The young produced were 8 blue chequers and 2 silver chequers. Two of the blue chequers and one of the silver chequers had no white feathers. Five blue chequers were classed as having very few white feathers, and the other one had several white feathers. The remaining silver chequer had only a very few white feathers.

Exp. 68. Silver chequer, some w. fs. ♀ 11 × Silver, no white ♂ 28.

This experiment shows the dominance of the chequer character, the chequer ♀ being obviously homozygous in this character. Eight young were produced, all being silver chequers. Of these six had no white feathers, whilst two had a very few white feathers on the vent and thighs.

Series E. (v. Table V.)

Exp. 69. Silver, no white ♀ 43 × Silver, no white ♂ 28.

This mating was made to test the recessive silvers. Seven young were produced, all being silver with no white. The character, therefore, breeds true.

Exp. 70. Blue, no white ♀ 31 × Blue, no white ♂ 9.

A similar experiment to test the extracted blues with no white feathers. These birds, mated in my aviaries, produced six young, and were afterwards sent to Mr J. L. Bonhote and produced five more, making a total of 11 birds. All these were true to the *C. intermedia*, or blue

11—2

with no white type. Silver may have been carried by ♂ 9, but being recessive to Blue would not appear in this cross.

TABLE V.

Extracted blues and silvers respectively mated together, and also mated to typical C. livia and to white.

Exp. No.	Female	Origin	Also used in Exp.	Male	Origin	Also used in Exp.	Blue no wh.	Blue few wh. fs.	Typical *Columba livia*	Blue much wh.	Silver no wh.	White
69	Silver, no wh. 43	47	48, 56, 57, 58, 59	Silver, no wh. 28	61	68	—	—	—	—	7	—
70	Blue, no wh. 31	53	—	Blue, no wh. 9	59	—	11	—	—	—	—	—
71	Silver, no wh. 22	61	73	Typical *C. livia* 27	—	—	—	—	2	—	—	—
72	Blue, no wh. (no number)	70	—	Typical *C. livia* (no number)	—	—	—	—	2¹	—	—	—
73	Silver, no wh. 22	61	71	White, 10	55	—	—	—	—	2	—	—
74	Blue, much wh. 42	73	—	Blue, much wh. 43	73	—	—	1	—	1	—	1

Exp. 71.   Silver, no white ♀ 22 × Typical blue *C. livia* ♂ 27.

In this small experiment an extracted silver was mated to one of the Irish *C. livias* sent me by Mr J. L. Bonhote.   Only two young were raised, both being typical blue rocks with white rumps.

Exp. 72.   Blue, no white ♀ (no number) × Typical blue *C. livia* ♂ (no number).

This mating was made by Mr Bonhote.   The blue, no white ♀ was raised by him from the two birds mated together in Exp. 70.   The typical ♂ was one of his Irish Rock pigeons.   Two young were raised, one being a typical *C. livia* in all respects, the other however had two blue feathers on the white rump.   Although, as has been seen, the white-rumped form is dominant to the blue-rumped form, this dominance is not always quite complete.

Exp. 73.   Silver, no white ♀ 22 × White ♂ 10.

This experiment, in which an extracted silver is mated to a white, is a contrast to Exps. 56, 57, and 58, from the fact that the white ♂ used, bred from two unchequered birds in Exp. 55, is devoid of the chequer character.   The two young produced were blue with several white feathers.   A very critical examination showed slight darkening near the shafts of twelve wing coverts in one bird and nine in the other, but apart from this there was no trace of chequering.

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¹ One of these birds had two blue feathers on a white rump.

Exp. 74.   Blue, several white feathers ♀ 42 × Blue, several white feathers ♂ 43.

These two birds, produced in the last experiment, were mated together, and three young were raised from them.   Of these one was blue with a very few white feathers on the rump and thighs, one blue with several white feathers like the parents, and one white.   No traces of chequering were observed on these birds.

Carriers, Dragoons, Owls and Fantails used in Experiments.

This series of experiments was commenced in 1910, and in only one case has the F_2 generation been raised as yet.   The following birds were obtained.

Black Fantails.   Some birds of this variety were obtained from Mr Stopford of Tichmarsh Rectory, Thrapston, who informs me that he has had his strain for over twenty years, during which time the birds have bred true.   They have been crossed with other strains from time to time, but do they throw birds of any other colour, or birds having white or any other than black feathers in their plumage? I have reason to believe that these are homozygous blacks.   The colour of the black in Fantails is duller and not so deep as that of some other breeds of pigeons, e.g. Carriers.

Dun Carriers.   Two dun Carrier ♀s were obtained from Mr C. F. Bescoby of Fernleigh, Romford, a well-known breeder of this variety. They were bred from the mating of Black ♀ × Dun ♂, and they have Black and Dun only in their ancestry ever since Mr Bescoby obtained the strain.   A dun ♂ was also obtained from the late Mr Wiltshire's strain, bred from two duns.   It is believed that the parents of these birds were also dun, but the more remote ancestry is unknown. These birds are of a deep rich dun colour except the flight and tail feathers which are lighter in shade [1].

Blue Dragoons.   A pair of this variety has been obtained from Mr Richard Woods of Mansfield.   The ♂ bird has been bred from blues for two generations, and the ♀ for several generations.   There is

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[1] Stress must be laid on the fact that the rich deep dun colour found in the Carrier is not found in the Owl.   The dun colour in the latter variety is much lighter in shade, and answers to the description given below of the dun F_1 type produced from the mating of Black Fantail ♀ with Silver Owl ♂.   There are therefore, apparently, two types of dun, a dark and a light variety; and also two types of silver, one of which has black bars and the other dun bars.

no dun or silver in their ancestry as far as Mr Woods knows. He has had 40 years experience of pigeons. The colour of the birds resembles that of the Rock Dove, except that the rump is light blue instead of white. The wing and tail bars are black.

Silver Dragoons. A pair of these birds was obtained from Mr F. Smalley, a joint author of the paper on colour inheritance alluded to in the introduction to the present report. Mr Smalley stated that they had been bred from silvers since 1903, and that he had reason to believe they had been so bred further back still, but he warned me that it is practically impossible to obtain a silver that does not possess some blue and grizzle blood in its ancestry. The colour of these birds is similar to that of the Silver Rock Doves already described, but the shade of the wing and tail bars is much darker. This matter has been discussed in the introduction.

Silver Owls. It was originally intended to use only Dragoons for the silver birds in these experiments, but on mating a Dragoon ♂ to a Fantail ♀ it was found that they did not breed, and so a smaller variety was considered necessary. A pair of Silver Owls was therefore procured from Mr Reid of Barnsly which had been bred from Silvers for two and four generations respectively. The colour of these birds is identical with that of the Silver Dragoons.

Crosses between Silver Owls and Black Fantails.

This cross was made both ways, and the results are given in Tables VI, Exps. 75 and 76.

It will be seen that when the ♂ was black only one type was produced in F_1, which was black, whereas when the ♂ was silver, blacks and duns were produced, the blacks being ♂s and the duns ♀s[1]. (*v.* Table VI, Exps. 75 and 76.)

TABLE VI.

Exp. No.	Female	Origin	Male	Origin	Offspring			
					Black	Dun	Blue	Silver
75	Silver Owl 4	—	Black Fantail (no number)	—	9	—	—	—
76	Black Fantail 933	—	Silver Owl (no number)	—	6	5	—	—
77	Dun Carrier 209	—	Blue Dragoon 877	—	9	—	—	—
78	Black C. D. 919	77	Black C. D. 920	77	5	1	1	?1

[1] Six pairs of black F_1's have been mated together this year. The result so far (April 23rd) is Black 6, Dun 5, Blue 2, Silver 2. Further two dun F_1 ♀s have been mated with two black F_1 ♂s and have produced Black 3, Dun 2, Blue 0, Silver 1.

Types produced in F_1.

A. *Blacks*. In every case the birds were of a dull sooty black colour. Traces of wing and tail bars were visible on handling the birds, and in many cases when the birds were flying in an aviary. In Exp. 75 five of the birds produced showed a very few white feathers on the thighs, and two of these had 3 or 4 white feathers on the rump as well. In Exp. 76 none of the young had any white feathers at all. I have shown in my previous paper (Exp. 13) that a coloured bird having a few white feathers is not necessarily heterozygous for white, and the birds raised here in Exp. 75 are doubtless comparable in that respect with the blue ♂ 19 raised in Exp. 5 from the mating of two F_1 Barb-Fantails.

B. *Duns*. The dun ♀s raised in Exp. 76 are of a very much lighter colour than the dun Carriers. They possibly bear the same relation to the dark duns that the sooty blacks described above do to the dark "raven-blacks" of the fancy varieties. I therefore propose to call them "smoky duns." The head, neck, breast, rump and tail feathers are the darkest parts of the birds. The wing coverts and flights are lighter. The wing and tail bars can be made out, but the wing bars are more obvious than the tail bar. The wing coverts present a somewhat blotchy appearance, but neither Mr Bonhote, who examined these birds, nor I could satisfy ourselves that chequering was present.

I have this year obtained some pure bred dun owls and find that they are identical in plumage with the smoky duns here described. Presumably, therefore, the full rich dun colour found in the Carrier and some other varieties is not found in the owl.

Cross between Dun Carrier ♀ and Blue Dragoon ♂.

Exp. 77 (*v.* Table VI). A pair of these birds have been mated together for two years, and a uniform F_1 generation of nine blacks resulted. These blacks were of a darker shade than the black F_1 Owl-Fantails, but, nevertheless, indications of the bars could be seen. In one case only were a few white feathers present on the thighs.

Exp. 78 (*v.* Table VI). A pair of the F_1 blacks, raised in Exp. 77, were mated together and produced 5 Blacks, 1 Dun, 1 Blue, and 1 bird which died in the nest at an early age. It was believed to be a silver, but was too young to be definitely identified as such, as it is difficult in the case of very young birds to distinguish between a blue and a

silver. The bird is therefore entered in the Table with a query. The F_2 blacks were identical with the F_1''s.

The dun died young, but there was no doubt as to its colour.

The blue resembled the blue Dragoon in plumage, having no white feathers.

Three pairs of the black F_1''s are put up this year[1].

Crosses between dark and white races of Doves.

For these crosses specimens of the common Turtle Dove (*Turtur turtur*), the Barbary Cage Dove or Collared Turtle Dove (*T. risorius*) and a white variety of the latter known as the White Java Dove were used.

The Turtle Dove. Several specimens of this bird were obtained from the neighbourhood of Fairford, Gloucestershire, where they were taken from the nests of wild birds. (Plate X, fig. 1.)

The Barbary Dove. This is the ordinary well-known cage dove. The general plumage of this bird is of a dark cream colour. The flights, rump and upper tail feathers are greyish drab. The under parts and under tail coverts are white. There is a black collar round the sides and back of the neck. (Plate X, fig. 4.)

The White Java Dove. The plumage of this variety is, in the majority of specimens I have seen, quite white. But in some specimens I have noticed, on handling the bird, a very light cream collar in the position of the black collar in the Barbary dove. (Plate X, fig. 2.)

Several specimens of the Barbary and White Java doves were procured for these experiments from bird shops and advertisements in Fanciers' papers. Unfortunately, however, for our purpose the breeders are in the habit of crossing these two varieties, and since, as is described below, the F_1 bird, if dark, produced from the mating of the varieties is absolutely indistinguishable from the pure bred Barbary dove, it is impossible to know whether the Barbary contains white or not until it is tested. Further, the breeders do not appear to have any system of marking their birds and I have been unable to ascertain the colour pedigrees of the birds obtained. Mr F. J. Rogers of Ipswich, from

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[1] These three pairs have so far produced Black 2, Dun 3, Blue 1, Silver 0. The colour of the duns appears at present to resemble closely that of the Carrier.

A blue Dragoon ♀ has been mated this year to a dun Carrier ♂, and four young have been produced of which one is black and three are dun. The sexes of the young birds cannot at present be ascertained.

whom I have obtained several specimens of both varieties, has been kind enough to give me his experience of breeding them. He has known whites to be produced from the mating of two Barbaries but never Barbaries from the mating of two whites. He has been in the habit of crossing the two varieties, the cross usually made being that of a Barbary ♀ × White ♂, and has obtained young of both varieties from such a cross. He has noticed that Barbary young mature much quicker than do white young. This has also been my own experience.

It was my original intention to use only the wild Turtle Doves and the White Java Doves, but they were found not to breed readily together, and only a small number of offspring were obtained from each pair. On referring to Table VII it will be seen that in Exps. 79—82 only two or three young were obtained from the pairs, whilst in

TABLE VII.

I. *Matings of Turtle Doves and White Java Doves.*

(a) White ♀ × Dark ♂.

Exp. No.	Female	Male	Dark Male	Dark Female	Dark, Sex uncertain	White Female
79	White, No. 9	Turtle, No. 8	—	2	—	—
80	White, No. 11	Turtle, No. 12	—	2	—	—
81	White, No. 43	Turtle, No. 8	1	—	2	—
82	White, No. 5	Turtle, No. 12	1	3	—	—

(b) Dark ♀ × White ♂.

Exp. No.	Female	Male	Dark Male	Dark Female	Dark, Sex uncertain	White Female
83	Turtle, No. 6	White, No. 33	—	—	4	—
84	Turtle, No. 53	White, No. 33	4	—	—	2

II. *Matings of Barbary Doves and White Java Doves.*

(a) White ♀ × Dark ♂.

Exp. No.	Female	Male	Dark Male	Dark Female	White Male	White Female
85	White, No. 1068	Barbary, No. 1293	2	5	—	—
86	White (no number)	Barbary (no number)	5	2	—	—
87	White, No. 29	Barbary, No. 1069	1	4	2	3
88	White (no number)	Barbary, No. 1061	3	1	1	—

(b) Dark ♀ × White ♂.

Exp. No.	Female	Male	Dark Male	Dark Female	White Male	White Female
89	Barbary, No. 45	White, No. 1893	10	—	—	8
90	Barbary, No. 2016	White, No. 45	4	1	—	7
91	Barbary, No. 2015	White, No. 2043	4	—	—	2

Exps. 83 and 84, in which cases the birds were mated for two years, generations of only four and six young resulted.

The sexes of Doves are not so readily distinguished as those of pigeons in my experience, consequently all birds recorded in the Table were dissected to ascertain the sex. It will be noticed that six birds from the Turtle cross are recorded as of uncertain sex, these died when quite young in the nest.

An attempt was made to cross a dark F_1 Turtle Java ♀ with a wild Turtle ♂, and sixteen eggs were laid every one of which proved unfertile. No further matings of the F_1's were made.

The experiments, all of which are recorded in Table VII, are divided into two parts: (i) The matings of Turtle × White Java, (ii) The matings of Barbary × White Java. Reciprocal matings have been made in each case. The following are the descriptions of the birds of the F_1 generation.

(a) *Dark F_1 Turtle Java.* The upper parts, mantle, and lesser wing coverts are reddish-brown somewhat resembling the young of the Turtle Dove. There is a conspicuous patch of black feathers tipped with white on each side of the neck. Secondary coverts bluish grey tinged with brown. Primary coverts blackish tinged with grey. Rump and upper tail coverts brown. Tail feathers brown tipped with white except the two central ones. Throat and breast vinaceous shading to blue grey on the flanks. Abdomen and under tail coverts white. The red of the coverts is not so pronounced as in the Turtle Dove, and the dark centres of the mantle feathers, so conspicuous in that species, are entirely absent in the hybrid. (Plate X, fig. 3.) In making the foregoing description I have had the kind assistance of Mr Bonhote.

(b) *White F_1 Turtle Java* ♀s, identical with White Java Dove.

(c) *Dark F_1 Barbary Java*, identical with Barbary Dove.

(d) *White F_1 Barbary Java*, identical with White Java Dove.

An examination of Table VII gives us the following results:

White Java ♀ × *Turtle* ♂. (Exps. 79—82.) Eleven young birds produced which differed in no respect from the general description of the dark F_1 type given above.

Turtle ♀ × *White Java* ♂. (Exps. 83 and 84.) Of these two matings the first is very unsatisfactory. The birds were mated for two years. In the first year only two pairs of eggs were laid. From the first pair two young were produced but they died four days after hatching. They were not feathered but as the skins, beaks and legs

were dark it was concluded that they would have been of the dark type had they lived. It was noticed that dark birds had dark beaks and legs, and white birds had white beaks and legs which could be distinguished in the next. From the second pair of eggs one bird was obtained which died twenty days after hatching, and was definitely of the dark type. The second year five pairs of eggs were laid, but from these only one bird was hatched. This died the next day but it was seen that the beak and legs were dark. All the other eggs were either broken by the parents or forsaken by them at an early age, and it was considered useless to attempt to breed from these birds again.

From the second experiment (No. 84) a very clear result was obtained. Six birds were hatched and all matured and were dissected for sex. There is absolutely no question of the purity of the Turtle ♀ as it was taken from the nest of a wild bird, and the mating conclusively shows the sex-limited inheritance of white in these species, for the result obtained was 4 dark ♂s and 2 white ♀s.

White ♀ × Barbary ♂. (Exps. 85—88.) The first two of these matings give the expected result, viz. a uniform generation of dark F_1's. Fourteen birds were produced, all of the Barbary type. From Exps. 87 and 88, however, a mixed generation occurred consisting of 4 dark ♂s, 5 dark ♀s, 3 white ♂s and 3 white ♀s when the offspring of the two pairs were added together. I think there is little doubt but that the Barbary ♂s were in both cases heterozygous and contained white. The family produced from Exp. 87 is exactly what would be expected from such a mating, and no doubt had Exp. 88 been continued more whites would have been produced, so that an equality of dark and white birds would have resulted.

Barbary ♀ × White ♂. (Exps. 89—91.) With one exception the results obtained from these matings are dark ♂s and white ♀s. Eighteen Barbary ♂s and seventeen white ♀s were produced. But in addition one dark ♀ was raised in Exp. 90. This is a parallel case to the black-eyed ♀s which Miss Durham obtained from the mating of black-eyed Canary ♀s with cinnamon Canary ♂s, as recorded by her in Report to the Evolution Committee of the Royal Society, iv. 1908. The occurrence of the dark female form in F_1 is exceptional, and further work is required to explain its appearance.

The foregoing experiments are, however, sufficient to show that a sex-limited inheritance of white occurs in the races of doves under consideration.

Conclusion.

EXPLANATION OF PLATE X.

Fig. 1.   Turtle Dove.
Fig. 2.   White Java Dove.
Fig. 3. F_1.   Turtle Java.
Fig. 4.   Barbary Dove.

PLATE X

1   2   3   4

GIGANTISM IN *PRIMULA SINENSIS*.

By FREDERICK KEEBLE, Sc.D.,

Professor of Botany in University College, Reading.

CONTENTS.

I. *Introduction.*

MANY of our cultivated plants are to be met with in giant and dwarf, as well as in normal forms, and anyone who will consult the pages of nurserymen's catalogues may learn that the plant breeder has exercised his genius no less successfully in moulding the form of plants than in modifying the colours of their flowers. The universality of the phenomenon of gigantism justifies the belief that a comparative study of the physiology of giant and normal plants will lead to results of interest and value. But such a comparison must be accompanied by a study of the genetical relations which exist between giant and normal forms; for although, so far, only one type of giant has been observed among plants, analogy with animals would suggest the possibility of the occurrence of more than one type of gigantism in the vegetable kingdom. (*Cf.* Gilford, 1911.)

The plants in which the phenomenon of gigantism has been investigated include *Oenothera gigas* (de Vries 1901, Gates 1909), *Lathyrus odoratus* (Bateson 1909 A) and *Pisum sativum* (Mendel, see Bateson 1909 B and Keeble and Pellew 1910). In all these cases the giant is

dominant to the normal form. Thus, as de Vries (*op. cit.*) has shown, *Oenothera gigas*, a giant mutant from *O. Lamarckiana*, when crossed with the latter form (*O. Lamarckiana* × *O. gigas*) yields an F_1 consisting of plants all possessing giant characters. In both *Lathyrus odoratus* and *Pisum sativum* the character of gigantism is known to be determined by two factors, the presence of both of which is necessary for the manifestation of the character.

The purpose of the present communication is to record the origination of a giant form of *P. sinensis* from a normal form the pedigree of which is known, to describe the histological characteristics which distinguish this giant, to present the results of the experiments which have been made on the genetics of gigantism and to discuss a few of the problems which are suggested by the results of these experiments.

II. *Gigantism in Primula sinensis.*

Among the many varieties of *Primula sinensis* in cultivation at the present day there are not a few which are characterised by an imposing massiveness of both floral and vegetative organs. The races thus distinguished are known to florists as giants and are prized by them on account of the remarkable size and substantiality of their flowers. Associated with the gigantic habit are a somewhat slow rate of growth, a certain leisureliness of flowering, and, not infrequently a considerable measure of infertility. It is probable that the last mentioned characteristic accounts for the opinion, not uncommon among gardeners, that giants are more difficult of cultivation than are the normal races. This opinion, however, is not well founded, nor is there any ground for the assertion, which is often made, that the gigantism of these strains depends on methods of cultivation. The fact that seedsmen are able to offer giant races year after year is well nigh sufficient evidence that gigantism is a "fixed" character, and that giants, when self-fertilized, breed true to form.

Beyond these facts little is known either of the origin of the giant races of *P. sinensis* (*cf.* Gregory 1909 and 1911) or of their genetical behaviour. The physiological peculiarities of giants are likewise unknown.

The giant races of *P. sinensis* which are offered by seedsmen are almost invariably of the *sinensis* type both with respect to habit and flower: that is to say the flowers are produced in a compact and

massive head and bear large petals the outer margins of which are not notched (*stellata* type) but fimbriated.

There is however no necessary connection between gigantism and the *sinensis* type of petals: indeed Messrs Sutton's remarkable collection of Primulas contains at the present time numerous giants which bear flowers of the *stellata* type.

Further, although the giants in general cultivation have the *sinensis* style of inflorescence, there is no essential connection between gigantism and that type of inflorescence. For example, the giant (Giant White Queen Star), the origin of which is about to be described, is characterised by the possession of a typically *stellata* habit of inflorescence (*cf.* Plate XI, fig. 2).

III. *The Mode of Origin of Giant White Queen Star.*

Among the Primulas which have been grown at University College, Reading, during the last ten years is a well-known variety, White Queen Star (Plate XI, fig. 1). This variety—a reddish stemmed, palm-leaved strain with white flowers of *stellata* form and habit—was raised from seed presented by Messrs Sutton in 1903.

Plants of the first generation of White Queen Star raised from these seeds were self-fertilized in 1904 and yielded an F_1 generation which, so far as the records show, presented no departure from the normal form.

Of the plants of the F_2 generation raised from seed obtained by self-fertilizing F_1 plants, some bore an occasional 6-petalled flower among the other normal 5-petalled flowers. Fluctuations of this kind are of course common in *P. sinensis*, and also in many other cultivated plants. They appear suddenly in one generation, may be lost sight of in the next; and their advent and disappearance are generally ignored.

Notwithstanding the strength of the evidence that such fluctuating variations arise and fade away without leaving recognizable after-effects in the descendants, experiments were undertaken with the object of testing the degree of permanence of the variation which results in the production of flowers with supernumerary petals.

As may be seen from the results of the experiment summarised in Table I the attempt to produce a race characterised by the possession of flowers with more than the normal number of petals was not successful. Inasmuch however as the experiment of breeding from

abnormally petalled plants was continued only for a few generations the negative results cannot be claimed as decisive.

<div align="center">TABLE I.</div>

Selection Experiments with White Queen Star (W. Q. S.).

	1903	W. Q. S. from seed		
	1904	no abnormality observed. Selfed W. Q. S.		
F_1	1905	some plants with occasional 6-petalled flowers selfed a 6-petalled flower		
F_2	1906	plants with most flowers normal, a few with 6 or 7 petals selfed a		
		7-petalled flower 5A	ditto 5B	ditto 5D

F_3	1907	10 plants petals slightly fimbriated 8 with 1 or more 6-petalled flowers *flowers large* selfed	12 plants 11 normal 1 with 1 flower with 6 petals selfed	4 plants some flowers with supernumerary petals selfed 2 plants	

F_4	1908	11 plants *all giant* (see Table II)	6 plants all normal	13 plants all normal	12 plants 5 normal 7 with occasional 6-petalled flowers
		selfed	selfed	selfed	
F_5	1909	=10 plants all giant selfed	*all normal*	all normal selfed	
F_6	1910	=giant		=normal	
F_7	1911	=giant		=normal	
F_8	1912	=giant		=normal	

It may be added that, as shown in Table I, the habit of forming supernumerary petals has not manifested itself in later generations to so marked a degree as it did during the time of the selection-experiment, and that the race of White Queen Star now growing at Reading, which consists of descendants of the 6- or 7-petalled plants, is not distinguished to any marked degree by this abnormality.

The interest attaching to the series of experiments which has just been described lies, however, in another direction. For it was in the course of these experiments that a Giant variety of White Queen Star was obtained. The mode of origin of the Giant race is set forth in detail in Table II.

As indicated in Table II, gigantism manifested itself in the F_4 generation (1908). That generation, which was composed of 11 plants, consisted of giant forms only. The striking appearance presented by

TABLE II.

The Origin of Giant White Queen Star.

1903			White Queen Star (W. Q. S.) raised from seed
1904			=normal W. Q. S.
			selfed
1905	F_1		plants with occasional 6-petalled flowers: most flowers normal
			selfed
1906	F_2		plants with occasional 6- and 7-petalled flowers: most flowers normal
			selfed
1907	F_3	(5A)	10 plants, all with slight fimbriation of edges of petals, 8 with 1 or more 6-petalled flowers: *petals large*, selfed 1 plant
1908	F_4	5A 1	11 plants: flowers large, of good substance; petals meeting or over-lapping. Flower slow in opening; slight fimbriation of edges of many petals= *Giant White Queen Star*, see Plate XI, fig. 2.
			selfed
1909	F_5	5A 1/1	3 plants all true to Giant form and habit
		5A 1/2	7 ,, ,, ,, selfed
1910	F_6	5A 1/2/7	8 plants all true to Giant form and habit
		5A 1/2/8	1 plant ,, ,, ,,
		5A 1/2/9	2 plants ,, ,, ,, selfed
1911	F_7	5A 9	6 plants all true to Giant form and habit selfed
		5A 10	1 plant ,, ,, ,,
1912	F_8	G. W. Q. S.	30 plants all true to Giant form and habit

these "mutants" is illustrated in Plate XI, figs. 1, 2—and a comparison of the giant and normal forms shows how markedly they differ the one from the other. The differences between the two forms are not confined to the flowers, though they are most patent in the petals (*cf.* Fig. 1). Giant White Queen Star, the origin of which is now
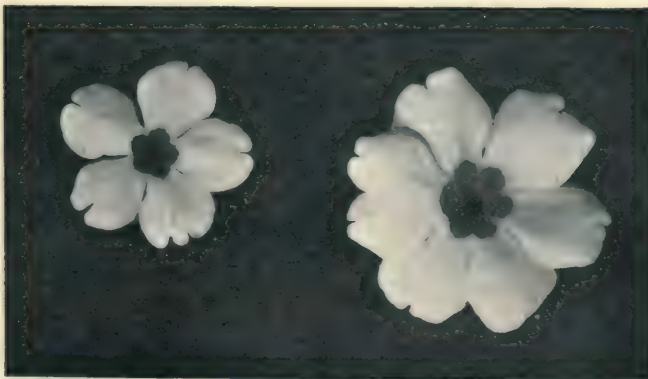
Fig. 1. Corolla of Normal and of Giant White Queen Star.

under consideration, is a more massive plant, with slower growth than that of the ordinary form of White Queen Star from which it arose. The corolla of the Giant is about half as large again as the corolla of the normal form and adjacent petals of the former either meet or overlap instead of leaving a narrow space between them as is the case with the normal plant.

The overlapping of the contiguous petals is a characteristic of giants both of the *sinensis* and *stellata* type of flower, and is due to the fact that the oblate petals are much broader basally than are the more ovate petals of varieties of normal stature. In one respect only does Giant White Queen Star differ from most giants. It has retained the tiering habit of the typical *stellata* form of inflorescence.

This example of a giant form characterised by a typically *stellata* habit of inflorescence is not unique. Thus the Giant White Star which has been investigated by Gregory (1909 and 1911) possesses also the *stellata* type of inflorescence. Giant White Queen Star conforms in all respects with the known giant races of *P. sinensis*. Its flowers are large, of good substance, and slow to open. Its notched petals—the edges of which are apt to be slightly fimbriated—are rounded in the bud stage, grow slowly, and as they grow broaden basally till those adjacent to one another meet or overlap. The flowers are much more lasting than are those of the normal parent, and the massive stigma persists for weeks if pollen be denied access to it.

IV. *A Histological Comparison of Giant with Normal White Queen Star.*

The most comprehensive examination of the histological characteristics of a giant form of plant is that made by Gates (*loc. cit.*) in the case of *Oenothera gigas*.

Comparison of *O. gigas*, a giant mutant of *O. Lamarckiana*, with the normal form from which it arose has led Gates to the conclusions that the cells in the giant are conspicuously larger than in *O. Lamarckiana*; that the cells of certain tissues of the giant are almost exactly twice the size of those in the corresponding tissues of *O. Lamarckiana*; and that the giant, *O. gigas*, has double the number of chromosomes present in *O. Lamarckiana*.

Histological comparison of Giant and Normal White Queen Star, shows at once that the former is a giant because its constituent cells are gigantic. The marked difference between Giant and Normal White
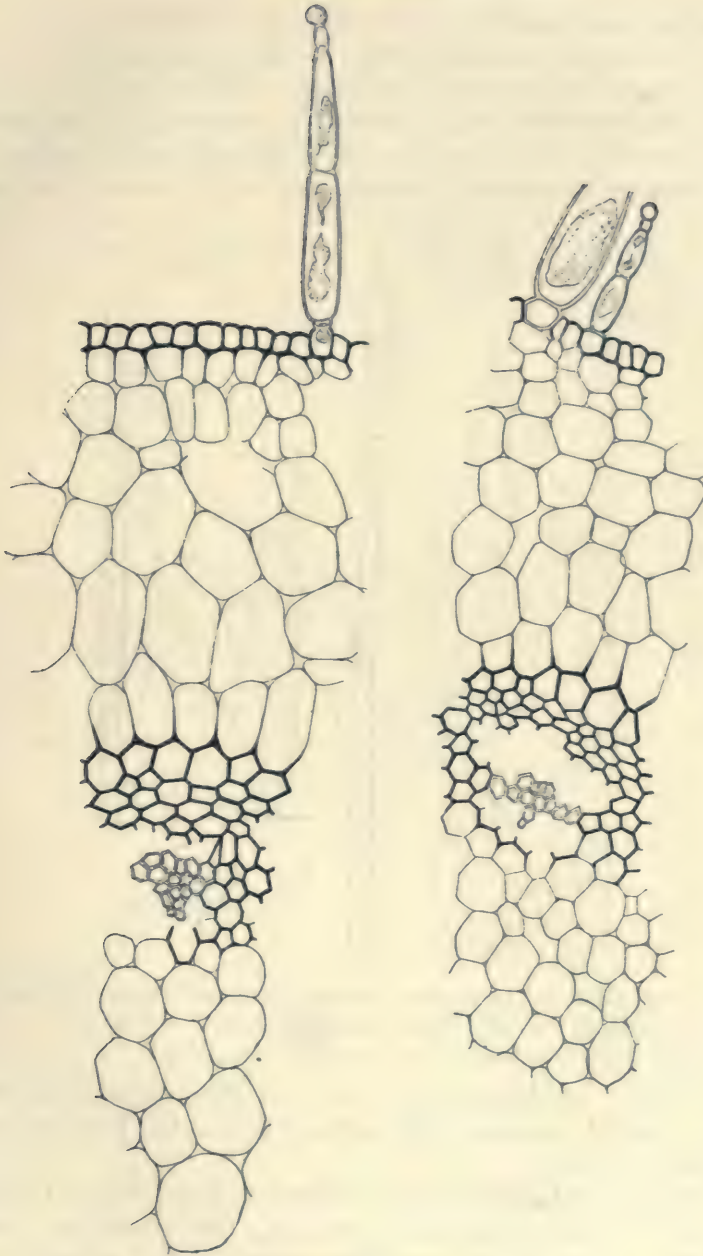
Giant 5/a/10. Normal 5/d/4.

Fig. 2. *Primula sinensis.* Cross sections of the flower peduncles of Giant White Queen
Star and of Normal. Magnification the same in both.

12—2

Queen Star with respect to size of cells is demonstrated by the Camera
Lucida drawings reproduced in Figs. 2—5, and appears to be in every
way similar to that obtained by Gregory (1909) in the case of the
Normal and Giant Star Primulas which he compared with one another.
The difference in size between the nuclei of the giant and normal form
which Gregory demonstrated, obtains also in the example now under
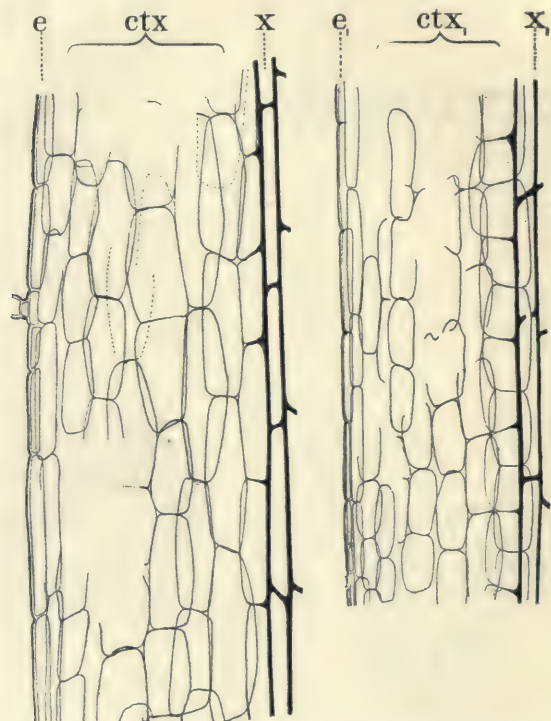
Fig. 3. *Primula sinensis.* Longitudinal sections of the flower peduncles of Giant White
Queen Star and Normal. Magnification the same in both.

e = epidermis, ctx = cortex, x = pericycle.

consideration. The larger size of the nuclei of the giants is well shown
in Fig. 5, which represents the pollen grains of the pin-eyed normal
and giant varieties.

The histological aspect of the phenomenon of gigantism in *P. sinensis*
deserves more detailed examination than has been devoted to it, and in
particular an enquiry into the relation between size of nucleus and size
of cell should prove of interest; but beyond confirming Gregory's con-
clusion that the numbers of chromosomes (12 and 24) are the same
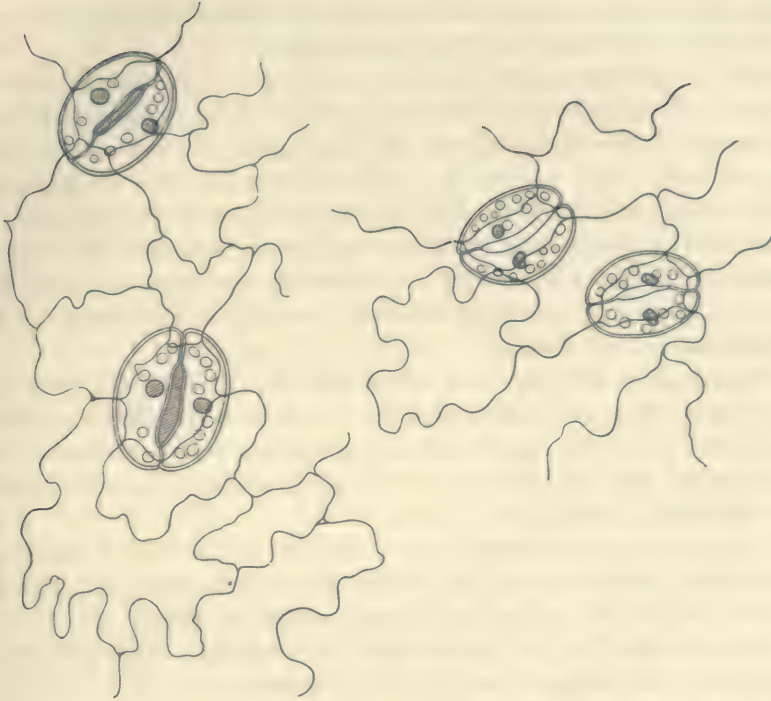
Fig. 4. *Primula sinensis*. Stomata and Epidermal cells of Giant and Normal White Queen Star. Magnification the same in both.
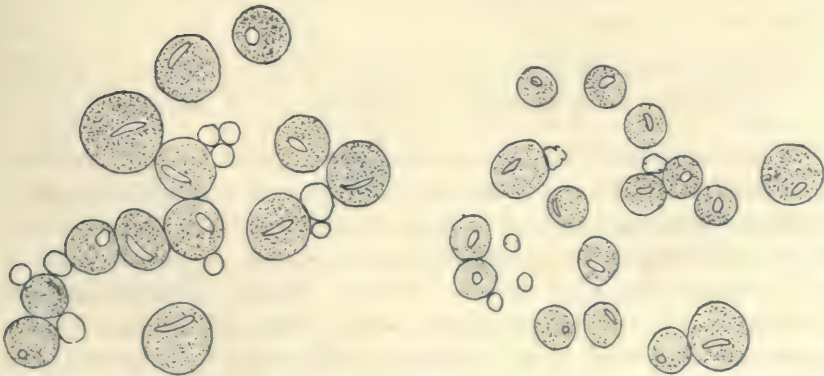
Fig. 5. *Primula sinensis*. Pollen grains of Giant and Normal White Queen Star. Both Pin-eyed. Magnification the same in both. Grains swollen and stained by Methyl green Acetic.

in the giant and in the normal varieties, no detailed histological study of the two forms has, as yet, been undertaken.

The rough histological analysis, which has been made, suffices, however, to prove that in the variety Giant White Queen Star, the gigantism of the individual is the expression of that of its constituent cells. The mutant is a giant because its cells are gigantic. Further, the facts that, in the cortex, the number of cell-layers is fewer in the giant than in the normal plant, and that the rate of growth of the plant as a whole is slower in the former than in the latter, suggest that cell gigantism may be due to a reduction in the normal rate of cell-division.

Examination of Figs. 2—5, shows that the cells of the giant are larger than those of the normal form in all three dimensions—radial, tangential, and longitudinal, and that gigantism is not peculiar to the elements of any one tissue-system, but is common to the cells of all—epidermal, cortical, and stellar.

The extent of the differences in size between the cells of the giant and ordinary White Queen Star is illustrated by the following figures, in which the size of a giant's cell is represented by 100.

Flower peduncles (see Figs. 2 and 3, and Plate XI). Cortical cells of the layer immediately external to the endodermis :—

	Giant	:	Normal
Radial measurement	100	:	48
Tangential „	100	:	81
Longitudinal „	100	:	57

V. *The Genetical Behaviour of Giant White Queen Star: with Notes on the Genetics of Gigantism in P. sinensis.*

The race, Giant White Queen Star, the origin of which is described in Section III, differs markedly from the parent race with respect to fertility. Whereas plants of normal White Queen Star produce seed freely both when self-fertilized and when crossed with other varieties of *P. sinensis*, the giant race is relatively sterile. Nevertheless self-fertilization of the original giants (1908, Table II) resulted in the production of enough seed to continue the strain. The partial self-sterility which characterised the first generation of giants has not been maintained, and the descendants of the original Giant White Queen Star now yield when self-fertilized a fair amount of seed.

As indicated in Table II, the giant race has been continued by self-fertilization for four generations, and several hundreds of descendants have been cultivated. All of these have been observed with care, and in the case of 58 plants (Table II) written records of their characters have been kept. All have without exception been true to the giant habit.

On the other hand all attempts (see Table III) to cross Giant White Queen Star with other varieties have—with one doubtful exception to be mentioned immediately—proved abortive. Whether the giant race be used as the seed parent or as the pollen parent; whether it be crossed with normal or giant forms, with *stellata* or *sinensis* forms, the result is the same. Even when it is crossed with the parental race of White Queen Star, and this cross has been repeated many times, no seed has been obtained except on one occasion, when two fertile seeds were produced (see Table III). The last-named cross has been repeated often and advisedly, because it was recognized that in the success thereof lay by far the best chance of obtaining material suitable in all respects for an experimental study of the genetics of gigantism. Despite the negative results obtained by crossing Giant White Queen Star with other varieties, and inasmuch as it would appear probable, from analogous cases, that this refractoriness to cross-fertilization will disappear, it seems worth while to record the varieties (see Table III) with which the attempts to cross fertilize Giant White Queen Star have been made.

The Mendelian phenomena presented by the offspring produced by crossing giant and normal plants are not altogether easy of interpretation. This, however, is due rather to the difficulties of experimentation and particularly of observation, than to the complexity of the problem. Since it is evident from the facts recounted already that gigantism is a cell phenomenon, it follows that the genetics of gigantism should be investigated with reference to the cell, and that, if Mendelian results of certain value are to be obtained, the attention of the observer must be directed not only to the dimensions of the individual plants or flowers, but also to those of the cells of the plants with which breeding experiments are made.

The experiments, the results of which are now to be discussed, were undertaken before this need was realised, and the experiments which were suggested by the advent and cell characteristics of the mutant Giant White Queen Star have never been made, owing to the obstinate sterility of that form. Hence it must be admitted at

TABLE III.

Record of Crosses between Giant White Queen Star and other varieties of P. sinensis.

	Year	Catalogue Number of the Variety	Name of Variety	Character of the Variety		Result
Giant White	1908	5 B	Normal White Queen Star	Normal stellata		no seed
Queen Star	,,	15	Ruby Star	,,	,,	,,
= ♀	1909	5 B2	Normal White Queen Star	,,	,,	2 seeds[1] which germinated
	,,	5 D3/2	Normal ,, ,, ...	,,	,,	no seed
	,,	5 D3/2	,, ,, ,, ...	,,	,,	a few seeds, none germinated
	,,	16/1	White Star	,,	,,	no seed
	,,		Ivy Leaf	,,	,,	,,
	,,	6Bp 7	Cambridge Blue	,,	,,	,,
	,,	20/3/1	Snow King	,,	,,	,,
	1908	130 B/2	F₃, Reading Pink × Crimson King	Normal sinensis		,,
	1909	25	Snow Drift	,,	,,	,,
	,,		Orange (Coral) Pink	,,	,,	,,
	,,	190 E	Carnation Mauve Flake	,,	,,	,,
	1911		Crimson King	,,	,,	,
	,,		Duchess	,,	,,	,,
	,,		Queen Alexandra	,,	,,	,,
	,,	180	Giant *Primula sinensis*	Giant form		,,
	,,	62/6/1	A Blue, Semi-giant	Semi-giant form		,,
Giant White	1908	80 B3	F₃ of Giant Pink × Ld. Roberts Star	?	?	no seed
Queen Star	,,	80 A6	,, ,, ,,	Giant form		,,
= ♂	,,	3 e1	Giant Pink	,,	,,	,,
	1909	180	Giant *Primula sinensis*	,,	,,	,,
	1911	180	,, ,, ...	,,	,,	,,
	,,		Giant White (Sutton)	,,	,,	,,
	,,	14/2/1 G	Giant F₂, Royal White × Snow King	,,	,,	,,
	1908	130 A1a	F₃, Reading Pink × Crimson King	Normal sinensis		,,
	,,	2 A1	A White Sinensis	,,	,,	,,
	1909	25	Snow Drift	,,	,,	,,
	1911		Duchess	,,	,,	,,
	1908	5 B	Normal White Queen Star ...	Normal stellata		,,
	,,	5 Bp	Cambridge Blue	,,	,,	,,
	1909	20/3/1 and 20/5	Snow King	,,	,,	,,

[1] The plants thus raised were typical Giant White Queen Star. Though possibly they were due to accidental selfing of the female parent, they yielded *no seed* neither when self-fertilized nor when crossed with normal White Queen Star.

the outset that the method by which the following records were made—that of appraising gigantism by reference to the gross appearances presented by individuals—is open to grave objections. For example, a large and massive flower may, if seen among a family of small-flowered plants, be classed unhesitatingly as a giant, although if it were judged side by side with indubitable giants, it might be relegated to the class of doubtful or semi-giants.

On the other hand an observer who spends a considerable amount of time among plants of a certain kind gains in some measure a sureness of judgment with respect to the "points" of those plants which is not likely to lead him into very serious errors of observation.

In spite of the drawbacks of the method of classification which has perforce been adopted in these experiments, the results which have been obtained appear to be worth recording both for their inherent interest and for the purpose of demonstrating that the phenomena of gigantism presented by *P. sinensis* are less simple than those exhibited by other plants which have been the subject of like investigation.

For example, the genetics of the gigantism of sweet peas (Bateson, 1909 a) and of culinary peas (Keeble and Pellew, 1910) is of a fairly simple kind. Thus in *Pisum sativum* gigantism depends for its expression on the presence of two factors. Of these growth-factors, the one induces excess of growth in length (factor for long internode), the other induces excess of growth in thickness (factor for thick internode).

In the presence of both these factors, either in a pure or heterozygous state, the plant is a giant (6 ft.), in the absence of either it is of mid-stature (3—4 ft.), and in the absence of both it is a dwarf (1—1½ ft.).

The gigantism of *P. sinensis* is built on less simple Mendelian lines.

The experiments which lead to this conclusion, recorded in Tables IV, V, and VI, were made mainly at University College, Reading; though some records which are included in the tables are those of breeding-experiments carried out by Messrs Sutton and Sons at their trial grounds. The records derived from the latter source are indicated in the tables; those in which the source is either not mentioned or indicated by the letters U. C. R. were carried out at University College.

For access to the records of Messrs Sutton and Sons' experiments

the very cordial thanks of the writer are due to Mr Leonard Sutton and to the firm's Primula experts, Messrs Macdonald and Tufnail.

From what has been said already with respect to the mode of inheritance of gigantism in *Pisum sativum* it follows that the F_1 generation resulting from a cross between a pure giant and any other form consists of giants. A like result is not exhibited by *Primula sinensis* in the F_1 generation derived from a similar cross.

In some instances (see Table IV) the F_1 progeny of a cross between a giant and a normal form are of giant type : this is the case, for example, in the F_1 of the crosses Giant Pink × Reading Pink and Duchess × Giant Lavender. In other instances, for example, in the

TABLE IV.

Crosses between Giant and Normal P. sinensis. Results in F_1.

I. Cases in which Gigantism appears to be more or less completely dominant.

Source of Record	Experiment No.	Nature of the Parents	F_1 Results
U. C. R.[1] 06—7	31	Giant Pink × Reading Pink (Normal)	12 plants of giant type
,, ,,	32 *A*	Royal White (Giant) × Pink Stellata (Normal)	14 plants, flowers like those of Royal White (Giant) but freer in habit and on longer pedicels
,, ,,	32 *F*	Pink Stellata (Normal) × Royal White (Giant)	11 plants, flowers like those of Royal White (Giant) but freer in habit and on longer pedicels
Sutton[2] II, p. 71		Duchess (Normal) × Giant Lavender	Giant

II. Cases in which the F_1 is intermediate with respect to Gigantism of flowers.

Sutton II, p. 20		Giant Royal White × Crimson King (Normal)	Semi-giant
,, II, p. 32		Crimson King × Giant White ...	8 plants, semi-giant
,, I, p. 22		Royal White × Crimson King ...	"Habit of flowers spoiled"

III. Cases in which Gigantism of flower appears to be recessive.

U. C. R.	80/07	Giant Pink × Lord Roberts Star (Normal)	Petals slightly fimbriated ; flowers not recorded as showing giant habit
,,	39/08	Lord Roberts Star × Giant Pink	ditto ditto

[1] U. C. R. = record of experiment carried out at University College, Reading.

[2] Sutton = record of experiment from the record books of Messrs Sutton and Sons, Reading.

cross Giant Royal White × Crimson King the F_1 family consists of semi-giants, and in yet other cases the F_1 plants were not recorded as showing giant habit of flower: whence it is to be inferred that if they possessed any symptoms of gigantism those symptoms were too slight to attract notice.

The one case in which seed was obtained as a result of crossing Giant White Queen Star, though it must be recorded, is open to suspicion. The cross in question (Table III) was one between Giant White Queen Star and its parent, the normal White Queen Star. Two seeds only were obtained, and yielded plants of giant habit. It is highly probable that they were the result of chance self-fertilization of the giant, although it is to be noted that these F_1 plants were sterile both with their own pollen and that of normal White Queen Star.

A superficial consideration of the differences which subsist between the F_1 generations might lead to the conclusion that two types of gigantism occur in *P. sinensis*: one type in which the gigantism behaves as a dominant character; and the other in which it behaves as a recessive. Such a conclusion is not open to objection on general, theoretical grounds, and meets with support from the known facts of gigantism in human beings. For as Gilford (*op. cit.*) shows, overgrowth in man may be of a normal type or of a pathological nature. In the former it is due to an exaggerated but normal development: in the latter it would appear to be the consequence of the lack of a growth-controlling factor. So in plants, excessive growth may be the outcome of the presence of a factor for growth-acceleration (or for inhibition of cell-division), or may be due to the absence of a factor which in the normal plant controls and limits the amount of cell-growth in the interests of the several organs or of the organism as a whole.

More careful examination of the results obtained with *P. sinensis* serves to show, however, that to apply such an hypothesis to their interpretation is certainly premature and probably unnecessary.

Hence in the argument now to be developed it will be assumed that there is but one type of gigantism in *P. sinensis* and that gigantism is dominant to normality. The variable extent to which the giant character manifests itself in F_1 generations must therefore be susceptible of explanation in terms of this hypothesis.

An inspection of Table V shows that, as with the F_1 generations, so with F_2 generations, there is a remarkable lack of uniformity with respect to the manifestation of gigantism. Thus the F_2 and subsequent

generations produced from self-fertilized F_1 plants may show either a considerable preponderance of giants or an even more marked excess of normal (non-giant) forms.

TABLE V.

Crosses between Giant and Normal P. sinensis. Results in F_2 and subsequent Generations.

I. Cases in which Giants appear in large numbers.

Source of Record	Experiment No.	Nature of the Cross	Results in F_2 and F_3
Sutton II, p. 32		Crimson King × Giant White F_1 = Semi-giant	F_2 = 18 giant : 5 non-giant. Several F_3 = plants true to giant
Sutton I, p. 71		Duchess × Giant Lavender F_1 = giant	F_2, giant, F_3, 18 plants all giant

II. Cases in which Giants appear in small numbers.

Source of Record	Experiment No.	Nature of the Cross	Results in F_2 and F_3
U. C. R. ...	120/07	Royal White × Pink Stellata F_1 = flowers in massive heads	F_2, 67 plants of which 1 = giant, another F_2, 54 plants *no* giants
U. C. R. ...	80/07	Giant Pink × Lord Roberts Star F_1 = flowers slight sinensis none recorded as giant	F_2, 211 plants of which 9 = giant
U. C. R. ...	14/2/1	Royal White × Snow King F_1 = no flowers recorded as giant	F_2, 13 plants, 11 non-giant, 2 giant F_3, *of giant* F_2 = 7 plants all giant, petals overlapping with very fimbriated edges F_3, *of giant* F_3 = 14 plants all giant, petals overlapping with very fimbriated edges F_3, *of non-giant* F_2 = 6 plants, 5 non-giant, 1 giant F_3, *of non-giant* F_2 = 11 plants, none giants F_3, *of non-giant* F_2 = 7 plants, none giants

A clue to the significance of this diversity of behaviour is provided by Exp. 120/07 (Table V). In this experiment a true-breeding giant, Royal White crossed with Normal Pink Stellata yielded an F_1 in which the flowers though non-giant were borne in massive heads—owing, as shown in Table VI, to dominance of the *stellata* habit of free-tiering.

TABLE VI.

Dominance of Stellata over Sinensis Habit of Inflorescence.

Royal White × Pink Stellata
F_1 = habit freer than Royal White, pedicels longer
F_2 = 22 stellata habit : 6 sinensis habit
in 3 : 1 ratio = 21 stellata habit : 7 sinensis habit

The F_1 plants yielded an F_2 generation which in one case consisted of 54 non-giants and no giants and in another case was composed of 67 plants of which *one* was a giant.

The ratio 66 non-giant : 1 giant suggests the further hypothesis that gigantism depends for its expression on three factors. Let it be assumed that a giant differs from a Normal Pink Stellata in the possession of at least three factors, which factors are either different in nature or —and more probably—similar in nature and of different distribution in the germ-plasm. Then when the giant of constitution **AABBCC** is crossed with a form which by virtue of its size and habit may be assumed to lack all three factors the result is an F_1, the members of which are heterozygous for all three factors. Thus :

$$\text{AABBCC} \times \text{aabbcc},$$
$$F_1 = \text{AaBbCc non-giant,}$$

and $\quad F_2 = 1\text{AABBCC} : 63$ plants of other constitutions,
$\qquad\quad = 1$ indubitable giant : 63 other plants,

none of which appears as a giant when viewed beside the pure dominant giant form.

Results which point to ratios of this order are exhibited in Table V. Thus in No. 120/07 the F_2 of the cross Royal White × Pink Stellata consists of 67 plants of which 66 are non-giant and 1 is giant. In another F_2 family of the same origin no giant appeared among 54 plants.

The case of Snow Drift × Snow King (Table VII, I) is similar and of interest in another direction also, inasmuch as it affords an example of the origin of a giant race as the result of crossing two non-giant varieties. The F_2 generation consists of 33 plants of which one was recorded as a "doubtful giant" (51/2/1). This plant yielded an F_3 consisting of seven plants of which five are non-giant and two are giants. Four of the non-giant F_2 plants yielded F_3 families which together contained 59 plants none of which are giants.

The results of this experiment and those of the experiments described previously conform with the requirements of the hypothesis that floral gigantism is determined by three factors, all of which must be present in the homozygous condition for the phenomenon to be exhibited.

The results now to be described require a slight modification, or rather extension, of this hypothesis.

As mentioned already the "doubtful giant" (51/2/1) obtained in the F_2 of the cross Snow Drift × Snow King (Table VII, I) yields an F_3 consisting of 5 non-giants and two giants. Whence it follows that

the floral habit of a plant may approach so nearly that of a giant as to be recorded as a "doubtful giant" although, as shown by its progeny, it is not pure for the giant character.

<div align="center">

TABLE VII.

The Production of Giants by the Crossing of Non-Giant Forms.

</div>

I　　1909　　　　*Snow Drift* × *Snow King*[1]

　　　1910　　$F_1=$　22 plants : no giants recorded
　　　　　　　　　　　　selfed 2 plants

　　　1911　　$F_2=$ $\begin{cases} 51/1=17 \text{ plants : no giants} \\ 51/2=16 \text{ plants of which } 1=(?\text{ giant}) (51/2/1) \end{cases}$
　　　　　　　　　　　　selfed (? giant) and 4 non-giants

　　　1912　　$F_3=$ $\begin{cases} 51/2/1 \ (\textit{from } (? \textit{ giant}) \ F_2)= \text{ 7 plants, viz. 5 non-giant, 2 giant} \\ 51/1 \ (\textit{from non-giant } F_2)=13 \\ 51/H \quad\quad,, \quad\quad\quad ,, \quad = 5 \\ 51/M \quad\quad,, \quad\quad\quad ,, \quad =14 \\ 51/W \quad\quad,, \quad\quad\quad ,, \quad =27 \end{cases}$ 59 plants : none giant

II　　1909　　　　*Snow Drift* × *White Queen Star* (Normal)

　　　1910　　$F_1=$　9 plants, no giant

　　　1911　　$F_2=$　8/2/2=15 plants, no giant

　　　1912　　$F_3=$ $\begin{cases} 8/2/11 =1 \text{ plant non-giant} \\ 8/2/2/11 =9 \text{ plants, no giant} \\ 8/11 =18 \text{ plants} \begin{cases} 1 \text{ with giant flowers} \\ 1 \text{ ? giant} \end{cases} \end{cases}$
　　　　　　　　　　　　　　　　=17 (? 16) non-giant : 1 (? 2) giant

It must therefore be assumed that combinations of the factors **A, B, C** other than the combination **AABBCC** may give rise to giant-like forms.

The assumption which appears to fit the facts most nearly is as follows: Of the three factors **A, B, C** two, *but not any two*, must be present in the homozygous state for the definite manifestation of gigantism. Provided that the plant have the constitution **AABB**, it may exhibit well-marked gigantism even though the third factor **C** be present in the heterozygous condition. Thus the three factorial combinations **AABBCC, AABBCc, AABBcC** may all produce giant-like plants, albeit the homozygous giant is recognizably more gigantic than the heterozygous giants.

The genetical behaviour of the "doubtful" giant 51/2/1 (Table VII, I) demonstrates that it is not pure to gigantism. If there be ascribed to it a constitution **AABBCc**, it should yield an F_3 of

$$1\text{AABBCC} : 2 \begin{cases} \text{AABBcC} \\ \text{AABBCc} \end{cases} : 1\text{AABBcc.}$$

---

[1] Snow King has flowers of fair size (*stellata* type) and should perhaps be classed as a semi-giant.

The first is an undoubted giant, the last is an undoubted non-giant, the two others are of the constitution of the F_2 parent, namely heterozygous for **C**, and although the parent arising in a family of smaller forms was classified as a doubtful giant, these plants now that they are seen side by side with the more massive (**AABBCC**) plant are thrown unceremoniously into the category of minor forms. Classified thus the F_2 consists of:

$$1 \quad \text{giant} : 3 \quad \text{non-giant}$$

in seven plants $\qquad 1\cdot75 \quad \text{„} \quad : 5\cdot25 \quad \text{„} \qquad expected$

$$2 \quad \text{„} \quad : 5 \qquad \text{„} \qquad found.$$

If the ultimate object of Mendelian analysis were merely to fashion constitutions on slender experimental bases, an hypothesis such as that now in course of formulation would be scarcely worth the making; but it must be remembered that one of the prime objects of Mendelian analysis is to provide classification with more subtle methods than those on which it relies at present; and, as is shown immediately, the hypothesis now in course of adumbration does lead to a better system of classification of the cultivated forms of *P. sinensis* than could be obtained by any other method whatsoever.

The validity of the assumption that well-marked gigantism may only be manifested by plants which, whilst possessing a certain factor **C** in the homozygous or heterozygous condition, are pure with respect to the presence of the other two factors (**AA**) and (**BB**) is borne out by the results of experiment 80/07 (Table V). In this experiment a true breeding giant (**AABBCC**) Giant Pink was crossed with Lord Roberts Star which by reason of its delicate habit of flower may be regarded as of the constitution **aabbcc**.

Giant Pink × Lord Roberts Star,

AABBCC × aabbcc

$= F_1:$ **AaBbCc,**

and such an F_1 on self-fertilization yields an F_2 composed of:

giant and $\qquad$ ⎰ 1**AABBCC**
approximately ⎰ 1**AABBcC** : 61 non-giants
giant forms ⎱ 1**AABBCc**

$= $ in 64 plants: $\quad 3 \quad : 61$

$= \text{„} \ 211 \quad \text{„} \qquad 9\cdot9 : 201\cdot1$

actual $F_3 = 9 \quad : 202.$

Although not directly germane to the subject of gigantism it may be recorded here that the form of inflorescence of the cultivated varieties of *P. sinensis* appears also to be determined by the mode of distribution of three factors in the zygote.

Thus in the cross just described (Giant Pink × Lord Roberts Star) the somewhat delicate and few-flowered type of inflorescence character-istic of Lord Roberts Star disappears in the F_1 generation and in the F_2 generation there are produced :

212 non-"Roberts" inflorescence : 8 "Roberts":

which ratio points to the conclusion that general type of inflorescence is determined by three factors (XYZ); that the Lord Roberts Star type which is patently more feeble than either the *sinensis* or *stellata* types is produced in pure form only when the zygote has the constitution $xxyyzz$; and that Roberts-like inflorescences are also produced in plants of the constitutions $Xxyyzz$ and $xXyyzz$.

If this be so then in 64 F_2 plants there are to be expected :

	3	Roberts : 61	non-Roberts		
and in 211 plants	9·9	„ : 201·1	„		
whereas	8	„ : 203	„	were found.	

The finer details of form of inflorescence, length of peduncle, length of pedicel etc., appear also to be determined by Mendelian factors. The evidence in support of this statement must be reserved for a further communication, although the fact, that the Mendelian method may aid the plant-breeder to trim up a plant to almost any desired form—to straighten the leaves, to elongate the pedicels, to lower or heighten the inflorescence—deserves to be brought to the attention of professional plant-breeders.

To return to the subject of gigantism : the hypothesis that this phenomenon depends on not less than three factors drives us directly to the conclusion that the classification of varieties of *P. sinensis* into giant and non-giant forms is illusory. For it is a necessary corollary to that hypothesis that the mode of distribution and combination of these factors must be very different in the different races. In other words the conclusion is inevitable that, as is notoriously the case in other cultivated plants, *P. sinensis* must contain not only giant and dwarf strains but also semi-giant races. Further the hypothesis enables us to understand our failure to recognize, previous to Men-delian experiment, the existence of such diverse races. For with three factors concerned in the determination of stature the number of

intermediate forms must necessarily be large, and these forms must also produce the illusion of a continuous series rather than of a series made up of a large but definite number of forms, each of definite constitution and each therefore distinct from the others in genetical behaviour (*cf.* Nilsson-Ehle (1909) and Baur (1911)).

Mendelian analysis thus leads to the recognition that just as with antirrhinums, peas, sweet-peas and hosts of other cultivated plants, so with *Primula sinensis* we have to deal with giants, dwarfs and middle races.

The case already described in which giants arose as the outcome of the mating of non-giants is at once intelligible when this fact is grasped. Two instances of the appearance of giants in this manner are recorded in Table VII. In one, Snow Drift × Snow King a doubtful (F_2) giant yielded an F_3 of 5 non-giant : 2 giant ; in another, an F_3 from Snow Drift × White Queen Star, the ratio is 16 (? 17) non-giant : 1 (? 2) giant.

Now although both Snow King and White Queen Star are alike in their *stellata* flowers and *stellata* habit of inflorescence, the flower of Snow King is distinctly larger and the petals more massive than is the case with White Queen Star. In other words Snow King is a semi-giant. It must therefore contain more of the factors for gigantism than are borne by White Queen Star.

By ascribing factorial formulae consistent with their apparent constitutions to the varieties Snow Drift, Snow King and White Queen Star it is possible to account for the several results obtained by crossing each of the two latter varieties with the former variety.

Thus and by way of illustration only, if the constitution of Snow Drift be **aabbCC** and that of Snow King be **AABBcc** then

<p style="text-align:center">Snow Drift × Snow King</p>

$$= \textbf{aabbCC} \times \textbf{AABBcc}$$

and $\qquad F_1 = \textbf{AaBbCc}$

$F_2 = 3$ giant and giant-like : 61 non-giant

	$= 3$	,,	$: 61$	,,	
as compared with	1	,,	$: 15$	,,	found
	$= 4$	,,	$: 60$	,,	,,

A further point worth bearing in mind is that the conception of three factors admits of the explanation of minor but constantly recurring variations in shape and size of flower. For in a family which lacks the

C factor, one of the other factors may be present in homozygous condition
in some members and in heterozygous condition in others. In such a
family, which can never produce a giant form, the constituent individuals
may be characterised according to their respective factorial constitutions
by different modes of growth of the corolla and other parts. In the
variety Mont Blanc Star for example there are to be met with constantly
plants which bear smaller flowers than the type. These peculiar flowers
are characterised not only by their smaller size but also by the fact
that the basal parts of the petal-lobes are more fused with one another—
approaching slightly to gamopetaly—than are the corresponding parts
of the flowers typical of the variety. The assumption of the existence
of three factors for size of corolla throws light on this phenomenon.
Lacking altogether one of the three factors for gigantism the variety
Mont Blanc Star cannot throw giants but if its constitution be **AaBBcc**
it may throw both **AaBBcc** and **AABBcc** forms, the former in larger
numbers than the latter. If the **AABBcc** form differs, as differ it must,
from the **AaBBcc** form it is described as a fluctuation. In other words
fluctuations or minor variations may owe their origin to the hetero-
zygousness (for one or more factors) of a factorial complex which is
completely lacking in one factor essential for the production of a given
Mendelian character.

On the assumption that growth factors may condition cell chemistry
this hypothesis of the origin of fluctuations may be found to supply
the key to an explanation of the facts discovered by H. E. and
E. Frankland Armstrong (1912) with respect to the sporadic dis-
tribution of cyanophoric glucoside in herbage plants, such as *Lotus
corniculatus*. Their studies have brought to light the interesting fact
that the glucoside may be present in one plant or group of plants and
absent from another, and although it may be that climatic conditions
may play a part in the phenomenon it seems also probable that this
fluctuation is dependent on the genetic constitutions of the individual
plants.

Again it will be at once evident that the cases enumerated in
Table IV, in which giants appear in F_1, are susceptible of explanation
on the hypothesis which has been put forward.

Thus Crimson King (see Table IV) is itself a fairly massive plant
and may be supposed to contain two of the three growth factors.
Hence when crossed with a giant it gives an F_1 which the expert
describes as semi-giant and an F_2 (Table IV) composed of 18 giant : 5
non-giant. Thus :

AABBcc × AABBCC

yields $F_1 = $ **AABBCc**

F_2 of 3 giant and giant-like forms : 1 non-giant

= in 18 plants : 13·5 giant : 4·5 non-giant

as compared with   13   „   : 5   „   found (Table V).

In supposing that plants which, in F_1, were ranked as semi-giants are liable to be classed with giants in F_2 no violence is done to probability; for in the first place the judgment is a rough judgment and in the second place the habit of inflorescence in F_1 is apt to be free, and the flowers borne in such an inflorescence are less likely either to appear or to be gigantic than those which are borne on a more massive flower-stalk. Indeed the records of the behaviour in subsequent generations of plants recorded as giants show that not all plants to which gigantism is ascribed with confidence prove to be pure to that character.

Finally with respect to the Mendelian phenomena of gigantism it appears reasonable to conclude that gigantism in *P. sinensis* depends for its full expression on the simultaneous presence of three factors: that pure giants are homozygous for these factors; that giant-like forms occur when the plant is heterozygous for the **C** factor; and that an intergrading series of semi-giant races occurs in which the grades are represented by appropriate combinations of factors and their "absences."

Theoretical Considerations.

Numerous considerations, some of no small interest and importance, arise out of the results which have been described in the previous sections; but of these considerations only few can be discussed in the present paper.

First among them is the question concerning the origination of Giant White Queen Star. Is the fact that it arose in course of "selection" of flowers with supernumerary petals a mere coincidence or did the selection process play any part in the liberation of the giant? If the hypothesis on the nature of fluctuation (see p. 184) be accepted it is evidently susceptible of application in the present case. For a form of *P. sinensis* of the type of constitution **AaBbcc** though, for lack of the **C** factor, it may not produce giants, may produce gametes of various constitutions and these in turn combining in the various ways open to

them may give rise to zygotes characterised by minor peculiarities which are the outcome of the several constitutions.

However this may be, the origin of Giant White Queen Star appears to provide an example of the appearance of a "new," dominant character and is noteworthy because of the small number of cases in which this form of evolution has been observed. For, as is well known, the "dropping out" of a factor is common enough in the descent by reduction which cultivated and wild plants are undergoing; whereas the number of known examples of the appearance of new dominant characters (Punnett, 1911) is much fewer and none is known in which the phenomenon has, as it were, been witnessed in a pure strain.

Second, the complete infertility of the giant when crossed either with its parent or with other strains and its original relative infertility on self-fertilization are remarkable and suggestive facts.

Third and last: the phenomena of gigantism appear to have a bearing on those which concern the origin and nature of certain of our cultivated plants such as fruit trees and shrubs. Thus a cultivated variety of apple, pear or plum is evidently a giant with respect to its fruit. It may well prove that this cell-gigantism is the origin of all the differences between the large and luscious fruit of the cultivated apple and the astringent puny fruit of the crab. Alter the size of the cell-laboratory and the operations of that laboratory are altered. Events which mark the waning of the life of the small and rapidly maturing cell of the crab may never—for reasons of time or space—occur in the large and slow growing cell of the apple. The character of astringency would seem to have been lost by the dropping out of a factor for that character; whereas on the view now presented it is only lost because under the new conditions of growth the character cannot appear. How far all or most Mendelian characters depend directly or indirectly on such growth acceleratory and growth inhibitory factors must be left for subsequent consideration.

Summary.

1. A giant form of White Queen Star originated from a normal strain of known pedigree.

2. The giant arose in the course of selection-experiments made with plants possessing flowers with supernumerary petals.

3. Histological comparison indicates that the gigantism of the mutant is due to that of its cells.

4. The giant arose suddenly and breeds true.

5. It is now moderately fertile with its own pollen but proves absolutely sterile when crossed with all other varieties (including the parent form) of *P. sinensis*.

6. Gigantism in *P. sinensis* is due to three factors and the character is dominant to normal-character.

7. Owing to the number of factors involved in the production of the character of gigantism numerous semi-giant races exist. These races intergrade one with another and hence their existence is not generally recognized.

8. Giants which breed true may be produced by crossing non-giant races of *P. sinensis*.

9. Fluctuating variations may owe their origin to the heterozygous state of one or more factors in a form from the genetic constitution of which is lacking entirely one of the factors for the production of a Mendelian character.

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EXPLANATION OF PLATE XI.

Fig. 1.   White Queen Star normal variety.

Fig. 2.   Giant White Queen Star—a mutant from the normal variety.

Fig. 2.

Fig. 1.

THE CHROMOSOMES IN THE OOGENESIS AND SPERMATOGENESIS OF *PIERIS BRASSICAE*, AND IN THE OOGENESIS OF *ABRAXAS GROSSULARIATA*.

BY L. DONCASTER, M.A.,

Fellow of King's College, Cambridge.

IN a recent note[1] on some stages in the spermatogenesis of *Abraxas grossulariata* I showed that there is apparently no inequality in the chromosomes of the spermatocyte divisions, corresponding with the heterochromosomes which have been found in other orders of insects. The same result has been found by others in Lepidoptera. I know, however, of no careful study of the oogenesis in this order, and since in *Abraxas* the sex-limited character is transmitted by the female only to her male offspring, it seemed possible that there might be inequality in the chromosomes of the eggs, corresponding with the male-determining and female-determining eggs, which are shown to exist by the facts of sex-limited inheritance. In *Abraxas* the chromosome number is large (the reduced number being 28), and in my first attempts to investigate the subject I failed to find oogonial mitotic figures which were suitable for accurate observation. On searching for other Lepidoptera which might provide more suitable material, I found that the Large White Butterfly (*Pieris brassicae*) has a much smaller number of chromosomes, with clearer mitotic figures. After following out the available stages in *Pieris*, the experience so gained, and especially the fact, unknown to me in my earlier attempts with *Abraxas*, that the oogonial divisions take place chiefly in the larva, enabled me to return to the study of *Abraxas* with more success, and to work out the earlier stages of oogenesis with some completeness. In the following

[1] *Journal of Genetics*, Vol. I. p. 179.

account I shall describe first the behaviour of the chromosomes in
the oogenesis and spermatogenesis of *Pieris brassicae* in which the
phenomena are more easily followed, and then return to the oogenesis
of *Abraxas*.

<div align="center">PIERIS BRASSICAE.</div>

Ovaries and testes were dissected out of fresh adult larvae and
young pupae (1—5 days) fixed immediately in Flemming's fluid, cut
into sections of about 6μ in thickness, and stained with Heidenhain's
iron haematoxylin.   Some also were stained with Breinl's process[1]
for comparison, especially for the study of nucleoli.   A point of some
interest is the fact that in adult larvae in August and September,
from which the imago will not emerge until the following spring, the
testes have attained their full size, and contain every stage of spermato-
genesis from spermatogonia grouped around a conspicuous Verson's cell
in each compartment to practically mature spermatozoa.   The ovaries in
adult larvae, on the other hand, are extremely small, consisting of four
parallel tubes or rather columns of cells showing as yet no division into
egg-chambers, and with no deposition of yolk in the eggs.   That almost
fully formed spermatozoa should exist in the larval testis before the six
months' hibernation of the pupa is a somewhat surprising fact.

Oogenesis.   In the tubes of the ovary—if they may be called tubes
when they are in no sense hollow—when the tube is cut longitudinally,
a continuous series may be seen from early oogonia through multipli-
cation stages to the earlier growth-phases of the oocytes before the
deposition of yolk.   Since all the stages occur in fairly regular order
from the apex of the tube downwards, they are easier to follow than
are the corresponding stages of spermatogenesis, for in the testis the
cells are grouped in follicles which are very irregularly arranged.   An
examination of the stages of spermatogenesis, however, shows that they
are closely similar to the series found in the egg-tubes.

The apex of the tube is packed with small oogonia, of which the
nucleus in the resting stage shows a faint reticulum and a conspicuous
nucleolus.   The latter in favourably-stained cells appears to consist of
a double or bilobed chromatin mass associated with a plasmosome, or
enclosed in a mass of achromatic material; not infrequently the two
halves of the chromatin mass may lie apart, and sometimes appear
compound.   Division figures commonly occur in groups; when equatorial

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[1] *Ann. Trop. Med. and Parasitology*, Vol. I. 1907, p. 470.

plates are seen in face, 30 chromosomes are easily counted (Fig. 1).
They include eight which are smaller than the rest, and 22 larger
chromosomes. The larger ones, however, are not all of exactly equal
size, so that it is not easy to separate the smallest of them from the
larger members of the eight small ones. The eight small ones usually
appear to be graded into two smallest, four rather larger, and two
somewhat larger still, but these pairs cannot always be recognised with
certainty. Pairs similar in size often but not always appear near to-
gether in the equatorial plate; this is most conspicuous in respect of
the two smallest. Some figures give the appearance of an odd number
of small chromosomes, but more careful study suggests that this is due
to one member of a pair being seen end-wise, and the other more side-
ways. I conclude therefore that there is no evidence for the existence
of an unequal pair, and still less for an unpaired chromosome in the
female of *Pieris brassicae*. Outside the circle of chromosomes in
the equatorial plate, two or more small chromosome-like bodies are
often visible; their position and size usually distinguish them without
difficulty from true chromosomes. Miss Cook[1] has described similar
" chromatin granules " in the spermatocytes of Lepidoptera.

After the last oogonial division, the nucleus begins to enlarge, the
reticulum becomes more clearly visible, and then, apparently suddenly,
the " synizesis " stage supervenes. The nucleus now contains a very
fine thread or group of threads, tightly coiled on one side of the cavity;
the chromatin nucleolus at this stage is single, small, with sharp outline
(Fig. 2).

The synizesis condition ceases as suddenly as it began, giving place
to nuclei with the chromatin thread in separate segments, much inter-
twined, and noticeably thicker than in the preceding stage (Fig. 3).
The transition from this to the following stages is gradual; the nucleus
enlarges, the threads become less interwoven, and finally become ar-
ranged not quite regularly but approximately in a meridional manner
round the nuclear membrane (Fig. 4). In the earlier stages it is im-
possible to count them, but in favourable cells at this stage it is
sometimes not difficult to see that there are 14 separate threads, and
as the unravelling of the segments is a quite gradual process there are
probably 14 at the close of synizesis, although some of the earlier cells
give the impression of a larger number (possibly twice as many). The
nucleolus during this process has enlarged and finally again become
conspicuously double; occasionally the two parts are separated; it is

[1] *Proc. Acad. Sci. Philadelphia*, 1910, p. 294.

now more obviously composed of an achromatic sheath enclosing a double mass of chromatin. There are thus at this stage fourteen chromatin threads and a double nucleolus. The last stage represented in my series is the .contraction of the threads into short thick chromosomes, which at the close of the process are conspicuously split (Fig. 5), so that the ordinary chromosomes are no longer clearly distinguishable from the "chromatin nucleolus" which must be regarded as a double, equally paired, heterochromosome such as is described by Miss Cook (*loc. cit.*) in the spermatogenesis of several species. I have not been able to determine with certainty how the doubleness of the thick chromosomes arises; in *Abraxas*, and also in *Pygaera bucephala*, the ovary of which I have examined for comparison, and in which the double chromosomes are most beautifully shown, the appearance strongly suggests that the doubleness is produced by the contraction of looped threads, which break apart at the bend of the loop and give rise to a double rod.

Certain exceptional conditions should be mentioned. At various stages from the oogonia onwards degenerating cells, singly or in groups, are not infrequent, in which the nucleus develops into a more or less compact staining mass. The same thing is not infrequent in spermatogenesis of various insects.

Among cells shortly after the synizesis stage in one ovary a single nucleus is present in which, in addition to chromatin-nucleolus and plasmosome, there are about 25 short thick chromatic threads like those of the last stage in the production of the 14 double chromosomes described above.

As will be seen below in the description of *Abraxas*, such cells with the diploid number of shortened chromosomes are abundant in the latter insect, and appear to arise by the more or less complete separation of the chromosomes which have paired in synapsis. In *Abraxas* these cells probably do not give rise to eggs, but become nutritive or follicle-cells. In *Pieris* the nuclei of all cells at this stage, whether they will ultimately become eggs or follicle-cells, normally show the reduced chromosome-number; the single cell found with the double number is quite exceptional.

Finally it should be mentioned that in cells of the ovarian epithelium enclosing the developing oocytes, mitotic figures occur with many more than thirty chromosomes; a similar reduplication of the chromosomes in the ovarian sheath appears also in *Abraxas*, and has been seen in other orders of insects.

Spermatogenesis. The testis is divided into about three compartments, and at one side of each of these is a large Verson cell round which the spermatogonia are packed, not as yet visibly arranged in follicles. Here and there groups of spermatogonia may be found in division, indicating that the grouping which later shows itself by the arrangement in follicles already exists. The equatorial plates of the spermatogonia are so crowded that I have found no case in which accurate observation of the chromosomes is possible; the best figures merely indicate that the number is about 30. A little distance away from the Verson cell, beyond the zone in which mitoses are found, the early spermatocytes, now clearly grouped in follicles, are seen to pass through phases comparable with those described in the young oocytes. Since the arrangement of the follicles is irregular, and all the cells in any follicle are nearly at the same stage, it is less easy to place the stages in their right order, but by comparison with the oocyte stages no doubt remains that the cells pass through a similar synizesis followed by a stage with long intertwined chromosomes, and then appear to contract into chromatic bodies round the nuclear membrane, connected by a fine reticulum. I have not found anything exactly comparable with the short double chromosomes found in the oocytes, but the last stage mentioned, which persists until the spermatocyte is ready for division, doubtless represents it. The chromatin nucleolus is also similar to that of the oocyte at the corresponding stage. During these processes the cells enlarge considerably, and the follicles grow still more rapidly, so that each comes to contain a considerable cavity. In some testes, perhaps occasionally in all, certain follicles and their contained cells fail to grow, and when these cells come to divide in the spermatocyte divisions, the mitotic figures are irregular and very similar to the abnormal divisions which I have described in *Abraxas*. They are much less frequent in *Pieris*, and I have not followed the subsequent fate of the spermatids in this form.

The normal spermatocyte divisions are remarkably regular and clear, and show with great regularity 15 chromosomes when the equatorial plate is seen in face (Figs. 6, 7). Three of these are always smaller than the rest, and usually one somewhat less small is distinguishable; these four correspond with the eight smaller chromosomes observed in the oogonial equatorial plates. Side views of the metaphase and early anaphase stages of the first division show that the chromosomes divide in the heterotype manner, the separating halves being connected for a time by two strands.

The second spermatocyte division is easily recognisable by the smaller size of the cells and of the chromosomes (Figs. 8, 9). Fifteen chromosomes can always be seen in well-placed equatorial plates, of which usually four, sometimes only three, and occasionally as many as five, are noticeably smaller than the rest. The two smallest are often, but not always, lying side by side. In some figures, these two small ones are very conspicuous; in others, often in the same follicle, only one very small one is found. This led me at first to suppose that in the first spermatocyte division there must be an unequal pair of heterochromosomes such as Wilson has described in *Lygaeus* and *Euschistus*, but if it exists, the difference in size between the two members of the pair is not sufficient to reveal itself in a side view of the metaphase and early anaphase. I have examined many such figures with great care, and have never found a dividing chromosome in which the two halves were certainly unequal. In some figures a very slight inequality is suggested occasionally, but in others, where every chromosome is visible, no inequality can be seen. Further, the fact that there may be three or four all equally small, and in other equatorial plates in the same follicle only one (or rarely none at all) which is conspicuous by its small size, makes the interpretation of these differences as being due to unequal heterochromosomes very doubtful. It may be concluded therefore that in both sexes of *Pieris brassicae* the somatic number of chromosomes is 30; these vary in size and include one pair which differ from the rest in the growth-phases in constituting a "chromatin-nucleolus," so resembling heterochromosomes. The reduced number in both sexes is 15, and although there is a suggestion that the heterochromosomes form an unequal pair in the male, the evidence for this is quite inconclusive, and the appearance is very probably deceptive.

ABRAXAS GROSSULARIATA.

The material consisted of ovaries removed from larvae varying from somewhat more than half-grown up to nearly full-grown, and treated similarly to those of *Pieris*. The ovarian tubes are shorter, but the stages follow each other nearly as regularly as in *Pieris*. The oogonia contain a nucleolus more or less conspicuously double, which consists chiefly if not entirely of chromatin. Among the oogonia, especially near the apex' of the tube, groups of cells are constantly found undergoing degeneration; their number is often considerable.

Oogonial mitoses, even in rather young larvae, are less numerous

than in *Pieris*, and it was not easy to find examples cut so that the chromosomes could be counted with perfect accuracy. Counts were always made by drawing the chromosome group, not by eye. In the most satisfactory figures the number appears to be 56, *i.e.* twice the number found in the spermatocyte divisions. Two of these equatorial plates are figured in Figs. 10 and 11; the only doubt about the number 56 in these cases consists in the facts that in Fig. 10 a pair of chromosomes (at the left upper edge, apparently consisting of a larger and smaller member) might possibly be two halves of a dividing chromosome, but the only reason for this suggestion is that they are at slightly different levels; and in Fig. 11 the double body outside the circle at the bottom might conceivably not be a chromosome, for other stained bodies occur in the cytoplasm outside the spindle. Careful examination has convinced me, however, that in fact there are 56 in each figure. The same number has been found in three other figures in which the number 56 is quite clearly seen, and in a further three in which part of the equatorial plate was seen in the next section to the main group, so that a chromosome might possibly be cut so as to appear in two sections, although this is very improbable when they are so small. In a number of other figures it was impossible to decide with certainty between 56 and 55, and in some only 54 were clearly visible.

Since in the best figures obtained there is no reasonable doubt that 56 is the true number, and since an even number is certainly present in *Pieris brassicae*, it may be concluded with some confidence that in the female *Abraxas grossulariata* the unreduced number of chromosomes is 56.

With regard to the variety *lacticolor* I am less certain; in all my figures of this form counts may be interpreted as 55 or 56, according to whether a double chromosome is regarded as one or two. As described in my paper on the spermatogenesis, the *lacticolor* male does not differ recognisably from the *grossulariata* ♂ in its chromosome group, the spermatocyte number being 28 in each. It is to be expected therefore that the number in the female should not differ from that of *grossulariata*. That the number in *lacticolor* is either 55 or 56 is certain; a final decision on the matter can only be arrived at when more material is available. It should be said that the chromosomes of the oogonial divisions in both forms are not all equal in size; in some cases two are noticeably larger than the others, but these two are not always recognisably different from the next in size, so that accurate identification of chromosomes is hardly possible.

After the oogonial stage, the nuclei begin to enlarge and pass through a synizesis stage closely similar to that of *Pieris*, except that the nucleolus is less conspicuous and is sometimes difficult to find. On emerging from this condition the nuclei, now considerably enlarged, contain interlaced chromatic threads, the number of which cannot be accurately determined. The nucleolus at this stage varies somewhat in appearance; it usually consists of an achromatic mass enclosing a double mass of chromatin, but may be almost or completely divided into two parts, and each half is sometimes seen to contain a compound mass of chromatin. In the rather later stages this compound nucleolus, consisting of a number of globules or irregular lumps of chromatin, and usually clearly divided into two more or less separate parts, is very commonly seen in sections from which the stain is rather thoroughly washed out or which are stained with Breinl's stain (Figs. 13 *a*, 15 *a*). The individual chromatin globules sometimes appear double, but I doubt whether this is more than accidental.

The chromatin threads contract and thicken, but do not in general assume the meridional arrangement often seen in *Pieris*. They appear to become bent into loops (Fig. 12), and these shorten to form horseshoe or even ring-like figures (Fig. 13). The nuclei are now so large as to extend through two sections, but careful counts show that the number of the contracted loops is not far from 27. From this stage onwards, or possibly from one somewhat earlier, a differentiation appears to set in among the cells. In the majority, the chromosomes contract still further and appear to break apart at the bend of the loop, giving rise to a double body, the halves of which are commonly completely separated, so that the nucleus contains about 27 pairs of short, thick chromosomes, and an additional larger pair apparently derived from the chromatin-nucleolus. Each member of a pair may itself show signs of doubleness, possibly owing to the precocious appearance of the longitudinal split of the next division, traces of which may sometimes be seen in an earlier stage (Fig. 13). In many nuclei the two members of each pair are completely separated, giving the diploid number of small irregular chromosomes scattered in the nucleus (Fig. 14). I was at first inclined to believe that these single chromosomes came together in pairs, giving rise to the stage in which the reduced number of doubles is seen, but a careful study of the various stages has convinced me that in the nuclei here described the paired condition precedes that with the full number of singles.

The nuclei just described are the most numerous class at the lower

end of the egg-tube, but are not, I believe, true oocytes. Among them, fewer in number, are somewhat larger nuclei, which always lie in the middle of the egg-tube, either surrounded by those of the type just described, or extending further down the egg-tube than the latter. I believe these larger nuclei are those which will give rise to the egg-nuclei; the kind described just previously probably belong to the cells of the egg-follicles or the nutritive cells. The large nuclei, which appear to be those of the true oocytes, contain a very large nucleolus and interlaced chromatin threads, which have not contracted into short loops. Their number is difficult to count, but appears to be the haploid number (27) rather than the diploid (54). The nucleolus appears to consist chiefly of chromatin; it is either double or divided into two separate parts, and each part consists of a number of masses of chromatin apparently embedded in achromatic material, which, however, is not very easy to demonstrate. The irregularity of the chromatin masses composing the two parts of the nucleolus makes it difficult to determine whether the two parts are equal or unequal; in some nuclei no difference in size is visible, in others one mass is certainly larger than the other. The nucleolus appears rather as a store of chromatin than as a definite chromosome.

In addition to the two classes of cells described in the lower region of the ovary-tube, there are cells of varying size with many scattered chromatin granules in the nucleus. These are occasionally found in division, and show about 56 chromosomes; if the cells described above give rise to the nutritive cells and true oocytes, these last are probably the follicle-cells.

In conclusion, then, it appears that *Abraxas*, like *Pieris*, has an even number of oogonial chromosomes, with no evidence of an unequal pair. In the early stages of the meiotic phase two of these give rise to a double chromatin-nucleolus, and the remainder undergo synizesis, from which they emerge in the haploid (reduced) number of chromatin threads. In cells which probably do not become true oocytes, these threads then become contracted into short loops, which break at the bend and give rise to the reduced number of pairs of chromosomes. The members of the pairs may then separate. In the true oocytes the bivalent threads persist to the latest stage observed—possibly till the prophase of the polar divisions. The halves of the chromatin-nucleolus, though not always identical in size, do not show any constant differences which would justify the assumption that it may be regarded as an unequally paired heterochromosome. The

chromosomes in the earlier stages of oogenesis therefore do not provide any visible basis for the sex-limited transmission of characters. If Spillman's suggestion be correct, that in the normal *grossulariata* male there are two *G*-bearing chromosomes, while in the female one of these is replaced by a sex-chromosome ("*X*") which does not bear *G* (the factor for *grossulariata*), this is what would be expected; but since, in the male at least, the variety *lacticolor* has the same number of chromosomes as *grossulariata*, the *G*-bearing chromosomes would have to be supposed capable of losing the factor *G* without becoming visibly different.

Note.—Since the above was written, I find that Payne (*Journ. Morphol.* Vol. XXIII. 1912, p. 331) has come to conclusions closely similar to mine about both the constitution of the nucleolus and the origin of the oocytes in Reduviidae; and Miss P. H. Dederer has published a preliminary note on the maturation of the eggs of *Philosamia cynthia* (*Biol. Bull.* XXIII. p. 40, June 1912) in which she finds no dimorphism among the egg-chromosomes.

Postscript, Sept. 26, 1912.

In ovaries of larvae derived from the 1912 pairings, although I have no perfectly trustworthy figures in individuals derived from the cross *lact.* × *lact.*, I have several which show 56 chromosomes quite clearly in larvae from the cross *gross.* ♀ × *lact.* ♂. Since this cross always gives only *lacticolor* females, it may be concluded with confidence that the chromosome number in the *lacticolor* female is not different from that in the *grossulariata* female.

EXPLANATION OF FIGURES.

All the figures except 13*a* were drawn from sections stained with iron-haematoxylin; for all Zeiss apochromat n. a. 1.40, 3 mm. and oc. 12 were used.

In figs., 3, 4, 5, 13*a*, 15*a* the shaded area round the nucleolus represents the non-chromatic portion from which chromatin stains are easily washed out.

Figs. 1—9.　*Pieris brassicae.*

　1.　Oogonial division, equatorial plate.
　2.　Synizesis stage.
　3.　Shortly after synizesis. The upper half of the nucleus is not included in the section.
　4.　Rather later stage; complete nucleus with 14 threads and chromatin-nucleolus.
　5.　Contraction of threads to form double chromosomes; only 13 of these are visible in this nucleus, the fourteenth is probably hidden by the nucleolus.

6, 7.   1st spermatocyte equatorial plates; four small chromosomes in fig. 6, three in fig. 7.

8, 9.   2nd spermatocyte equatorial plates : three small chromosomes in fig. 8, only one conspicuously small in fig. 9.

Figs. 10—15.   *Abraxas grossulariata.*

10, 11.   Two oogonial equatorial plates, each with 56 chromosomes.

12.   Oocyte after synizesis.   Reduced number of chromatin loops.   The nucleolus and a number of loops are not included in the section.

13.   Slightly later stage ; not all the chromosomes are shown.   (26 double chromosomes and two nucleoli were visible in this nucleus.)

13a.   Nucleolus of similar nucleus stained with Breinl.

14.   Later stage, probably a nutritive cell.   Somatic number of short chromosomes, most of them split, some lying in pairs.   (54 chromosomes and two nucleoli were visible in this nucleus.)

15.   Oocyte, at about the stage of fig. 14, with reduced number (approximately, not all shown) of chromatin threads.

15a.   The two halves of the nucleolus of a nucleus in about the same stage, from which the stain is so far washed out that the chromatin threads were nearly colourless.

SOME RECENT WORK ON MUTATION IN MICRO-ORGANISMS.

By CLIFFORD DOBELL.

MUCH work has been done in the last few years upon mutation[1] in several different groups of micro-organisms. This work has been published in many different places, and has been largely carried out in connexion with investigations of a medical nature. The records are therefore somewhat diffuse, and not always easily accessible to the biologist who applies himself mainly to the study of genetics. In the compass of the following few pages I shall endeavour to chronicle— in a somewhat critical spirit—some of the more important observations which have been recently recorded in this branch of biology.

I. MUTATIONS IN TRYPANOSOMES.

In this first section, I shall describe some recent work upon mutation phenomena observed in several species of flagellate Protozoa belonging to the genus *Trypanosoma*. The mutations may be grouped in two different classes—morphological and physiological.

A. *Morphological Mutations.*

In several cases, structural modifications have been induced in Trypanosomes, and found to be permanent and transmissible for a variable number of succeeding divisions. These cases will now be described.

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[1] I use this term—as others have already done—to denote those heritable modifications which have been induced in various ways in various micro-organisms. I believe that a "mutation" in a Trypanosome is essentially the same sort of thing as a "mutation" in a multicellular organism. But I must also point out that I use the words "inheritance," "heritable," and similar terms in the customary manner—applying them to the transmissible characters of such organisms as Trypanosomes, Bacteria, etc. I do not wish to assert, however, that "inheritance" in Trypanosomes means exactly the same thing as "inheritance" between parent and offspring in sexual multicellular organisms.

The Trypanosomes are Protozoa possessing a very remarkable structure. It is necessary, therefore, to recall at the outset the structures present in a typical animal of this sort. This can be most clearly done with the aid of a diagram (Fig. A), an inspection
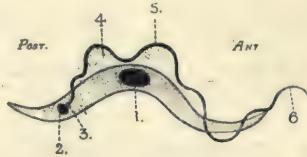
Fig. A. Structure of a typical Trypanosome.

(1) Trophonucleus, or chief nucleus. (2) Kinetonucleus, or smaller nucleus, in connexion with locomotory apparatus. (3) Blepharoplast—a minute basal granule at the root of the flagellum. (4) Undulating membrane, used in locomotion. (5) and (6) Flagellum—marginal (to undulating membrane) and free parts. *Ant.*, anterior or flagellar end. *Post.*, posterior or aflagellar end of organism.

of which will, I think, make further explanation unnecessary. The terminology of the parts is that of Minchin. To avoid any confusion I should mention that the organ which is called *kinetonucleus* throughout this paper is called *centrosome* by Laveran, and *blepharoplast*[1] by the majority of German workers. It should also be added that Trypanosomes reproduce by longitudinal division—both nuclei dividing into two.

I will now describe the curious structural changes which have been brought about in certain Trypanosomes, and will begin with the work of Wendelstadt and Fellmer (1910).

It has been found by these workers that Trypanosomes—they used two species, *Trypanosoma brucei* and *T. lewisi*—which normally live in the blood of certain mammals (*e.g.* rats) may be inoculated into coldblooded vertebrates and invertebrates, in which they can live for a certain time. The Trypanosomes used were from well-known strains which had been cultivated in rats and under observation in the laboratory for several years.

Trypanosoma brucei was, after some difficulty, passed from the blood of rats into the blood of grass snakes (*Tropidonotus natrix*)[2]. In the snake's blood, the Trypanosomes become smaller (Fig. B, 2), as compared with the initial forms in the blood of the rat (Fig. B, 1).

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[1] Frequently—and incorrectly—also written "blepharoblast."

[2] The authors refer to the snake merely as the "Ringelnatter," but presumably this animal is meant.

Frequently no parasites could be demonstrated microscopically in the snake's blood, although subsequent inoculation experiments proved them to be present. The small forms were apparently formed by the divisions of the original larger forms, and themselves underwent
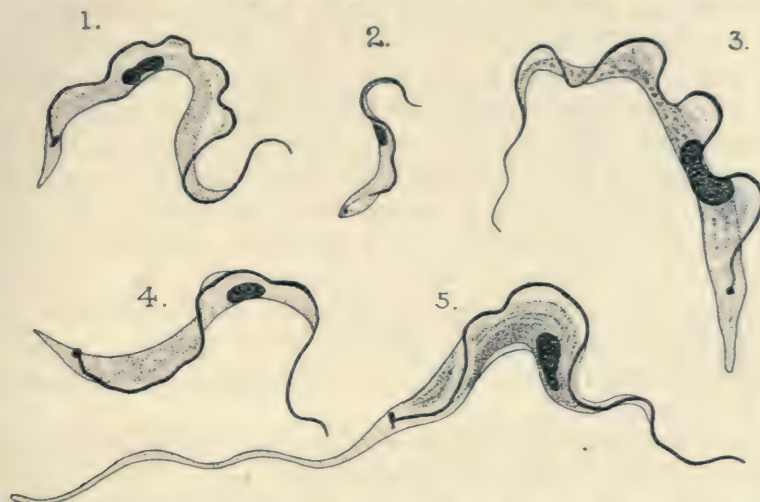
Fig. B. (1) Normal *T. brucei* in blood of rat. (2) *T. brucei* in blood of grass snake— eight days after inoculation. (3) *T. brucei*, giant form produced by inoculation from grass snake back into rat. (4) *T. lewisi*, normal form during chronic infection in blood of rat. (5) *T. lewisi*, form produced by passing the strain from rat through grass snake, then frog, and then back into rat. Fourth rat passage, five days after inoculation. [From Wendelstadt and Fellmer (1910), slightly diagrammatized.]

The organisms are all drawn to the same scale, so that the differences in size are correctly shown.

division. They showed a slight change in their staining capacity. When these small forms in the snake were inoculated back into rats, they became very large, thus giving rise to a race of giant Trypanosomes (Fig. B, 3). The increased size persisted for many divisions, during passage through several rats[1]. In later passages, however, the Trypanosomes diminished in size, and returned to their normal dimensions.

Closely similar results were obtained by passing the Trypanosomes through tortoises ("europäische Sumpfschildkröte") and lizards ("graue und Smaragdeidechsen"): but no difference in size was observed after passage through the salamander ("Erdmolche"). A temporary increase

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[1] When Trypanosomes (or other micro-organisms) are passed into a fresh host, or culture medium, the new race which thus arises is frequently termed a new "generation "— a vicious usage of the word borrowed from bacteriology.

in the size of the Trypanosomes was also brought about by passing them through beetles (*Cychrus rostratus* and *Aphodius* sp.), or through a slug (*Arion impiricorum*), and then back again into rats[1]. Several other passages (axolotl, caterpillars, etc.) were unsuccessfully attempted.

Wendelstadt and Fellmer made similar experiments with *T. lewisi*. They succeeded in passing this species from the rat through lizards, frogs, and grass snakes. In the cold-blooded host, no Trypanosomes could be found microscopically after inoculation: but inoculation of the blood back into uninfected rats gave rise in them to an infection with parasites of increased size[2]. When the normal Trypanosomes (Fig. B, 4) from the rat were passed through a snake, or through a snake and then a frog, and then back into a rat, a remarkable modification was finally produced. The Trypanosomes were not only much larger, but they were also greatly elongated at the aflagellar end[3] (Fig. B, 5). No divisions were observed in these forms. Moreover, they showed certain differences in their staining properties as compared with the original forms.

It may be added that no results similar to these of Wendelstadt and Fellmer have been recorded by other workers.

A much more interesting—because more thoroughly investigated—morphological mutation in Trypanosomes has been discovered by Werbitzki (1910). In the course of some researches on the effects of certain organic dyes upon living Trypanosomes, this worker made the following observations. (The researches were carried out in Ehrlich's laboratory, on a strain of *T. brucei* known as "Nagana ferox," and cultivated in mice.) When certain dyes were injected into infected mice, the Trypanosomes (Fig. C, 2) lost their kinetonuclei (Fig. C, 1). The modified Trypanosomes were found to remain permanently devoid of this organ during subsequent divisions. They divided normally and actively, and could be passed in the usual way through other mice by subinoculations. A race of Trypanosomes with a permanent morphological modification has been thus produced. The dyes used successfully

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[1] The blood containing the Trypanosomes was injected into the body of the invertebrate, which was subsequently ground up in salt solution and the liquid so obtained injected into a rat. It is somewhat surprising that any positive results were obtained by such crude methods. Besides the Trypanosomes, very many other things must have been injected into the rats.

[2] The incubation period in the rat was also found to be shortened.

[3] Forms similar to these are of constant occurrence during the multiplication period of normal *T. lewisi* in the rat (Minchin). They have been described as a distinct species ("*T. longocaudense*") by Lingard.

by Werbitzki were chiefly substances belonging to the pyronin, acridin, and oxazin groups (*vide infra*)—the best results having been obtained

Fig. C.   *T. brucei*, strain "nagana ferox."   (1)   Form without kinetonucleus, after treatment with pyronin.   (2)   Normal form.   [From Werbitzki (1910), slightly diagrammatic.]

with oxazin.   The action of the dye upon the Trypanosomes is rapid. In one experiment in which oxazin was injected into a mouse on the second day after infection with the Trypanosomes, the following observations were made :—

Hours after injection of dye	Number of Trypanosomes without kinetonucleus
1—2	Isolated specimens
4	10—12 $\%$
6	25—30 $\%$
8	40—50 $\%$
10—12	70—80 $\%$
24	80—90 $\%$

The strain containing about 80 $\%$ of individuals devoid of a kineto-nucleus, when inoculated into other mice, shows a smaller—but still large—percentage of the modified organisms.   By passing this strain through mice 6—10 times, however, and treating with oxazin each time, a strain of Trypanosomes in which *every* individual is devoid of a kinetonucleus has been obtained.   This strain remains constant after numerous subsequent passages through untreated mice[1].

The Trypanosomes devoid of kinetonuclei are—as regards motility, general appearance and behaviour, etc.—indistinguishable from normal organisms save in this one feature.   Their rate of multiplication is, moreover, unchanged.   They show, however, a slight difference in resistance.

[1] Kudicke (1911 A) reports that one such strain has been passed through 115 mice, without any treatment, and still retains its morphological peculiarity unaltered.

It should be noted that it is the kinetonucleus only which has been removed from these organisms.   The blepharoplast (end-knob, or basal granule) and the rest of the locomotory apparatus remain intact[1].

Werbitzki endeavoured to obtain a race of Trypanosomes *with* kinetonuclei by further treatment of the race from which this organ had been removed.   The parasites were treated with various dyes, and passed through various animals (rats, guinea-pigs, rabbits); but the results were not always the same.   In one case, passage through 50 animals, and treatment with dyes, left the strain quite unaltered. In another case, however, it was found that 7 $\%$ of the Trypanosomes had acquired kinetonuclei at the 16th passage: and this percentage increased during subsequent passages, until at the 27th practically every individual possessed a kinetonucleus.   How the kinetonuclei were "regenerated" was not determined.   Microscopically, the individuals of the new race did not differ in any way from normal Trypanosomes. But it was found that their new kinetonuclei were susceptible to the action of drugs which were without effect upon ordinary organisms. For example, the kinetonuclei in the new race were removed by the action of arsacetin—a drug which has no action in this respect on normal Trypanosomes.

An important question now arises as to the exact way in which the kinetonucleus is removed from the strain of Trypanosomes which has been subjected to the action of dyes of a certain sort.   Werbitzki suggested that its disappearance might be accounted for in three different ways.   First, the kinetonucleus might have been destroyed by the dye, or eliminated from the organism; secondly, it might have fused with the trophonucleus; thirdly, it is possible that the kinetonucleus has really not been removed, but its apparent absence is due to the fact that it no longer takes up chromatin stains in the usual way—owing to the action of the drug—and therefore is invisible in microscopic preparations.   The second and third suppositions were shown by Werbitzki to be unsupported by any direct evidence.   He inclined to the supposition that the kinetonucleus had been destroyed in some way.   He figured, moreover, dividing forms of the Trypanosomes in which one daughter individual contained a kinetonucleus, whilst the other contained none.   The suggestion therefore seemed justified that the new race arose in this manner—by an irregular distribution of the organ during division.   No definite conclusions in this respect were arrived at, however, by Werbitzki.

[1] This is stated on the authority of Dr v. Prowazek, to whom the strain was submitted for a careful cytological examination.

Kudicke (1911) made a further attempt to discover how Werbitzki's strain had lost its kinetonuclei. He says that even in normal races of Trypanosomes—that is, in organisms *untreated* with dyes—as many as 5 °/₀ of the individuals may lack kinetonuclei. It is therefore possible that the drug selects these organisms: they may be more resistant to the drug, and therefore survive after treatment and so give rise to the new race. Kudicke was unable, however, to discover exactly how the kinetonucleus disappeared. He found that acridin would remove the kinetonucleus from *T. lewisi*—in a certain percentage of cases— but here again he was unable to decide with certainty how the removal was brought about. Kudicke's work, on the whole, did not show whether the races of Trypanosomes without kinetonuclei were produced by selection, by an irregular division, or in some other way.

An important sequel to Werbitzki's work has been furnished by Laveran and Roudsky. Laveran (1911) obtained the "nagana ferox" and "Werbitzki" strains of *T. brucei* from Ehrlich. He was able to confirm Werbitzki's observations on the structure of the individuals composing these strains. In collaboration with Roudsky (1911), he reinvestigated the action of oxazin upon *T. brucei*. These workers found that the dye removed the kinetonucleus—as Werbitzki had stated. They were able to extend the investigations, moreover, to seven other species of Trypanosomes (*evansi, soudanense, gambiense, dimorphon, pecorum, congolense, lewisi*). In all of these, oxazin caused a disappearance of the kinetonucleus: and the peculiarity was transmitted hereditarily in subsequent divisions, so that strains were produced in which the individuals were—to a greater or less extent—deprived of kinetonuclei.

Laveran and Roudsky (1911, 1911 A) appear to have decided how the kinetonucleus is removed. They have found that when oxazin[1] is injected into a mouse infected with Trypanosomes, the kinetonuclei of the latter are stained pink or violet with the dye. The rest of the Trypanosome is uncoloured, and it remains actively motile—provided that the dye is not present in such concentration as to kill. The action of the dye can be observed in a drop of infected mouse's blood under the microscope. It seems certain, therefore, that the dye has a special affinity for the kinetonucleus. It can be seen further that the kinetonuclei which have been stained by the dye—in the living Trypanosomes —dwindle in size, and finally disappear. Laveran and Roudsky accordingly believe that the dyes used have a direct and specific action

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[1] Similar results were obtained with acridin.

upon the kinetonucleus, which they attack and finally remove. They suggest further that the actual destruction of the kinetonucleus is brought about by autoxidation *in situ*. Certain experiments appear to support this view. It is known that potassium cyanide and alkaloids—when present in very small quantities—retard autoxidation processes in the tissues. Laveran and Roudsky made a number of different preparations of heavily infected mouse blood. To some they added oxazin alone: to others oxazin with minute quantities of KCN or certain alkaloids[1]. The results were very striking. Oxazin alone coloured and removed the kinetonuclei of the Trypanosomes (as usual): oxazin + KCN, or oxazin + alkaloid, did not affect the kinetonuclei, which remained quite colourless.

It appears certain, from the above observations of Laveran and Roudsky, that the production of a race of Trypanosomes devoid of kinetonuclei by the action of dye-stuffs, is due to the specific action of the dye upon the kinetonucleus. The latter is attacked by the dye—as is shown by its becoming coloured—and then removed, probably by autoxidation. Laveran and Roudsky find no evidence to show that the kinetonucleus is ever removed by an irregular division—as suggested by Werbitzki and Kudicke. They suggest that "if in certain cases the kinetonucleus does not divide at the moment of bipartition, it is probably because it is already dead or altered"—through the action of the dye.

Werbitzki (1910) found that the only difference—save as regards the nuclei—between his strain and normal *T. brucei* was that the former was less resistant to pyronin. Laveran (1911, 1911 A) and Laveran and Roudsky (1911) found that the two strains differed in that the Werbitzki strain had an attenuated virulence for laboratory animals. They also found that injections of oxazin caused the appearance of giant forms of the Trypanosomes in infected mice (*T. brucei*, *T. evansi*, *T. soudanense*). All the species of Trypanosomes without kinetonuclei appear to possess a diminished virulence.

Laveran and Roudsky (1911 A), by imitating Werbitzki's procedure, have now obtained by oxazin injections a strain of *T. evansi* which has no kinetonuclei and is apparently fixed in this respect. It breeds true in untreated mice. From *T. soudanense*, however, they have only succeeded in obtaining a race which—at the 50th passage—contains 66 % of individuals without kinetonuclei.

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[1] The authors do not state which alkaloids they used.

There is a point of considerable interest in connexion with the dyes which bring about the disappearance of the kinetonucleus in Trypanosomes: for there appears to be a definite relation between the chemical structure of the dye and its action upon the Trypanosome. Werbitzki found that those dyes which destroy the kinetonucleus are substances belonging to the pyronin, oxazin, and acridin groups ("aniline" dyes with acid chromophores). These dyes all possess a structure which Ehrlich (1909) calls an *orthoquinoid* structure. Orthoquinoid substances possess a structure which is essentially thus:

R
R
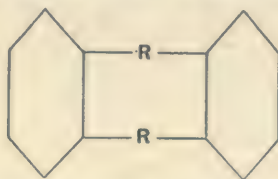
That is to say, they consist of two benzene rings united together as shown. Dyes of the pyronin series have the general structure:

O
C
H
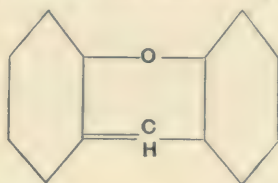
Those of the acridin series have the structure:

N
C
H
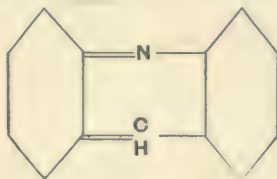
Both the pyronins and the acridins are derivatives of diphenylmethane. This is not the case with the dyes of the oxazin group, however, which have the general structure:

O
N
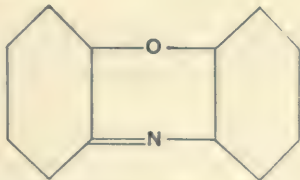
and are diphenylamine derivatives.

The orthoquinoid linkage appears to be the important thing in these substances. Those drugs with it act upon the kinetonucleus: those without it have no action. (Many dyes and other substances were tried in this respect, *e.g.* atoxyl, arsenophenylglycin, trypan-red, etc.) It must be noted, however, that dyes with a structure which Ehrlich (1909) terms *paraquinoid* (*e.g.* parafuchsin) also have a slight action upon the kinetonucleus. These dyes have the general structure:

R
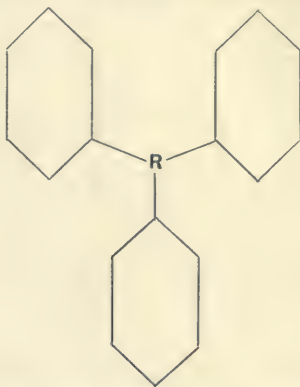
parafuchsin being a derivative of triphenylmethane with the structure:

NH_2 NH_2Cl

C

NH_2
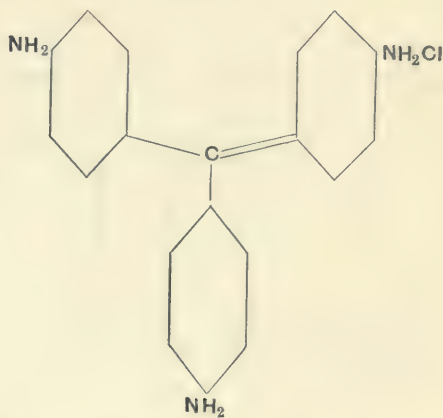
When parafuchsin is employed in doses large enough to affect the kinetonucleus, it also injures the rest of the Trypanosome, and finally kills it[1].

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[1] The parafuchsin-resistant strain of Ehrlich, kept at the Speyer Haus, has its kinetonuclei intact. The strain was produced by acting upon normal *T. brucei* with increasingly large doses of the dye.

It seems legitimate to conclude, therefore, that dyes with an ortho-quinoid structure have a specific action upon the Trypanosome kineto-nucleus[1]. They fix themselves to it in some way, and bring about its disappearance.

Concerning the Trypanosomes without kinetonuclei there are but a few additional facts of importance to record. These are results of the work of Kudicke (1911 A). He has not found it possible to obtain from the Werbitzki strain—either by drug treatment or transplantation into other animals—a strain in which the kinetonucleus is present once more. (But compare Werbitzki, p. 206, supra.) From certain immunity experiments, he has concluded that the original strain of "nagana ferox" and the Werbitzki strain derived from it, are—as regards immunity reactions—alike. Kudicke has also made some interesting observations on relapse strains of "nagana ferox." He inoculated a mouse with the strain together with trypan-blue[2]. Four days later, all the Trypanosomes had disappeared from the blood of the mouse. But four days after this, many Trypanosomes were found in the blood—that is, a relapse race arose. Nearly all the individuals of this race were devoid of a kinetonucleus. After passage through a second, and then a third mouse, *all* the Trypanosomes were without kinetonuclei. They persisted in this condition during 79 subsequent passages. An explanation of this phenomenon was not arrived at, but it seems that something different from what occurs in the case of ortho-quinoid drugs must have happened[3].

It is perhaps of some interest to recall here—in connexion with the experiments just recorded—certain observations which have been made upon some flagellate Protozoa closely related to the Trypanosomes. Several observers have found occasional individuals which have lost their *trophonuclei*. Hartmann and Prowazek (*Arch. Protistenk.* x Bd. 1907) noted, for example, that 5-day cultures of the kala-azar parasite contain individuals which have lost this organ. Similarly, Flu (*ibid.* XII Bd. 1908) and Berliner (*ibid.* XV Bd. 1909) describe the occurrence of individuals with a similar defect in *Crithidia melophagia* and

[1] Ehrlich has found that Trypanosomes which have become resistant to orthoquinoid substances are also resistant to arsenic compounds—a very curious phenomenon. He also found, conversely, that races resistant to arsenic are resistant to the orthoquinoid diphenyl-methane derivatives.

[2] Not an orthoquinoid substance, and usually without action upon the kinetonucleus.

[3] The relapse race may have been formed by selection—a chance individual with no kinetonucleus having been more resistant to the drug than the normal forms, and having survived the injection and given rise to the new race.

Herpetomonas jaculum respectively. It is unfortunate that the origin of these forms is as yet quite unknown[1]. Probably they were merely degenerate individuals.

B. *Physiological Mutations.*

It has now been known for some years—largely through the work of Ehrlich and his collaborators—that drugs and antibodies may modify profoundly the physiological properties of Trypanosomes. As early as 1907, Ehrlich showed that treatment of Trypanosome-infected animals with atoxyl[2] might cause—under certain conditions—the Trypanosomes to acquire an immunity to the drug. By subjecting the Trypanosomes to the action of minute but increasing quantities of atoxyl or other drugs, Ehrlich has succeeded in obtaining strains of the parasites which are highly resistant[3] to these poisons. In 1909 he stated that he had produced an arsenic-resistant strain of *T. brucei*, which had, in the course of three years, undergone passages through some four hundred untreated animals—without any loss of resistance to arsenic. Many similar observations have since been recorded, so that it may now be stated as a fact that physiologically modified races of Trypanosomes can be made by artificial means from the races which occur normally in nature.

Mesnil and Brimont (1908) also succeeded in obtaining a race of Trypanosomes resistant to atoxyl. But they pointed out that the resistance was only manifested "in a given organism" (*i.e.* host). More definite in this respect, however, were the statements of Breinl and Nierenstein (1908)[4]. From inoculation experiments, they concluded that a Trypanosome's resistance to the drug is manifested in that

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[1] It should be remembered that Trypanosomes which have grown in artificial culture media frequently display morphological peculiarities—as regards size, shape, relative position of nuclei, etc. These modifications are, however, transitory: they do not persist after the organisms have been reinoculated into the animals which are their normal hosts.

[2] Atoxyl is sodium arsanilate—the Na salt of *p*-aminophenyl-arsenic acid (Ehrlich and Bertheim).

[3] A Trypanosome which tolerates the action of a drug is generally said to be "fast" to the drug in question, *e.g.* a Trypanosome which has been rendered tolerant to atoxyl or other organic arsenicals is spoken of as "arsenic-fast." The word "fast" has, however, an older and very different usage in bacteriology. For instance, tubercle Bacilli—and certain others—are called "acid-fast." This does not mean that the *living* organisms tolerate, or are resistant to, acids: it means that *dead* organisms when stained with carbol-fuchsin are stained "fast" (in the dyer's sense) against mineral acids. I therefore prefer to use "resistant" rather than "fast" when discussing the phenomena of living Trypanosomes.

[4] These workers, it may be noted, give an incorrect account of the results of Mesnil and Brimont.

species of host animal alone in which it was acquired. For example, they found that *T. brucei* in donkeys became resistant to atoxyl after injections of the drug. Transplanted into rats, however, it rapidly lost its resistance and became susceptible again.

Races of Trypanosomes with a changed virulence, produced by passages through a different host, have several times been recorded. Fellmer (1907), for example, stated that the virulence of *T. brucei* was diminished by passage through the hedgehog. The structure of the parasites was also stated to be modified by a sojourn of the race in this animal. Fellmer's experiments were repeated by Gonder and Sieber (1909), who used both *T. brucei* and *T. equiperdum.* They completely failed, however, to produce any change in either the virulence or the structure of these Trypanosomes in this way.

Wendelstadt (1909) and Wendelstadt and Fellmer (1909, 1910) also announced that the passage of *T. brucei* and *T. lewisi* through cold-blooded vertebrates—to which reference has already been made—greatly modified their virulence. They found, for example, that *T. lewisi* when passed through the grass snake becomes modified into a race which is pathogenic for rats—in which the infection is normally harmless. Inoculation of Trypanosomes from the snake back into the rat kills the latter. Twenty-four passages with a similar result were thus made. Laveran and Pettit (1909) repeated these experiments. They injected both *T. lewisi* and *T. evansi* from rats into snakes, and then back into clean rats. But they failed entirely to produce any change in the virulence of the Trypanosomes. They state, moreover, that the blood of the snake is very toxic to rats—which may account for the results of Wendelstadt and Fellmer.

It seems, therefore, that these experiments in which changes of virulence are said to have been produced in Trypanosomes should be regarded with considerable scepticism for the present[1].

Levaditi and Mutermilch (1909) found that they could produce races of *T. brucei* which were resistant to certain antibodies. Later, Levaditi, in collaboration with Twort (1911), has shown that a race of *T. brucei* can be made which is resistant to the toxin produced by *Bacillus subtilis*—a substance which is usually very toxic to *T. brucei.* If a normal race of this Trypanosome (from the blood of the mouse) is subjected *in vitro*—even for only a few minutes—to the action of

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[1] It should be recalled, however, that the Werbitzki races of Trypanosomes have undergone a diminution in their virulence—a fact which appears to be established (Laveran and Roudsky).

the toxin, it is found, after reinoculation into a mouse, to have acquired a marked resistance to it. A *subtilis*-toxin-resistant strain of *T. brucei* has been thus produced which remains as such during subsequent animal passages—that is, the acquired resistance of the Trypanosomes is trans-mitted hereditarily.

Most important and extensive work in this direction has been recently published by Gonder (1911), whose results may now be con-sidered in some detail. The work was carried out in Ehrlich's laboratory, where it was begun by Werbitzki.

In his experiments, Gonder used two strains of *T. lewisi*. These were, first, a strain from wild rats, grown in tame laboratory animals and susceptible to arsenic. An injection of 0·1 gm. of arsenophenyl-glycin per kilogram body-weight of rat sufficed to kill all the Trypano-somes in its blood. This strain, after numerous passages through normal rats, always remained susceptible to arsenic. The second strain was one which had been made arsenic-resistant[1]. It was made by accustoming the Trypanosomes to minute but gradually increasing doses of arsenophenylglycin. Finally—after two years—a strain of *T. lewisi* was obtained which was resistant to the drug to such an extent that it was unaffected by injections of 0·2 gm. per kilo. Passage of this strain through untreated rats showed that the arsenic-resistance had become fixed, and was transmitted hereditarily. At the twentieth passage, the resistance was unchanged[2]. As regards structure, and behaviour in other ways, the Trypanosomes were found to be indis-tinguishable from the normal race.

Gonder found that both the non-resistant and the resistant race could be transmitted from rat to rat by the rat louse, *Haematopinus spinulosus*—which is supposed by some workers to be the usual inter-mediate host of *T. lewisi* in nature. The incubation period in the louse was found to be 15—17 days in the case of the normal race (3 rats); 25—30 days (6 rats) in the case of the arsenic-resistant race. The two races of Trypanosomes were then tested as regards their resistance to arsenic. And the results showed that *all* the Trypanosomes—whether they had previously been resistant or not—were non-resistant to arsenic after development in the body of the louse.

[1] Arsenic-resistant strains of Trypanosomes can be rapidly produced by treatment with a number of different organic arsenic compounds, and also by the action of dyes of an orthoquinoid type (Ehrlich, 1911).

[2] Similar arsenic-resistant races of other Trypanosomes had, of course, been previously produced by Ehrlich and others.

In two cases, where mechanical transmission by the louse was believed to have occurred—that is, where no development of the Trypanosomes in the louse intervened—it was found that the transmitted Trypanosomes had retained their power of resisting arsenic. The incubation period in the louse in these cases was only 5 days.

By daily injecting emulsions made from the bodies of lice—in which Trypanosomes were developing—into uninfected rats, Gonder was able to determine how long the arsenic-resistance of the Trypanosomes persists. He found that it persists for 12 days in the body of the louse. After this period, the Trypanosomes lose their resistance to arsenic, and become normal.

Cultures of normal and arsenic-resistant races of *T. lewisi* were made in artificial media[1]. Both races behaved exactly alike. The non-resistant races, when reinoculated into rats, were still non-resistant: the arsenic-resistant races remained arsenic-resistant. Both races underwent similar structural changes in the cultures—being gradually converted into *Crithidia*-like forms in the course of some 3 months. These forms, when injected back into rats, assumed the normal Trypanosome form once more—the incubation period being 3—11 days. Multiplication occurred in the artificial cultures.

Ehrlich (1911) and Gonder (1911) have interpreted the foregoing facts in the following way. They suppose that the development which *T. lewisi* undergoes in the louse constitutes a *sexual* cycle in the life-history of this species. They suppose that the resistance to arsenic, which the Trypanosomes have been made to acquire, persists only so long as the asexual cycle endures—that is, during the period when the Trypanosomes are in the blood of the rat, or in artificial culture media. When the sexual cycle takes place in the body of the louse, the acquired resistance of the race is lost, and the individuals revert to their original non-resistant condition. The "acquired character" is thus "inherited" in asexual reproduction only[2].

This extremely interesting and suggestive idea cannot be regarded at present as anything more than a hypothesis. For in the first place,

[1] *T. lewisi* was first successfully cultivated in an artificial medium by Novy and MacNeal, in 1903. Since then, many other workers have succeeded in cultivating a number of other species.

[2] Far-reaching conclusions regarding "the inheritance of acquired characters" can be drawn from these experiments only by those who are content with words and unable or unwilling to analyse the facts.

it has not been proved that the louse is the normal intermediate host of *T. lewisi*. There is much evidence to show that it is the flea, and not the louse, which is the normal carrier of the Trypanosome from rat to rat: though the louse may occasionally be the means of infection. Secondly, it has never yet been proved that the development which the Trypanosomes undergo in the gut of the louse is a *sexual* development. A sexual cycle in the louse was first described by Prowazek, and has since been alleged to occur by Baldrey, Rodenwaldt, and others, whose results Gonder says he can confirm "almost entirely." To prove the existence of a sexual cycle in the louse, however, something more than the arbitrary seriation of certain stained specimens is requisite. Until the publication of more convincing evidence—derived from a study of the living organisms, and from careful cytological research—it is not justifiable to conclude that conjugation of the Trypanosomes occurs in the body of the louse. It is, moreover, obvious that Gonder's own results cannot be held to prove that conjugation occurs in the louse: his interpretation is based on the supposition that conjugation *does* occur. And there is really no reason why the development—which the Trypanosomes appear undoubtedly to undergo—in the louse, should be regarded as necessarily of a sexual nature.

Since it has been found (Mesnil and Brimont [1908], Breinl and Nierenstein [1908]) that the resistance of a race of Trypanosomes to arsenic is manifested only so long as the race remains in a given host, it is not impossible that Gonder's results are explicable on the same principle. *T. lewisi* may remain arsenic-resistant so long as it continues in the blood of the rat, or in an artificial medium: but a change of host (*i.e.* from rat to louse) may abolish the resistance—just as *T. brucei*, arsenic-resistant in donkeys, becomes non-resistant when transplanted into rats. (Cf. p. 213.) If one substance can bring about arsenic-resistance, it is at least conceivable that another substance can remove it. And it is possible that the body of the louse may furnish such a substance. At all events, there is no need to assume the existence of sexual phenomena to account for the results of the experiments.

Ehrlich and his followers regard resistance to drugs or sera as a direct consequence of the action of the substances in question upon the living protoplasm. That is to say, they suppose that when a Trypanosome is treated with a minute quantity of arsenic, its protoplasm becomes changed in such a way as to make it resist the drug when applied subsequently. New races of Trypanosomes are thus

supposed to be directly produced by a modification of the individuals of the old race[1].

Erhlich's views in this respect are not shared by some other workers. Levaditi, with Mutermilch (1909) and Twort (1911), interpreted his own results as due to selection by the poison employed. The toxin of *B. subtilis* was found to kill or affect many Trypanosomes, when observed *in vitro*. And it was concluded that "certain races of Trypanosomes, considered as homogeneous, are only, in reality, a mixture of a large number of individuals endowed with unequal susceptibility towards a given trypanocidal poison." (Levaditi and Twort [1911].) In other words, resistant pure lines may be formed from a mixed population by the selective action of a poison—only those naturally most tolerant Trypanosomes being able to survive, and to perpetuate the race.

It therefore seems uncertain how resistant races of Trypanosomes arise. It is possible, however, that both a direct action of the drug and an indirect selection by it play a part in their formation.

In conclusion the more important results noticed in the foregoing pages may be very briefly summarized. I will limit myself to only those conclusions which appear to me to be justified at the present moment.

(A) It has been stated that the passage of certain Trypanosomes, which normally occur in mammals, through cold-blooded vertebrates and certain invertebrates, causes them to undergo certain structural changes which persist during subsequent divisions (Wendelstadt and Fellmer). This work has not yet been confirmed.

It has further been stated (Werbitzki) and confirmed (Laveran and Roudsky, Kudicke) that certain dyes can destroy a definite organ (kinetonucleus) in a Trypanosome, without killing or injuring it or impairing its power of propagation. Thus new races of Trypanosomes may be produced which completely lack this organ. It has, moreover, been rendered highly probable that the dyes which have this power possess a certain chemical structure (orthoquinoid substances of Ehrlich):

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[1] According to Ehrlich (1911), resistant races of Trypanosomes are of two quite different sorts: (1) Serum-resistant, *i.e.* resistant to specific antibodies; (2) Chemo-resistant, *i.e.* resistant to various chemicals. Such races are supposed to arise in different ways. In the terms of Ehrlich's theory, a serum-resistant race is formed by the serum causing a certain receptor (nutriceptor) to disappear, when it is replaced by an altogether new kind of receptor. A chemo-resistant race, on the other hand, is produced, not by the replacement of one receptor by another, but by the diminution ("Herabminderung") of a certain chemical function.

and that the dyes have a specific action upon the kinetonucleus
—but upon no other organ in the Trypanosome—and bring about its
destruction by autoxidation (Laveran and Roudsky). New races of
Trypanosomes are thus produced by modifying the individuals of the
old—not by selection.

(B) Races of Trypanosomes without kinetonuclei possess a lowered
virulence (Werbitzki, Laveran and Roudsky).

By the action of various drugs and antibodies, races of Trypanosomes
may be obtained which are resistant to these substances (Ehrlich, Mesnil
and Brimont, Breinl and Nierenstein, Levaditi and Twort, etc.). These
races subsequently breed true—though it may be a necessary condition
of this that they be kept in the same sort of host as that in which they
originally acquired their resistance.

Races of Trypanosomes with a changed virulence are said to be pro-
duced by passage through certain animals (Wendelstadt and Fellmer):
but this has been denied (Gonder and Sieber, Laveran and Pettit).

By treating *T. lewisi* with arsenophenylglycin, a race may be
obtained which is resistant to this drug. This race breeds true—
retaining its resistance during numerous passages through untreated
rats. Resistant and non-resistant races remain unchanged, as regards
this character, when grown in artificial cultures. When the resistant
race undergoes a development in the louse—the exact nature of which
is not determined, though it is possibly sexual—resistance is gradually
lost, and the race returns to the original non-resistant condition
(Gonder).

It has not been definitely determined whether resistance is brought
about by the direct action of the poison on the living Trypanosome
(Ehrlich, etc.), or whether it is the result of selection (Levaditi, etc.).

That some of the observations noticed in the course of this review
are of great interest, I think nobody would deny. And that they may
lead to a better comprehension of the phenomenon of mutation in
general is at least possible. In his Dresden address in 1911 Ehrlich
said : " ...Aber, meine Herren, in der Natur ist nichts spontan, alles
hat seine Ursache, und wenn es sich um biologische Fragen handelt,
meistens eine chemische Ursache....So glaube ich, dass gerade diese
Studien an Parasiten, an künstlich herbeigeführten Mutationen durch
bestimmte biologische Eingriffe, deren Mechanismus genau erklärbar
ist, uns auch ein helles Licht über die so dunklen Fragen der Mutation
überhaupt bringen werden." (Ehrlich [1911], p. 95.) Though all may

not take so confident and hopeful a view, this expression of opinion is noteworthy, and indicates the vast possibilities which the future still holds for one branch of biological research.

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INHERITANCE OF COAT-COLOUR IN RABBITS.

By R. C. PUNNETT, M.A., F.R.S.

CONTENTS.

INTRODUCTION.

WHEN the following experiments were started in 1907 we were already familiar, through the work of Castle (1) and Hurst (5), with certain phenomena in the inheritance of coat-colour and pattern among rabbits. These investigators had shewn that the wild grey, or agouti, is dominant to black, and that these two full colours are respectively dominant to the two dilute forms, yellow and tortoise (= the "sooty-yellow" of Castle). Moreover with regard to pattern Hurst had published a brief statement (6) to the effect that Dutch marking is recessive to self-colour, the heterozygote being variably marked, and that the Himalayan pattern is recessive to the self-coloured form. Castle also (1) had given an account of a few experiments with the Himalayan in which this form was shewn to be dominant to the pure albino.

More recently (1909) Castle has published a general account (3) of the colour varieties in the rabbit that have so far been analysed. With that account my own work is in general agreement, and in so far as is possible I have adopted his system of nomenclature for the various factors concerned [1].

[1] Of the four colour varieties agouti, yellow, black, and tortoise, the two former are regarded as containing the agouti factor **A** which is absent from the black and the tortoise. The agouti and black again differ from the yellow and the tortoise in containing a factor **E** for the extension of the pigment. Melanic pigment occurs also in the tortoise and the yellow but in much smaller amount and is chiefly localised in the nose, ears, tail, and feet.

General Scheme of the Experiments.

My own experiments were started with the idea of investigating more fully the genetics of the Dutch and Himalayan patterns, but they had not proceeded far when it became evident that certain phenomena in connection with the inheritance of colour were unlike any hitherto met with, and promised results of unusual interest. It is with these colour phenomena that the present paper is concerned. At the same time I can confirm Hurst's statement as to the recessive nature of the Himalayan pattern, though with regard to the nature of the Dutch marking and its relation to the self-coloured form I am inclined to think that the matter is more complicated than his account appears to imply. Experiments on the inheritance of coat-pattern are still in progress and 1 hope to publish them when more complete. Pattern and colour appear however to be quite independent of one another and in the present paper the results will be treated solely from the standpoint of colour.

The subjoined scheme provides a general view of the experiments.

The qualitative result of the various matings is alone indicated. The quantitative results will be found in the various tables to which reference is given.
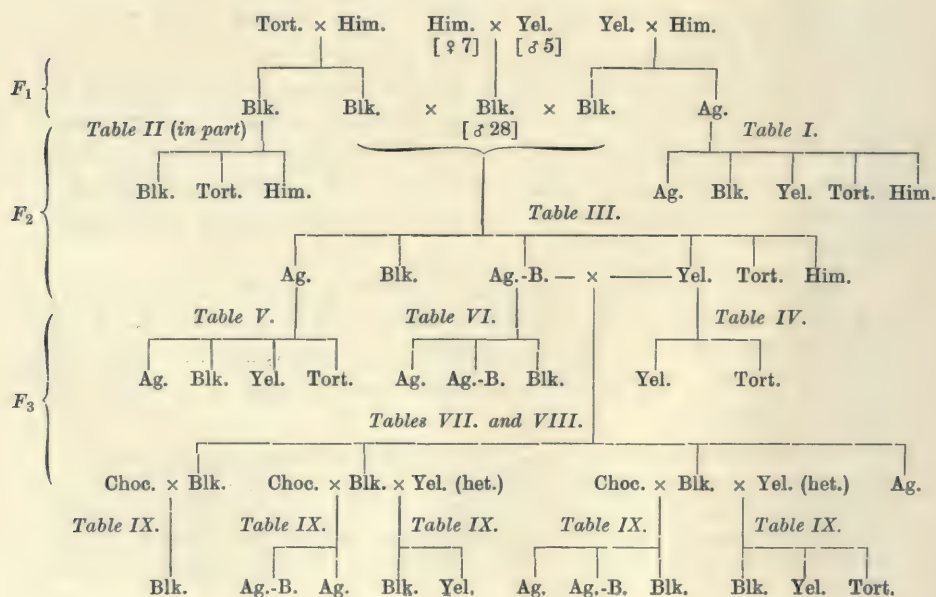
The F_1 generation.

The experiments were started with three yellow Dutch rabbits (♀ 1, ♀ 2, and ♂ 5), three tortoise Dutch (♀ 3, ♀ 4, and ♂ 6), and two Himalayans (♀ 7 and ♂ 8). As a description of these breeds is readily accessible in any work dealing with fancy rabbits it is unnecessary to say more than that the animals used were in each case fair specimens of their respective breeds. It should however be mentioned that each of the three yellows turned out to be heterozygous for the recessive tortoise. This was not only indicated when they were crossed with the Himalayan, but was shewn to be the case by breeding them together and by crossing them with the tortoise. Thus:

Yellow ♀ 1 × yellow ♂ 5 gave 12 yellows and 2 tortoise
Yellow ♂ 5 × tortoise ♀ 3 gave 2 ,, ,, 3 ,,

The tortoises when bred together gave only tortoise.

The cross between the yellow and the Himalayan was made in three cases with the following results:

		Agouti	Black
Yellow ♀ 1 × Himalayan ♂ 8 gave		7	6
,, ♀ 2 × ,, ♂ 8 ,,		5	6
,, ♂ 5 × ,, ♀ 7 ,,		2	3
	Totals ...	14	15

The black Himalayan, though recessive as far as pattern is concerned, supplies the factor for extension of colour (**E**), and as the yellows were all heterozygous the expectation from such crosses is equal numbers of agoutis and blacks. This expectation, as the F_1 figures shew, is closely realised.

Since the tortoise does not contain the agouti factor (**A**) the natural expectation from the cross between tortoise and Himalayan is blacks only. This, as the figures shew, was actually realised experimentally:

Tortoise ♀ 3 × Himalayan ♂ 8 gave 4 blacks
,, ♀ 4 × ,, ♂ 8 ,, 21 ,,
Total ... 25 ,,

The F_2 generation from the F_1 agoutis.

The reversionary agoutis from the manner of their making must be heterozygous both for the agouti factor (**A**) and for the intensity factor (**E**). Hence when bred together they should give agoutis, blacks, yellows, and tortoises in the ratio $9:3:3:1$. At the same time one-quarter of their offspring should be Himalayans. Five F_1 does were

mated several times with the same agouti ♂ and, as Table I shews, the results are in fair accordance with expectation:

TABLE I.

	Agouti	Black	Yellow	Tortoise	Himalayan
Agouti F_1 ♀ 10 × F_1 ♂ 9	9	4	4	1	9
,, 11 × ,,	13	8	4	2	6
,, 12 × ,,	13	8	3	—	5
,, 16 × ,,	10	4	2	1	6
,, 27 × ,,	12	3	2	1	3
Totals ...	57	27	15	5	29
Expectation	*56·0*	*18·8*	*18·8*	*6·2*	*33·2*

F_1 ♀ 10 was also crossed with the Himalayan ♂ and gave 2 agouti, 1 black, and 5 Himalayans, expectation here being 2 agouti, 2 black, and 4 Himalayans.

The F_2 generation from the F_1 blacks.

Though the F_1 blacks originated from two different sorts of cross, viz. from yellow × Himalayan as well as from tortoise × Himalayan, they should nevertheless all behave similarly. For there is no reason to suppose that there is any difference between a tortoise gamete

TABLE II.

	No. of Female	Male 35 Black F_1			Male 20 Black F_1			Male 74 Tortoise		Male 116 Himalayan	
		Blk.	Tort.	Him.	Blk.	Tort.	Him.	Blk.	Tort.	Blk.	Him.
Black F_1 ♀♀ ex Tortoise × Himalayan	22	8	4	2	—	—	—	—	—	—	—
	23	—	—	—	8	—	4	—	—	—	—
	24	—	—	—	6	3	3	—	—	—	—
	25	—	—	—	—	—	—	—	—	3	2
	26	—	—	—	—	—	—	4	4	—	—
	45	—	—	—	—	—	—	3	—	—	—
	47	—	—	—	—	—	—	4	5	—	—
	53	—	—	—	—	—	—	2	2	—	—
	56	—	—	—	—	—	—	—	—	2	4
	106	—	—	—	2	1	—	—	—	—	—
Black F_1 ♀♀ ex Yellow × Himalayan	17	2	—	3	—	—	—	—	—	—	—
	37	—	—	—	—	—	—	3	4	—	—
	39	—	—	—	—	—	—	—	—	5	4
	43	—	—	—	—	—	—	1	6	—	—
	102	—	—	—	—	—	—	2	4	—	—
Totals ...		10	4	5	16	4	7	19	25	10	10
			26 : 8 : 12								
Expectation			*27·7 : 6·7 : 11·6*					*22*	*22*	*10*	*10*

produced by a tortoise and one produced by a heterozygous yellow. And with a single exception, which will subsequently be dealt with more fully, the breeding results indicate that all the blacks are similar. These results are set out in Table II which shews not only the matings between the F_1 animals but also the effect of crossing them with pure tortoise ($\male$ 74) and pure Himalayan ($\male$ 116). All the results accord closely with expectation.

Agouti from Black × Black.

So far the experiments had merely served to confirm the work of previous writers and offered nothing of novelty beyond the fact that the black of the Himalayan rabbit can behave like the black of the ordinary self-coloured. All the F_1 blacks hitherto dealt with were the progeny of a yellow or a tortoise doe by a Himalayan buck ($\male$ 7) and with two exceptions all the F_1 animals used were made in this way. The two exceptions were an agouti ($\female$ 27) and a black ($\male$ 28) reared from the mating of the Himalayan doe ($\female$ 7) with the yellow buck ($\male$ 5). The agouti doe behaved like the other F_1 agoutis and her progeny have been included in Table I. $\male$ 28 however proved to be a remarkably interesting rabbit.   He was a pure self-coloured black to look at, and, unlike most of the F_1 animals, shewed no touch of white marking in spite of a Dutch parent.   For this reason I reserved him out of a number of F_1 bucks for the breeding of the F_2 generation. The mating of this animal with the F_1 black does led to a striking and entirely unlooked for result, *for from this mating of black × black came not only the expected blacks and tortoises but also yellows and agoutis*[1]. Further, among the blacks certain individuals were characterised by the development of some agouti hairs, a feature which was especially well marked in the area between the nape of the neck and the shoulder blades (cf. Pl. XII, fig. 2).   The amount of agouti hairs was somewhat variable and on the whole they were rather more abundant in the males than in the females.   Generally speaking these hairs only became conspicuous about the third week after birth and until then it is not possible to distinguish with certainty between these "black-agoutis" and true blacks.   Both also are black bellied.

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[1] Woods (8) also obtained some agoutis from the mating of black with black. His experiments were made in pre-Mendelian days and the data are insufficient for analysis. But as yellows and apparently also tortoises appeared in his litters it is quite likely that the hypothesis given later would serve to explain his results also if we knew more about them.

In Table III are collected the results of crossing ♂ 28 with a series of black F_1 does. The does have been classified according as their mother was a yellow or a tortoise, but the figures, as in Table II, make it evident that there is no difference between the two lots.

TABLE III.

	No. of Female	Agouti	Black	Agouti-Black	(?)[1]	Yellow	Tortoise	Himalayan
	17	1	3	1	—	1	1	4
	18	2	3	—	1	3	1	2
	36	—	5	2	—	—	1	5
Black F_1 ♀ ♀ ex	37	3	3	1	2	1	4	4
Yellow × Himalayan	39	1	4	3	—	2	1	2
	40	2	5	3	—	3	—	2
	42	—	6	—	1	1	—	1
	43	2	5	1	1	—	2	5
	102	5	8	4	—	1	2	3
	22	1	3	2	2	—	—	2
	23	2	5	1	2	2	1	4
	25	3	10	4	—	—	1	6
	26	1	4	1	—	2	3	2
	45	2	4	1	—	2	1	5
Black F_1 ♀ ♀ ex	46	—	12	1	2	2	3	6
Tortoise × Himalayan	47	2	5	1	5	2	—	3
	52	1	2	—	—	—	2	2
	53	1	6	2	1	2	3	3
	56	5	3	1	—	2	2	3
	106	2	2	1	2	1	2	1
	107	—	—	—	—	1	—	2
		36	98	30	19	28	30	67
			15	4				
Totals ...		36	113	34	—	28	30	67

The interpretation of these results was for some time obscure. At first it was tempting to look upon the difference between agouti and black as a difference of two factors. On such a view it must be supposed that all the agoutis and blacks previously used were homozygous for one of these factors, and that the simple Mendelian relation hitherto found depended upon the presence or absence of the other factor. The appearance of agoutis from blacks would then depend upon the absence of one of the two factors on the one side and on

[1] The animals recorded in this column were blacks which died too early to determine whether they were true blacks or agouti-blacks. They have been ultimately distributed among the blacks and agouti-blacks according to the proportions in which these two classes were definitely determined.

the absence of the other factor upon the other side of the cross. Without going into detail I may say that this hypothesis proved incapable of affording a complete explanation of the facts. Indeed the matter turned out to be considerably more complicated than at first appeared and it was only after three years work that a satisfactory hypothesis was eventually built up. Nevertheless the subsequent experiments will be easier to follow if at this point I set out the explanation which I have been led ultimately to frame.

The Hypothesis.

In the set of experiments into which ♂ 28 and his descendants enter we are dealing essentially with three separate factors[1]:

A, the "agouti" factor which turns black into agouti, and tortoise into yellow.

E, a factor for the extension of the melanic pigment which turns yellow into agouti and tortoise into black.

D, a factor of which the effect is to produce a deepening in the melanic pigment[2].

The effect produced by **D** depends (1) upon whether this factor is present in a homozygous or in a heterozygous condition, and (2) upon whether the animal is homozygous or heterozygous for **E**. The addition of one dose of **D** to an agouti which is homozygous for **E** turns it into an agouti-black, while the addition of a second dose results in a full black. If however the agouti is heterozygous for **E**, the addition of either one or two doses of **D** produces the same visible effect, viz. a full black. The presence of **D** in a black makes no difference to the appearance of the animal. For reasons which will appear later it is probable that **D** does not occur in yellows or tortoises.

The factor **D** must be supposed to have been brought in by the Himalayan doe (♀ 7), and her zygotic constitution was probably **DdEEaa**. The yellow buck (♂ 5) with which she was crossed was known to be heterozygous for **A** and was therefore **ddeeAa**. Of the two animals from this mating which were subsequently bred from, ♀ 27 was evidently **ddEeAa** (cf. Table I), having been formed by

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[1] The factor whose presence or absence determines whether the animal shall be self-coloured or Himalayan may for the present be left out of account.

[2] It is possible to look at the matter from a rather different point of view and to regard **D** as a factor which prevents the working of the agouti factor. The agouti factor is itself in a sense an inhibitor of black. Consequently on this view we should have to regard the factor **D** as an inhibitor of an inhibitor.

gametes **dEa** from the Himalayan and **deA** from the yellow. The black F_1 buck ($\male$ 28) on the other hand we shall assume to have been formed by the gametes **DEa** from the Himalayan, and **deA** from the yellow, and his zygotic constitution was therefore **DdEeAa**[1]. To give the results set out in Table III he was mated with a series of F_1 does, presumably all of the constitution **ddEeaa**, there being no grounds for supposing that their Himalayan father ($\male$ 8) carried the factor **D**. To explain the peculiar proportions which occur in that F_2 generation one further assumption must be made, viz. that the factors **D** and **E** are coupled in the formation of gametes by $\male$ 28. This assumption I shall hope to justify fully a little further on when I come to deal with the results of other experiments. Whether the coupling is complete or partial there are at present no data for deciding. For the moment it will be assumed to be complete.

On this scheme then the gametes formed by the F_1 $\male$ are of four kinds, viz. **DEA**, **DEa**, **deA**, **dea**, and the gametes formed by the F_1 $\female\female$ are of two kinds, viz. **dEa** and **dea**. The results following the bringing together of two such series of gametes are shewn in Fig. 1.

DEA dEa Agouti-black	**DEa** dEa Black	**deA** dEa Agouti	**dea** dEa Black
DEA dea Black	**DEa** dea Black	**deA** dea Yellow	**dea** dea Tortoise

Fig. 1.

In other words, the F_2 generation should consist of agoutis, blacks, agouti-blacks, yellows, and tortoises in the ratio $1:4:1:1:1$. The subjoined figures shew how closely the expected results on this scheme tally with the numbers actually found by experiment.

	Agouti	Black	Black-Agouti	Yellow	Tortoise
Experimental data from Table III	36	113	34	28	30
Expectation ...	*30·125*	*120·5*	*30·125*	*30·125*	*30·125*

[1] It is of course possible that $\female$ 7 may have been **DdEEAa**, in which case $\male$ 28 would have been formed by an ovum **DEA** fertilised by a spermatozoon **dea**.

The F₃ generation.

The above hypothesis can be tested by the results of breeding the various types which appeared for a generation further. For if it is true, three of the F_2 groups, viz. the yellows, the agoutis, and the agouti-blacks, should each consist of homogeneous material. We should be in a position to predicate of the yellows that they are all heterozygous for **A**, of the agoutis that they are all heterozygous for both **A** and **E**, and of the agouti-blacks that they are all homozygous for **E** but heterozygous for **D** and **A**.

A. The Yellows.

Seven F_2 does were mated to two F_2 bucks and the results, which are tabulated in Table IV, shew that, as was to be expected on the hypothesis, all nine were heterozygous for **A**.

TABLE IV.

	Male *B* 111			Male *E* 31		
	Yellow	Tortoise	(Himalayan)	Yellow	Tortoise	(Himalayan)
♀ *B* 79	13	4	(5)	—	—	(—)
♀ *B* 93	12	6	(3)	—	1	(3)
♀ *B* 169	18	12	(—)	—	—	(—)
♀ *B* 185	10	2	(4)	3	—	(1)
♀ *C* 14	12	1	(3)	—	—	(—)
♀ *C* 15	5	3	(4)	—	—	(—)
♀ *D* 139	3	1	(—)	—	—	(—)
Totals	73	29		3	1	

B. The Agoutis.

Of the F_2 agoutis five does and two bucks were mated together in the way indicated in Table V. The results shew that all were hetero-

TABLE V.

	Male *B* 104 [1]					Male *D* 125				
	Agouti	Yellow	Black	Tortoise	Hima-layan	Agouti	Yellow	Black	Tortoise	Hima-layan
♀ *B* 146	9	5	3	2	(—)	—	—	—	—	(—)
♀ *B* 188	19	2	6	2	(—)	—	—	—	—	(—)
♀ *B* 217	12	4	3	—	(—)	—	—	—	—	(—)
♀ *C* 55	—	—	—	—	(—)	4	—	1	—	(1)
♀ *C* 102	—	—	—	—	(—)	2	1	—	—	(1)
	40	11	12	4	(—)	6	1	1	—	(2)
Totals ...	46 Agouti		12 Yellow		13 Black		4 Tortoise			
Expectation	42·1		14·1		14·1		4·7			

[1] ♂ *B* 104 was evidently homozygous for full-colour as opposed to Himalayan pattern.

zygous for **A**, while both the bucks and four of the does were certainly heterozygous for **E**. The remaining doe, *C* 55, mated with ♂ *D* 125 gave four agouti and one black among her self-coloured offspring, though as the expectation is only one dilute (yellow or tortoise) in four it is not at all improbable that further testing would have shewn her to be also heterozygous for **E**.

C. *The Agouti-blacks.*

On the scheme suggested all the agouti-blacks should be of the constitution **DdEEAa**, and should all produce the four kinds of gametes **DEA**, **DEa**, **dEA**, **dEa** in equal numbers. The effect of two such similar series of gametes meeting is shewn in Fig. 2 from which it will be seen that the expected result of mating the agouti-blacks together is blacks, agouti-blacks, and agoutis in the ratio 7 : 6 : 3.

DEA **DEA** Black	**DEA** **DEa** Black	**DEA** **dEA** Agouti-black	**DEA** **dEa** Agouti-black
DEa **DEA** Black	**DEa** **DEa** Black	**DEa** **dEA** Agouti-black	**DEa** **dEa** Black
dEA **DEA** Agouti-black	**dEA** **DEa** Agouti-black	**dEA** **dEA** Agouti	**dEA** **dEa** Agouti
dEa **DEA** Agouti-black	**dEa** **DEa** Black	**dEa** **dEA** Agouti	**dEa** **dEa** Black

Fig. 2.

There should be no yellows or tortoises from these matings. It must be expressly stated here that this result is based on the assumption that an animal which is homozygous for **D** is black whether it contains **A** or not. The test will of course be to cross a series of these F_3 blacks with pure black (**ddEEaa**). In one case out of seven on the average an F_3 black (viz. **DDEEAA**) should give only agouti-blacks as the

result of such a mating. It is hoped to make this experiment in the course of the present year[1].

The results of the matings among the F_2 agouti-blacks are shewn in Table VI, from which it appears that the results obtained from this series of matings are in fair accordance with the theoretical expectation.

TABLE VI.

No. of Female	Male B 144				Male C 32				Male D 122				Male D 128			
	Blk.	Ag.-Blk.	Ag.	?²	Blk.	Ag.-Blk.	Ag.	?²	Blk.	Ag.-Blk.	Ag.	?²	Blk.	Ag.-Blk.	Ag.	?²
B 76	9	7	3	—	—	—	—	—	—	—	—	—	—	—	—	—
B 151	8	1	—	—	—	—	—	—	2	2	2	—	—	—	—	—
B 198	5	1	1	3	5	8	5	2	—	—	—	—	—	—	—	—
C 144	—	—	—	—	3	4	1	4	—	—	—	—	—	—	—	—
D 20	—	—	—	—	6	4	1	—	—	—	—	—	4	3	3	—
D 21	—	—	—	—	4	3	2	3	—	—	—	—	5	5	1	4
D 145	—	—	—	—	13	7	2	—	—	—	—	—	—	—	—	—
D 156	—	—	—	—	4	4	3	—	—	—	—	—	—	—	—	—
D 191	—	—	—	—	4	2	1	—	—	—	—	—	4	2	1	—
D 237	—	—	—	—	5	5	5	—	—	—	—	—	6	1	3	1
E 35	—	—	—	—	5	6	3	—	—	—	—	—	4	2	2	1
	22	9	4	3	49	43	23	9	2	2	2	—	23	13	10	6

	Black	Agouti-Black	?²	Agouti
Totals	96	67	18	39
	11	7		
Amended totals ...	107	74	—	39
Expectation ...	96·25	82·5		41·25

In order to test the agouti-blacks further with respect to the factor **E** both does and bucks were crossed with F_2 heterozygous yellows, and the does also with pure tortoise. Since the gametes of the agouti-black are **DEA**, **DEa**, **dEA**, **dEa**, and those of the F_2 yellow **deA**, **dea**, the expected result of such a cross is given in Fig. 3.

The expectation is blacks and agoutis only in the ratio 5 : 3. Tables VII and VIII shew that only blacks and agoutis resulted from such a cross, the actual numbers being 69 black and 33 agouti where the expectation was 64 black and 38 agouti.

[1] Since the above was written an F_3 black ♀ made in this way has been shewn to be of the constitution **DDEEAa**. Crossed with a buck of the constitution **ddEEaa** she has given only blacks and agouti-blacks. Among the 14 young so bred from her there was no agouti.

[2] The rabbits placed in this category were blacks which died too early to determine whether they were true blacks or agouti-blacks. In making the amended total they have been distributed *pro rata* among these two classes.

DEA deA Black	DEa deA Black	dEA deA Agouti	dEa deA Agouti
DEA dea Black	DEa dea Black	dEA dea Agouti	dEa dea Black

Fig. 3.

TABLE VII.

		F_2 Agouti-black Males					
		Male *C* 32		Male *D* 128		Male *D* 122	
	No. of Female	Agouti	Black	Agouti	Black	Agouti	Black
F_2 ♀ ♀ Yellow (heterozygous)	*B* 79	—	4	2	5	—	—
	B 93	—	—	—	3	—	—
	B 169	—	—	1	6	1	4
	B 185	—	—	3	4	4	5
	C 14	3	2	—	—	—	—
	C 15	—	—	—	—	8	9
	D 139	—	—	1	3	1	3
		3	6	7	21	14	21

Totals   ...   24 Agouti   48 Black

Expectation   *27* ,,   *45* ,,

TABLE VIII.

		Male *E* 31 (heterozygous yellow)		Male 74 (pure tortoise)	
	No. of Female	Agouti	Black	Agouti	Black
F_2 Agouti-black ♀ ♀	*B* 76	—	—	2	6
	B 151	—	—	2	4
	B 191	—	5	1	6
	C 144	—	—	2	10
	D 20	4	4	—	—
	D 21	—	2	—	—
	D 133	3	4	—	—
	D 145	2	6	—	—
	D 156	—	—	3	3
	D 191	—	—	1	14
	D 237	—	—	2	4
	E 35	—	—	2	4

Totals   9   21   15   51

Expectation   ...   *11·25*   *18·75*   *16·5*   *49·5*

Eight of the agouti-black does were also crossed with a pure tortoise buck ($\male$ 74), and the numbers obtained, 51 black and 15 agouti, tally closely with the expected 3 : 1 ratio.

It may therefore be fairly claimed that the constitution of the F_2 generation from $\male$ 28 as tested by further breeding from them is in accordance with the hypothesis framed above.

The Synthesis of agouti-bearing blacks.

So far the evidence that a rabbit, visibly pure black, can carry the agouti factor rests entirely upon a single individual—the F_1 $\male$ 28. But if our hypothesis is correct, there should be no difficulty in synthesising the agouti-bearing black from the material to hand, and as a matter of fact this has actually been done within the past year. Reference to Fig. 3 (p. 232) shews that of the five blacks resulting from the mating of agouti-black with yellow three carry the factor **A**, two being heterozygous and one homozygous for that factor. Neither of the two remaining blacks carries **A**. The simplest way to find these blacks would be to test them by crossing with a pure black of the constitution **ddEEaa**. As however I had not such an animal ready to hand, I used for testing purposes a chocolate $\male$ which had previously been shewn to be homozygous for **E**, but to contain neither **D** nor **A**[1]. In this way 13 black does *ex* agouti-black × yellow have been tested, with the result that three proved to be homozygous for **A**, four proved to be heterozygous, while in the remaining six **A** was absent. The details are given in Table IX.

TABLE IX.

	No. of Female	× Chocolate male (**ddEEaa**)				× Male E 31 (heterozygous yellow=**ddeeAa**)				
		Blk.	Ag.-Blk.	Ag.	(Him.)	Blk.	Ag.	Yellow	Tort.	(Him.)
DdEeAA	F 63	—	4	3	(3)	2	—	2	—	(1)
	F 67	—	4	5	(4)	1	—	2	—	—
	F 148	—	(1 ?)	5	—	2	—	4	—	—
DdEeAa	F 22	6	—	1	—	4	—	1	1	—
	F 62	3	—	1	(4)	1	—	1	1	(1)
	F 66	1	2	1	(1)	3	—	2	2	(1)
	F 69	2	1	—	—	2	—	4	3	(1)
DdEeaa or **ddEeaa**	F 9	10	—	—	—					
	F 10	11	—	—	—					
	F 19	9	—	—	—					
	F 60	5	—	—	—					
	F 61	7	—	—	(2)					
	F 64	5	—	—	—					

[1] For a further account of this animal see later, p. 235.

At the same time five black ♂♂ bred similarly to the above black ♀♀ were also tested. Of these one turned out to be homozygous for **A**, two turned out to be heterozygous, while the remaining two did not carry **A**. In all therefore 18 blacks from the mating of agouti-black × yellow were tested for the presence of **A**, and as the subjoined list shews the proportion of those homozygous for **A**, heterozygous for **A**, or homozygous for its absence tallies closely with expectation:

	AA	**Aa**	**aa**
Proportion found by testing ...	4	6	8
Proportion expected	3·6	7·2	7·2

The test of the coupling between **D** *and* **E**.

The possession of a number of agouti-bearing blacks renders possible the carrying out of a critical test to decide whether the suggested coupling between **D** and **E** occurs. On this hypothesis the gametes produced by the agouti-bearing black are **DEA, DEa, deA, dea** if it is heterozygous for **A**, and **DEA, deA** if it is homozygous. Such animals when mated with yellows or tortoises should be able to give yellow offspring *but no agouti*. And as one of the parents in such a mating lacks the factor **E**, no agouti-blacks should appear from such matings. In Table IX are shewn the results of crossing the agouti-bearing black ♀♀ with a heterozygous yellow ♂ (*E* 31). Hitherto from such matings have come 15 blacks, 16 yellows, and 7 tortoises, but no agoutis or agouti-blacks. A few families have also been raised from agouti-bearing black ♂♂ and tortoise ♀♀, viz.:

	Black	Agouti	Yellow	Tortoise
♂ 28 × tortoise ♀	3	—	1	2
♂ 54 × ,, ♀	7	—	3	—
♂ 20 × ,, ♀	2	—	3	—
♂ 20 × orange ♀	3	—	2	—

In all there have been raised from the mating of agouti-bearing black with either yellow, tortoise or orange, 64 young[1]. Of these 30 were black, 25 yellow, and 9 tortoise. No agoutis and no agouti-blacks have appeared from such matings. These results, as has already been shewn, are explicable on the hypothesis of coupling between the two factors **D** and **E**. On the explanation suggested, and with such facts as are at present available, the coupling must be supposed to be complete. Analogy with cases of possibly similar nature in the sweet pea and other plants suggests that the coupling, though intense, may yet be but partial. Larger numbers however are necessary before a decision

[1] Excluding Himalayans.

can be arrived at, and the matter must remain open until more material has accumulated. The data with regard to coupling and repulsion in animals are still scanty and in most cases sex is one of the characters concerned. Morgan's experiments with *Drosophila* (7) suggest coupling of some kind between factors for eye colour and shape of wing, though both of these factors may shew sex-limited inheritance in other families.

Hagedoorn (4) has described a case of what may be termed complete repulsion between the factors for agouti and for colour in mice, but so far as I am aware no other instance of repulsion or coupling between characters independent of sex has yet been met with in the vertebrates.

The chocolate series.

Mention was made above (p. 233) of a chocolate buck which has been used extensively for purposes of testing during the course of these experiments. This animal was very kindly sent to me from Holland by Dr Hagedoorn in the spring of 1910. It proved to be heterozygous for Himalayan, a fact not without interest in view of Dr Hagedoorn's statement that the chocolate colour was first met with in this variety ((4), p. 107). My various experiments with this animal have amply confirmed Hagedoorn's view of the recessive nature of chocolate as opposed to black. In the course of the experiments I have made the chocolate bearing the agouti factor, the so-called cinnamon (Pl. XIII, fig. 3); also the dilute chocolate which lacks **E** and corresponds to the tortoise in the black series. This dilute chocolate, which, following Hagedoorn, may be termed orange (Pl. XIII, fig. 1), is of a clearer richer colour than the ordinary yellow rabbit (Pl. XIII, fig. 2). The belly is also orange, whereas in the dilute cinnamon it is white. Otherwise these last two are not readily to be distinguished from one another.

Corresponding to the agouti-black there is a form in the chocolate series which may be called the deep cinnamon. This is a cinnamon which is heterozygous for the factor **D**, and, as the illustration shews (Pl. XII, fig. 3), it is almost as deep in colour as the pure chocolate. Analogy with the black series would lead us to expect the deep cinnamous which are homozygous for **D** to be similar to chocolates in appearance, but whether this is so or not I cannot say as I have not yet been able to make the necessary experiments.

There is one further point of interest in connection with the effects produced by the factor **D**. A rabbit which is heterozygous for **D** and is at the same time heterozygous for black (**B**) is an agouti-black, but of a distinctly different type to one which is homozygous for **B**. The

agouti markings are much more in evidence and are spread more evenly over the whole coat (cf. Pl. XII, fig. 1). The various corresponding forms in the black and in the chocolate series may be set out in tabular form as follows :

Black Series			Chocolate Series		
Agouti	**AABBEEdd**		Cinnamon ... (Pl. XIII, fig. 3)	**AAbbEEdd**	
Black	**aaBBEEdd** or **AABBEEDD** or **AABBEeDd**		Chocolate ... ... (Pl. XII, fig. 4)	**aabbEEdd**	
Yellow ... ... (Pl. XIII, fig. 2)	**AABBeedd**		Dilute Cinnamon...	**AAbbeedd**	
Tortoise ... ... (Pl. XIII, fig. 4)	**aaBBeedd**		Orange ... ... (Pl. XIII, fig. 1)	**aabbeedd**	
Agouti-black ... (Pl. XII, fig. 2) or (Pl. XII, fig. 1)	**AaBBEEDd** **AaBbEEDd**		Deep cinnamon ... (Pl. XII, fig. 3)	**AabbEEDd**	

The Himalayan pattern.

It has already been pointed out that the Himalayan pattern is recessive to self-colour, a fact which had been previously demonstrated both by Hurst (6) and Castle (1). Castle has also shewn that the Himalayan pattern behaves as a dominant to the complete albino. My experiments have enabled me to add a few more points of interest in connection with this peculiar pattern.

In the first place there is a Himalayan form corresponding to each full-coloured variety, i.e. there are black Himalayans, chocolate Himalayans, agouti Himalayans, etc., etc. The black Himalayan is indistinguishable in appearance from the agouti-black Himalayan because the distinctive hairs of the agouti-black occur only on those parts of the body where the Himalayan is entirely white. The agouti Himalayan is quite distinct from the black Himalayan as may be readily gathered from the figures on Plate XIV[1]. The Himalayans corresponding to agouti, tortoise, and yellow are not readily distinguished until they are nearly half grown, and even then the distinction is not always very clear. This is largely because the Himalayan shews no yellow. A "Himalayised" agouti for example contains in its points only the melanic pigment of the agouti, those parts of the hair which are yellow in the agouti being

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[1] Castle's "intermediate" Himalayans were probably of this nature, the albino ♀♀ used by him being heterozygous for **A** ((1), p. 70). Indeed in his latest paper (3) he recognises the distinction between the Himalayan bearing **A** and that without it.

colourless in the Himalayan. This peculiarity of the Himalayan is strikingly brought out in some experiments made by my friend Mr T. H. Riches. An ordinary black Himalayan was crossed with a black-and-tan rabbit, and in F_2 came normal black Himalayans together with " Himalayised" black-and-tans. These last were characterised by the markings of the black-and-tan with the difference that the yellow of the nose, ears, and paws of the full-coloured form was replaced by white in the corresponding Himalayan. The facts seem to suggest that the Himalayan pattern is determined by the absence of yellow, the presence of this pigment being necessary for the full development of the melanin series. Yet tempting as such a hypothesis is, there are difficulties in the way of its acceptance—difficulties which are at once evident when the relations of the Himalayan and the true albino are considered. Since the albino behaves as a simple recessive to the Himalayan the lack of colour in the two cases must be regarded as resulting from the same cause, the only necessary difference between them presumably being in the presence or absence of a factor which determines the pigmentation of the points. Again the self-coloured rabbit behaves as a simple dominant to the Himalayan, and consequently must be supposed to contain the factor producing the pigmented points in the Himalayan in addition to the factor for yellow, which on this hypothesis the Himalayan lacks. Hence the ordinary self-coloured rabbit should contain two factors both lacking in the full albino, viz. the Himalayan point factor, and the yellow factor. The F_2 from self × albino should consequently contain Himalayans as well as true albinos. But among the large number of animals reared from such matings no Himalayans have hitherto been recorded, and for the present the relations between these various forms remain obscure.

Lastly there is a small point in connection with what Castle has termed "mosaic" Himalayans ((1), p. 70). My experiments have shewn that in F_2 from Dutch × Himalayan the majority of the coloured animals are more or less marked with white, the amount of which varies between that found in typical Dutch to a small white patch on the tip of the nose or one of the paws. These white markings, generally irregular, occur also in the F_2 Himalayans from the Dutch cross. Sometimes there is a white spot only on the nose, sometimes one or more paws are tipped with white. In other cases an F_2 Himalayan may be a full Dutch Himalayan when the only pigment found is on the ears and tail together with a small strip on either side of the nose, all the feet in such animals being completely white as in the Dutch. Such animals

are simply "Himalayised" animals with white markings, and are strictly paralleled by their white marked coloured brothers and sisters. It is probable that the "mosaics" which appeared in Castle's experiments were due to the fact that the albino mothers used were potentially white marked. The inheritance of these white markings is not yet clear but their genetics are at present under investigation.

In conclusion I desire to acknowledge a gift of £10 from the Master and Fellows of Gonville and Caius College towards the cost of these experiments in 1908, and in the present year a grant of £50 from the Government Grant Committee of the Royal Society.

EXPLANATION OF PLATES.

PLATE XII.

Fig. 1.   Agouti-black (heterozygous for black).
Fig. 2.   Agouti-black (homozygous for black).
Fig. 3.   Deep cinnamon-agouti.
Fig. 4.   Chocolate.

PLATE XIII.

Fig. 1.   Orange.
Fig. 2.   Yellow.
Fig. 3.   Cinnamon-agouti.
Fig. 4.   Tortoise.

PLATE XIV.

Fig. 1.   Himalayan with black points.
Fig. 2.   Himalayan with agouti points.

The above are all photographed directly from the original specimens.

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Fig. 1. Agouti-black
(heterozygous for black).

Fig. 2. Agouti-black
(homozygous for black).

PLATE XII

Fig. 3. Deep cinnamon-agouti.

Fig. 4. Chocolate.

Fig. 1. Orange.   Fig. 2. Yellow.

PLATE XIII

Fig. 3. Cinnamon-agouti.

Fig. 4. Tortoise.

ON THE INHERITANCE OF CERTAIN CHARACTERS IN THE COMMON GROUNDSEL — *SENECIO VULGARIS, LINN.*—AND ITS SEGREGATES.

By A. H. TROW, D.Sc., F.L.S.

INTRODUCTION.

THE common groundsel is a plant of world-wide distribution and one might reasonably expect that its various forms would be well known to botanists. In Koch's *Synopsis*, Ed. III. 1902, two varieties only are recognised, *sordidus* and *radiatus*, and the descriptions are unfortunately too brief and too vague to be of much value. Rouy, in the *Flore de France*, Vol. VIII, p. 343, adds two more—*crassifolius* and *Motelayi*, which are also badly defined and inadequately investigated. The majority of botanists recognise two forms only: (*a*) the type form *S. vulgaris, Linn.* which is non-radiate, and (*b*) a variety of this with ligulate ray florets, *S. vulgaris, Linn.*, var. *radiatus, Koch.*

It is noteworthy that although the Compositae constitute about one-tenth of the phanerogamic flora of the world, they have received comparatively little attention so far from those who are engaged in experiments on plant breeding.

Moreover, numerous plant hybrids are recognised in every modern flora, but comparatively few of them have hitherto been tested by the methods of genetics.

Certain plant genera, such as *Hieracium, Rosa* and *Rubus* have been studied intensively to such an extent that old well-known species like *Rubus fruticosus* and *Rosa canina* have been split up into numerous smaller units recognisable with certainty only after much painstaking study, yet there has been practically little effort, except in *Hieracium*, to trace by experimental means the genetic relationships of such forms. Indeed the study of " critical " species is carried on still almost entirely

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by morphological methods alone. Such methods, valuable and even indispensable though they may be, are nevertheless inadequate for the complete solution of such intricate problems as need investigation in these cases.

Few weeds, moreover, have been as yet studied by experimental methods; the classical work in genetics has been done on species and varieties of plants long cultivated in gardens—peas, sweet peas, snap-dragons, stocks, etc.

Such considerations as these induced me, six years ago, to undertake a genetic study of the common groundsel and its segregates. Although it soon became obvious that I should have to register annually two to three thousand plants, and sacrifice the greater part of each long vacation, the results were of sufficient interest to cause me to extend the work year by year. Although the investigation, as a whole, is still very incomplete, it seems desirable that some of the chief results of the last six years' work should now be placed on record.

Origin of the Experimental Material. Methods. The Radiate Character *RR*.

In the year 1891 or 1892 I observed and examined hundreds of specimens of a groundsel growing in abundance near Cardiff and Penarth, which was chiefly remarkable for its conspicuous capitula, each of which bore from eight to 13 large ray florets. In January, 1894, Dr C. T. Vachell called attention to this plant at a meeting of the Biological Section of the Cardiff Naturalists' Society and specimens were submitted to W. B. Hemsley, who reported on them as follows:— "I cannot make anything of this but *vulgaris b. radiatus*." I had already expressed the same view, with certain reservations; based on the fact, that although these plants were clearly radiate groundsels, they did not agree well with the description and figure of the Channel Islands plant in Sowerby's *Botany*.

The same groundsel occurs in abundance near Swindon, Cork, and Northwich, where, as at Cardiff, it is frequently accompanied by *S. squalidus*. It has also been sent to me from the Cambridge Botanic Gardens by Mr I. B. Pole-Evans and Prof. Yapp. It has spread widely in the counties of Glamorgan and Monmouth in recent years, and specimens have been received recently through Dr Moss from North-wich, Cheshire, and Freshfield, Lancashire. The only other locality recorded for it in Great Britain appears to be Bigbury, S. Devon. It

certainly flourishes in localities where *S. squalidus* does not occur, and as we shall see, these two species appear to have no necessary association with each other.

A capitulum of this radiate groundsel, from one of 49 plants of the pure strain cultivated in 1912 (7th Generation) which I have named *erectus, radiatus*, is represented in Pl. XVI, fig. 8. This strain occurred originally as a weed in my own garden along with a non-radiate form, indistinguishable from it until the opening of the flowers, when of course the difference is very obvious. The non-radiate type may be styled *erectus*. Its capitulum is shewn in Pl. XVII, fig. 14. Either form may thus be regarded as a true variety of the other, using the term variety in the sense suggested by De Vries.

In the year 1904, I noticed a single plant whose capitula differed from those of the two preceding. Each capitulum had eight to 13 ligulate ray florets, but the ligules were only one-half the usual length and were conspicuously three-toothed at the apex (Pl. XVI, fig. 5 and text-figure 29, p. 274). Perhaps influenced by the prevalent view that groundsels are autogamous plants (self-pollinated), this was regarded as a mutation, and the supposed mutant was allowed to seed freely, but no seeds were collected. In 1905, as a result of the natural sowing, a small crop of groundsels appeared in which the three types were present, and I resolved to start a set of pedigree cultures to determine their relationships to each other.

It will be convenient to denote the three types as follows:—(1) the radiate plants with the ray character fully developed by RR (Pl. XVI, fig. 4), (2) the non-radiate plants by NN (Pl. XVI, fig. 6), and (3) the intermediate plants with the ray character partly developed by NR (Pl. XVI, fig. 5). The figures apply equally well to the *erectus* and *multicaulis* types.

In all the experiments methods were employed to secure the absence of all foreign seed from the cultures. The soil and seed-pans were sterilized by submission to one hour's steaming. So effective were these precautions that from 1905 to 1911 I have only found two invading seedlings in the seed-pans; one of a *Trifolium*, the other of *Poa annua*, while the controls were invariably sterile.

Preliminary experiments, Nos. 1, 2, 3, 4 *and* 5. The first experiments were more or less tentative and were made to test the inheritance of the full ray-character in RR plants. *Exp.* 1. A single head of seeds (really fruits) was collected from a plant growing as a weed in the garden in September, 1905, and the seeds were sown at once. Thirty-

six seedlings were raised, eight of these ultimately flowered, and all proved to be *RR*. *Exp.* 2. One of these eight plants was isolated in the greenhouse, and from its seeds one hundred and nineteen seedlings were raised in the following year. Of these one hundred and sixteen flowered, and all were *RR*. *Exp.* 3. Another experiment carried out under somewhat different conditions gave the following result. Five plants flowered in 1905, all *RR*. *Exps.* 4 *and* 5. From two of these five plants seeds were obtained (*a*) after isolating in the greenhouse, (*b*) after selfing in the open air. These seeds produced respectively 74 and 13 plants, all *RR*. Altogether 216 plants were raised in two generations, and all were *RR*, breeding perfectly true to the parent type.

Exps. 6, 7 *and* 8. Further experiments were set up to test the inheritance in all three types under natural conditions—cross pollination by natural agents being possible. Three plants growing close together, but not in contact, in the open garden, of types *NN*, *RR* and *NR* were marked down for seed-saving on September 16th, 1905.

Seed was collected as follows :—

From *NN*. From Oct. 16th to Nov. 17th. 33 Heads on 17 separate days.
From *RR*. From Sept. 16th to Nov. 17th. 43 Heads on 20 separate days.
From *NR*. From Sept. 20th to Nov. 17th. 30 Heads on 14 separate days.

The results are summarised in

TABLE I.

Exp.	Type of Seeds	Date of Sowing	No. of plants raised	No. of plants flowered	Distribution of Types		
					NN	*RR*	*NR*
Exp. 6	*NN*	11.4.06	463	439	436	0	3
Exp. 7	*RR*	3.4.06	660	617	0	616	1
Exp. 8	*NR*	3.4.06	491	449	109	114	226

The interpretation of these three experiments is clear: *NN* and *RR* are homozygotic forms so far as concerns the ray character, and *NR* is the corresponding heterozygotic form. In addition to this main conclusion it may be noted that cross-fertilization, with the production of natural hybrids, takes place in both ways, $N \times R$ and $R \times N$, and may produce an error of about 1 °/$_\circ$ unless measures are taken to prevent it. This error is so small that it might be neglected, if constant. Subsequent experiments, however, shew that the error due to the absence of selfing may reach 10 °/$_\circ$ or more. Such free natural crossing as is represented by a 10 °/$_\circ$ error is due to (1) *vicinism* in its various forms, (2) *visits by honey-bees*—in a bad honey season, the groundsels, radiate and non-radiate, are freely worked by these insects, (3) *Aphis*, the

winged form of which doubtless carries pollen freely, and (4) *various other insects*.

Selfing is therefore necessary and has been carried out in various ways; viz. (1) under a framework covered with muslin, (2) inside muslin bags, the mouths of which have been carefully closed with cotton wool—the most effective open-air method, (3) by isolation in the greenhouse—the most effective method of all, and perhaps absolutely safe when muslin bags are used as well. Before making crosses, it is of course necessary to self *both* parents—a precaution neglected in some of the earlier experiments.

Constant handling of groundsels soon trained the eye to the perception of comparatively slight differences of form, and at an early stage in the investigation (1904) distinct strains were collected and separated which can be regarded provisionally as *elementary species*. Three of these, named *praecox*, *latifolius* and *multicaulis*, were non-radiate, and were soon subjected to the test of experimental culture.

Praecox was first noted in 1904, growing in the Cathays Park, Cardiff, in a colony of about a hundred plants, associated with an *erectus*-like and much larger form. In 1905 a single head of seeds was collected, and the type has since then been cultivated and maintained as a pure strain for six generations (1906 to 1911). The plants are early and dwarf, with dark-green slightly cut leaves and reddish glabrous stems with long internodes and few nodes. (Pl. XV, fig. 1 and Pl. XVII, fig. 13.)

Latifolius is of garden origin and has been maintained pure in type for five generations. It has broad incurved leaves, glabrous and shining above, and is a tall erect plant like *erectus*.

Multicaulis was first observed near Barry, and has been cultivated for five generations. It is rather late, generally produces many strong branches from the axils of the basal rosette of leaves, has glabrous green stems, dark-green leaves, yellowish-cream coloured flowers, and large capitula. (Pl. XVII, fig. 12.)

Genevensis is the name given to a non-radiate type which occurs abundantly, apparently unmixed with other forms, in the vineyards about Montreux, and which resembles *praecox*. It has small capitula. (Pl. XVII, figs. 15 and 16.)

The full pedigrees of these types and a more detailed consideration of their characters are not required for our immediate purposes.

But it seemed to me of some importance to test whether the rayed character RR was transferable to these four non-radiate types. If one

could produce the hybrid in each case and from its progeny select the corresponding radiate form, one would be able to demonstrate two facts of importance : (1) the general transmissibility of the RR character, and (2) the reality and permanence of the characters which serve to define the four types.

This transference has been successfully made and the new types thus produced have been maintained as pure cultures for various periods.

a. *Praecox, radiatus* was produced in 1908 and has been kept pure for 3 generations. (Pl. XVI, fig. 7.)

b. *Latifolius, radiatus*   „   1908   „   „   3   „

c. *Multicaulis, radiatus*   „   1909   „   „   2   „ (Pl. XVI, fig. 4.)

d. *Genevensis, radiatus*   „   1911.   (Pl. XVI, fig. 9.)

Methods of crossing adopted to secure the F_1 generation in each case. It is of course possible to cross radiate groundsels by removing the disc florets while they are still young and crossing the ray florets with foreign pollen.   Seeds have been actually obtained from *S. viscosus* and from *S. squalidus* when endeavouring to obtain hybrids in this way, but the plants raised from them were in each case identical in character with the mother plants.   There are two possible interpretations of such results :—either (1) the ray florets are apogamous and are thus independent of fertilization, or (2) pollen of the same type as that of the mother plant accidentally reached the stigmas.   It is quite likely that the ray florets of one of these species are apogamous. Though then it may be *possible* to cross the individual flowers of the common groundsel, it is not *practicable* in the open air, and in the greenhouse it is quite unnecessary to do this in order to secure the best result. The two plants selected as parents are isolated as much as possible, and the flowers protected from insect visits.   If the capitula are then rubbed together cross-fertilization is effected in both of the possible ways, seeds are set freely, and the parentage of the offspring is readily determined. All the seeds of a crossed head are used to produce a fresh colony of the next generation.   Two kinds of plants are to be expected in this :— (*a*) plants like the mother produced by self-pollination, and (*b*) hybrids produced by the crossing.   The hybrids when *praecox* is the mother plant are much larger and later plants than their pure-bred neighbours. In the large number of crosses so far made, there has never been any difficulty in distinguishing the hybrids soon after the plants are bedded out.   When *praecox* is one of the parents, the hybrids may often be

distinguished at a very early stage. When two forms of the types NN and RR are used for crossing, as in the cases under consideration, the ray florets in the hybrids are of the NR type and the previous recognition is amply confirmed.

Hybrids are formed quite readily in this way as Table II shews.

TABLE II.

Exp.	Date	Cross	Total No. of plants raised	No. of F_1 hybrids secured	Per cent. of hybrids
Exp. 9	1906	Praecox × erectus, radiatus	16	9	56
Exp. 10	1906	Latifolius × erectus, radiatus	19	1	5
Exp. 11	1907	Multicaulis × erectus, radiatus	172	40	23
Exp. 12	1909	Genevensis × erectus, radiatus	36	12	33

The number of hybrids produced averages 25 %.

The F_1 plants derived from any cross are always very much alike and so are the pure-bred plants. In the beds the two types have invariably been very obvious, so much so that inexperienced visitors to the garden easily recognise them. Hence *provided that both parents have been adequately protected* there is no difficulty either (1) in raising hybrids or (2) being certain of their parentage. It is scarcely possible to secure absolute certainty as to the effectiveness of the isolation and protection, but generally there would be no great difficulty in recognising the introduction of some foreign strain by the agency of a misplaced pollen grain. There can only be doubt as to the paternal parentage. One must remember that the ordinary method of selfing, involving castration, is at least as fallible as the one outlined above. Hence the labour of crossing individual flowers is not only tedious and unprofitable but unnecessary.

Analysis of the F_2 generation. The second generation, so far as concerns the characters RR and NN, in the four crosses under consideration, is very easily analysed. In each case the three types of plant—RR, NN, and NR—are recognisable at the first glance. We have before us a very simple case of segregation without dominance, as the ray florets of the heterozygotes, NR, are typical intermediates. With respect to the other characters, of which there are several, one finds, as a rule, even after days of analysis, a variety which seems at first to defy classification. For the present it will be well to confine our attention to the ray character. The results of the analysis are given in Table III.

The only remarkable feature of this analysis is the constant tendency to a slight excess of NN forms. This may have some importance and is at any rate interesting, as the typical groundsel is also NN.

TABLE III.

Exp.	Date	Type	Total No. of plants flowered	Nos. actually found, distributed amongst the three types			Nos. calculated, distributed amongst the three types		
				NN	RR	NR	NN	RR	NR
Exp. 13	1908	F_2 of praecox × erectus, radiatus	548	161	117	270	137	137	274
Exp. 14	1908	F_2 of latifolius × erectus, radiatus	108	39	23	46	27	27	54
Exp. 15	1909	F_2 of multicaulis × erectus, radiatus	461	121	122	218	115	115	230
Exp. 16	1911	F_2 of genevensis × erectus, radiatus	234	66	53	115	58	58	117
		Totals	1351	387	315	649	337	337	675

The radiate groundsel of the Channel Islands. Mr Marquand, to whom living specimens of *erectus, radiatus* were forwarded, with a request that he would send back the corresponding Channel Islands form, obliged me by forwarding in April, 1907, several living specimens in fruit. These were, as he also recognised at that time, quite distinct from the Glamorgan *erectus, radiatus*. The ligulate ray florets (see Pl. XVI, fig. 10 and text-figure 30, p. 274) are not only shorter and so less conspicuous, but more distinctly 3-toothed at the apex. Moreover, the plants were nearly covered with a somewhat coarse type of hair, and hence distinguishable by that character alone from all the preceding types, which are glabrous. The plants had obviously been obtained from a sandy habitat and consequently it was anticipated that there would be some difficulty in raising normal colonies in the heavy soil of my garden. *Exps.* 17 *and* 18. Cultures were started on the day of the arrival of the plants, and at a later date as well, and the parent plants preserved for comparison. The plants raised on the Lias clays of Glamorgan differ in many particulars from the parent plants, but the *RR* character remains unchanged and the hairiness becomes so pronounced as to form a whitish felt sufficiently dense to make the plants conspicuous at a distance and to prevent, near at hand, the easy recognition of the stem-colour (Pl. XV, fig. 2, Pl. XVIII, fig. 24). Pedigree cultures have been continued, not without difficulty, for four generations, and the plants adhere strictly to one type. They are always relatively unhealthy, late (requiring about 110 days to mature seeds, while *praecox* requires only 72 days), and demand extra care in selfing. Unselfed plants may produce 20 °/₀ of hybrids. This constant hairy, radiate type has been named *lanuginosus*.

It seemed desirable to test once more the inheritance of the *RR* character (and also of course its relations to other characters) in the hybrids between *lanuginosus* on the one hand and *praecox, erectus* and *multicaulis* on the other. It was certainly advisable to produce, if

possible, a non-radiate *lanuginosus*. The necessary crosses were made
in 1908 and the F_1 generation was raised in 1909. The result
appears in

TABLE IV.

Exp.	Cross	Plants flowered	Distribution between		Per cent. of Hybrids
			Lanuginosus	Hybrid F_1	
Exp. 19	Lanuginosus × praecox	99	53	46	46
Exp. 20	Lanuginosus × erectus	100	61	39	39
Exp. 21	Lanuginosus × multicaulis	42	18	24	57
	Totals	241	132	109	45

Owing to the ease with which crossing takes place in this type, not
anticipated, and of course unknown in 1908, an unexpected result came
to light with the flowering of the hybrids: *some of these were of RR
type.*

The results were similar in each of the three crosses.

In Exp. 19 (lanuginosus × praecox) with 46 Hybrids there were 38 *NR* and 8 *RR* plants
In Exp. 20 (lanuginosus × erectus)   ,,   39   ,,   34 *NR* and 5 *RR*   ,,
In Exp. 21 (lanuginosus × multicaulis) ,, 23   ,,   20 *NR* and 3 *RR*   ,,

As it was important to determine whether the appearance of the *RR*
plants was due to inefficient selfing, and it was desirable for other
reasons to repeat the experiment, the cross *lanuginosus × praecox* was
again made in 1910 (Exp. 22) with plants very carefully isolated and
protected in the greenhouse, with the result that, in 1911, 31 plants
of the second generation were raised, *and all of them were hybrid and
the thirty which flowered were of NR type.* In this case *praecox* pollen
completely supplanted the much more abundant pollen of *lanuginosus*.
We have therefore, however vexatious the conclusion may be, no alter-
native but to suppose that the mixture of *RR* and *NR* hybrids in the
first three experiments was due to pollination with two kinds of pollen
grain of *R* and *N* type respectively. It is of interest to note that these
RR F₁ hybrids had the large ray of *erectus, radiatus* and that the plants
were of intermediate hairiness. The *R* pollen grains were probably
already on the inadequately selfed *NN* plants used for pollination.

The analysis of the F_2 and F_3 generations of the NR hybrids. In
these cases the analysis even of the ray character presents some diffi-
culty. The *RR* type varies considerably,—some plants have rays as
conspicuous as those of *erectus, radiatus*; others have the rays of
lanuginosus. The clue to the solution of the difficulty is furnished
when one notices that hairiness appears to have a depressing influence

on the development of the ray florets. The NR plants, the original
F_1 hybrids, as well as those appearing in F_2 and F_3, have the rays so
feebly developed that each head must be examined under a lens. With
this help and when special attention is paid to very hairy examples, the
results need not be ambiguous. (See notes on the illustrations, p. 275
and Pl. XV, figs. 25, 26, and 27.) There were seven beds altogether in
1910 and 1911 in which the segregation of the ray character could be
followed, and the result of their examination is given in

TABLE V.

Exp.	Cross	Gen.	Year	Parent Type	Plants flowered	Nos. found, of the three types			Nos. calculated, of the three types		
						NN	RR	NR	NN	RR	NR
Exp. 23	Lanuginosus × praecox	F_2	1910	NR	83	22	26	35	21	21	42
Exp. 24	Lanuginosus × praecox	F_3	1911	NR	92	25	26	41	23	23	46
Exp. 25	Lanuginosus × erectus	F_2	1910	NR	88	22	23	43	22	22	44
Exp. 26	Lanuginosus × erectus	F_3	1911	NR	92	17	23	52	23	23	46
Exp. 27	Lanuginosus × multicaulis	F_2	1910	NR	202	49	44	109	50	50	100
Exp. 28	Lanuginosus × multicaulis	F_3	1911(1)	NR	48	12	11	25	12	12	24
Exp. 29	Lanuginosus × multicaulis	F_3	1911(2)	NR	97	38	20	39	24	24	48
			Totals	...	702	185	173	344	175	175	350

The only noteworthy deviation from the normal ratio occurred in
the last bed, where the plants were uniformly hairy (homozygous for
hair) and where it might be suspected that some NR plants had been
counted as NN. It is just possible (not probable) that some of the
apparent NN plants were in this case really heterozygous for the ray
character. If R be taken as recessive in this case, we get the proportion
for this bed of Dominant : Recessive :: 77 : 20.

The complete result of the analysis of the segregation of the ray
character is summarized in

TABLE VI.

Experiment	Date	Plants flowered	Nos. found of the three types			Nos. calculated of the three types		
			NN	RR	NR	NN	RR	NR
Exp. 8. Table I.	1906	449	109	114	226	112	112	224
Exps. 13 to 16. Table III.	1908—11	1351	387	315	649	337	337	675
Exps. 23 to 29. Table V.	1910—11	702	185	173	344	175	175	350
	Totals ...	2502	681	602	1219	625	625	1250

While these results prove that the radiate character is generally
transmissible, they also shew that the theory of dominance has to be
accepted with some reserve. The experimenter who analysed only the

results summarized in Table III would be impressed with the obvious rays of the NR plants, and adopting the theory of dominance would count the NR and RR types together, and regard R as dominant; but in the analysis of the results summarized in Table V, he might quite easily overlook the NR individuals altogether and count them as NN, in which case he would be obliged to regard R as recessive. The association of the factors for hair and rays probably effects a reduction of the ray character. It appears then to be of little consequence whether we regard N or R as dominant, or neither. Whatever signs we adopt, the interpretation remains the same. The theory of dominance has probably been often pushed too far. Under these circumstances it will suffice to choose the most convenient system of nomenclature. The simpler ratios associated with the theory of dominance often give the clearer view of the experimental results.

Neither is it quite clear in such cases as these whether the presence and absence hypothesis strictly applies. The original type of flower in the Compositae was doubtless actinomorphic. In the section Tubuliflorae we recognise the introduction of the radiate character or zygomorphy into the outer florets, and in the Liguliflorae, possibly the same
· character, certainly a similar character, into all the florets. At least two factors would be necessary to effect this. In the genus *Senecio* the presence of typical ray florets is the rule, but the common groundsel is generally described as non-radiate, the radiate type being regarded as a variety. The groundsel must have assumed its peculiar non-radiate character either by the loss of the factor for rays (absence) or by the acquisition of some new factor (presence) which made itself manifest by suppressing the rays. We have at present no experimental means of testing the relative value of these two hypotheses.

It seems fairly certain however that the non-radiate type of the common groundsel is more recent than the radiate types found in the related species, *Senecio viscosus*, *squalidus* and *sylvaticus*. Admitting this, we are still unable to decide whether the radiate groundsels *lanuginosus* and *erectus*, *radiatus* are newcomers or relicts of the older original type: they are apparently aggressive invading forms and are certainly, to a considerable extent, replacing the non-radiate forms.

This kind of mutability is not confined to the common groundsel. In *Senecio Jacobaea* there is a comparatively rare non-radiate form. It is abundant in one part of Ireland, according to Praeger. *Exp.* 30. A single specimen was collected near Cardiff, and was cultivated in my

garden for two or three years, crossed with a local radiate form in 1908, and 48 plants were brought to the flowering stage in 1911. As the results of analysis are peculiar and difficult of interpretation, it is necessary to state that of the 112 specimens which were originally planted, 64 perished of a disease induced probably by the rich soil and close planting. The disease had no selective action; it simply destroyed the plants in the middle of the bed. The 48 plants which matured consisted of two types: 24 were RR and 24 NR plants. The seed-bearing plant was certainly NN in appearance; the pollen was from an ordinary RR plant specially introduced into the garden for the purpose of making this cross. The expectation was, as self-pollination was not excluded, that a large number of non-radiate (NN) plants would be secured and a few F_1 hybrids, NR in type. It was rashly assumed that the non-radiate type would produce non-radiate plants only, if selfed, as is invariably the case in non-radiate forms of *Senecio vulgaris*. This ambiguous result may be tentatively explained by assuming (1) that the non-radiate (NN) plant produced equal numbers of R and N ovules, was indeed in reality a heterozygote (NR), and (2) that the pollen of the radiate type (RR) is prepotent and, in the presence of both types, is the only one that is effective. Accepting . these assumptions, all the N ovules would be fertilized by R pollen grains and produce NR plants, and all the R ovules by R pollen grains and produce RR plants. The explanation is purely provisional, and not the only possible one. The experiment is recorded at this stage to show how necessary it is to avoid the inference that what is proved to be true for one species is also true for others, however closely allied the species may be.

The ray character has hitherto been examined experimentally in *Senecio vulgaris* and *S. Jacobaea* only. *Centaurea nigra*, as is well known to many botanists, possesses radiate and non-radiate forms, and the mode of occurrence of these in Glamorgan suggests that the ray character segregates as in the groundsel. Other similar variations are by no means infrequent in the Compositae, and are well worth investigation by the experimental methods of genetics. The problem of the inheritance of the ray character in groundsels can, however, be regarded as solved, and it seems desirable, with a view to securing uniformity and brevity of notation, to adopt the theory of dominance to the extent of using the signs RR, Rr and rr instead of RR, RN and NN for the three types of ray character. R signifies *radiate, r non-radiate.*

Investigation of a Colour Character in the Flowers.

The colour of the *multicaulis* flowers was, in the first generation raised, appreciably different from that of the other forms, and it was described in 1908 (*Flora of Glamorgan*) as of "a soft yellow." It was not anticipated that so subtle a distinction would admit of further analysis. However it can now be shewn that the ray florets may, in certain types at least, be either *yellow* or *cream* coloured, and that cream behaves towards yellow as a recessive. It will be of interest to produce the evidence for this statement, especially as it demonstrates that there must be a second factor which acts when present along with that for cream colour in such a way as to inhibit more or less completely the development of the cream-colour character.

Exp. 15. *Multicaulis* was crossed by *erectus, radiatus* in 1907, and 461 plants of the F_2 generation were examined in 1909. At a late stage in the examination, some of the RR plants attracted notice on account of the cream colour of the flowers. At first the colour was not treated as a character deserving of much attention, especially as it was not perfectly uniform in all the cream-flowered individuals. The total number of creams of which a definite record was kept is five, but there were certainly more. Fortunately seeds were saved from the two most typical creams—Nos. 174 and 346. These plants, however, had not been properly selfed. The nature of the progeny of these two plants is given below in the form of genealogical tables.

1909. F_2.   No. 174.   Cream, not selfed.

1910. F_3.   *Exp.* 31.   33 plants, of which 5 were NR hybrids due to ordinary crossing.
        5 Deducting these, there were left

        28 plants, all Cream, of which No. 5 was selfed.

        No. 5

1911. F_4.   *Exp.* 32.   47 *plants, all cream.*

1909. F_2.   No. 346.   Cream, inadequately selfed.

1910. F_3.   *Exp.* 33.   108 plants, of which 4 were NR hybrids.
        4 Deducting these, there were left

        104 plants, all RR.

97 plants, Cream   7 plants, Yellow, no doubt due to the inadequate selfing.

No. 8.   Selfed.

1911. F_4.   *Exp.* 34.   49 *plants all cream.*

These experiments indicate that *cream* is recessive to *yellow*; the seven rogues in Exp. 33 must have been heterozygous for yellow and cream, and due to an accidental cross with a yellow RR form.

Seeds were also saved from several of the yellow types of the F_2 generation, and as it sometimes proved advisable (colour was not the sole problem under investigation) to select other parents than those originally selfed, the results must be read with the necessary caution.

1909. F_2.   No. 2.   Yellow, selfed.

1910. F_3.   *Exp.* 35.   108 plants.

85 yellows   23 creams; of these Nos. 9 and 37 were selfed.

No. 9.   No. 37.   Slight yellow tinge in cream, and tested for that reason.

1911. F_4.   *Exps.* 36 *and* 37.   49 plants, cream.   49 plants, cream.
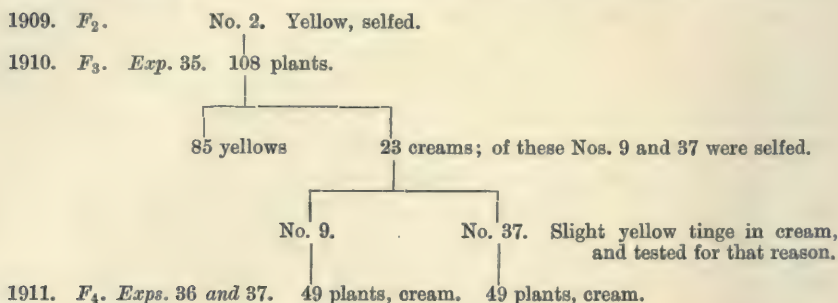
This result proves the dominance of yellow. No. 2 was clearly a heterozygote.

Two other yellows, Nos. 243 and 297, were tested and proved to be homozygotes. The record for one is given below; that for the other is similar.

1909. F_2.   No. 297, yellow, not selfed.

1910. F_3.   *Exp.* 39.   36 plants, of which 3 were NR and were destroyed.
                              3   Deducting these, there were left
                              ――
                              33 plants, all yellow, of which No. 34 was selfed.
                              No. 34.

1911 F_4.   *Exp.* 40.   49 *plants, all yellow.*
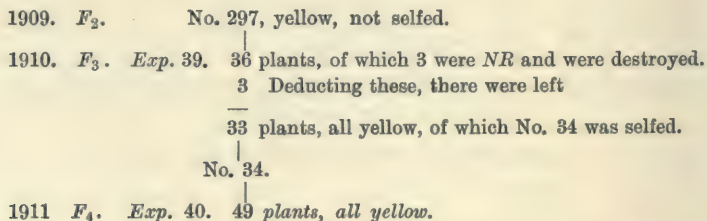
We can now accept the following notation, CC, Cc, cc, for the three types found. cc signifies creams; CC yellows.

The progeny of a sixth plant, however, was the means of elucidating the cause of the *rarity* and *variability* of the creams in the F_2 generation. If there is a factor X dominant over another x and cream colour can only appear in the plant when cc is associated with xx, being wholly suppressed by XX and almost wholly by Xx, then it is clear that in a generation where these two pairs of factors are segregating normally, there will be one-fourth of the individuals of the type xx and one-fourth only of these xx individuals also of the type cc, and therefore one-

sixteenth only of the individuals constituting the whole colony will be of the type $xxcc$. These $xxcc$ plants will alone be *pure creams*.

The progeny of this sixth plant is shewn below.

1909. F_2.   No. 368 yellow, not selfed.

1910. F_3. *Exp.* 41.   37 plants, of which 4 were *NR* and 2 others did not flower
                    6 Deducting these, there were left
                    ——
                    31 plants

         24 yellows   7 creams

                          No. 1 selfed. *Exp.* 44.

     No. 13, selfed   No. 30, selfed

1911. F_4. *Exps.* 42 *and* 43.   90 yellows, *CC.*   98 creams. *(cc xx)*

                       88 plants, which seemed at first all yellow. No. 96 was a typical cream, and close examination led to the discovery of three types of plant

| 7 Creams. *(cc xx)* | 4 Cream to Yellow, the colour difficult to estimate, but certainly with some cream. *X x cc* | 77 Yellows, the colour rather faint and sometimes shewing a creamy tinge on the under side of the corolla |

As one-sixteenth of 88 may be taken as 6, the result corresponds fairly with the view that the recessive cream only appears as a definite character as the result of the association of the cream factor with some other recessive factor. At first, I suspected foliage-leaf colour to be concerned in producing this result, but the observations so far made seem to shew that cream-coloured flowers may appear on plants with either dark-green or yellow-green foliage leaves. For the present we must be content to assign to this unknown pair of factors the signs X and x. X, when present, more or less prevents the development of the cream-coloured character.

MUTATION. A FIMBRIATE TYPE.

The 461 plants of the F_2 generation of *multicaulis* × *erectus, radiatus* (*Exp.* 15) included one plant—No. 43—which was not only markedly unlike all the others, but unlike any other radiate groundsel that I had ever seen. The ray florets, in this case, were of the usual number, but

instead of being very obscurely 3-toothed at the tip, the ligules were often divided down to the basal tubular portion into three (sometimes two) long narrow segments. The capitula, as a whole, thus acquire a tasselled or fimbriate character. (Pl. XVII, figs. 19 and 20.)

The study of the inheritance of this character is beset with difficulties. Although these have not yet been surmounted, it will be of interest to record the results hitherto obtained. No. 43 was selfed and a few seeds ultimately collected. It was noteworthy that the basal branches of this plant produced normal flowers, causing me to suspect that the anomalous fimbriate appearance was due to *Aphis* attack. Fortunately the experiment was carried on, and it was soon found that perfectly healthy plants quite free from *Aphis* might be fimbriate. The nature of the progeny of this plant is given in the following table:

1909. F_2.

No. 43, fimbriate, selfed.

1910. F_3. *Exp.* 45.

17 plants, of these three were Rr plants.
3 Deducting these, there were left

14 plants, all RR

9 fimbriate

5 non-fimbriate

No. 17 selfed. *Exp.* 47.

No. 4 selfed   *Exp.* 46. No. 6 not selfed

No ripe seeds obtainable. Therefore self-sterile

47 plants, all non-fimbriate

1911. F_4. *Exps.* 46 *and* 47.

49 plants

22 fimbriate

27 non-fimbriate
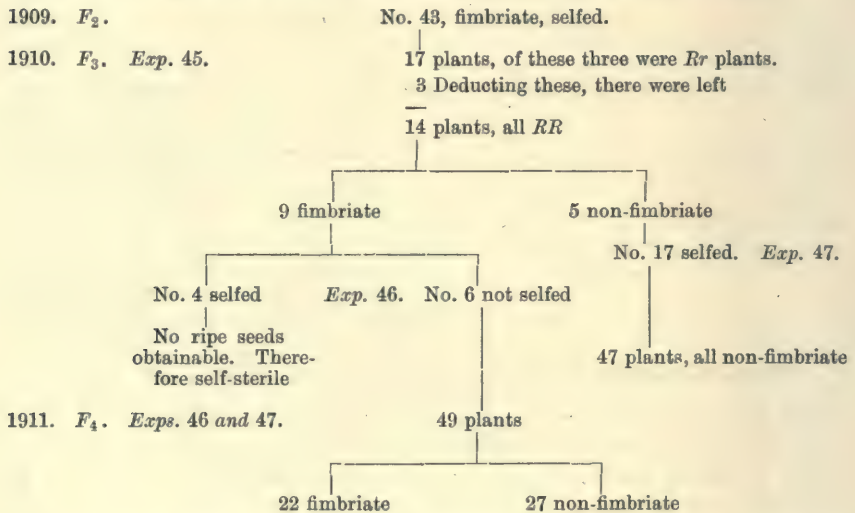
It is clear that the fimbriate character is inherited, and it may be assumed that a corresponding factor F suddenly dropped out of the constitution of the original plant, No. 43. The new character, represented let us say by the recessive factor f, as far as known, does not occur in any other local form of *Senecio*. Yet it probably occurs in many Compositae, e.g. in chrysanthemums. Ultimately time may be found to trace its development and to make the necessary comparative studies. In the meantime, we may safely assume that we have to deal in this case with a mutation (and possibly a segregation also).

It is clear that the plants are either self-sterile or very infertile to their own pollen. In this connection it may be stated that on

several occasions during this study of groundsels, the difficulty of self-sterility has been encountered. *Exp.* 48. In one case, that of a plant isolated in the greenhouse (it was a monstrosity with fused cotyledons and other malformations) which did not set a single seed, it was noteworthy that all the flowers were *long-styled*. Examples of long-styled capitula are reproduced in Pl. XVII, figs. 16, 17 and 18. Long-styled flowers have been frequently observed during the last three years, and there is little doubt that the further study of them and their distribution will help to clear away several difficulties. In another case, which occurred in the F_2 generation of *lanuginosus* × *praecox*, the plant was a giant, the only one of the kind, and provided with very large ray florets. *Exp.* 49. Yet not a single perfect seed was obtained from it, although ultimately exposed to the pollination of all the other plants in the garden.

How are we to explain the results detailed in the table on page 254? The 47 non-fimbriate plants of the F_4 generation, as well as the parent plant No. 17, were all clearly homozygous and *FF* plants. No. 6, one of the parents ($\female$) of the 49 plants of the F_4 generation, was certainly a *ff* plant. If we suppose that the chance pollen grains which in this case effected pollination were either f (22) or F (27), we get an explanation of the mixed offspring. This provisional explanation is to be tested in 1912 by isolating two *ff* plants and obtaining seed from them[1]. It is quite possible that the fimbriate character is such that we shall always get under ordinary conditions nearly equal numbers of *ff* and *FF* individuals. We have to assume in this case that the ray character depends not only upon the factor f but also upon the environment. The heredity would follow the same rule as prevails in teazels with twisted stems, and in other " umschlagende Sippen."

HAIRINESS.

Hairiness is one of the most interesting and puzzling of the characters which serve to define these elementary species of groundsel, and has been investigated sufficiently to justify the publication and discussion of some of the results. In the last edition of Koch's *Synopsis*, 1902, a single hairy variety is described under the name of *sordidus*, with the brief description "Pfl. ganz spinnwebig-wollig." Rouy, in the

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[1] This was done, but the plants produced no ripe pollen. The 1912 examination leads simply to the conclusion that all the fimbriate plants are female $\female$. It remains doubtful whether it is possible to secure male fimbriate plants.

Flore de France, 1903, recognises no hairy forms. Until colonies of *lanuginosus* had been grown in my own garden, I had assumed, like most other botanists, that hairiness in groundsels was the result of a more or less direct response to changes in the external conditions, belonged, in short, to the category of modifications. *Lanuginosus*, however, *is always very hairy*, whatever the external conditions may be. It is almost certainly impossible to raise a colony of these plants in the glabrous condition. Most of the other types are normally so glabrous that hair can, as a rule, only be found by looking carefully for it. The most glabrous forms, however, produce a little hair in the leaf axils and the buds are consequently often slightly hairy. Such hair is, however, soon lost, and the plants appear to be, as indeed they practically are, quite glabrous. In this sense *praecox, genevensis, erectus, multicaulis, latifolius* and *erectus, radiatus* are glabrous.

Intermediate between these two types are two other forms of hairiness. In these hair is constantly to be found on most parts of the plant until full maturity is reached, when it generally becomes greatly reduced in amount. The hair, even when abundant, however, is never dense enough to mask the stem-colour, and appears white at a distance, as in *lanuginosus*, yet it is generally recognisable with certainty, except perhaps in very old plants. These forms of hairiness occur in several incompletely investigated types that have been cultivated for several years in the garden. We thus have to recognise four standards of hairiness :—(1) very hairy (H^3), as in *lanuginosus*; (2) distinctly hairy (H^2), as in three types from Burry Green, Horton, and Cross Common respectively; (3) slightly hairy (H^1), as in two types from Cardiff and St Bride's respectively, and (4) the glabrous types (H^0). (Pl. XVIII, figs. 21, 22, 23 and 24.)

Grown under constant conditions, side by side in the same garden, the standard of hairiness is maintained for generations. It naturally varies a little in response to seasonal changes, but as all the plants are subject to the same change, the same relative differences appear year after year.

It may perhaps be advisable to put on record the extent of the experiments which have led to these generalizations.

The *lanuginosus* type of hairiness is very distinct, and so is the glabrous condition of such types as *praecox* and *erectus*. The intermediate types—H^2 and H^1—are more variable. They are sufficiently definite to admit of classification when pure colonies are examined. When segregation of hair factors is taking place, however, it is almost

impossible to arrange the forms in their respective groups with strict accuracy.

The observations summarized in Table VII suffice to demonstrate that hairiness and its opposite are transmissible characters, and there-

TABLE VII.

Exp.	Type	Standard of Hairiness	No. of generations maintained in pure culture	Date of first generation	Years in which hairiness was tested	No. of plants examined
Exp. 48	Lanuginosus	H^3	3	1907	3	234
Exp. 49	Burry Green type	H^2	3	1909	2 last	135
Exp. 50	Horton type	H^2	3	1909	2 last	122
Exp. 51	Cross Common type	H^2	3	1909	2 last	89
Exp. 52	Cardiff type	H^1	5 (possible cross in 2nd generation)	1907	1st and last	209
Exp. 53	St Bride's type	H^1	3	1909	2 last	111
Exp. 54	Praecox	H^0	6	1906	6	361
Exp. 55	Erectus, &c.	H^0	a very long series of experiments, the results agreeing with those opposite praecox			

fore probably represented in the constitution of these plants by at least one pair of factors. We shall therefore make use of the signs HH, Hh, and hh to represent the three types of constitution. Glabrous forms are hh. We shall find that the variability of the hairy character is due (1) to the influence of other factors, and (2) to a second pair of hair factors[1].

Exps. 19, 20 *and* 21. In 1908 *lanuginosus* was crossed by *praecox*, *erectus* and *multicaulis*. *Lanuginosus* is hairy (HH) and radiate (RR). The other three types are each glabrous (hh) and non-radiate (rr). The objects in view were :—(1) to secure the non-radiate form of *lanuginosus*, (2) to secure hairy varieties of *praecox*, *erectus* and *multicaulis*, and to determine whether the hair factors could be transposed from type to type with the same facility as the ray factors, and (3) to note the effect on each other of the factors for hair and rays. The results were unexpected. At the close of the examination even of the F_3 generations of these three crosses, it was still somewhat doubtful whether either of the first two objects had been secured: the third however had been investigated with at least considerable success.

The F_1 hybrids in each of these three crosses are of intermediate hairiness; in the F_2 generation segregation takes place, and at least three types of hairiness are noticeable. Taking the hairiness of the F_1 hybrid as the standard, we find (1) some plants conforming to this,

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[1] See notes to the illustrations for evidence of a third pair of hair factors.

while others are either (2) *more*, or (3) *less* hairy. Arranging the plants in three groups accordingly, and commencing our analysis with the F_2 generations of three RR plants (probably the result of an accidental cross of *lanuginosus* by *erectus, radiatus* (see p. 247)), we get the following result:

TABLE VIII.

Exp.	Cross	Ray Character in F_1	No. of Plants	Types		
				HH	Hh	hh
Exp. 56	Lanuginosus × erectus, radiatus ?	RR	108	21	51	35
Exp. 57	Lanuginosus × erectus, radiatus?	RR	166	45	79	42
Exp. 58	Lanuginosus × erectus, radiatus?	RR	160	41	78	41
Totals ... Found			434	107	208	118
Expected			434	108	217	108

Segregation apparently takes place normally in each of these three cases.

If we now examine in the same way the F_2 generations of the three NR types of known parentage, we get the result shewn in

TABLE IX.

Exp.	Cross	Ray Character in F_1	No. of Plants	Types		
				HH	Hh	hh
Exp. 23	Lanuginosus × praecox	Rr	84	22	47	15
Exp. 25	Lanuginosus × erectus	Rr	88	19	43	26
Exp. 27	Lanuginosus × multicaulis	Rr	203	61	79	63

The result of Exp. 27 is clearly aberrant. Deferring its consideration, we may summarize the results in the other five experiments, thus:—

Exp.	No. of plants	Types		
		HH	Hh	hh
Nos. 56, 57 and 58	434	108	208	118
Nos. 23 and 25	172	41	90	41
Totals Found	606	149	298	159
Expected	606	151	303	151

No thoroughly satisfactory explanation can be given of the result in Exp. 27. Not only are the numbers very aberrant, but the mode of obtaining them is liable to criticism. All the HH plants were certainly more hairy than the original F_1 heterozygote. There were certainly 61 HH plants present. But all the other plants had some hair, and at the first examination only 20 were marked as hh. Extracting these and re-examining the remainder, this was found to

include apparently two types more and less hairy respectively; the less hairy were added to the *hh* group, and the more hairy constituted the presumed *Hh* heterozygotes. Under these circumstances, it should be understood that the result shews little more than that segregation actually occurs in this case. The aberration is probably due to transgressive variability, which makes it impossible to fix exactly the limits of the three types. A nearer approximation is gained by applying the law of dominance, but this procedure reveals nothing new. All groundsels, even the most glabrous types, vary a little in hairiness, and in such sunny weather as that of last summer (1911) to a considerable extent. It is possible that the *multicaulis* strain introduced an unknown disturbing factor.

We may now turn our attention to the relation of the factors for rays and hairiness when they act together. *Lanuginosus* (*RRHH*) × *praecox* (*rrhh*), *erectus* (*rrhh*) or *multicaulis* (*rrhh*) might be expected to produce nine types of plants in the F_2 generation. Table X shews both the expectation and what was actually found.

TABLE X.

		No. of plants flowered			RR		Rr			rr		
Exp.	Cross			HH	Hh	hh	HH	Hh	hh	HH	Hh	hh
Exp. 23	Lanuginosus × praecox	83	Found	13 !	10	3 !	6	23	6	2 !	14	6 !
		—	Expected	5	10	5	10	21	10	5	10	5
Exp. 25	Lanuginosus × erectus	88	Found	14 !	8	1 !	4	25	14	1 !	10	11 !
		—	Expected	5	11	5	11	22	11	5	11	5
Exp. 27	Lanuginosus × multicaulis	202	Found	34 !	6	3 !	25	51	24	1 !	22	26 !
		—	Expected	12	25	12	25	50	25	12	25	12

Confining our explanations to Exps. 23 and 25, in which *Hh* and *Rr* taken separately segregate normally, we note that the heterozygotes for hair are distributed fairly, according to the usual law, among the three types *RR*, *Rr* and *rr*. There are, however, too many plants of *HHRR* type and too few of *hhRR*, and also too many plants of *hhrr* and too few of *HHrr*. In other words, hairy radiate types and smooth non-radiate types are produced in greater relative abundance than glabrous radiate or hairy non-radiate types. In Exp. 27, which although anomalous, agrees with Exps. 23 and 25 in these respects, only one plant was *HHrr* and could therefore be of the type of a *hairy multicaulis* or *non-radiate lanuginosus*. In Exp. 25 there was only one *HHrr* plant, and in Exp. 23 only two. Hence it is clear that the production of a hairy *praecox*, *erectus* or *multicaulis*, or of a non-radiate *lanuginosus*,

is a matter of difficulty, for the factors for hair and rays are by no means the only ones present.

There are at least two possible explanations of this behaviour, viz. (1) that the four types of gamete RH, Rh, rH, rh are not produced in equal numbers—gametic coupling; and (2) that whether produced in equal numbers or not, certain unions are preferred to others, e.g. $RH \times RH$ and $rh \times rh$ to $rH \times rH$ or $Rh \times Rh$. This is not an improbable explanation, for certain combinations might easily have special advantages; e.g. more rapid growth of the pollen tube or quicker response to the chemotactic stimulation of the ovule.

Let us assume that R and H are really dominant, and simplify Table X to

TABLE XI.

Exp.	Cross	No. of plants raised		4 Types			
				HR	Hr	hR	hr
Exp. 23	Lanuginosus × praecox	83	Found	52	16	9	6
	Ratio 9 : 3 : 3 : 1	—	Expected	45	15	15	5
	Ratio 22 : 5 : 5 : 4	—	Expected	51	11	11	9
Exp. 25	Lanuginosus × erectus	88	Found	51	11	15	11
	Ratio 9 : 3 : 3 : 1	—	Expected	50	16	16	55
	Ratio 22 : 5 : 5 : 4	—	Expected	54	12	12	10
Exp. 27	Lanuginosus × multicaulis	202	Found	116	23	37	26
	Ratio 9 : 3 : 3 : 1	—	Expected	113	37	37	12
	Ratio 22 : 5 : 5 : 4	—	Expected	123	28	28	22
	Totals ...	373	Found	219	50	61	43
	Ratio 9 : 3 : 3 : 1	—	Expected	210	70	70	23
	Ratio 22 : 5 : 5 : 4	—	Expected	228	52	52	41

The agreement with the latter ratio is remarkably close, and the inference may fairly be drawn that coupling takes place according to the system $2HR : 1Hr : 1hR : 2hr$. There is, however, more than this to be deduced from the experiments. The excess of $HHRR$ plants suggests that the presence of R helps the factor H to assert itself more effectively in the development of the hair character.

The analysis of the F_2 generation of these *lanuginosus* hybrids made it desirable that further tests should be made by utilizing the F_3 generation. The following cultures were therefore undertaken in 1911, with seeds produced on selfed plants of 1910 (F_2).

The three objects already described were still in view—to secure new hairy and non-radiate types, to test the general transmissibility of hairiness, and to determine the relationships of the factors for hair and rays—but there was now a further one, viz. to test the accuracy of the

analysis of the F_2 generation. It is well known that the safest test (as well as the most troublesome) for a presumed heterozygote is to raise a colony of plants from it.

Ten of the eleven colonies raised shewed that the constitution of the parent plants had been correctly estimated with respect to hairiness.

TABLE XII.

Exp.	Culture No. for 1911	Cross	Hair type of parent F_2	Hair type of progeny F_3	Ray type of parent	No. of plants required
Exp. 59	28		hh	—	RR	100
Exp. 60	29	Lanuginosus × praecox	Hh	Hh	rr	100
Exp. 61	30		HH	HH	Rr	100
Exp. 62	31	Lanuginosus × erectus	Hh	HH	rr	100
Exp. 63	32		HH	HH	Rr	100
Exp. 64	33		Hh	Hh	Rr	100
Exp. 65	34		Hh	Hh	rr	100
Exp. 66	35		Hh	Hh	RR	100
Exp. 67	36	Lanuginosus × multicaulis	HH	HH	RR	50
Exp. 68	37		HH	HH	Rr	100
Exp. 69	38		HH	HH	rr	100
Exp. 70	39		HH	HH	RR	50

The eleventh (Exp. 62) marked Hh, proved to be constant for hairiness, all the F_3 plants conforming to the standard H^1. The plants were all rr; if we assume that the non-radiate condition has the effect of depressing hair development, we secure at least a provisional explanation of this case. The original analysis, however, becomes as a whole subject to an error of $10\,^{\circ}/_{\circ}$, and one must admit that the results are to be accepted only with such reservations.

Let us now note the result of the examination of the segregation of the hair in the Hh types of Table XII, and take in order the rr, RR and Rr groups. The results are presented in Table XIII. The numbers accord fairly well with the expectations. The divergences are partly due to the attempt to recognise the heterozygotes.

The most hairy types in No. 29 were as hairy as *lanuginosus*—in the three cultures Nos. 34, 35 and 33 this was not the case. We may assume that typical *multicaulis* carries a factor Y which depresses the development of the hair character. In its absence (y) hair is fully developed if the factor for hair, H, is present. Pure *praecox* is a yy plant, *multicaulis* is YY. This hypothesis will also enable us to explain the result of Experiment 36. Two types of hairy plants appeared in this case: (a) very hairy—H^3—like *lanuginosus*, and (b) hairy—H^2—like the HH plants of No. 35. The 13 very hairy

TABLE XIII.

Exp.	No.	Cross	Hair type	Ray type	No. of plants	*HH*	*Hh*	*hh*	Remarks
Exp. 60	29	Lanuginosus × praecox	*Hh*	*rr*	97	22	49	26	Very hairy individuals present in *HH*
Exp. 65	34	Lanuginosus × multicaulis	*Hh*	*rr*	90	23	44	23	The hair in *HH* plants relatively weak
Exp. 66	35	Lanuginosus × multicaulis	*Hh*	*RR*	96	31 (H^2)	33	32	The hair in *HH* plants well developed
Exp. 67	36	Lanuginosus × multicaulis	*Hh*	*RR*	49	13(H^3)	36 H^2		The H^3 plants as hairy as lanuginosus
Exp. 64	33	Lanuginosus × multicaulis	*Hh*	*Rr*	49	14	20	15	Hair fairly well developed

plants were apparently of the same physiological constitution as *lanu-ginosus*—the soil did not suit them—the 36 hairy individuals were fairly well adapted to their environment. It was doubtless no accident that caused one-fourth of the plants of this colony to behave in this way. The plant selected as the parent for this colony happened to be an individual with the constitution *Yy*. The thirteen very hairy plants were *yy* in constitution. This explanation may possibly suffice to account for the occurrence of a certain percentage of weakly plants in other cultures derived from the cross *lanuginosus* × *multicaulis*. These weaklings are recognisable in the seed-pans and boxes, and invariably perish when planted out. May they not have been to a great extent the *yy* plants of the different generations? They are always so hairy that the possibility has always been kept in mind that ill health in itself, whatever its cause, promotes hair development by its indirect influence on certain regulating factors. These weak, hairy plants were carefully grown and watched in 1912, and proved to be invariably long-styled and relatively infertile. Fig. 17, Pl. XVII shews the capitulum of one of them.

TABLE XIV.

Exp.	Culture No.	Cross	Hair type	Ray type	H^3		H^2		H^1		Remarks
Exp. 61	30	Lanuginosus × praecox	*HH*	*Rr*	34	22*RR* 10*Rr* 2*rr*	52	4*RR* 27*Rr* 21*rr*	6	0*RR* 4*Rr* 2*rr*	No *rr* plant was very hairy, although 2 were placed under H^3
Exp. 63	32	Lanuginosus × erectus	*HH*	*Rr*	0		92		0		Rather weak type of H^2 hair. Unaffected by segregation of *Rr*
Exp. 68	37	Lanuginosus × multicaulis	*HH*	*Rr*	0		98		0		Hair tending to disappear with age; unaffected by the segregation of *Rr*

Let us now examine the variability of the hair character in the six remaining groups of Table XII, in which the parents were all homozygous for hair (HH). Table XIV gives the result for those groups whose parents were Rr, Table XV for those whose parents were RR or rr.

In Exp. 61 the hairiness varies in degree, shewing the three grades H^3, H^2 and H^1, and the RR, Rr and rr types are irregularly distributed amongst these. There are so few H^1 types and these are so loosely distinguished from H^2 types that the colony may be arranged thus:—

```
                        92 plants
                           |
          ┌────────────────┴────────────────┐
        34H³                               58H²
   ┌──────┼──────┐                  ┌────────┼────────┐
 22RR    10Rr   2rr               4RR      31Rr      23rr
```
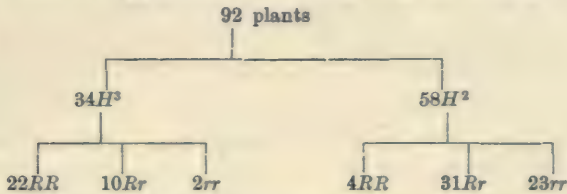
The numbers, although $58:34$ *might* represent $9:7$, shew that there is apparently no real segregation of hair factors in this case. The difficulty of arranging the plants in definite groups according to their hairiness supports this view. We have therefore to explain as best we can the remarkable association of RR and H^3, and rr and H^2. We are perhaps justified in assuming that the greater type of hairiness—H^3— is due to the association of the hair factor H with R. On this assumption

$HHRR$ plants should be   very hairy   and of the type H^3,
$HHRr$ „   „   „   less hairy   „   „   „   „ H^2,
and $HHrr$ „   „   „   slightly hairy   „   „   „   „ H^1.

The facts revealed by Exp. 61 agree very well with this assumption.

Applying the hypothesis to the explanation of the results of Exps. 63 and 68, we are at once met with a difficulty, the standard of hairiness is constant at the grade H^2 and the segregation of the ray character does not affect it.

This difficulty may be removed by amplifying the original hypothesis. Let Z be an unknown factor which when present nullifies the stimulating effect of R on H. Then, since the presence of R promotes hairiness in Exp. 61, *praecox* is probably a zz plant, and since the hair character is constant in Exps. 63 and 68, *erectus* and *multicaulis* are probably ZZ plants.

Such a hypothesis is somewhat involved and perhaps unnecessary; but as it explains the results hitherto obtained and can readily be tested

by experiment, it deserves provisional acceptance. It is possible that the presence of the Y factor alone in Exps. 63 and 68 might serve to explain the result. Further experiments are necessary.

The remaining three experiments are of greater interest, as they were intended to test the behaviour of the progeny of plants homozygous for hair and rays. An unexpected segregation occurred in one of them.

TABLE XV.

Exp.	Culture No.	Cross	Hair type	Ray type	Variability in hairiness			Remarks
					H^3	H^2	H^1	
Exp. 62	31	Lanuginosus × erectus	HH	rr	—	—	$93YY$	Hairiness of the lowest type and constant. Hairiness probably depressed by YY
Exp. 69	38	Lanuginosus × multicaulis	HH	rr	$20yy$	$49Yy$	$27YY$	All H^3 plants unhealthy. Hairiness upon the whole less than in the praecox cross. Exp. 61
Exp. 70	39	Lanuginosus × multicaulis	HH	RR	—	$49YY$	—	Hairiness not quite equal to H^3. Hence YY plants. R raises the grade of hairiness

The plants of Exp. 62 were all of a very low standard of hairiness. This may be partly accounted for by the absence of R and the presence of Y.

Exp. 69 is comparable with Exp. 67. The Y factor is involved, and the heterozygotes are recognisable. The slight loss of hair may be due to the absence of R. The numbers found correspond very well to the expectation.

$$\text{Found:—} \quad YY : Yy : yy :: 27 : 49 : 20$$
$$\text{Expected:—} \quad\quad\quad :: 24 : 48 : 24$$

In Exp. 70 the plants shewed constant hairiness; they were probably of YY type, the presence of R raising the grade of hairiness from H^1 to H^2.

The study of the inheritance of hair is no doubt incomplete; but it is clear that (1) the transmissibility of hair from one type to another is possible; (2) that two pairs of factors at least are involved, H, h and Y, y; (3) that consequently there must be at least several grades of hairiness; and (4) the presence (R) or absence (r) of the ray factors modifies the hairiness due to the proper hair factors. The difficulties of the study are accentuated by the transgressive variability of the segregating characters and by the direct influence of the environment in producing slight non-heritable modifications.

OBSERVATIONS ON STEM COLOUR.

At an early date in the course of these experiments it was noted that some of the pure types were green-stemmed, viz. *erectus, erectus, radiatus* and *multicaulis*; others had stems which were more or less reddish in colour, viz. *praecox, genevensis* and *lanuginosus*. No attempt was made to study the behaviour of this pair of characters in segregation until 1911, when certain families of the F_3 generation of the crosses *lanuginosus × erectus* and *lanuginosus × multicaulis* were found to consist either of green-stemmed individuals only, of red-stemmed individuals only, or of individuals of several types of stem-colour. It seemed that stem-colour varied independently of the other characters under investigation, and was therefore probably represented by definite factors in the constitution of the plants.

There are very great difficulties in arranging the plants in definite categories according to stem-colour, for green-stemmed plants tend, under certain conditions (exposure), to develop some red colour, and red-stemmed plants lose some of their red colour in shade. Moreover, the red-stemmed types are not red-stemmed throughout—the upper internodes are apt to be green, although these parts are subject to the most intense illumination. Green-stemmed plants, if they develop red colour at all, do so most freely on the lowest internodes. Let GG denote red-stemmed and gg green-stemmed plants. When G is present (GG) and certain standard conditions are maintained, at least one-half of the main axis should be of a reddish colour; if G is absent (gg), there should be little more than traces of red colour except at the very base of the main stem; the intermediate condition (Gg) is represented by varying degrees of redness, both as regards the intensity of the colour and its extent. The pure types grown in adjacent beds under similar conditions furnish a convenient standard of reference.

Perhaps the simplest proof of the existence of factors for stem colour is furnished by the investigation of the F_2 generation of *genevensis × erectus, radiatus*. Both parent plants are glabrous, but differ with respect to two pairs of characters. *Genevensis* is red-stemmed and non-radiate ($GGrr$); *erectus, radiatus* is green-stemmed and radiate ($ggRR$).

Exp. 16. A colony of 234 plants was raised and brought to the flowering stage without the loss of a single plant.

Segregation took place as follows:—

For stem colour:— $GG : Gg : gg :: 53 : 124 : 57$

For the ray character:— $RR : Rr : rr :: 53 : 115 : 66$

The expectation in each case was $:: 58 : 117 : 58$

But nine types should be present in the F_2 generation. If these occur in the expected proportion, we have a fair proof that there are two pairs of factors and that they not only segregate normally, but that the method of investigation is fairly accurate. Table XVI gives the result of the analysis made from this point of view.

TABLE XVI.

	RR			Rr			rr		
	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Nos. found	10	30	13	28	57	50	15	37	14
Nos. calculated	15	29	15	29	58	29	15	29	15
Differences	−5	+1	−2	−1	−1	+1	0	+8	−1

The approximation is as close as could be reasonably expected. The greatest difference amounts to a divergence of 33 °/₀.

A similar result was obtained with the F_3 generation of the cross *lanuginosus × erectus*, the F_2 parent plant having apparently the constitution $HHYYRrGg$. The F_3 generation in this case consisted of 92 plants, and the segregation proved to be as follows:—

For the ray character:—$RR : Rr : rr :: 23 : 52 : 17$

For stem colour:— $GG : Gg : gg :: 20 : 48 : 24$

For both characters, see Table XVII.

TABLE XVII.

	RR			Rr			rr		
	GG	Gg	gg	GG	Gg	gg	GG	Gg	gg
Nos. found	5	9	9	11	29	13	4	10	2
Nos. calculated	6	12	6	12	23	12	6	12	6
Differences	−1	−3	+3	−1	+6	+1	−2	−2	−4

When we consider the small number of plants experimented with, the result agrees fairly well with the expectation. The deviations in the types $RRgg$ and $rrgg$ are however worthy of note. They may mean more than mere chance aberrations. Treating R and G as dominant, the segregation appears as follows:

Found:— $RG : rG : Rg : rg :: 54 : 14 : 22 : 2$

Calculated:—$RG : rG : Rg : rg :: 52 : 17 : 17 : 6$

There appears to be a distinct impediment to the production of green-stemmed, non-radiate plants, and therefore a tendency to the formation of a $9:3:4$ ratio.

The examination of the F_3 generation of the cross *lanuginosus × multicaulis* for stem colour was attended with difficulties. It is difficult to arrange the plants under the three types GG, Gg and gg, owing possibly to the direct colour modifications due to the environment. Much more work is required before a clear and complete explanation can be given of the results in the case of this cross. Six colonies were examined, all growing together in the open air, under conditions as similar as possible. The outside plants of each colony might have been expected to shew differences when compared with the inside ones, but none were noticed, although specially looked for. On the other hand, a certain effect of shading was very obvious; when the earlier plants were uprooted for examination from some of the beds, the later ones received considerably more light and air, and their stems quickly acquired a certain amount of red colouring matter. This kind of response is well known to botanists, and appears to be more or less independent of the colour factors for stems that we are considering here. The result of the examination of these six colonies for stem colour is presented in

TABLE XVIII.

Exp.	Culture No.	Cross	Gen.	Estimated constitution of parent	No. of plants	Types of Stem Colour			Remarks
						GG	Gg	gg	
Exp. 64	33	Lanuginosus × multicaulis	F_3	$HhRrGg$	49	13	20	16	—
Exp. 66	35	Lanuginosus × multicaulis	F_3	$HhRRGg$	96	77		19	Difficult to distinguish GG from Gg
Exp. 67	36	Lanuginosus × multicaulis	F_3	$HHYyRRGG$	49	—	49	—	All plants, reddish green
Exp. 68	37	Lanuginosus × multicaulis	F_3	$HHYYRrGg$	97	13	49	35	Colony difficult to sort. GG not sharply defined
Exp. 69	38	Lanuginosus × multicaulis	F_3	$HHYyrrGg$	92	57		35	Possibly all plants were gg
Exp. 70	39	Lanuginosus × multicaulis	F_3	$HHYYRRGg$	48	37		11	Possibly all plants were gg

It does not seem desirable to spend much time in analysing further these incomplete results. Additional experiments are necessary. Nevertheless all are of interest. Green is apparently recessive. It is certainly easier to pick out the pure greens (gg), for the pure reds are apt to pass over more or less gradually into the reddish greens.

Cultures 39 and 35 suggest the possibility of a single pair of factors; cultures 38 and 37, of two pairs of factors, giving the ratio 9 : 7. The difference between the numbers found and those calculated is considerable. For 97 plants the ratio should be 55 : 42 and not 62 : 35; for 92 plants, 52 : 40 and not 57 : 35.

Culture 36 did not segregate, although apparently of an intermediate type. It is probably to be regarded as a modified pure red (GG). The facts, as a whole, point unmistakably to segregation for stem colour taking place in the hybrid *lanuginosus* × *multicaulis*.

Culture 33 is of special interest, as there were three pairs of characters involved. Theoretically, there should have been 27 different kinds of plants in this colony. Although there were less than 50 plants, 15 of these kinds were found. The segregation for rays, stem colour and hair separately considered was as follows:—

$$RR : Rr : rr :: 11 : 25 : 12$$
$$GG : Gg : gg :: 13 : 20 : 16$$
$$HH : Hh : hh :: 14 : 20 : 15$$

The detailed analysis of this result suggests that there is possibly a gametic coupling of the form $1HG : nHg : nhG : 1hg$ where n is a fairly large number. The demonstration of such a coupling, including the determination of the value of n, involves further experiments on a larger scale, and the discussion of this result must be postponed until these have been completed.

Leaf Colour Factors.

A brief reference may be made to the factors for leaf colour, the detailed examination of which has scarcely commenced. It is certain that segregation of leaf colour characters takes place in these groundsel hybrids. The foliage leaves of *erectus* and *erectus, radiatus* are of a yellowish-green colour, those of *multicaulis* are of a dark green. Each pure type can be almost said to have its own distinctive shade of green, so that one pair of factors can scarcely suffice to explain the facts unless we assume that the leaf colour factors are affected by other factors. In the F_2 generation of the hybrid *multicaulis* × *erectus, radiatus* the leaf colour was observed to segregate, but the apparatus necessary for estimating the types exactly was not available and therefore no definite analysis was made. It may be provisionally assumed that there is a factor for leaf colour L which determines the darker leaf colour, its

absence (*l*) may then represent the yellow-green colour. It is note-worthy that in one culture of hybrids of *Senecio sylvaticus* × *S. viscosus* —a species hybrid—a number of seedlings with white cotyledons were observed, which of course soon perished. It is probable that the occur-rence of these marked the segregation of another leaf colour character, one which is very likely to occur in *Senecio vulgaris* also[1].

The most exacting and perhaps most interesting work of the last five years, consisting of a long and very tedious series of measurements of the vegetative organs, still remains to be discussed, but must form the basis of a second contribution to our knowledge of the common groundsel.

Summary.

The common groundsel, *Senecio vulgaris, Linn.*, is an aggregate species which has been found to include many segregate or ele-mentary species.

Twelve of these elementary species have been cultivated and main-tained pure and true to type for at least several generations. Six of them have been studied in detail, and are distinguished by more or less descriptive names—*praecox, erectus, multicaulis, latifolius, genevensis* and *lanuginosus*. Of these *lanuginosus* is radiate, the other five non-radiate. *Lanuginosus* was obtained from the Channel Islands, *genevensis* was collected in the vineyards near Montreux, and the others were found in the neighbourhood of Cardiff. Another form, collected near Cardiff and equally well studied, is *erectus, radiatus*—the radiate variety of *erectus*. Five other forms have proved true to type in cultures, but have been so incompletely studied that they are for the present simply designated with their place of origin—all Glamorgan localities: (1) Car-diff, (2) Burry Green, (3) Horton, (4) Cross Common, and (5) St Bride's.

It has been shewn by experiment that the radiate character of *erectus, radiatus* can be transferred by hybridization and subsequent segregation to *praecox, multicaulis, latifolius* and *genevensis*. A radiate variety of each of these elementary species has in fact been produced in this way, and is now being cultivated. In *multicaulis* there are at least three kinds of radiate varieties, with yellow, cream and fimbriate florets respectively. Hence, if we accept the usual terminology and persist in regarding *S. vulgaris* as the species, we have to make varieties of

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[1] This prophecy was fulfilled in 1912. Seedlings with white cotyledons were observed in the F_2 and F_4 generations of the cross *lanuginosus* × *praecox*.

varieties of a variety; thus, *Senecio vulgaris*, Linn., var. *multicaulis*, var. *radiatus*, var. *fimbriatus*.

Hairy varieties, in contrast to radiate varieties, are not produced so readily. The non-radiate type of *lanuginosus* has not yet been certainly obtained, nor a typically hairy *praecox, erectus* or *multicaulis*. All, or nearly all, non-radiate types are less hairy than *lanuginosus*. Consequently it is still somewhat doubtful whether the hair of *lanuginosus* can be transmitted to the glabrous types quite unchanged.

Analyses of the various segregations reveal the presence of eight or nine factors, which may be represented in the usual way, accepting provisionally, on account of its convenience, the theory of dominance and the presence and absence hypothesis; thus

(a)   Flower-factors.

1.	$R, r.$	Factor for the radiate character.	R = radiate. r = non-radiate.
2.	$C, c.$	Factor for flower colour.	C = yellow. c = cream.
3.	$X, x.$	Factor for determining the development of the cream colour.	X inhibits the development of cream colour. xc = cream. Xc = yellow.
4.	$F, f.$	Factor for the development of the fimbriate character.	F = normal rays. f = fimbriate rays.

(b)   Hair-factors.

5.	$H, h.$	Main factor for hairiness.	H = hairy. h = glabrous.
6.	$Y, y.$	Factor modifying hairiness.	Y depresses hairiness. y allows full development of hairiness.
7.	Z, z (?)	Factor affecting the stimulation of H by R.	Z reduces hairiness in R plants. z allows normal hairiness in R plants.

(c)   Stem Colour factor.

8.	$G, g.$	Factor for red and green colour in stems.	G = red. g = green.

(d)   Leaf Colour factor.

9.	$L, l.$	Factor for leaf colour.	L = dark green. l = yellow green.

These factors behave normally in segregation, except that in certain cases gametic coupling has been recognised, viz. a gametic coupling of the form $2HR : 1Hr : 1hR : 2hr$ and possibly one of the form

$$1HG : nHg : nhG : 1hg$$

where n is a fairly large number. The latter type of coupling suggests that certain combinations of characters may either be unrealizable or realizable only with difficulty.

The constitution of the pure types has not been adequately investigated, but already the results are of interest. The constitution of the twelve already referred to is given below:—

Praecox—rrCChhyyzzGGLL.

Erectus—rrCChhXXYYZZggll.

Multicaulis—rrcchhxxYYZZggLL.

Latifolius—rrhhggll.

Genevensis—rrhhGG.

Erectus, radiatus—RRCChhXXZZggll.

Lanuginosus—RRHHyyGGLL.

Cardiff Groundsel
Burry Green Groundsel
Horton Groundsel } *rrHHGG.*
Cross Common Groundsel
St Bride's Groundsel

Crosses are apparently possible between any pair of these. The cross *multicaulis* × *erectus, radiatus* gives a hybrid of the constitution *RrLlCcXx*...... heterozygotic for four pairs of factors, and there should thus be at least 18 distinct types of plant in the F_2 generation, recognisable by inspection, without further experiment. The cross *lanuginosus* × *multicaulis* gives a hybrid of the constitution *HhGgRrYy*, and there should be at least 54 recognisable types in the F_2 generation. Moreover several other factors affect the form and habit of the plants, producing still further diversity of type. Consequently there must be several hundreds of these groundsel forms still unidentified, but nevertheless recognisable. Such facts shew how inadequate the older methods of study are for the investigation not only of hybrids, but of critical species also.

After an investigation extending over six years, including the critical examination of about 10,000 groundsel plants, I still often find it very difficult to estimate, even provisionally, the constitution of a casual wild plant. Yet the methods of genetics, diligently applied, obviously give one the power to replace loose speculation and guesswork by irrefutable inductions, and so to lay down a foundation upon which the evolutionist and taxonomist can build with safety.

April 12th, 1912.

ADDITIONAL NOTE (*August* 14, 1912).

Two F_2 colonies of the cross *lanuginosus* × *praecox* of 213 and 215 plants respectively were raised during the current year, and further careful observations made of the segregations for hairiness and ray characters.

It seemed desirable for several reasons to verify the type of coupling or reduplication of the form $2 : 1 :: 1 : 2$ described at p. 260. A close study of the various types of coupling may give valuable clues as to the time, mode or place of segregation. The type represented by the above ratio has not hitherto been definitely recognised. Gregory (*J. of Genetics*, Vol. I, p. 128), however, publishes a result which he suggests may be due to coupling in one sex only, according to the ratio $7 : 1 : 1 : 7$. While one must admit the possibility and even the probability of the occurrence of differences of this kind in the sexes, such an interpretation becomes doubtful when we find that the hypothesis of a coupling of the form $2 : 1 : 1 : 2$ in each sex explains the result equally well; thus

	MG	*Mg*	*mG*	*mg*
Nos. observed by Gregory	411	98	97	78
Nos. expected when coupling is of the form $7 : 1 : 1 : 7$, and confined to one sex	417	96	96	75
Nos. expected when coupling is of the form $2 : 1 : 1 : 2$, and occurs in both sexes	418	95	95	76

The results obtained by me this year may be summarized as follows :—

	HR	*Hr*	*hR*	*hr*
Culture 23. Nos. found	128	36	31	18
Culture 24. ,, ...	132	32	25	26
Both cultures ,, ...	260	68	56	44
Expectation, on the basis of coupling of the form $2 : 1 : 1 : 2$ in both sexes. Ratio:—$22 : 5 : 5 : 4$	262	59	59	48
Expectation, on the basis of coupling of the form $7 : 1 : 1 : 7$ in one sex. Ratio:—$39 : 9 : 9 : 7$	261	60	60	47

Further experiments are necessary to determine which of these forms of coupling is actually operative in such cases. In *Primula*, such experiments would be comparatively easy ; in *Senecio*, they would be somewhat difficult If the F_1 plants are crossed with the recessive parents, thus

(1) F_1 ♀ (*HRhr*) × P ♂ (*hr*) and (2) P ♀ (*hr*) × F_1 ♂ (*HRhr*),

we should expect in *each* case (if the coupling occurs in both sexes in the form $2:1:1:2$) families of the constitution $2HR:1Hr:1hR:2hr$. If, however, coupling occurs in one sex only in the form $7:1:1:7$, one family should have the constitution $7HR:1Hr:1hR:7hr$, and the other the constitution $1HR:1Hr:1hR:1hr$.

In *Senecio*, the F_1 ♀ plants would need isolation, and the bisexual disc-florets would have to be removed while in bud. The P ♀ plants unfortunately have no ♀ flowers, and it is practically impossible to remove the stamens without injury to, or accidental pollination of, the stigmas. It would therefore be necessary to allow self- and cross-pollination to take place, and to allow for the additional hr types produced by self-pollination. The ratios to be looked for are:—

$$2:1:1:2\,;\,7:1:1:7\,;\,1:1:1:1\,;\,2:1:1:2+x\,;\,7:1:1:7+x;\text{ and }1:1:1:1+x,$$

where x represents the additional hr types produced by self-pollination. In *Primula*, where the necessary castrations are easily made, the three ratios $2:1:1:2$, $7:1:1:7$ and $1:1:1:1$ should alone occur. Small families of 16 to 32 plants should suffice to determine the type of ratio. I regret to say, however, that the pure strain of *lanuginosus* has become so weak and unhealthy—perhaps by successive selfings— that it is no longer available for experiment. The experimental proof in the case of *Senecio* cannot be furnished before the end of 1915[1].

The assumption of dominance, although often of great assistance, as in this case, in giving a clear and condensed view of an experimental result, often tends to obscure facts of importance. The nine types which result from the interaction of two pairs of characters are, I think, recognisable in these cultures.

The following table gives the numbers found and the expectation on the assumption of coupling of the form $2:1:1:2$ in both sexes. Compare also Table X, p. 259.

	RR			Rr			rr		
	HH	Hh	hh	HH	Hh	hh	HH	Hh	hh
Culture 23	35	11	1	15	67	30	3	33	18
Culture 24	47	5	0	17	63	25	2	30	26
Total (23 and 24) ...	82	16	1 !	32	130	55	5 !	63	44
Expectation. Coupling of the form $2:1:1:2$	48	48	12	48	119	48	12	48	48

[1] I find that Gregory (*Proc. Roy. Soc.* B., Vol. 84, p. 14) has made the necessary crosses and proved that coupling takes place in each sex. The exact form of the coupling, however, still remains somewhat uncertain. *Primula sinensis* appears to be a somewhat difficult type for such a determination. Upon the whole, the evidence clearly points to the occurrence in this case of the $2:1:1:2$ ratio in each sex.

Some of the discrepancies may be due to the difficulty of recognising the various types. *RRhh* plants cannot however be mistaken, yet there was only one and not 12 as expected. Although five plants are marked *rrHH*, not one of these was a typical *non-radiate lanuginosus*—the production of which was one of the chief objects of the experiment. The single *RRhh* plant was a perfect example of a *radiate praecox*.

Treating *H* as dominant over *h*, the table becomes

		RR		*Rr*		*rr*	
		HH and *Hh*	*hh*	*HH* and *Hh*	*hh*	*HH* and *Hh*	*hh*
Found	...	98	1	162	55	68	44
Expected	...	96	12	167	48	60	48

Treating *H and R* as dominant over *h* and *r*, we get (compare Table XI):

		R		*r*	
		H	*h*	*H*	*h*
Found	...	260	56	68	44
Expected	...	263	60	60	48

The process gets rid of all discrepancies of importance, but at the same time obscures the very important facts revealed by the fuller analysis—the great dearth of *RRhh* and *rrHH* plants. This is a point of interest to which students of genetics might pay greater attention. Now that the heterozygotic forms are being more acutely studied, it is probable that many similar results will be brought to light and help to increase materially our knowledge of the actual mechanism of segregation.

Fig. 28.   *RR* × 3   Fig. 29.   *Rr* × 3   Fig. 30.   *Rr* × 3   Fig. 31.   *rr* × 3

Enlarged drawings of individual flowers by Miss M. Brockington. Fig. 28—a ray floret of *erectus, radiatus, RR* ; Figs. 29 and 30—ray florets of the hybrids *praecox × erectus, radiatus* and *lanuginosus × erectus* respectively, *Rr*; Fig. 31—a disc floret of *erectus, rr*. Note the presence of the five corolla lobes in Fig. 30.

NOTES ON THE ILLUSTRATIONS (Plates XV—XVIII).

All the photographs were prepared from the crops of 1912, which included colonies of all the types referred to in the paper. Certain new features of interest were observed for the first time in 1912, such as the unexpected appearance of the single specimen of a *cream coloured, fimbriate, multicaulis* represented in Fig. 20, Pl. XVII. These are reserved for further consideration and a later report. Two points, dealing with the ray character and hairiness, deserve a brief notice here.

The examination of two large colonies of the F_2 generation of the cross *lanuginosus* × *praecox* shewed that there were present in each, two distinct RR types (Figs. 7 and 10, Pl. XVI.) and three Rr types (Figs. 25, 26 and 27, Pl. XV.). The difference in the degree of ray development is due doubtless to the presence or absence of the hair factors. Comparison of the figures 25, 26, 27, and of the figures 3, 4, 7, 8, 9, 10, and 11, will prove that the character due to the presence of the same factor may be a variable one.

The examination of the F_4 generations of the cross *lanuginosus* × *multicaulis*, in the current year (1912), led to the conclusion that the four types of hairiness referred to as H_0, H_1, H_2, and H_3 are mainly due to the existence of *three* factors for hairiness. In addition to H and Y there is a third factor which made itself evident by certain colonies from a H_2 parent segregating in the proportion $H_1 : H_2 :: 3 : 1$. H_0, H_1, and H_3 plants do not segregate apparently; $H_0 = hh$, $H_1 = HHDD$, where D represents the third hair factor, $H_3 = HHyy$. H_2 may be homozygotic and is then probably always $HHYY$. It is often heterozygotic, and may throw either H_1 or H_3 plants, but not both, in the proportions $H_1 : H_2 :: 3 : 1$ or $H_2 : H_3 :: 1 : 3$. It must not be forgotten that other factors apparently influence the development of hair (see p. 263).

Fig. 1. *Praecox.* 45 days old, photographed as it commenced to flower. The neck of the flask seen in Figs. 1, 2, and 3 is 26 mm. in diameter. The whole plant is practically glabrous.

Fig. 2. *Lanuginosus.* 103 days old, photographed as it commenced to flower. The whole plant is very hairy. The flowers are not easily recognisable in the illustration. Most botanists will, I think, be prepared ultimately to concede specific rank to the two types shewn in Figs. 1 and 2.

Fig. 3. One of the H_2 types of Exp. 67, specially remarkable for the peculiar character of the rays, which were originally recorded as Rr? but proved to be RR. A single head is shewn enlarged as Fig. 11. It will be noted that the plant differs in other respects from *praecox* and *lanuginosus*.

Figs. 4, 5, and 6. Microphotographs of single capitula of *multicaulis, radiatus, multicaulis, radiatus* × *multicaulis*, and *multicaulis*. Radiate and non-radiate forms, if glabrous, produce in all the generations one type of heterozygote only, that represented in Fig. 5. All the microphotographs in Plates XV—XVIII, are magnified about four diameters.

Figs. 7, 8, and 9. The radiate forms of *praecox, erectus*, and *genevensis*.

Fig. 10. Capitulum of *lanuginosus*.

Fig. 11. Capitulum with peculiar RR rays, liable to be mistaken for the Rr type. Compare this with Fig. 25, which is that of a Rr head.

Figs. 12, 13, 14, and 15. Side views of capitula of *multicaulis, praecox, erectus*, and *genevensis* to shew size and shape of capitula and normal length of styles. Such types set seed freely when selfed.

Figs. 16, 17, and 18.   Various long-styled types.   These are very sterile, if selfed, but
fertile to their own pollen, yielding seed quite freely when pollinated artificially with
their own pollen or that of neighbouring long- or short-styled capitula.   The capitulum
represented in Fig. 16 was taken from an ordinary plant of *generensis*.   Fig. 17 illus-
trates the type of head alone found in the sickly, hairy, individuals referred to on
p. 262, and was taken from an individual of the F_2 generation of *lanuginosus × praecox*.
Fig. 18 was taken from a plant which may almost be described as a *non-radiate lanugi-
nosus* (F_4 generation of *lanuginosus × multicaulis*).

Fig. 19.   *Multicaulis fimbriatus*, yellow.

Fig. 20.   *Multicaulis fimbriatus*, cream.

Both these are apparently ♀, the anthers producing no good pollen, hence the difficulty
in clearing up the mode of inheritance of the fimbriate character.

Figs. 21, 22, 23, and 24.   These figures illustrate the various types of hair development
denominated H_0, H_1, H_2 and H_3 respectively.   The portions were carefully selected
from young stems at the same phase of development.   21 is from *multicaulis*; 22 from
the Burry Green groundsel (in 1911 this type had the higher grade of hairiness H_2);
23 is from a very late flowering type (F_4 generation of *lanuginosus × multicaulis*);
and 24 is from *lanuginosus*.

Figs. 25, 26, and 27.   Three types of *Rr* rays produced in the F_2 generation of *lanugi-
nosus × praecox*.   In the F_1 generation there is one type only, that represented in
Fig. 26.   Compare Fig. 25 with Fig. 11.   In such cases as these mistakes would be
made unless the influence of the hair factors was considered and allowed for.

Figs. 28, 29, 30, and 31 (in text, p. 274).

Fig. 32 (cp. Pl. XV).   A nearly ripe head of *praecox, radiatus*, shewing the revolute ligules
of the old withered florets, a condition often erroneously credited to the flowers at the
period of pollination.

University College of South Wales
and Monmouthshire, Cardiff.
July 22, 1912.

Fig. 1.

Fig. 2.

Fig. 25.
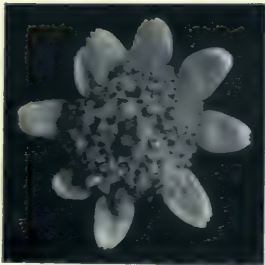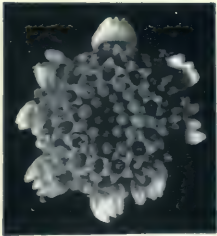
Fig. 26.

Fig. 3.

Fig. 32.
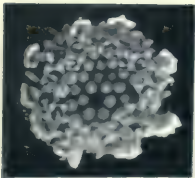
Fig. 27.

Fig. 4. Multicaulis, radiatus.   Fig 5. Hybrid.   Fig. 6. Multicaulis.
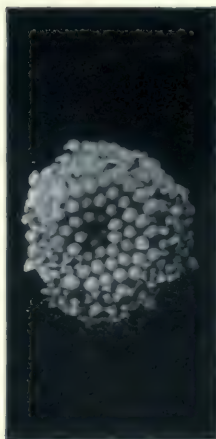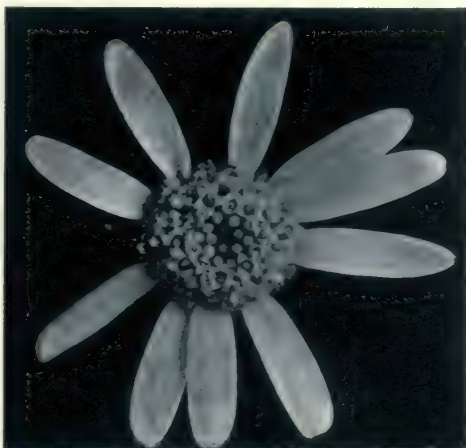
Fig. 7. Praecox, radiatus.   Fig. 8. Erectus, radiatus.
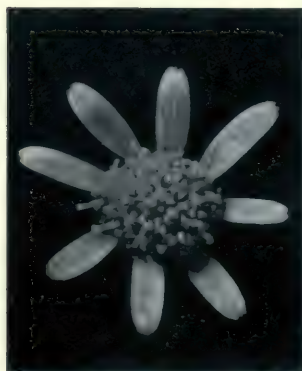
Fig. 9. Genevensis, radiatus.   Fig. 10. Lanuginosus.   Fig. 11.

Fig. 12.   Multicaulis.   Fig. 13.   Praecox.   Fig. 14.   Erectus.   Fig. 15.   Genevensis.
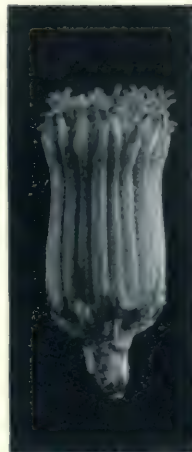
Fig. 16.   Genevensis.   Fig. 17.   Fig. 18.
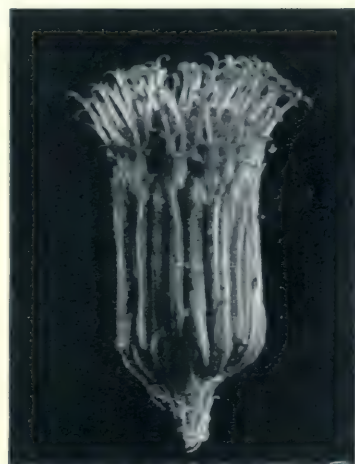
Fig. 19.   Fimbriate type, Yellow.   Fig. 20.   Fimbriate type, Cream.

PLATE XVIII
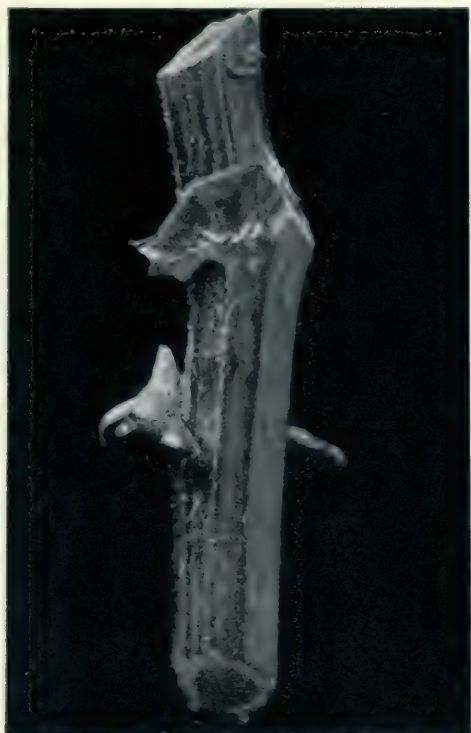
Fig. 21. H_0 Multicaulis.

Fig. 22. H_1 Burry Green Groundsel.

Fig. 23. H_2, F_4 of Lanuginosus × multicaulis.
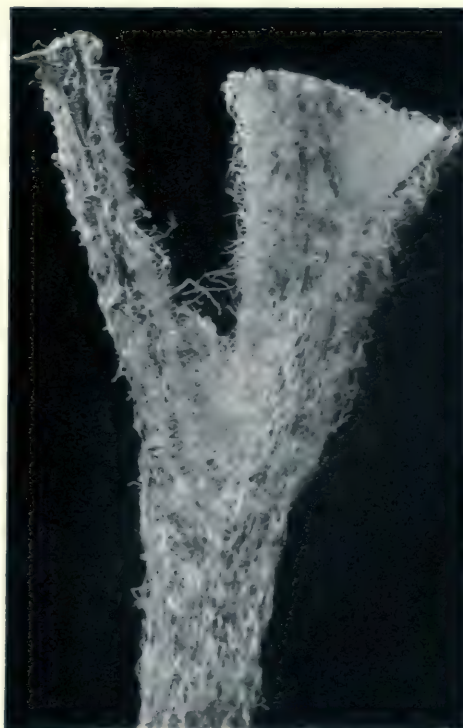
Fig. 24. H_3 Lanuginosus.

THE RÔLE OF OXYDASES IN THE FORMATION OF THE ANTHOCYAN PIGMENTS OF PLANTS.

By FREDERICK KEEBLE, Sc.D.,

Professor of Botany, University College, Reading,

AND E. FRANKLAND ARMSTRONG, D.Sc., Ph.D.

CONTENTS.

I. INTRODUCTION AND METHODS.

THE progress of discovery with respect to the genetics of colour has been so rapid of recent years as to outpace that of our knowledge of the chemistry of pigment-formation in plants and animals. Thanks to the labours of Bateson, Miss Saunders, Punnett, Gregory, Baur and many

other workers, the modes of inheritance of colouration are known with detailed accuracy in many species.   This rapid advance has stimulated enquiry into the chemical aspect of the phenomena of pigmentation, and the investigations of Miss Durham (1904), Palladin (1908), Combes (1910), Miss Wheldale (1910) and Gortner (1910) have resulted in the production of a large body of evidence in favour of the current hypothesis that pigmentation is the outcome of the action of oxydase on chromogen.

We propose in the present communication, some of the results of which have been published in abstract form elsewhere (Keeble and Armstrong, 1912), to present further and as we think convincing evidence in favour of this hypothesis, to describe the distribution of oxydases in *Primula sinensis* and certain other plants, to produce experimental evidence of the fact that the pigment-forming activity of oxydases may be inhibited in various races of plants (Dominant White races) and to show that the oxydase-content of a plant is modified by such external conditions as light and darkness.

The Rôle of Oxydase in Pigment Formation.

Inasmuch as the literature relating to oxydase is very extensive and has been summarised recently in monograph form by Kastle (1909) and by Clark (1911) we need do no more here than state briefly the hypothesis of Bach and Chodat, the acceptance of which is implied in the present communication.

This hypothesis holds that an oxydase is of a dual nature.   The constituents of an oxydase are a peroxydase and a peroxide.   The peroxide behaves as an activator to peroxydase in the sense that it supplies the latter with oxygen which may then be transferred to an oxidisable body.   This activating action may be effected by hydrogen peroxide.   The nature of the peroxides of the plant is unknown.

Peroxydases are classed generally with enzymes ; they differ, however, from hydrolytic enzymes in that they undergo destruction, at least *in vitro*, in consequence of the exercise of their oxidising function.

There seems reason to believe that iron, manganese and other elements in combination with organic substances play some part as yet obscure in the oxidation processes of the plant.

The hypothesis that oxydases are concerned in the formation of plant-pigments was first suggested by Pick (1883), and evidence in favour of this hypothesis has been put forward by many observers—

particularly by Palladin and Miss Wheldale. The latter investigator
(1910 *loc. cit.*) has formulated in a clear and schematic manner the
following hypothesis as to the course of events which leads to the
formation of pigment in plants. The colourless chromogen by the
oxidation of which pigment is produced occurs in the plant as the
constituent of a glucoside. In this combined form it resists oxidation.
Enzymes of the emulsin type hydrolyse the glucoside and liberate
chromogen which is then oxidised by atmospheric oxygen made active
by the oxydase. Thus an anthocyan or soluble sap pigment is formed.
Such anthocyan pigments, which are common in the flowers and other
parts of plants, are of very different chemical constitution and origin
from the plastid pigments which are also of common occurrence. We
are concerned here only with the former. The anthocyan pigments
are generally red, violet or blue.

The chemical nature of the anthocyan pigments is obscure. They
are regarded by Miss Wheldale as flavone derivatives which are known
to be widely distributed in plants in the form of glucosides.

An illuminating paper suggesting a mode of formation of organic
pigment has been published recently by Chodat (1912). He shows
that when tyrosinase acts on *p*-cresol in the presence of one or other
of the products of protein hydrolysis a series of diversely coloured
pigments resembling the natural pigments is produced. Chodat believes
that a pigment of a given kind is produced by the action (1) of an
oxydase on (2) a phenolic compound in the presence of (3) an amino
compound, and suggests that, as the compositions of the phenolic
compound and the amino compound vary, so the composition and hence
the colour of the pigment varies.

Gortner (1910 and 1911) working independently has already pub-
lished conclusions which in some respects are similar to those of Chodat.
In a series of valuable papers Gortner has produced evidence in support
of the view, put forward previously by Miss Durham (1904) and other
observers, that the black or brown melanin pigments of animals are
formed by the action of tyrosinase on a product of protein hydrolysis,
namely tyrosine. He holds further, as described in the text, p. 299, that
inhibition of pigment formation may be brought about by the con-
version of part of the tyrosine into a closely allied substance which is
not only itself resistant to tyrosinase but also checks the action of
that substance on tyrosine. Gortner applies this hypothesis to the
elucidation of the suppression of pigment which is known on genetical
grounds to occur in certain white forms of plants and animals.

Such forms have been investigated by Bateson and are described as dominant whites.   Although they may be identical in appearance with true albinos they differ fundamentally from the latter in gametic composition and hence in genetical behaviour.   The true albino lacks colour, the dominant white possesses the power of pigment formation, but that power is held in abeyance by an inhibitor.

Inasmuch as the phenomenon of dominant whiteness is dealt with somewhat fully in the text, we need not devote attention to it here, beyond remarking that attempts to demonstrate by chemical means the truth of the Mendelian hypothesis with respect to dominant whites have not as yet been wholly successful.

Miss Wheldale (1910), whose researches have contributed so much to our knowledge of this subject, regards inhibition as due to the action of deoxidising substances such as sugars, tannins, and the like, and has brought forward experimental evidence in favour of this opinion.   In cases of partial inhibition, which is illustrated in *P. sinensis* by the dominance of pale over more deeply coloured varieties, it is suggested that the effect is due to a reductase.

Methods.

The methods in general use for detecting the presence of oxydase in plants depend on the addition of a colourless chromogen to the solution or extract obtained from the plant.   If the result of the operation is to produce a pigment it is concluded that oxydase is present.   If the pigment be produced only after the addition of hydrogen peroxide the oxidising substance is described as a peroxydase.   The method has many disadvantages.   Certain of the chromogens used as tests for oxydases undergo oxidation with more or less rapidity when exposed to the air.   The extract or solution may contain reducing or inhibitory substances which interfere with the reaction, and in any case the localisation of the oxydase in the tissues of the plant is wellnigh impossible by this method.

Clark (1911) has carried out recently an elaborate series of tests with different chromogens and finds that certain of them may be used for the microchemical determination of oxydase in plant tissues.   Among the reagents used by Clark are guaiacum, phenolphthalein and α-naphthol. Pyrogallol in the presence of glucose is employed by Chodat.   Schreiner has obtained valuable results with respect to the oxydases of living roots by the use of benzidine.

As the result of an extended trial of the many reagents which have been employed for the purpose of demonstrating oxydase we find that the most serviceable for our purpose are α-naphthol and benzidine. We make use of the fact demonstrated by one of us (Armstrong, 1910) that substances dissolved in alcohol penetrate rapidly into plant cells, and the method which we employ is as follows:

In the case of benzidine, a 1 per cent. solution is made in 50 per cent. alcohol which is then diluted with so much water as not to cause a precipitate. The object to be examined, for example, an intact corolla or a section of a petal of *P. sinensis*, is taken from the plant and placed immediately in the reagent in a corked specimen tube. The tube is incubated at 37° C., and if no direct oxydase reaction is obtained the material is removed from the tube, washed lightly with water and treated with 1 to 2 drops of 10 vols. hydrogen peroxide, or the latter reagent may be added directly to the tube containing benzidine. In the case of α-naphthol, the solution may be used of even less alcoholic strength. Sections may be prepared either with a dry razor or with one moistened with a solution of cane sugar of suitable strength. No inhibitory action appears to be produced by this substance. The first effect of treating coloured flowers with either of these reagents is a decolourisation of the pigmented parts. This decolourisation appears to be due to the action of a catalase, but we have not made it a subject of special investigation.

As we show in detail in Section II. the result of treating a coloured or recessive white flower of *Primula sinensis* is the demonstration of peroxydase in the epidermal layer of the petals and also in the bundle sheath which surrounds and accompanies the veins in their finest ramifications.

Preparations of a considerable degree of permanence may be made for demonstration purposes in the following manner:—The flower in which the oxydase reaction has developed is washed well in running water, the corolla tube is cut away, the petals floated on water, whence they are transferred by means of filter paper to a glass lantern slide. The superfluous water is removed by means of filter paper, and dry filter papers are placed over the flattened flower. A glass slide is laid on the filter paper, the preparation is placed in the incubator at 37° C. and pressed down by means of a weight. After a day or two, when the preparation has become dry, it is treated as a lantern slide, bound and kept in a dark place. The degree of permanence seems to depend first, on the thoroughness with which the preparations are washed,

and second, on the extent to which they are preserved from the access
of light.   Many of our preparations with benzidine retain their
colour for months, but the colours produced by the use of α-naphthol
may show signs of fading within a shorter period.   Sections may be
mounted either in glycerine jelly or may be taken up rapidly through
the alcohols and mounted in Canada balsam.

In the case of large objects such as fruits the reagent may be painted
on to the surface to be studied and the reaction examined by reflected
light.

It is noteworthy that, as we show in the text and illustrate in
Figs. 4 and 5, Plate XIX, the only reagent which gives a satisfactory
reaction with the epidermal oxydase is benzidine, and that benzidine
and α-naphthol discriminate as it were between epidermal and bundle
oxydases.   The former is picked out by benzidine but is untouched or
almost untouched by α-naphthol.   The latter gives strong reactions
with α-naphthol and benzidine.   The behaviour of α-naphthylamine is
very similar to that of α-naphthol.

The colour of the reaction with benzidine is a rich brown due to the
deposition of the oxidised product in the cells.   Under certain circum-
stances, and in early stages, this reagent produces a blue or blue-green
colour which however passes more or less rapidly into brown.   The
reaction with α-naphthol takes the form of a delicate lilac blue or
lavender colour, and that with α-naphthylamine, a pink.

It remains to mention that the reagents should be made up
fresh for use and that the flowers or other subjects to be investigated
should be fresh, uninjured, and in good growing condition.   Late formed
flowers, for example, are apt to give uncertain results and this is in
conformity with the fact that such flowers show often considerable
departure from their normal colouration.   The length of time of
exposure to the several reagents varies with the object and can be
decided only by trial.   In the case of sections, the reaction takes place
almost immediately ; but solid objects such as whole corollas require to
be incubated for 1, 2 or more hours.

II. The Distribution of Oxydases[1] in Plant Tissues.

A. *The Oxydases in the Vegetative Members of P. sinensis.*

The methods described in the previous section permit of the mapping out of oxydases in the tissues of a plant with very considerable accuracy. Hence it should be possible, by determining the distribution of pigment and of oxydase in a given species, to obtain evidence as to the validity of the hypothesis that the formation of anthocyan pigment depends on the action of an oxydase on a chromogen. For, if oxydase is specifically concerned in pigment formation a certain parallelism is to be expected between the distributions of pigment and of oxydase.

Before describing the results of our observations on the localisation of oxydase in the tissues of *Primula sinensis* it is necessary to point out that we must not expect to find an exact coincidence of pigment and oxydase in each and every variety of this species. For, as shown by Bateson, Gregory, and others, the range of pigmentation in both vegetative and floral members of *P. sinensis* is very considerable. Some varieties have white flowers and others have coloured flowers of widely differing depths and shades; some varieties possess green stems, others reddish stems, and others, again, deep red stems.

Certain white-flowered, green-stemmed varieties, such for example as Snow Drift (see Gregory, 1911) are lacking altogether in anthocyan pigment. In other green-stemmed varieties the small amount of sap-pigment which they contain is confined to special parts of the plant, for example the roots, root-stocks, and bases of the petioles where it occurs either in the epidermal cells only or in the sub-epidermal cells as well. Of such minimally pigmented varieties, some possess no pigment in their other vegetative parts, for example the flower-peduncle, others, for instance, Sirdars, contain anthocyan pigment in isolated epidermal and sub-epidermal cells of the flower-peduncle.

Between the pure green-stemmed varieties and those with reddish stems is a series of forms characterised by a progressively widening distribution of epidermal pigment in the vegetative members; and in the reddish stemmed plants the pigmentation is wellnigh continuous throughout the epidermal layer. At the other end of the colour series are the dark red-stemmed varieties in which anthocyan pigment is

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[1] For the sake of brevity we use the term oxydase to connote both peroxydase and oxydase and reserve for sub-section G (p. 304) the description of the distribution of these bodies in the several tissues.

much more widely distributed than in the green and reddish stemmed forms. For example the flower-peduncle of the dark red-stemmed variety Mt. Blanc Star contains pigment not only in the epidermis and in three or more outer layers of the cortex but also in the tissues of the stele (vascular cylinder).

If the investigator has these facts of variety of pigment distribution in mind he will not be surprised to discover when he proceeds to determine the distribution of oxydase that, although oxydase is present in every pigmented cell, it is not necessarily confined to these cells. This fact might appear at first sight to militate against the hypothesis which associates pigment formation with oxydase action; but further consideration shows that it does not. For it is evident that oxydase and chromogen, though they interact to form pigment, may be produced independently one of the other and therefore the failure of certain tissues, rich in oxydase, to produce pigment may well be due to the absence of chromogen. Or, to express the idea in other terms, chromogen and not oxydase is the factor the lack of which limits pigmentation in *P. sinensis*. The evidence offered by the known facts of the Genetics of this species lends weighty support to this conclusion and indeed leads directly to the expectation that one of the two agents concerned in the production of anthocyan pigment is more generally distributed than the other.

For if the two agents, chromogen and oxydase, were equally susceptible of restriction of distribution in the plant then it would be reasonable to expect that two types of green-stemmed plant exist; one, green-stemmed because it lacks chromogen, the other, because it lacks oxydase. In point of fact, and in spite of the large amount of breeding work which has been carried out with this species, only one type of green-stemmed plant is known in *P. sinensis*.

The evidence from Genetics in support of this statement is clear. Some plants, for example Stocks and Sweet Peas, show by their genetical behaviour that either of two factors for pigment production may be lacking from a variety.

In such plants, as Bateson, Miss Saunders and Punnett (1906) have proved, it is possible, by mating white-flowered individuals of the two types, to bring the complementary colour factors together and thus to produce a reversionary, coloured F_1 generation. But with *P. sinensis* a like result has never been obtained. A pure green-stemmed variety mated with any other similar variety gives rise to a green-stemmed F_1 generation, and pure breeding white-flowered varieties, each of which

lacks a factor for colour, give rise when crossed with one another to white-flowered offspring only.

The simultaneous study of the genetical behaviour of *P. sinensis* and of the distribution of oxydases in the tissues of the plant provides an explanation of this behaviour. For the latter line of inquiry demonstrates that all varieties of *P. sinensis*, including the pure green-stemmed varieties, contain oxydase in certain of their tissues. Whence it follows that at least enough oxydase for a definite amount of pigment production is a possession common to all Chinese primroses. We have in this fact the explanation of the failure of green-stemmed varieties to yield a red-stemmed F_1 when crossed with one another. It is also clear why the cross, green-stemmed variety by reddish-stemmed variety, yields a reddish-stemmed F_1 generation. Thus if we represent the chromogen producing factor by **C**, its absence by **c**, and the oxydase producing factor by **O**, and its absence by **o**, then green stem × reddish stem

$$= \textbf{Oc} \times \textbf{OC},$$

and $$F_1 = \textbf{OOCc},$$

and since both chromogen and oxydase are present in F_1 the plants of that generation are pigmented.

F_1 plants of this kind produce gametes **OC** and **Oc**, and hence when such plants are self-fertilised the F_2 generation is of the typical monohybrid kind and consists of 3**OC** : 1**Oc**, i.e. 3 reddish-stemmed plants : 1 green-stemmed plant.

As we shall show immediately there is a similar agreement between the results of genetical inquiry and those obtained from the study of oxydase distribution in the case of red-stemmed varieties.

But the broad features of the relation between oxydase and pigment may be seen in an equally striking manner when they are viewed from another standpoint.

If, instead of fixing our attention on one variety only, we consider the distribution of pigment and oxydase in the different coloured varieties we obtain clear evidence of the truth of the hypothesis that oxydases play a part in pigment formation. For when we adopt this procedure we discover that, as we pass stage by stage from the less pigmented to the more pigmented forms, the new additions of pigment occur in those tissues which in unpigmented and pigmented forms alike are most generally rich in oxydase. In other words, the cells which in general contain most oxydase are prepotentially pigment forming cells. Thus, although in pure green-stemmed varieties neither the ordinary

epidermal cells nor those of the epidermal hairs contain pigment, these elements give pronounced oxydase-reactions and they constitute the tissue to which pigment is confined in those pale reddish-stemmed varieties which have a minimum of pigment. Next to the epidermis, the sub-epidermal layer is, of all tissues outside the stele, richest in oxydase, and it is in the sub-epidermal layer that pigment occurs in the varieties somewhat more pigmented than those which belong to the category of pale reddish-stemmed forms. In the case of green-stemmed and reddish-stemmed varieties, the distribution of oxydase in a member of the series is indicative of the distribution of pigment in the next higher member of that series.

It is only in dark red-stemmed varieties, e.g. Mt. Blanc Star, that the pigment extends to any considerable depth into the cortex. These varieties are remarkable also for the fact that their anthocyan pigments are not limited to the epidermal and cortical tissues systems, but occur also in those of the stele (vascular cylinder). As is the case with all pigmented tissues of the epidermis and cortex, the pigmented tissues of the stele give clear evidence of a high oxydase content.

Therefore we have a further means of testing the hypothesis; for by analogy with the distribution of oxydase in the progressively less pigmented members of the colour series we may expect to find that the localisation of oxydase in those forms which are without pigment in their stelar tissues coincides with that of the pigment in the corresponding tissues of the dark red forms.

Appropriate examination shows that this anticipation is realised. For example, the pigment in the stelar tissues of the flower-peduncle of a dark red-stemmed plant occurs in the pericycle and in patches of pith cells which lie against the protoxylems of the wood; and these, together with the phloem, are the tissues which are rich in oxydase. In the root of a dark red-stemmed plant, large quantities of pigment occur in the pericycle, in the medullary rays which extend from near the periphery of the stele almost to the centre, and also in the phloem. The distribution of oxydase in the stele of the green and reddish varieties is very similar to that of the pigment of the red varieties. Thus the flower-peduncle of a green-stemmed plant contains oxydase in considerable quantity in the pericycle, phloem, and in the pith cells which abut on the protoxylems.

We conclude therefore that those tissues which in non-pigmented forms are richest in oxydase are the tissues in which in coloured forms pigment makes its appearance.

Although there can be no doubt but that this broad relation exists between oxydase and potentiality of pigment formation, it is not to be concluded that all varieties of *P. sinensis* have identical oxydase content.

Simple observations and experiments suffice to show that the extent of distribution of oxydase differs in different varieties. For example, in the superficial tissues of the flower-peduncle of Sirdar, oxydase is limited exclusively or almost exclusively to the epidermal layer; whereas the corresponding tissues of other green-stemmed varieties give a well marked oxydase reaction in both epidermal and sub-epidermal layers. There is good reason to believe that chloroplasts act as inhibitors of oxydase-formation in a cell and it is noteworthy that the sub-epidermal layer of Sirdar is specially rich in chloroplasts. Again in red-stemmed varieties, the petioles, which together with the roots appear to be the members richest in oxydase, are so rich in both chromogen and oxydase that pigment occurs in practically every cell; in green-stemmed plants, though the roots are rich in oxydase, the cortical cells of the petioles give no oxydase reaction. These phenomena and others to be referred to immediately lead us to the opinion that such localisation of oxydase as occurs in *P. sinensis* is at least in large measure a phenomenon of inhibition. We shall see in a later section, that, as postulated by Mendelians, flower colour may be inhibited. We know that the result of crossing a dark red- and a reddish-stemmed plant is the production of a reddish F_1, and that the F_2 generation from this cross consists of 3 reddish : 1 dark red-stemmed; that is, dark red stem behaves as a simple recessive to reddish stem. Nevertheless, as we have just seen, red stem differs from reddish stem in possessing pigment in certain cortical and stelar tissues which are not pigmented in the reddish-stemmed plants. This being so, it would be expected that the presence of pigment would be dominant to the absence and hence that red stem would be dominant to reddish stem. Since the reverse is the case we can scarcely escape the conclusions that reddish stems contain an inhibitor and that this inhibitor is not powerful enough to suppress pigment formation altogether though it suffices to suppress it in certain tissues.

It remains to ask whether partial inhibition of pigment formation is to be attributed to inhibition of the action of oxydase on the chromogen or to inhibition of the processes which lead to the formation of chromogen. We are not in a position to answer this question with respect to stem colour though, as we show in the following section, we can answer it

20—2

with respect to inhibition of pigment formation in the flower (see page 301). Some of the evidence which we possess as to the nature of the inhibition of pigment formation in the vegetative parts leads us to favour the view that this inhibition applies to the production of chromogen rather than to the activity of oxydase. This evidence is derived from the oxydase reaction given by the outermost cortical layer and various tissues of the stele of certain green-stemmed varieties. Instead of no, or at most a weak, reaction, which is to be expected if substances inhibiting oxydase action are present, the tissues just enumerated give a particularly strong oxydase reaction and hence it would appear probable that these cortical and stelar tissues contain an inhibitor which is capable of preventing the production of chromogen.

On the other hand the cortical tissues of the petioles of certain green-stemmed varieties give no reaction for oxydase although the corresponding tissues of red-stemmed forms are extraordinarily rich in that substance, and in this instance the failure to give the reaction for oxydase may be due to the inhibition of the latter substance.

Whether inhibition be of the nature of the suppression of oxydase activity, as we know it to be in the dominant white flowers of *P. sinensis*, or whether it consist in the prevention of chromogen production, it follows that negative results with respect to the oxydase content of a given tissue must be accepted with caution : this applies, of course, not only to qualitative results such as those with which we are dealing, but also and with even greater force to those obtained by quantitative estimates of the oxydase content of plant juices. Elaborate methods are in use for this purpose (cf. Bunzel, 1912) but, unless they are associated with methods for removing any inhibitors which may be present, the results which they yield must be accepted with reserve.

To sum up our observations on the distribution of oxydase in the vegetative parts of *P. sinensis*: We find that the methods described in Section I serve for the faithful mapping out of oxydases in the several tissues of the plant : that this mapping out leads to the conclusion, that although oxydase is more widely distributed than chromogen, the distribution is in conformity with that required by the oxydase-chromogen hypothesis and that, owing to the existence of inhibiting substances, caution must be exercised in interpreting the negative results obtained by the use of oxydase reagents as proof of the absence of oxydases.

B. *The Oxydases of the Flower of P. sinensis.*

(1) *Self-Coloured Varieties.*

The oxydases of the vegetative parts of *Primula sinensis* are located in two groups of tissues; one group—the epidermal—is superficial, the other—the stelar—is deep seated. The "epidermal" oxydase is confined to the epidermis in certain green-stemmed varieties but extends to the sub-epidermal layer in reddish-stemmed varieties and reaches its widest distribution in the dark red-stemmed races in the peduncles of which oxydase occurs not only in the epidermis but also in the two or three outer layers of the cortex. Hence it follows that the epidermal oxydase is separated widely by the intervening cortical cells from the bundle oxydase.

The epidermal and bundle oxydases of the vegetative members have their counterparts in the flower; but, inasmuch as the cortical tissues of the corolla consist only of some two layers of flattish cells, the epidermal and bundle oxydases of the petals lie in close proximity with one another. Nevertheless, and in spite of their proximity, it is possible to demonstrate macroscopically the presence of both oxydases in the petals. The discrimination between the two oxydases is aided by the fact that they do not react in precisely the same way to our reagents—α-naphthol and benzidine. The former reacts much more quickly and in most cases exclusively with the bundle oxydase to produce a lavender blue colour which picks out the veins in exquisite detail and for the most part leaves the epidermal oxydase unaffected. The selective action of benzidine is less precise. This reagent reacts with the epidermal oxydase to produce a rich brown colouration of the superficial layer of the petals and also produces a similar though darker colouration in the veins.

Examples of the colour reactions which are obtained by the use of these reagents are given in Figs. 2, 4, 5, 11, 12, 14, Plate XIX.

The coloured (blue) flower shown in Fig. 1 yields with benzidine the reaction illustrated in Fig. 2. Recessive whites give a precisely similar reaction (see Fig. 5) with this reagent. Unlike benzidine, which reacts with epidermal and bundle oxydase, α-naphthol reacts with the latter only both in the case of coloured and of recessive white flowers. Hence as shown in Fig. 4 the veins stand out prominently on an almost unstained ground.

The central part of the corolla of these varieties is characterised, as are many other varieties of *P. sinensis*, by a yellow eye, the colour of which

is due, not to anthocyan, but to plastid pigments; and it is a noteworthy and general fact that the region of the yellow eye shows no oxydase reaction except in the epidermal hairs which give with benzidine a deep brown-black colouration. The failure of the oxydases of this region of the eye to react with α-naphthol or benzidine is to be attributed to the inhibition of oxydase by the chloroplasts.

We have investigated the oxydase contents of the corollas of many other colour varieties of *P. sinensis*, e.g. Crimson King, Coral Pink and Giant Red among the reds; various magentas and lavenders, e.g. Giant Lavender; and Czar, Cambridge Blue, etc. among the blues, and though the extent of the reaction of epidermal and of bundle oxydases varies considerably in the several varieties it is characteristic of them all.

Of white-flowered varieties of *P. sinensis*, genetical research has shown that there are two kinds, which are known respectively as Recessive Whites and Dormant Whites.

(2) *Recessive White Varieties.*

The Recessive Whites show by their behaviour when crossed with coloured varieties that they lack a factor for colour. When crossed with a coloured variety they yield a coloured F_1 which on self-fertilization gives rise to an F_2 generation composed of 3 coloured : 1 white.

The usual and evidently proper interpretation of this result is that recessive whites lack a factor for colour which is possessed by the pigmented varieties. The cross is therefore to be represented thus:

$$c \times \mathbf{C}$$
$$F_1 = \mathbf{C}c$$
$$F_2 = 3\mathbf{C} : 1c$$
$$= 3 \text{ coloured} : 1 \text{ white.}$$

As we should expect from our study of the oxydases of the vegetative members of *P. sinensis* that which is lacking from recessive white flowers is not the oxydase forming factor but the factor for chromogen production. Treatment of the petals with benzidine demonstrates that this expectation is correct, for, as indicated already, the result of the treatment is a well marked oxydase reaction in both epidermis and veins (Plate XIX, Fig. 5). Whence we conclude that since the corollas of recessive whites contain both epidermal and bundle oxydases their lack of colour is due to the absence of the factor for chromogen production. Of all the varieties the flowers of which we have examined only those belonging to three categories show any departure from the

general rule that epidermal and bundle oxydases are present in the corollas.

These three categories are the dominant white, blue with white inhibitory patches and the flaked varieties.

(3) *Flaked (Ever-sporting) Varieties.*

We will deal first with the flaked varieties. Snow King and Mt. Blanc Star, the varieties of *P. sinensis* which we have investigated, bear white flowers marked more or less prominently by splashes of magenta. Sometimes a whole petal is magenta coloured and sometimes a magenta flower appears among neighbouring magenta flaked flowers. Although the amount of flaking varies very considerably the races breed wellnigh true to this habit. Thus Mt. Blanc Star produces offspring the great majority of which are flaked; but ıt throws occasionally a plant all the flowers of which are magenta coloured. These magenta flowered plants may bear darker flakes of magenta on a lighter coloured ground and the numbers in which they appear are said to be about two per cent. The genetics of these flaked forms which is in course of investigation by one of the present writers (see Keeble, 1910 a) need not concern us here except in so far that it provides evidence of the existence in the petals of a partial inhibitor of pigment formation.

Magenta-flaked white flowers of Mt. Blanc Star give much fainter and far less regular oxydase reactions than are exhibited by any coloured or recessive white varieties. They provide also a good illustration of the relation between oxydase and pigmentation. For, as is exemplified in the text-figure (Fig. 1), if, before treating the flower with the oxydase reagent, the pigmented areas are recorded it is found that the distribution of oxydase coincides very closely with that of the pigment. In the case depicted in the text-figure a flower was chosen which had, in addition to certain irregular magenta flakes, one petal of a uniform magenta colour. A comparison of the distribution of pigment with that of the oxydase shows that the magenta petal gave a well marked oxydase reaction, that the magenta patches on other petals were also the seat of a fair amount of oxydase and that the white areas gave no reaction.

The absence of oxydase-reaction from the unpigmented parts of the flower is noteworthy because it is the first piece of evidence which we have been able to produce to show that failure to form pigment may be connected with failure to yield the reaction for oxydase.

Flowers of this kind, characterised by a considerable degree of fluctuation of colour and by the localisation of such colour as they may have

in flakes or spots, are very common among cultivated plants, for example, Azaleas, Sweet Williams, Stocks and Carnations. They are known as ever-sporting and their genetical behaviour is difficult of interpretation. The observations of which we have just given a brief account indicate quite definitely that in the case of the white magenta-flaked flowers of Mt. Blanc Star the ever-sporting habit is associated with irregularities in amount or activity of oxydase in the tissues of the petals.
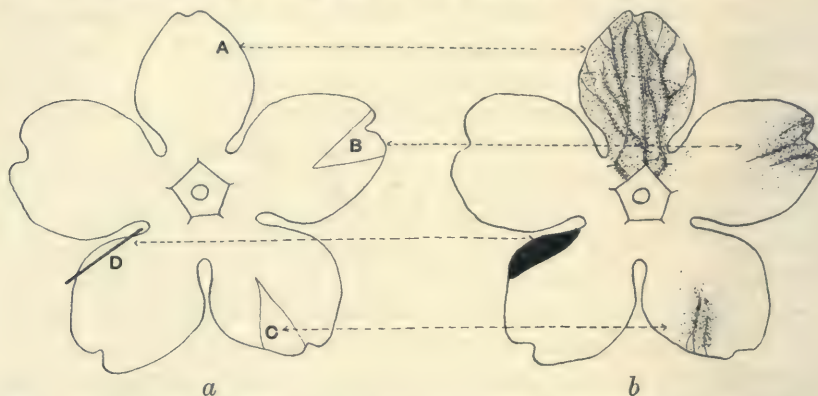
Fig. 1. The coincidence of peroxydase with pigment in the white magenta-flaked flower of Mt. Blanc Star. Diagrammatic. The distribution of pigment in the several petals was recorded with reference to the incision indicated at *D* (*a*). Petal *A* was uniformly magenta coloured, *B* and *C* indicate the position of magenta flakes. The peroxydase distribution is shown in fig. *b*. The copious wound peroxydase is indicated by the black patch, the position of which coincides with *D* which marks the place of the wound. (See text and Text-figure 5.)

Inasmuch as the problems presented by ever-sporting varieties are of considerable interest, we have extended our inquiry with respect to them to other species of plants. We were the more anxious to do so because, except for the difficult case of white magenta flaked varieties, in which, as we have seen, the amount of oxydase-reaction is small, we had discovered no race of *P. sinensis* the flowers of which lack oxydase. Now as pointed out already, this fact, though it might be predicated from our knowledge of the genetics of *P. sinensis,* is less likely to obtain with respect to certain other plants. Thus, the genetical behaviour of Sweet Peas is such that two distinct factors for colour must be assumed if this behaviour is to be accounted for in terms of Mendelian hypothesis. Hence the suggestion is bound to present itself that the two factors in question are an oxydase-producing and a chromogen-liberating factor. We have therefore an added reason in seeking among plants other than *P. sinensis,* for an example of a flower which lacks oxydase.

C. *The Oxydases of Dianthus barbatus (Sweet William).*

(1) *Ever-sporting Varieties.*

The nearest approach to a flower of this description which we have
found so far is that of the Sweet William (*Dianthus barbatus*). This
species presents a wide range of variety of flower colour. Pure white-
flowered races as well as races with purple, red and salmon colour are to
be found in almost every garden.   In addition to races with pure white
flowers, others occur in which the flower is white except for a ring of
fine pink dots or lines across the middle of the petals.   Ever-sporting
varieties are likewise common.   They bear on one and the same inflor-
escence flowers of very different colours.   Thus in the race with which
we have experimented the colours of the flowers of a single plant were
deep magenta, strawberry, pale and streaked pink on a white ground
and white.   In some of the white flowers a small amount of rose coloured
pigment occurs a little below the middle of the limb of each petal,
and in others the amount of pink colouration is so small that the
flowers are almost pure white.   If this series of differently coloured
flowers of an ever-sporting variety of Sweet Williams be examined
for oxydase it is discovered that the amounts of oxydase present in the
petals of the several members of the series are strictly proportional to
the amounts of pigment in those members.

As illustrated in Text-figure 2, all the coloured forms contain both
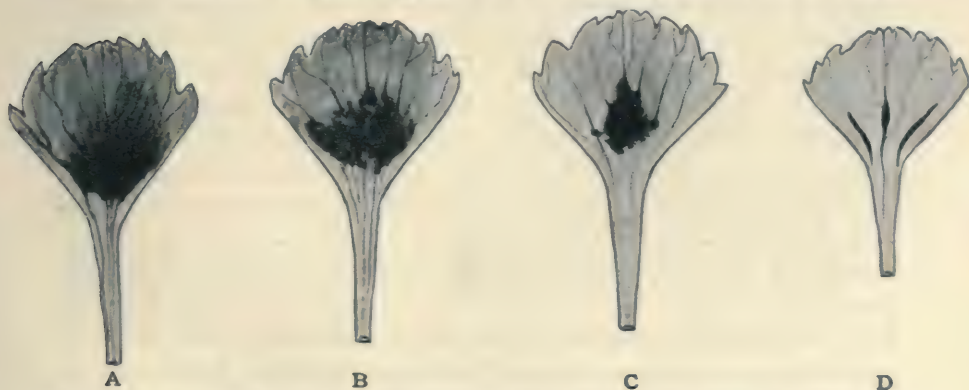
Fig. 2.   The oxydase (benzidine) reactions of the flowers of an ever-sporting variety of
Dianthus barbatus (Sweet William), illustrating the parallelism between pigmentation
and oxydase content.

Flower-colour :   A.   Deep red magenta.
B.   Light red magenta (strawberry colour).
C.   Pale rose, blotched.
D.   White with trace of rose in centre.

[The limbs of the petals contain no pigment and no oxydase.]

epidermal and bundle oxydases; the most deeply coloured flowers contain most, those of intermediate tint, less, and those which approach most nearly to whiteness, contain the least amount of oxydase. The coincidence of oxydase with pigment in the all but white forms of flower is most impressive. In such forms the pigment is confined to three short rosy lines at the base of the limb of each petal and it is only along these lines, which mark the position of the main veins, that oxydase is found. These approximately white forms give but a light brown reaction with benzidine; the pink pigmented forms give a rich brown and the fully pigmented forms give a brown-black reaction. Hence we conclude that in this example of an ever-sporting flower the extent and depth of pigmentation are determined by the quantitative distribution of oxydase.

(2) *White Varieties of Sweet William.*

Among the various, non-sporting varieties of Sweet Williams which we have examined are races with fully coloured, white and nearly white flowers. The fully coloured varieties all give pronounced oxydase reactions; the white and approximately white varieties behave to our reagents in one of two ways. The pure white race gives a very definite, albeit limited, oxydase reaction which is most pronounced in the central region towards the base of the petal limb. This variety is therefore colourless because it lacks chromogen. The all but white race, the petals of which bear a ring of rosy dots or lines about one-third of the way from the point of junction of limb and claw, contains no oxydase except in the region which in the fresh flower is occupied by the rosy ring. Hence this variety, unless indeed it prove to be a dominant white, owes its whiteness to lack of oxydase.

The final establishment of these conclusions must await the results of breeding-experiments which are being carried out.

Should these experiments lead to the production of a coloured F_1 generation, we shall have the proof of the hypothesis suggested first by Miss Wheldale, that, where two colour factors are involved in the production of pigment, one is a chromogen producing factor and the other an oxydase producing factor. But apart from these experiments we have in the cases of white, magenta flaked *P. sinensis* and of the various races of Sweet William clear evidence that pigment formation depends on oxydase action, that depth of colour is determined by amount of oxydase and that a lack of this substance results in an absence of pigmentation.

D.   *The Oxydases of Geranium sanguineum.*

We may cite further evidence in favour of the major proposition that the two factors for pigment formation are chromogen and oxydase. As has been shown by genetical research, with such plants as Sweet Peas (Bateson, 1906) and Orchids (Hurst, 1909), so we show by means of chemical methods that albino races are of two kinds.   The white flowered races of Sweet Peas (*Lathyrus odoratus*) and of culinary peas (*Pisum sativum*), so far as we have examined them, all contain oxydase of the type present in the coloured forms and hence the lack of colour in these races of the two species is due to lack of chromogen.   With respect to the former species, however, it should be remarked that we have not yet had an opportunity of investigating thoroughly the two types of white flowered plants which yield when crossed with one another a coloured F_1.   So far as our observations go at present we have found, neither in round-pollened nor in long-pollened albino Sweet Peas, no flower from which oxydase is absent.   It may be that further search will discover such flowers or it may of course be that the factors determining pigmentation are other than those suggested above.   In any case the evidence provided by the albino of another species of plants, *Geranium sanguineum*, is favourable to our hypothesis.   The petals of the flower of the purple type of *G. sanguineum* give with benzidine a definite epidermal oxydase reaction and a yet more marked reaction for bundle oxydase.   The pure white petals of the albino variety give with the same reagent a distinct bundle reaction, but no epidermal reaction. Hence this white form is either a dominant white or a true albino of the second type, namely one which owes its whiteness to deficiency of oxydase in the epidermal cells.   Although we have been unable, owing to lack of material, to determine absolutely to which of these categories it belongs, the fact that the bundles give a definite oxydase reaction appears to indicate that the white *G. sanguineum* is not a dominant white.

The intermediate form, *Geranium lancastriense*, which is characterised by a flower of pale flesh colour with darker pink veins stands midway between the type and the albino; for although it gives no or at most a very slight oxydase reaction in the epidermis of the flower, it gives a very distinct (violet-brown) bundle reaction.

It would appear therefore from the observations on ever-sporting races *Primula sinensis* and *Dianthus barbatus* and on the albino forms of Sweet Peas and of *Geranium sanguineum* that two types of albino

exist.   In the one type the white flowers are rich in oxydase and from the other, as judged by the reactions with benzidine and α-naphthol, oxydase is absent.   We hope by the application of these methods to the flowers of the many albinos which occur in cultivation and in the wild state to ascertain whether one or the other type is of more frequent occurrence in nature.

E.   *The Localisation of Oxydases in the Tissues of the Flower*
(Primula sinensis).

We return now to *Primula sinensis*.   In order to complete our survey of the oxydases of the flower, and before proceeding to discuss the oxydase content of dominant white flowers, we will describe briefly the localisation of the oxydases present in coloured and recessive white forms.   The distribution of the epidermal oxydase in the petals is definitely circumscribed.   The epidermal oxydase is confined to the epidermal cells and the hairs which are outgrowths from these cells.   The distribution of the bundle oxydase is less sharply defined.   By treating tangential sections of the petals with the reagent, oxydase may be seen to follow the veins throughout their whole course and to extend to their finest ramifications.   If a section which exposes one of the finer veins be treated with benzidine and then examined microscopically the brown granular or needle-like products of the interaction of the reagent and the oxydase are seen as dense masses in the elongated cells of the bundle sheath—which cells are more deeply stained than any others.   These elongated cells are wrapped around and also extend beyond the tracheids of the veins.   They give off short branches which make contact with corresponding branches of the stellate parenchyma of which the body of the petal is composed (see Text-figure 3).

In petals rich in oxydase the stellate parenchyma cells, the branches of which abut on those of the bundle sheath cells, also give oxydase reactions, sometimes as marked but generally less marked than those of the cells of the bundle sheath.   The reaction in the cells of the stellate parenchyma becomes more faint as the cells of that tissue are traced further from the veins (see Text-figure 3).

The appearance presented by the stellate parenchyma and the bundle sheath is as though oxydase were passing from the cells of the latter to those of the former.

We have given reason in a former communication (Keeble and Armstrong, 1912 B) for our belief that oxydase may be translated from cell to cell, and have offered the suggestion that the frequency with

which lines of colour are seen to demark the veins of petals is attributable to the action of oxydases which pass from the bundle to the epidermis.
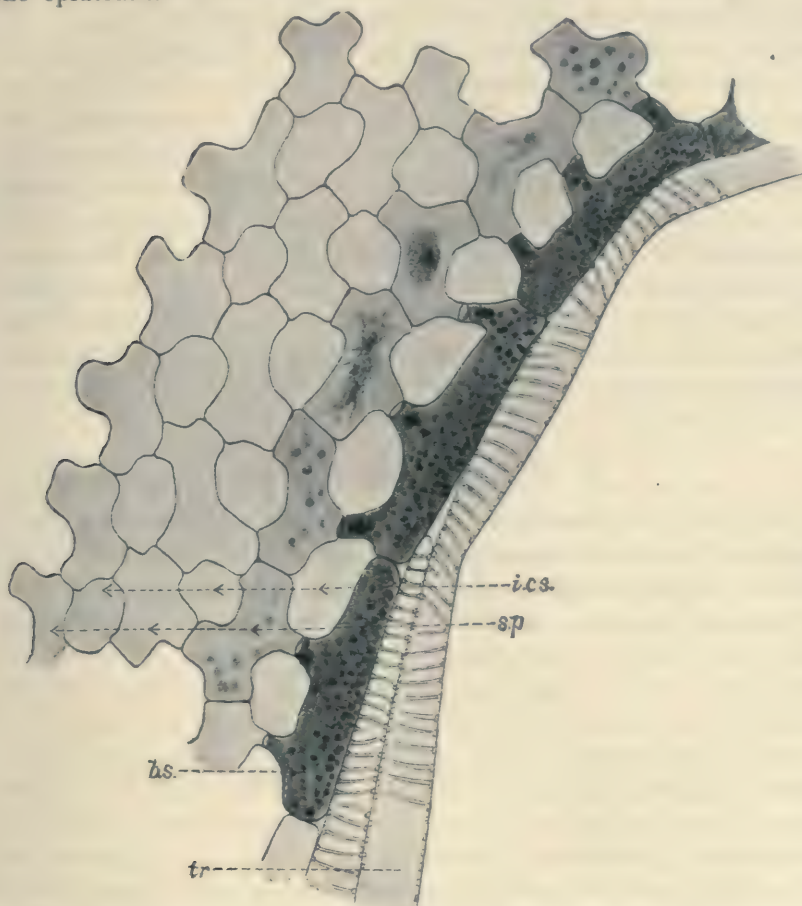
Fig. 3. The oxydase content of the cells of the bundle sheath of the terminal portions of a vein in a petal of *P. sinensis*.

    b.s.   Bundle sheath—rich in oxydase.
    s.p.   Stellate parenchyma, the cells in contact with those of the bundle sheath are rich in oxydase : those more distant contain less.
    tr.   Tracheid.
    i.c.s.   Intercellular spaces.

If preparations of sections treated with the benzidine reagent are examined microscopically, the localisation of the brown product of oxydase action may be seen in the cytoplasm of the tissues rich in

oxydase. It happens very frequently that the walls of these cells take on also a brown stain. Although this staining of the wall may be due to diffusion of the oxidized benzidine derivative from the cell, yet the possibility is not precluded that in the living plant oxydase may occur in the walls of the cells. We are not in a position to make a positive statement on this subject but may point out that changes, often of a remarkable nature, go on in cell walls and in some cases, for example, in the macrospores of species of Selaginella very considerable changes of structure and dimensions occur in cell walls which are far removed from contact with cytoplasm. Therefore it would seem not unreasonable to suppose that oxydases may play a part in the growth processes of cell walls, and that the occurrence of wall staining after the treatment of a tissue with an oxydase reagent may be due to the formation *in situ* of the colour substance indicative of oxydase and not to the passage of that substance from the cytoplasm to the wall.

F. *Dominant White Varieties.*

We have now to consider the oxydase content of those varieties of *P. sinensis* known to students of Genetics as Dominant Whites. As the term implies, Dominant Whites present the appearance of albinism but, as it also suggests, the albino-like appearance masks a distinct and remarkable genetical constitution. Hitherto the only test serving to discriminate between Recessive and Dominant Whites has been the breeding test. Recessive Whites crossed with coloured varieties give a coloured F_1 generation; Dominant Whites when mated with similar coloured varieties yield a white F_1 generation. The F_1 plants from the cross, recessive white × colour, give on self-fertilization or on inter-breeding an F_2 consisting of 3 coloured : 1 white. The F_2 generation derived from self-fertilized or inter-bred F_1 plants from a cross between coloured and dominant white consists of 3 white : 1 coloured. The phenomenon of dominant whiteness does not appear to be very common among plants. It is exemplified by the flowers of *Primula sinensis* (Bateson, 1906), and the Foxglove (*Digitalis purpureus*, Keeble, 1910 B). Among animals a similar phenomenon occurs with respect to coat colour in certain breeds of fowls, for example White Leghorns (Bateson, 1906). The interpretation which meets the facts of the genetical behaviour of Dominant Whites is well known and involves the existence of a factor which, even though the factor or factors for colour be present, prevents the development of pigment. In other

words the genetical hypothesis of the nature of dominant whites holds that the latter are due to the inhibition of pigment formation.

This Mendelian interpretation is so convincing that it scarcely needs the collateral support to be derived from chemical investigation; but, although this be the case, it is at once evident that the hypothesis suggests a promising line of inquiry into the physiology of pigmentation. Investigations on this subject made by Miss Wheldale (1910) supply confirmation of the hypothesis by indicating that an inhibitor of pigment formation exists in flowers the pale shades of which are dominant to deeper shades.

A further advance has been made by Gortner (*loc. cit.* 1911) who has produced experimental evidence confirming the conclusion reached by previous investigators, that the black pigment (melanin) of various insects and other animals is produced by the interaction of the oxydase, tyrosinase with a chromogen, tyrosin, and has shown that, when these substances are allowed to act *in vitro*, the addition of certain dihydroxy phenols such as phloroglucinol, orcinol and resorcinol prevents the reaction from taking place. These substances which exercise an inhibitory action on pigment formation Gortner terms antioxydases. He suggests further that the inhibition of melanin formation in animals may be due to a chemical change in the chromogen (tyrosin). Gortner's views may be expressed schematically thus:

Let $T =$ Tyrosin,

and $T^{ase} =$ Tyrosinase.

Then $T + T^{ase} =$ Melanin,

and $T +$ derivative of $T + T^{ase} =$ Inhibition (Dominant White).

We proceed now to consider dominant white flowers of *P. sinensis* with respect to their behaviour with oxydase reagents. The addition of α-naphthol or benzidine and the subsequent addition of hydrogen-peroxide to intact corollas of dominant white plants results in no oxydase reaction whatever. Even after prolonged action of the reagents both epidermis and veins fail to give the colouration characteristic of oxydase. It follows therefore that either oxydase is absent from the flower or it is inhibited from oxidizing the reagents. It so happens that among the Primulas which one of us has been breeding at Reading is a strain derived from a cross between a pure blue-flowered variety and the white, magenta-flaked Snow King to which reference has been made already. Among the descendants of this cross are certain plants illustrated in Plate XIX, Figs. 10 and 13, the flowers of which are

characterised by fairly regular and symmetrically placed white areas on an otherwise uniformly blue ground. We have ground for believing, both on account of the origin and of the genetical behaviour of these plants, that the white areas represent what may be called inhibitory patches.

It is further to be mentioned that these white-zoned blue flowers exist in two forms. In the one form, all the flowers of the plant are marked symmetrically with the white areas and remain in this state as long as they last. In the other form, certain of the flowers and particularly those which are produced late in the season show a blurred, pale blue colour extending from the blue perimeter into the white areas. The plants which exhibit the white areas sharply and permanently are homozygous, that is breed true to the character; those in which the areas tend to be blurred are heterozygous for the character; that is to say, they throw plants which bear uniformly blue flowers as well as others with blotched blue flowers.

The results of an investigation of the distribution of oxydase in the corolla of the true breeding, white-zoned blue flowers are depicted in Plate XIX, Figs. 11 and 12, and show in most striking and definite manner that whereas both the epidermis and vascular bundles of the blue areas give well marked oxydase reactions, no such reactions are given by the white areas. Careful observation of the preparations indicates (see Figs. 11 and 12, Plate XIX) that, although they exhibit no sign of epidermal reaction, a faint bundle reaction may be traced in some cases along the veins of the white areas. From the eye of the corolla to the edge of each white patch, the bundle reaction is very distinct; but as soon as the bundle enters the white area the reaction becomes either imperceptible or at most very faint; and when the veins pass from the white area into the blue region of the petals, the bundle reaction resumes its distinctness. A comparison of Figs. 11 and 12, Plate XIX with the illustration (Fig. 2, Plate XIX) of the oxydases of a uniformly coloured blue flower shows how remarkable is the definition of the white areas of the blue, white-patched petals. The curious attenuation of the bundle oxydase reaction over the white area recalls that which occurs over the yellow eye of the flowers of *P. sinensis*.

Dominant whites and the white areas of the white-zoned blue flowers are alike in that they yield no oxydase reactions, and they stand in this respect in marked contrast with Recessive whites which as we have shown give pronounced oxydase reactions. Recessive whites

contain oxydase but lack chromogen. Dominant whites would appear from our biochemical investigation to lack oxydase. Such a conclusion however is not compatible with the known results of crossing Dominant and Recessive whites, and we are driven therefore to seek the interpretation of the behaviour of the dominant white races in terms of inhibition of oxydase action.

It seemed possible that—if inhibition occur—the suppression of oxydase reaction over the white areas might be due to the presence of sugar; but the application of appropriate tests indicates that reducing sugars are present very generally in the petals of those varieties, coloured and recessive whites, which give good oxydase reactions. We conclude therefore that inhibition is not due to the presence of reducing sugars. Those (heterozygous) plants in which the white areas are ill defined, show the same readiness to give a colour reaction with benzidine or α-naphthol as to develop a blue colouration when in the living state: cf. Figs. 13 and 14, Plate XIX. Just as the blue ground colour of the petal may spread into the inhibitory area in the heterozygous plants, so the benzidine reaction may extend over the boundary between the blue, where it is well marked, and the white from which, at first, it is absent. Here again the suggestion of inhibition forces itself on the observer; for the phenomena are paralleled by those exhibited by heterozygous dominant whites. For whereas pure bred dominant whites have pure white flowers, plants which are heterozygous for the inhibition factor may show a faint tinge or flush of colour over the surface of their petals.

The results obtained by the investigation of the oxydase contents in dominant white and white-zoned blue flowers, though they suggest the presence of an inhibitor of pigment formation do not, of course, supply actual proof thereof, nor do they indicate the mode of action of the suppositious inhibitor. If the inhibitory substance exist, it should be possible either to remove or destroy it. If we assume the existence of an inhibitor of pigment formation, then we must suppose that it acts in one of two ways. It either inhibits chromogen formation or it checks the action of oxydase. The evidence which we have just produced points to the latter mode of action, for dominant white and the white-zoned blue flowers are unique among varieties of *P. sinensis* in not giving an oxydase reaction. We will assume therefore that the inhibitor exercises its influence on oxydase. That influence may be brought to bear in one of two ways: either the oxydase may be destroyed or it may be prevented from doing the work of pigment formation. If the

oxydase be destroyed, the only method by which inhibition may be demonstrated must consist in the isolation of the inhibitor, and the addition to it of oxydase. If the latter be destroyed, the evidence for inhibition is forthcoming. If, on the other hand, the inhibitor does not destroy, but only checks the action of oxydase, it should, perhaps, be possible to discover a reagent which, whilst acting destructively on the inhibitor, leaves the oxydase unharmed. Could this be done, the addition of the appropriate reagents should demonstrate the existence of oxydase in the dominant white flower.

We have pursued our inquiry along the lines suggested by these reflections and have succeeded in demonstrating experimentally the existence of an inhibitor of oxydase in the flowers of Dominant whites.

As the result of experimenting with various substances, we have found that dominant white flowers, after preliminary treatment with certain reagents, are no longer refractory to benzidine or α-naphthol, but give with them well marked reactions for epidermal and bundle oxydases. The reagent which is most efficient in producing this result is hydrogen cyanide. Thus, if a dominant white flower be treated for 24 hours with an aqueous solution of hydrogen cyanide and then washed with water and treated with the oxydase reagent, it gives a uniform and marked oxydase reaction. A comparative examination of Figs. 6 and 8, Plate XIX, shows how striking is the difference in behaviour with respect to oxydase reaction between a dominant white flower placed directly in the oxydase reagent and a similar flower treated previously with hydrogen cyanide. Whereas the former shows scarcely a sign of oxydase, the latter proves itself by the reaction to be rich in that substance. The most satisfactory results are obtained when the following method is practised:

Immersion of the dominant white flower in a 0·4 per cent. solution of hydrogen cyanide for 24 hours, washing with water, addition of benzidine (alcoholic solution), washing and then adding to the water a drop of hydrogen peroxide.

Instead of hydrogen cyanide, a saturated solution of carbon dioxide may be employed, but the removal or the destruction of the inhibitor takes place more gradually with this reagent than with hydrogen cyanide (see Fig. 7, Plate XIX).

Our white-zoned blue flowers provide us with material for verifying the conclusion that hydrogen cyanide brings about the removal or destruction of the inhibitor. If a blue flower with inhibitory patches be treated with hydrogen cyanide in the manner described above,

the subsequent addition of the oxydase reagent brings about a uniform colouration of the petals. The originally white areas are now as deeply stained as the blue regions, and the veins in the former, which as we have seen yielded at most a faint oxydase reaction give after this treatment as marked a reaction as those in the blue areas of the flower.

The results confirm the Mendelian hypothesis that dominant white flowers owe their lack of pigment to the presence of an inhibitor of pigment formation. They show moreover that the inhibition is exercised, not on the process which results in the liberation of chromogen, but on the oxydase and that the inhibitor acts not by destroying oxydase but by effective interference with its action.

Attention may be drawn to the fact that, as shown by the results obtained with dominant white flowers and contrary to the general opinion, hydrogen cyanide does not destroy oxydase.

We may summarise our observations on the oxydases of the flower (sections B—F) thus:

In all coloured and recessive white varieties of *P. sinensis* oxydase is present in the petals. It occurs in two situations, namely in the epidermis and in the bundle sheath of the veins. The epidermal and bundle oxydases react differently with our reagents (α-naphthol and benzidine). Dominant white varieties of *P. sinensis* contain an inhibitor of oxydase. On the removal of the inhibitor from the petals of dominant white flowers a strong oxydase reaction is obtained. The white areas in the petals of certain races of blue-flowered Primulas also contain an inhibitor which prevents the oxydase contained in those areas from reacting with oxydase reagents. When this inhibitor is removed the white areas give well marked oxydase reactions. Ever-sporting varieties of *P. sinensis* and of *Dianthus barbatus* show most epidermal oxydase in the most deeply pigmented flowers, less in the less pigmented and none in the white flowers.

The albino forms of *P. sinensis, Pisum sativum, Lathyrus odoratus* (in the forms as yet examined) all contain oxydase and their floral albinism is attributable to lack of chromogen. The white-flowered *Geranium sanguineum* lacks oxydase and we are of opinion that it owes its albinism to lack of oxydase.

Albino or approximately albino forms of *Dianthus barbatus* are of two forms; in one form oxydase is present and from the other it is absent.

G. *The Nature of the Oxydases in Flowers.*

(1) *The Oxydases and Peroxydases of Primula sinensis.*

As indicated in the footnote at the beginning of the section the term oxydase is used in the foregoing sub-sections (A—F) to include both oxydase and peroxydase, that is to say those oxidising substances which react directly with benzidine or α-naphthol (direct oxydase) and those which react with these reagents only after the addition of hydrogen peroxide (peroxydases). Our reasons for the practice which we have adopted are threefold: first to simplify description; second because a given tissue may yield, in one variety, an oxydase reaction and, in another variety, a peroxydase reaction; and third because there is evidence that a tissue of a given variety at one time and under one set of circumstances may contain peroxydase, and at another time and under other circumstances may contain a complete oxydase—namely peroxydase plus organic peroxide—and so give a direct reaction.

It is necessary therefore to describe the results of our observations on the nature of the oxydase content of the various tissues of *P. sinensis* and of the other plants which we have investigated.

In the case of *P. sinensis* direct oxydases are relatively rare and peroxydases are the rule. Thus the oxydase reactions of the petals of coloured and recessive white flowers are invariably or all but invariably indirect; in other words peroxydases are present in the flowers both in the epidermis and in the tissues of the veins: hence we may speak of epidermal peroxydase and bundle peroxydase as present in the petals of the flower. The only exception to this rule occurred in the case of the flower of a Sirdar, one petal of which gave on one occasion a well marked direct oxydase reaction. We have no evidence, except in the single example just recorded, of the existence of direct oxydase in the flower of *P. sinensis*. We have satisfied ourselves, however, that it occurs in the vegetative parts of normal plants. Thus sections of flower-peduncles of dark red-stemmed varieties, of green-stemmed Sirdars and of reddish varieties, may all possess direct oxydase in the phloem. In Sirdars and in dark red-stemmed varieties there is evidence that the pericycle contains a direct oxydase. On the other hand it is very rare indeed for the epidermal tissues of plants grown under normal conditions to exhibit a direct oxydase reaction. We have obtained such reactions occasionally in certain red-stemmed varieties; for example sections of flower-peduncles treated with benzidine may give the colouration characteristic of the oxydase reaction in the epidermal cells as well as in the phloem.

For reasons which will be apparent immediately there is no need to attempt to discriminate more closely between the distribution of peroxydase and that of oxydase in the normal flower. What is evident and of importance is the fact pointed out already by Miss Wheldale and by Clark that peroxydase is more widely distributed than the organic peroxide which activates it. Even in the case of the phloem which, as we have seen, gives a direct reaction there appears to be more peroxydase present than the organic peroxide is capable of activating; for the result of adding hydrogen peroxide to sections which have already given a direct reaction, is to increase that reaction. Hence it would appear that the efficiency of the system, peroxydase and organic peroxide, is determined by the extent to which the organic peroxide is present, and that whereas peroxydase is relatively stable, the organic peroxide is unstable.

We have satisfied ourselves not only that this is the case but that external conditions play an important part in determining the amount of peroxydase present in the tissues of a plant.

(2) *The Oxydases and Peroxydases of Other Flowers.*

Before proceeding to justify this statement we will describe briefly the nature of the oxydases in the various plants to which reference is made in the preceding pages and in certain other species which we have had occasion to examine.

Flowers of the Sweet Pea and of the Culinary Pea contain no direct oxydase in their epidermis and either none or a very small amount in the veins. They give, however, well marked peroxydase reactions. *Geranium sanguineum* possesses direct oxydase both in the epidermis and bundles of the petals. The pale flesh-coloured variety *G. lancastriense* contains direct oxydase in its veins and peroxydase in its epidermis. The white variety contains in its epidermis neither oxydase nor peroxydase, but its bundles give a distinct peroxydase reaction.

Coloured varieties of Sweet William give good oxydase reactions in both bundles and epidermis.

Species of *Prunus* and *Pyrus* are of interest in this connection. Those species the flowers of which are white, and remain white on fading, contain peroxydase only; those species which turn brown on fading contain direct oxydase.

The very general phenomenon of browning presented by dried plants is to be regarded in all probability as an indication of the presence of a direct oxydase, and in order to prevent the discolouration of herbarium

specimens some method must be devised whereby the oxydase is destroyed. It is probable that the method of preserving the colours of flowers by drying them in sawdust or sand at moderately high temperature owes its efficiency to a destruction of oxydase.

(3) *The Influence of Light and Darkness on the Oxydase Content of Plants.*

We now turn to the consideration of the influence of external conditions on the oxydase content of plants. From our experiments with *P. sinensis* it would appear that light and darkness play an all-important part in determining the amount of oxydase present in plant tissues. As demonstrated by the facts which we record below, light exercises a destructive influence on the organic peroxide constituent of oxydase. Thus under normal conditions of illumination a tissue may give no reaction with α-naphthol or benzidine, though it yields a well marked peroxydase reaction when hydrogen peroxide is added. When, however, a plant is maintained in darkness for 24 hours or longer, the tissue corresponding to that previously tested gives a pronounced and direct reaction with the oxydase reagents. In illustration of this fact we may cite the following examples :—Sections of the flower-peduncle of a reddish-stemmed plant which had been maintained in normal conditions of illumination gave no direct reaction with benzidine. Similar sections from a sister plant, which had been kept in the dark for 48 hours, gave a distinct oxydase reaction in the epidermal hairs. Other sections of the same two plants treated with α-naphthol gave the following results : the illuminated plant—a direct reaction (faint lilac) only in the phloem ; the dark kept plant—a much deeper reaction in the phloem (rose-colour to almost black) and a fair reaction in the epidermis and hairs. The same experiment shows further that the effect of darkness is also to increase the peroxydase content of the tissues. Thus, if flowers of sister plants, one exposed to normal illumination and the other maintained in the dark, be tested for peroxydase the reaction of the latter is found to be so definitely more considerable as to be appreciated by macroscopic examination. Text-figure 4 is typical of the results which were obtained in numerous experiments.

Whatever be the interpretation of these facts the phenomena themselves are definite. Darkness leads to the formation of peroxide and to an increase of peroxydase. We cannot say whether the latter result is to be interpreted as being due to a destructive action of light on oxydase or whether it is to be regarded as a consequence of the

continuous using up of oxydase in the production of new pigment to replace that which, for all we know, may be continuously destroyed when the plant is exposed to light. It is a well known fact that conditions of illumination influence the amount of anthocyan pigment which occurs in a plant. For example, it is a common practice among horticulturists to enclose choice fruits such as grapes and apples in translucent paper bags and it is claimed that this expedient, beside protecting the fruit from insects, improves its colour. This, if true, would point to the conclusion that light of high intensity exercises a destructive influence on anthocyan pigment.
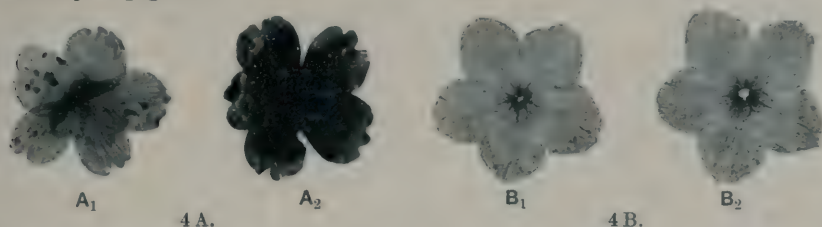
Fig. 4. (From photographs.) The effects of Light and Darkness on the Peroxydase of *Primula sinensis*.

A_1 and B_1, normally illuminated plants.

A_2 and B_2, plants kept in darkness for 48 hours.

A. A double, light magenta plant (8/2/2/11): flowers treated with benzidine and hydrogen peroxide.

B. A white magenta-flaked plant of Mt Blanc Star: flowers treated with α-naphthol and hydrogen peroxide.

The most casual observations in the garden show that the depth of colouration of flowers varies considerably during the course of development of the flower. Thus the petals of many varieties which are white in the mature stage are pink or pinkish in the bud stages and it is possible that we have in such instances an example of the pigment destroying action of light.

Although an adequate discussion of the facts just recorded would be out of place in the present communication, it will be evident that if they are shown to be true generally these facts may have an important bearing on many phenomena other than those connected with the pigmentation of plants. For if we ascribe to oxydases a general rôle in the metabolism of the plant as well as a special function in pigment production, the fact that the oxydase content of plant tissues waxes in darkness and wanes in the light may have bearings on the phenomena of periodicity which are at once such puzzling and general attributes of the Vegetable Kingdom. The work of Palladin (1911) and others

indicates that oxydases have such a general function as is postulated above and that they are part of the respiratory mechanism of the plant. Hence it is reasonable to suppose that the rate of respiration of a given cell is determined by the amount of oxydase present in that cell. If light exercise directly or indirectly a destructive action on the oxydase content of the cell the rate of respiration of the latter will fall off during the day and will rise again after a sufficient exposure to darkness has set going the oxydase secreting apparatus of the cell and allowed of the accumulation of peroxydase and organic peroxide.

It may be that, beside the phenomena of periodicity referred to above, the remarkable respiratory phenomena presented by succulent plants are attributable to their diurnal rhythm of oxydase destruction. During the night the respiration of such succulent plants as *Mesembryanthemum* results in a smaller output of carbon dioxide than that which takes place during the day. Instead of the respiratory substances becoming completely oxidised, they produce incompletely oxidised bodies, namely, organic acids. During the day the normal respiration is resumed and the organic acids which have accumulated at night disappear.

We propose to investigate this phenomenon in the light of our knowledge of the diurnal variation of oxydase.

Lastly a brief reference must be made to the influence of wounding on the liberation of oxydase. As is well known the effect of mutilating a tissue is to produce a speedy and copious liberation of oxydase. The benzidine reagent serves to indicate wound oxydase. That this is so is illustrated in Text-figure 5, which represents white corollas of

A   B
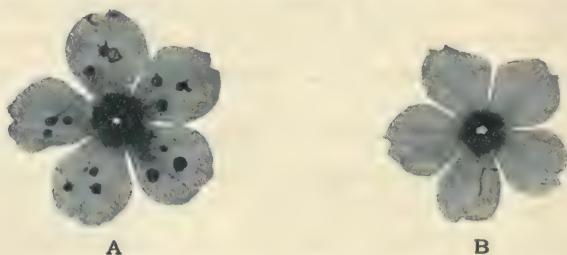
Fig. 5. (From a photograph.) The wound peroxydase of *Primula sinensis*. Each of the petals of a dominant white flower of *P. sinensis* (5 A) was stabbed in three places with a needle. The wounded corolla and also one from an uninjured flower (5 B) were subjected to the action of the benzidine reagent, then washed with water and treated with hydrogen peroxide. The unwounded corolla gave no peroxydase reaction: the mutilated corolla gave an intense reaction in the neighbourhood of each wound (see text).

P. sinensis the surface of one of which was wounded in several places by means of the point of a needle. The flowers were transferred at once to the benzidine reagent and treated subsequently with hydrogen peroxide with the result that the wounded areas gave an intense peroxydase reaction. It is therefore evident that the wound oxydase is in this case of the nature of peroxydase. Experiments of a similar nature made with other flowers lead to the conclusion that when a peroxydase only is present in the coloured or white parts of the petals the effect of wounding is to bring about copious liberation of peroxydase, but when a direct oxydase is present wounding results in the liberation of direct oxydase.

The main facts dealt with in the foregoing section may be summarised as follows :

Cells in which anthocyan pigment is present contain oxydase in one of two forms, namely peroxydase or complete (direct) oxydase. The latter is found in the flowers of Sweet William (*Dianthus barbatus*), of *Geranium sanguineum* and certain species of Pyrus and Prunus. The former, of more general distribution, occurs in *P. sinensis*, *Lathyrus odoratus*, *Pisum sativum* and many other plants.

The oxydase content of a plant varies with external conditions. A tissue of a normally illuminated plant contains less peroxydase than is contained in the corresponding tissue of a plant kept in darkness ; and the organic peroxide constituent of the complete oxydase, though it may be absent from the normal plant, makes its appearance after that plant has been maintained for some time in darkness.

The wound oxydases of plants resemble those which are concerned in the work of pigment production. Those plants which contain peroxydase only, liberate, when lacerated, wound peroxydase and those which contain both peroxydase and organic peroxide show in their wounds the complete oxydase.

For summaries of other subsections see pages 288 and 303.

DESCRIPTION OF PLATE XIX.

The illustrations are reproduced from water-colour drawings by Miss D. Richardson from natural flowers (Nos. 1, 3, 10 and 13) and from preparations made by treating the flowers with reagents for oxydases (see text).

1. A uniformly (self) coloured blue flower (natural colour).
2. The same after decolourisation with the benzidine reagent and subsequent treatment with hydrogen peroxide; showing epidermal and bundle peroxydases.
3. A white-flowered form (natural).
4. A recessive white (α-naphthol and H_2O_2); showing bundle peroxydase.
5. A recessive white (benzidine and H_2O_2); showing epidermal and bundle peroxydases.
6. A dominant white (benzidine and H_2O_2).
7. A dominant white (treated first with carbon dioxide, then with benzidine, and subsequently with H_2O_2). Cf. with 6.
8. A dominant white treated with hydrogen cyanide—benzidine—H_2O_2. Cf. with 6.
9. A dominant white treated with HCN, α-naphthol H_2O_2.
10. A blue with white inhibitory patches (54/2/1). F_3 of cross between Cambridge blue and Snow King (natural colour).
11. The same after decolourisation with the α-naphthol reagent and subsequent treatment with H_2O_2.
12. Another flower of 54/2/1 after decolourisation with the benzidine reagent and subsequent treatment with H_2O_2.
13. A heterozygous plant of the same race (54/2/1) natural colour.
14. The same after decolourisation with the benzidine reagent and subsequent treatment with H_2O_2.

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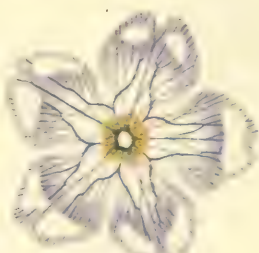
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FORMS OF REDUPLICATION :—PRIMARY AND SECONDARY.

By A. H. TROW, D.Sc., F.L.S.

ACCORDING to Bateson and Punnett (*J. of Gen.* Vol. I. 4, p. 293) all forms of reduplication hitherto discovered, with one possible exception, may be grouped in two classes, the gametic series for these being represented by the empirical formulae

$$n-1 : 1 : 1 : n-1 \text{ and } 1 : n-1 : n-1 : 1 ;$$

the single exception being the cases of complete repulsion, in which the end terms disappear, giving the series $n - 1 : n - 1$. Even this exceptional type however is now regarded by these authors as probably a special case of the series $1 : n - 1 : n - 1 : 1$, where n is large and therefore one of the zygotic types so scarce as not to be expected except in very extensive cultures[1].

My own studies of *Senecio vulgaris* have however revealed the existence of the ratio $2 : 1 : 1 : 2$, and Baur (*Vererbungslehre*, p. 124) appears to have found the ratio $6 : 1 : 1 : 6$ in an *Antirrhinum* cross. Neither of these ratios comes under the general formula given above. Such being the case, it seemed to me desirable to ascertain what the consequences of accepting the current hypothesis of reduplication would be, not simply as applied to a pair of factors **AB** (or two pairs of allelomorphs **Aa**, **Bb**), but to three or more factors **A, B, C, D** ....

The immediate problem which presented itself for solution was a comparatively simple one, yet one which has apparently been over-looked. Given three factors **A, B,** and **C** and the occurrence of reduplication between **A** and **B** in the form $n : 1 : 1 : n$ and between **A** and **C** in the form $m : 1 : 1 : m$, where n may be equal to, greater, or less than m, is there necessarily a form of reduplication between **B** and **C**,

---

[1] In these formulae n is a power of 2 and is equal to one-half the number of gametes in a series.

and if so, of what type must it be? We need only state now that the answer is that there is reduplication between **B** and **C** of the type

$$nm + 1 : n + m : n + m : nm + 1.$$

The mode by which this ratio is found is given below. We have however to note that this type of ratio also does not conform to the Bateson-Punnett formula.

Certain experimental results will, I believe, in view of these conclusions, repay further study. All the gold has not yet been extracted from the ore.

Reduplication clearly depends upon peculiarities in the mode of formation of the gametic series. As however it, so far as we know at present, affects pairs of factors only, it is convenient to ignore such possible cases of reduplication as might occur between, say, triplets or quartets. With this limitation and adopting the Bateson-Punnett hypothesis of reduplication (*Journ. of Genetics*, Vol. I. No. 4, p. 293), it is quite easy to construct the gametic series for any set of reduplications.

Let us consider first the simple case in which three factors **A**, **B** and **C** are involved, with reduplication between **A** and **B** only, and in the form $n : 1 : 1 : n$.

The gametic series if **A** and **B** are alone considered would be

$$n\mathbf{AB} + 1\mathbf{Ab} + 1\mathbf{aB} + n\mathbf{ab}.$$

To include the factor **C**, the series must consist of eight terms and be arranged so that each member of the above will be associated with **C** and **c**, without disturbing the established reduplication; thus

$$n\mathbf{ABC} + n\mathbf{ABc} + 1\mathbf{AbC} + 1\mathbf{Abc} + 1\mathbf{aBC} + 1\mathbf{aBc} + n\mathbf{abC} + n\mathbf{abc}.$$

By extracting the pairs separately from this series, we get

　　AB : **Ab** : **aB** : **ab** :: $2n$: 2 : 2 : $2n$　or　$n : 1 : 1 : n$.
　　AC : **Ac** : **aC** : **ac** :: $n+1 : n+1 : n+1 : n+1$　or　$1 : 1 : 1 : 1$.
　　BC : **Bc** : **bC** : **bc** :: $n+1 : n+1 : n+1 : n+1$　or　$1 : 1 : 1 : 1$.

Clearly a reduplication between two factors **A** and **B** does not alter the ratios for **A** and **C** and **B** and **C**.

An experimental illustration of this is furnished by Gregory's work on *Primula sinensis*, in what may be called the **MSD** group of experiments; where

　　　　　M = magenta　　dominant over **m** = red;
and　　　**S** = short style　　　　　　„　　　　„　　**s** = long style;
and　　　**D** = single flower　　　　„　　　　„　　**d** = double flower.

In these experiments there is reduplication between **M** and **S** of the form $7 : 1 : 1 : 7$; but **M** and **D** and **S** and **D** show no reduplication and give each the normal ratio $1 : 1 : 1 : 1$.

We may now consider the more important case, where there are three factors **A**, **B** and **C** and reduplication between **A** and **B** in the form $n : 1 : 1 : n$, and between **A** and **C** in the form $m : 1 : 1 : m$.

The gametic series when **A** and **B** are alone considered would be

$$n\text{ABC} + n\text{ABc} + 1\text{AbC} + 1\text{Abc} + 1\text{aBC} + 1\text{aBc} + n\text{abC} + n\text{abc}.$$

To secure reduplication between **A** and **C** as well, and of the form $m : 1 : 1 : m$, the terms involving **AC** and **ac** must be multiplied by m; the series thus becomes

$$nm\text{ABC} + n\text{ABc} + m\text{AbC} + 1\text{Abc} + 1\text{aBC} + m\text{aBc} + n\text{abC} + nm\text{abc}.$$

Extracting the three pairs separately from this series, we get

$$\text{AB} : \text{Ab} : \text{aB} : \text{ab} :: nm + n : m + 1 : 1 + m : m + nm$$
$$:: \quad n \quad : \quad 1 \quad : \quad 1 \quad : \quad n.$$
$$\text{AC} : \text{Ac} : \text{aC} : \text{ac} :: nm + m : n + 1 : 1 + n : m + nm$$
$$:: \quad m \quad : \quad 1 \quad : \quad 1 \quad : \quad m.$$
$$\text{BC} : \text{Bc} : \text{bC} : \text{bc} :: nm + 1 : n + m : m + n : 1 + nm.$$

From this procedure, it is clear that reduplication between **A** and **B** and between **A** and **C** involves reduplication between **B** and **C**. It is worthy of note that this derived or secondary type of reduplication has apparently been entirely overlooked, especially as there is good reason to suppose that it has already been observed experimentally. Moreover, it belongs to a fundamentally different series,—of the form $p : q : q : p$.

Gregory's interesting results on *Primula sinensis* illustrate this case. In the **MSG** group of experiments, where **M** and **S** have the same significance as above, **G** represents green stigma, dominant over **g**—red stigma. The best numerical results are given by the crosses in which the F_1—**MSGmsg** was crossed by the triple recessive **msgmsg**. In such cases it is clear that the ratios of the zygotic series coincide with those of the F_1 gametic series. The results may be grouped as follows:—

	MS	Ms	mS	ms
Nos. found	53	3	6	40
Expectation on ratio of $7 : 1 : 1 : 7$	45	6	6	45

	MG	Mg	mG	mg
Nos. found	39	17	18	28
Expectation on ratio of $2 : 1 : 1 : 2$	34	17	17	34

								SG	Sg	sG	sg
Nos. found ...	...	...	...	...	...	...	...	64	35	30	44

Expectation on ratio of $5:3:3:5$ derived from $\begin{cases}7:1:1:7 \\ 2:1:1:2\end{cases}$ *54 32 32 54*

The suggestion of the $2:1:1:2$ ratio in the second case is made on my own responsibility—Gregory does not assign one. The main interest lies in the fact that the derivative ratio $5:3:3:5$ explains fairly well the facts of the case.

The following diagram will serve to illustrate the hypothetical course of the segregations and the cell-divisions in this case.
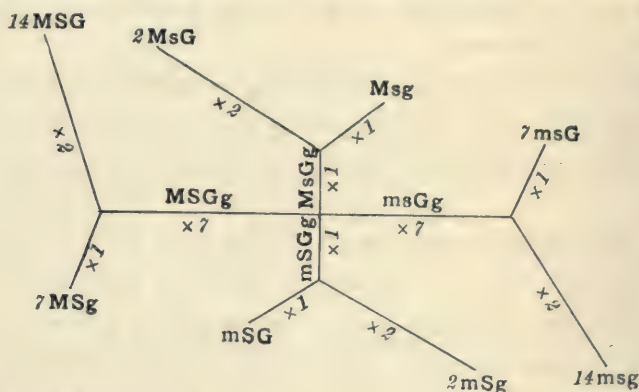
The sign × signifies increase in the number of gametes, or gametogenic, segregating cells, and the following number the relative amount of increase along the different axes.

The primary and secondary reduplications, three in number, are notable in that each represents a case of coupling. Let us therefore consider the case in which the primary reduplications are of the form $1:n:n:1$ for **A** and **B** and $1:m:m:1$ for **A** and **C**. Under these conditions the gametic series will be

ABC + m**ABc** + n**AbC** + nm**Abc** + nm**aBC** + n**aBc** + m**abC** + **abc**,

and the reduplication between **B** and **C** will be found (by extracting) to be of the form

$$\text{BC} : \text{Bc} : \text{bC} : \text{bc} :: 1 + nm : m + n : n + m : nm + 1.$$

We thus get the result that the reduplication between **B** and **C** is of the same form whether the ratios between **A** and **B** and **A** and **C** are

$1:n:n:1$ and $1:m:m:1$ or $n:1:1:n$ and $m:1:1:m$.

This case may be represented diagrammatically; thus

nm Abc

n AbC × m

× l

m abC

AbCc × n

ABC

× m

× l

ABCc abCc

× l × l

× l

abc

× m

aBCc × n

m ABc

× m × l

n aBc

nm aBC
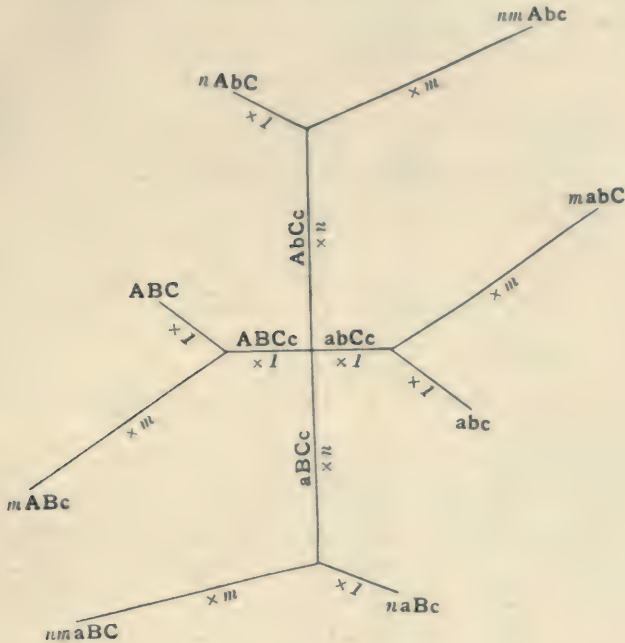
Since n and m are each greater than one, it may be shewn that whether n is equal to, greater than or less than m, $\dfrac{1 + nm}{m + n}$ is greater than one, and therefore that the type of reduplication between **B** and **C** is of the nature of a coupling. We have therefore established the rule that reduplications between **A** and **B** and between **A** and **C** whether of the form of couplings or of repulsions, give rise to a *secondary reduplication between* **B** *and* **C** *of the form of a coupling.*

We may now consider the case in which the types of reduplication between **A** and **B** and between **A** and **C** belong to the series $n : 1 : 1 : n$ and $1 : m : m : 1$ respectively. The gametic series in this case will be

n**ABC** + nm**ABc** + **AbC** + m**Abc** + m**aBC** + **aBc** + nm**abC** + n**abc**,

and the reduplication between **B** and **C** will be necessarily of the form

BC : **Bc** : **bC** : **bc** :: $n + m : nm + 1 : 1 + nm : m + n$,

and be graphically represented thus :—

AbC

m Abc

nm abC

n ABC

AbCc

ABCc
× *n*

abCc
× *n*

aBCc

n abc

nm ABc   *m* aBC

aBc
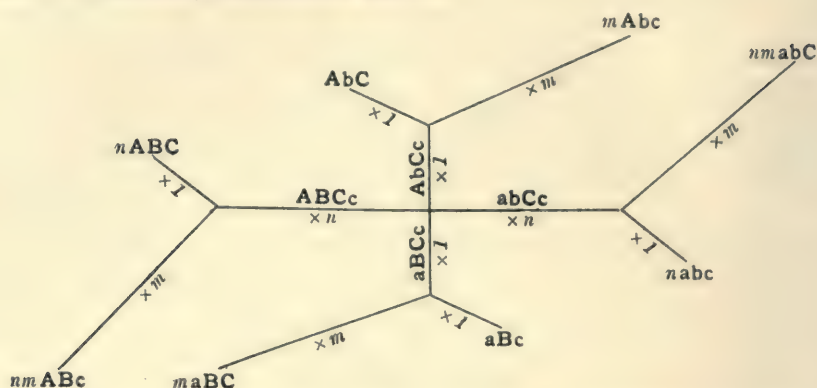
Since $n + m$ is less than $nm + 1$ this type of reduplication is of the nature of a repulsion.

The **EBL** group of experiments conducted by Bateson and Punnett and described in *Proc. Roy. Soc.* B, Vol. 84, p. 7, illustrates this case. The cross **Ebl** × **eBL** shews repulsion between **E** and **B** and coupling between **B** and **L**. It will simplify comparison to write the factors in the order **BLE**. It has been found that

$$\textbf{BL} : \textbf{Bl} : \textbf{bL} : \textbf{bl} :: 7 : 1 : 1 : 7,$$

and $$\textbf{BE} : \textbf{Be} : \textbf{bE} : \textbf{be} :: 1 : m : m : 1.$$

Hence it may be deduced that

$$\textbf{LE} : \textbf{Le} : \textbf{lE} : \textbf{le} :: 7 + m : 7m + 1 : 7m + 1 : 7 + m,$$

which indicates a repulsion. This appears to have been observed, but it is not clear to me from the description of the results whether these three types of reduplication have been observed in the same cross. They should certainly be looked for.

q AbC   *q* Abc

p ABC

p abC

AbCc

ABCc
× *p*

abCc
× *p*

aBCc

p ABc

p abc

q aBC   *q* aBc
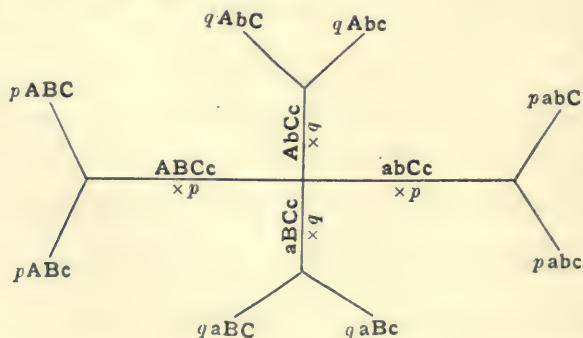
We have found that certain derivative reduplications are of the form $p : q : q : p$.   It seems probable that there may be primary reduplications also of this type.   When such a reduplication is confined to one pair of factors **A** and **B**, a third factor **C** being unaffected, the gametic series would be

$pABC + pABc + qAbC + qAbc + qaBC + qaBc + pabC + pabc$.

The diagram would take the form shewn at the bottom of p. 318.

If $p = q$ there is no reduplication.   If p is $> q$, we get coupling; if q is $> p$, we have repulsion.

But we may have reduplication between **A** and **B** of the form $p : q : q : p$ and between **A** and **C** of the form $r : s : s : r$.   In this event there will be a derivative reduplication between **B** and **C**, the form of which may be ascertained as follows:—

The gametic series will be

$prABC + psABc + qrAbC + qsAbc + qsaBC + qraBc + psabC + prabc$,

and, by extracting, the derivative reduplication is found to be

$$BC : Bc : bC : bc :: pr + qs : ps + qr : qr + ps : qs + pr,$$

or more simply

$$:: pr + qs : ps + qr : ps + qr : pr + qs.$$

This is the most general formula for a derivative reduplication and is of course applicable to all the preceding simpler cases.

The following diagram illustrates the course of the assumed segregations and cell-divisions :—
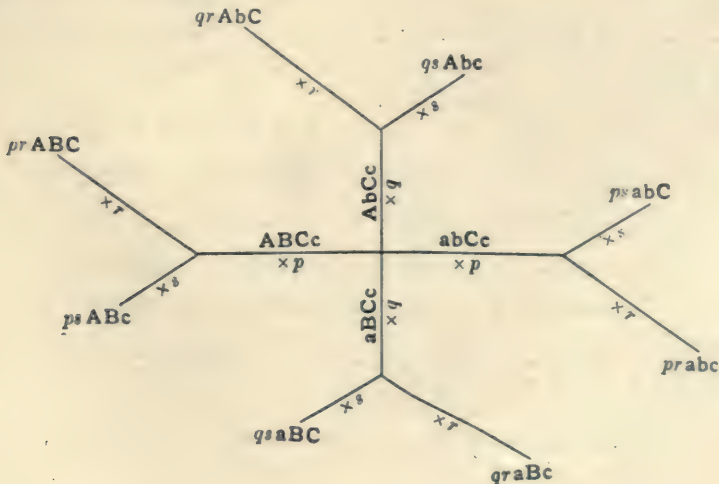
These considerations shew that the reduplication hypothesis adequately explains the occurrence of all the ratios hitherto determined. We perceive too how segregation and cell-division *may be* associated, and that these appear to be carried out symmetrically. In the complete absence of reduplication there is a typical radial symmetry of the segregation apparatus. When reduplication sets in, a bilateral structure is developed, and this may ultimately assume quite a complex form. There seems some reason, moreover, to believe that the development of cells at the two ends of the same axis may be unequal. This would produce a new form of symmetry—the structure becoming two-ended, enabling one to distinguish not only between different axes, but between the two ends of the same axis. In such a case, with a single pair of allelomorphs **A**, **a**, we should get a divergence from the gametic ratio,—1 : 1 and the normal zygotic ratio,—1 : 2 : 1. Such divergences are not infrequently met with in the literature of genetics. We may, therefore, ultimately find cases of asymmetrical types of reduplication, such as are represented by the ratios

$$(1) \quad w : x : y : w,$$
$$(2) \quad w : x : x : z,$$
$$(3) \quad w : x : y : z.$$

The experimental determination of such ratios would of course be difficult.

Finally let us consider the case of four or more factors **A**, **B**, **C**, **D** ... with reduplication between **A** and **B**, **A** and **C**, **A** and **D** ... of the form $n : 1 : 1 : n, m : 1 : 1 : m, p : 1 : 1 : p, \ldots$. In addition to the derivative reduplication between **B** and **C**, there will be now reduplications also between **B** and **D** and **C** and **D** ....

The gametic series for four factors **A**, **B**, **C** and **D** would be

$$nmp\textbf{ABCD} + nm\textbf{ABCd} + np\textbf{ABcD} + \quad n\textbf{ABcd}$$
$$+ \quad mp\textbf{AbCD} + m\textbf{AbCd} + \quad p\textbf{AbcD} + \quad \textbf{Abcd}$$
$$+ \quad \textbf{aBCD} + \quad pa\textbf{BCd} + \quad ma\textbf{BcD} + mpa\textbf{Bcd}$$
$$+ \quad n\textbf{abCD} + np\textbf{abCd} + nm\textbf{abcD} + nmp\textbf{abcd}$$

and it can be shewn, by extracting, that the reduplications between **B** and **D** and **C** and **D** have the form :—

$$\textbf{BD} : \textbf{Bd} : \textbf{bD} : \textbf{bd} :: np + 1 : n + p : n + p : np + 1,$$
$$\textbf{CD} : \textbf{Cd} : \textbf{cD} : \textbf{cd} :: mp + 1 : m + p : m + p : mp + 1.$$

This result may be reached more easily by making use of the general formula on p. 319.

For if $\quad$ **AB : Ab : aB : ab ::** $\quad n \quad : \quad 1 \quad : \quad 1 \quad : \quad n,$

and $\qquad$ **AD : Ad : aD : ad ::** $\quad p \quad : \quad 1 \quad : \quad 1 \quad : \quad p,$

then $\qquad$ **BD : Bd : bD : bd ::** $np + 1 : n + p : n + p : np + 1.$

Any number of derivative ratios may be ascertained in the same way by this method.

This more complex case may be represented graphically thus :—

According to this scheme of segregation (which however must not be regarded as the only possible one), each additional factor (or pair of allelomorphs) **Ee**, **Ff**, **Gg**, etc. will necessitate a further dichotomy of each branch. If these additional branches are equally developed it can readily be shewn that reduplication does not take place. We have the important rule that *equal dichotomies produce normal segregation; unequal dichotomies produce reduplications.* These two types of behaviour may occur in any order or at any stage in the phylogeny, but as Bateson and Punnett have already stated, they cannot occur simultaneously.

There seems to be no reason why the most various types of reduplication should not occur together in the same plant as the result of the same cross. The hypothesis of reduplication seems adequate to explain the occurrence of any type of ratio.

The most suggestive point which emerges from the analysis is the importance of the product nmp ... and of its constituent factors. From these, when all the factors and all the ratios in any one cross have been ascertained, it should be possible to compute the minimum number of successive cell-divisions needed to produce the complete system of segregation. It ought to be possible to determine also, in sweet-peas for example, the *number* of successive cell-divisions which normally intervene between the first division of the zygote and the last of the gametogenic divisions, and the *distribution* of these in the ontogeny. Comparison of the two results *might* serve to fix the stage at which segregation takes place.

It is then advisable to distinguish between *primary* and *secondary* reduplications. A ratio of reduplication ascertained by experiment may belong to either series. The gametic series is based upon the primary reduplications alone. Every observed type of reduplication must be assigned to its proper position. It is comparatively easy, as we have seen, to calculate the secondary from the primary reduplications.

The schemes on p. 323 will illustrate the relationships of primary and secondary reduplications.

It is perhaps advisable to add that systems of segregation will probably be seldom found in which all the primary reduplications take place between one factor **A** and a number of others **B, C, D, E,** etc. Primary reduplications may occur between any pair of factors, and the consequent secondary reduplications will undergo corresponding modifications.

The construction of the gametic series, when the ratios of primary reduplication are known, is easy, and from these any secondary reduplication is ascertainable. The following scheme illustrates such a system of reduplications:—

Primary reduplications	Secondary reduplications	
A and **B** $= n : 1$		
B and **C** $= m : 1$	**A** and **C** $= nm + 1 : n + m$	
C and **D** $= p : 1$	**A** and **D** $= nmp + n + m + p : nm + np + mp + 1$	**B** and **D** $= mp + 1 : m + p$

Factors A, B, C, D, E, F, etc. The ratios are halved for the sake of brevity.

Primary reduplications

A and B = n : 1
A and C = m : 1
A and D = p : 1
A and E = q : 1
A and F = r : 1
etc.

Secondary reduplications

B and C = $nm+1 : n+m$ C and D = $mp+1 : m+p$ D and E = $pq+1 : p+q$ E and F = $qr+1 : q+r$
B and D = $np+1 : n+p$ C and E = $mq+1 : m+q$ D and F = $pr+1 : p+r$
B and E = $nq+1 : n+q$ C and F = $mr+1 : m+r$
B and F = $nr+1 : n+r$

or more generally,

Primary reduplications

A and B = $n : m$
A and C = $p : q$
A and D = $r : s$
A and E = $v : w$
A and F = $x : y$

Secondary reduplications

B and C = $np+mq : nq+mp$ C and D = $pr+qs : ps+qr$ D and E = $rv+su : rw+sv$ E and F = $vx+vy : vy+vx$
B and D = $nr+ms : ns+nr$ C and E = $pv+qv : pw+qv$ D and F = $rx+sy : ry+sx$
B and E = $nv+mw : nw+mv$ C and F = $px+qy : py+qx$
B and F = $nx+my : ny+mx$

Two numerical examples may be added:

Primary	Secondary		
A and B = 2 : 1	B and C = 7 : 5	C and D = 13 : 7	D and E = 7 : 3
A and C = 3 : 1	B and D = 3 : 2	C and E = 2 : 1	D and F = 5 : 2 E and F = 31 : 11
A and D = 4 : 1	B and E = 11 : 7	C and F = 19 : 9	
A and E = 5 : 1	B and F = 13 : 8		
A and F = 6 : 1			

Primary	Secondary		
A and B = 2 : 1	B and C = 4 : 5	C and D = 8 : 7	D and E = 1 : 1
A and C = 1 : 2	B and D = 7 : 8	C and E = 1 : 1	D and F = 9 : 11 E and F = 1 : 1
A and D = 2 : 3	B and E = 1 : 1	C and F = 5 : 7	
A and E = 1 : 1	B and F = 7 : 5		
A and F = 8 : 1			

It is also noteworthy, that complex reduplications may arise owing to the combination of a primary and a secondary reduplication in the same gametic series; e.g. the primary reduplications may be of the following types:

$$\mathbf{A} \text{ and } \mathbf{B} = n : 1 : 1 : n$$
$$\mathbf{A} \text{ and } \mathbf{C} = m : 1 : 1 : m,$$
$$\mathbf{B} \text{ and } \mathbf{C} = p : 1 : 1 : p.$$

The first two alone involve a secondary reduplication between **B** and **C** of the type

$$nm + 1 : n + m : n + m : nm + 1,$$

and this combined with the primary reduplication between **B** and **C** gives the complex reduplication for **B** and **C** of

$$\mathbf{BC} : \mathbf{Bc} : \mathbf{bC} : \mathbf{bc} :: p\,(nm + 1) : n + m : n + m : p\,(nm + 1).$$

A careful study of systems of segregation will therefore, as soon as two or more reduplications have been discovered, furnish the student of genetics with data which will enable him on the one hand to test his hypotheses by further experiment, and on the other hand to extend and facilitate his researches.

It must be borne in mind however that cell-divisions, if they do really set up the phenomena of reduplication, must themselves depend upon the structure of the protoplasm. It may be that the systems of segregation will prove of some value in the analysis of this structure.

UNIVERSITY COLLEGE OF SOUTH WALES
AND MONMOUTHSHIRE, CARDIFF.

SOME RECENT WORK ON MUTATION IN MICRO-ORGANISMS.

II. Mutations in Bacteria[1].

By CLIFFORD DOBELL.

In the following article I shall try to give a coherent summary of some recent work in bacteriology in so far as it is of interest to the student of genetics. This work is very extensive and scattered through numerous papers dealing with medical or purely bacteriological matters. It is, moreover, to some extent beyond the reach of the general biologist on account of the phraseology in which it is couched. I shall try, therefore, to present the facts in such a way that they may be seen stripped of irrelevant detail and in language intelligible to the average reader.

Accordingly, the facts given in the following pages are to be regarded as a selection from a vast array recorded by many different workers, and not as a complete review of even those works given in the bibliography (p. 349). They represent, rather, certain personally chosen facts arranged in an orderly manner so as to interest workers in genetics. It must be remembered, therefore, that many additional facts—some of them perhaps of fundamental importance—are to be found not only in the works to which reference is made, but also in works which I have not considered[2].

I should like to point out that I employ the word "mutation" throughout in the sense of Wolf (1909), who follows Baur in this

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[1] In a previous article (this *Journal*, Vol. II. p. 201) I have given some account of recent work on mutation in Trypanosomes.

[2] Those desiring an account of the work hitherto done on variation in bacteria, will find it admirably analysed in the recent monograph by Pringsheim (1910).

matter. By *mutation*, accordingly, I mean a permanent change— however small it may be—which takes place in a bacterium and is then transmitted to subsequent generations. The word does not imply anything concerning the magnitude of the change, its suddenness, or the manner of its acquisition. The term denotes a change in genetic constitution. All other changes which are impermanent—depending generally upon changes of the environment—and not hereditarily fixed, are called *modifications*. The word "mutation" has been used with such different meanings by so many bacteriologists and others, that the foregoing statement seems called for. Indeed, discussions as to whether such and such a change is or is not a "mutation" might have been avoided in many cases if the opponents had defined their use of the word precisely. I do not wish to assert, however, that my usage is the correct one: I wish merely to state what I mean when I use the word in the following article.

It is not appropriate to discuss here, I think, the applicability of the word "mutation" to the Bacteria. I am well aware of the difficulties involved in applying the word—generally applied to certain changes in sexual multicellular organisms—to the Protista. I am also well aware of the difficulties involved in the above definition. Perhaps an imaginary concrete instance will serve to make its meaning clear. Let us suppose that a given *Bacillus* is coloured red under normal conditions. By growing it and its offspring upon a new medium they become—let us suppose—colourless. If the organisms and their descendants when transplanted again into the original medium are again found to be red, then the change (loss of colour) is a *modification*: if, on the other hand, they are found to be now permanently colourless, then the change is a *mutation*. By far the greater number of variations described in Bacteria are of the former type.

The mutations observed in Bacteria may be conveniently grouped into two classes—those in which the change is functional (e.g. changes in the power of producing ferments or pigments) and those in which the change is structural. Most of the mutations about to be recorded are of the former type—or physiological mutations, as I shall call them. I will therefore begin with a description of these, and consider some recent work on morphological mutations in a later section (p. 344).

A. PHYSIOLOGICAL MUTATIONS.

The first series of experiments which I shall describe in this section concerns changes in the powers of fermentation observed in the Bacteria belonging to the *coli-typhosus* group[1]. This is a very large group containing mostly gut-inhabiting organisms. It contains a large number of different "species" or "races," ranging from the common "harmless" *Bacillus coli* to the parasitic *Bacillus typhosus* of typhoid fever. It is convenient to regard these two organisms as the extreme limits of the group, and to place the dozens of other members in various intermediate positions according to their properties. Morphological differences between the members of the group are so slight and inconstant that a physiological classification is at present the only one possible. For our present purposes it should be remembered that—in addition to the difference in pathogenicity—*Bacillus coli* differs from *B. typhosus* in the following features among others: it is able to ferment lactose and glucose, to produce indol when grown in broth, and to clot milk. *B. typhosus*, on the other hand, can do none of these things. Owing to the differences in their powers of splitting sugars, we find that these two organisms shew characteristic differences when grown in certain test media. On the medium of Drigalski and Conradi, *B. coli* forms red colonies, whilst the colonies of *B. typhosus* are blue. On Endo's medium, similarly, *B. coli* forms

[1] For those unversed in bacteriology a few additional remarks concerning the naming of these organisms will perhaps be necessary. The common colon bacillus is variously known as *Bacillus coli* and *Bacterium coli commune*. The typhoid organism is called *Bacillus typhosus* or *Bacterium typhi abdominalis*. The other members are in part named on the binominal system, but frequently also on a trinominal or even quadrinominal or quinquenominal system (e.g. *Bacillus faecalis alcaligenes liquefaciens*). The strings of Latin names thus used are as a rule descriptive terms rather than ordinary specific or varietal names. In part, however, the members of the group are known by the names of their describers (e.g. Gärtner's bacillus, Flexner's bacillus). In part also they are designated by numbers or letters of the alphabet (e.g. paratyphoid bacillus B, etc.). Combinations of these methods are also resorted to. The vast numbers of these organisms, and the extreme difficulty of deciding upon the systematic status of the various "species," "varieties," "strains," etc. have thrown the nomenclature into a state of well-nigh hopeless chaos. Throughout this paper I shall call the common colon organism *Bacillus coli* and the typhoid organism *Bacillus typhosus*. In other cases I shall use the terms employed by the writers whose work I am considering.

red, *B. typhosus* colourless colonies[1]. In the absence of extraneous colouring matters, both organisms form colourless or whitish colonies.

We may begin our description of the mutations observed in the *coli-typhosus* group with a consideration of the fundamentally important work of Massini (1907)[2]. From a case of enteritis, this worker obtained an organism which at first grew as whitish colonies (like *typhosus*) on Endo's medium. After the third day of growth, however, minute red nodules appeared in the whitish colonies—the nodules increasing in number in the course of time until certain colonies contained as many as 200. These nodules were found to be solid masses of bacteria, constituting daughter-colonies within the parent-colony: and, as their colour indicated, they consisted of organisms which possessed the power of fermenting lactose (like *coli*). One would naturally suppose that this peculiar phenomenon was due to the impurity of the original culture—its two different constituents having simply separated out in the older cultures. By carefully plating out[3] the original culture, and by other tests, Massini convinced himself

[1] To understand what follows it is necessary to understand the principles involved in these methods, which are in everyday use for the identification of members of the *coli-typhosus* group. The principles upon which the Drigalski-Conradi and Endo mediums are compounded are similar. Both consist essentially of an agar medium containing lactose and a colour indicator. The medium of v. Drigalski and Conradi is slightly alkaline and contains litmus as an indicator. *B. typhosus* does not attack lactose, and therefore grows as a blue colony on the medium. *B. coli*, on the contrary, splits the lactose with the formation of lactic acid and gas. The acid produced by a growing colony turns the litmus red, the *coli* colonies thus being distinguished by assuming this colour. Endo's medium contains fuchsin, reduced by Na_2SO_3 to a colourless leuco-compound. *B. typhosus* accordingly grows on the medium in the form of colourless colonies: whereas *B. coli*, by splitting the lactose and forming acid, converts the reduced fuchsin back to its characteristic red colour. Colonies of *B. coli* on this medium are therefore distinguished by possessing the deep red colour with a green shimmer characteristic of fuchsin. It will be understood, therefore, that when in the following account a bacillus is said to form—let us say—red colonies on Drigalski-Conradi agar, what is implied is not that the bacteria themselves are red organisms, but that they possess the power of fermenting lactose, i.e. produce a lactase.

[2] Massini's investigations were carried out in the laboratory of Neisser. According to Pringsheim (1910) similar observations had been previously made upon yeasts by Hartmann, whose records I have not been able to consult (*Wochenschr. f. Brauerei*, 1903).

[3] "Plating out" is a method frequently used for testing the purity of cultures. It consists essentially in "diluting" a suspension of organisms with a large volume of culture medium, and then spreading it out on glass plates. The individuals are thus separated as far as possible, and the colonies which develop are known to be derived from single, or at most a few, individuals. By repeating the process a number of times a mixed culture can be detected and pure races isolated.

that this was not the case. Absolute proof of the matter could only have been obtained, of course, by isolating a single individual from the original culture and cultivating it further—a course which Massini could not follow owing to the great technical difficulties involved. The assumption that the original culture was pure was, however, rendered extremely probable. The organisms, when grown in media containing lactose (1 °/₀), always produced red daughter-colonies, but they never behaved in this way when grown in other media containing dextrose, mannite, or other similar substances instead.

Further cultivation of the colonies yielded remarkable results. The organisms in the red nodules had permanently acquired the power of fermenting lactose. They always produced red colonies on Endo's medium—never white[1]. Even after transplantation on to other media free from lactose, they never lost this power. Subsequent colonies again grown on Endo's medium were invariably pure red. The *typhosus*-like original, therefore, had given rise to a number of new individuals which closely resembled *coli*, having acquired the power of fermenting lactose.

Now the white parts of the colonies containing the red nodules behaved exactly like the original organisms. When transplanted, they produced colonies at first white but subsequently developing red nodules, the individuals in which bred true. White colonies, if transplanted every 24 hours, remained white: but they always produced red nodules if left for several days. The "white" race was therefore to all appearances constantly undergoing a partial mutation into a pure "red" race. Or, to put it in another way, the original non-lactose-fermenting race constantly split up into two daughter races—a pure lactose-fermenting race and a non-lactose-fermenting race of the same nature as itself (i.e. with the power of splitting into these two components again). The non-lactose-fermenting race might therefore be called an "ever-sporting variety," and it was called by Massini, in consequence, *Bacterium coli mutabile*. He regarded it as a *typhosus*-like organism which was constantly undergoing mutations into *coli* races[2].

The other characters of his remarkable race were carefully studied by Massini. He also tried to discover whether other *coli-typhosus*

[1] In one instance, under peculiar conditions, a single white colony was obtained from a red race. The red colonies never developed nodules.

[2] Massini regarded the "red" races as typical races of *B. coli*. See however the observations of Thaysen recorded on p. 333, footnote (*infra*).

organisms[1] behaved in a similar manner, but he could find no evidence that this was so. It may be mentioned that as regards their virulence and agglutination reactions the "white" and "red" races appeared to be identical.

It is somewhat remarkable that all the numerous workers who have subsequently studied these organisms have confirmed Massini's observations. But later workers have been able to amplify his work in several directions, and have discovered similar phenomena in many other races of *coli-typhosus* organisms.

Twort (1907) recorded independently that certain *coli-typhosus* organisms were able to acquire the power of fermenting certain sugars if grown in them for a sufficiently long time. The change took place slowly. In this manner he modified dysentery bacteria (Kruse and Flexner strains) so that they were able to ferment saccharose: and he was able to train *B. typhosus* to ferment lactose and dulcite. The organism which had acquired the power of splitting dulcite is stated to have retained this power permanently—even after passage through a guinea-pig and cultivation in a dulcite-free medium.

Burk (1908) isolated an organism almost exactly like that studied by Massini. He made similar observations upon it. Sauerbeck (1909) records closely similar results.

Benecke (1909) and Kowalenko (1910) were able to confirm Massini's results and to make an important addition to them. They succeeded in isolating and testing *individual* organisms. Both observers adopted Burri's Indian ink method[2], and obtained the same results, which completely agreed with Massini's original results. As Kowalenko's results are recorded in greater detail, they may be given here, though Benecke's were recorded first.

Kowalenko (1910) obtained both the races studied by Massini and

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[1] Amongst the forms investigated were typical *B. coli*, *B. typhosus*, Flexner bacilli, Shiga bacilli, and *paratyphosus* races.

[2] The introduction of this ingenious method has made it possible to isolate and cultivate individual organisms from a colony of bacteria with comparative ease. (See Burri, *Das Tuscheverfahren*, Jena 1909.) In principle the method is as follows. A drop of fluid containing a few bacteria is mixed with a sterile solution of Indian ink. A minute drop of the mixture is then placed under a cover-glass and examined under the microscope. The bacteria can be seen, under a comparatively low magnification, as white dots in a black field: and the small number of individuals in each preparation can be counted. Those preparations containing but a single individual are then selected, their contents transferred to a suitable culture medium, and cultivated further. In this way it may be known with certainty that the culture produced has sprung from one original individual.

Burk. When sub-cultures were made from them by isolating individual organisms, precisely the same results were obtained. That is to say, it was found that a single individual (belonging to either race) which at the outset was unable to split lactose, produced in presence of this substance offspring which were in part like itself and in part able to split lactose. The only possible objection to Massini's results was therefore removed. Single individuals from " white " colonies gave rise to mutating races: single individuals from " red " colonies bred true. Their properties were unaltered by passage through animals, by changes of temperature, or by phenol and other drugs.

A series of investigations parallel to those which have so far been mentioned has been conducted by Reiner Müller (1909, 1911), whose work appears to have been extremely thorough. Altogether he has studied several hundred races of organisms of the *coli-typhosus* group, especially as regards their powers of fermenting 18 different kinds of carbohydrates.

The most important outcome of Müller's work has been the demonstration that all typical races of *B. typhosus* behave towards rhamnose exactly as Massini's *B. coli mutabile* behaves towards lactose. That is to say, typical pure races of *B. typhosus* are unable to ferment rhamnose. When grown in a medium containing this sugar, however, the colonies develop daughter-colonies (nodules) consisting of individuals which have permanently acquired the power of splitting rhamnose. He claims that this is the most typical cultural character of the typhoid bacillus[1]. Mutations of this sort invariably occur, and under no conditions do the rhamnose-splitting organisms lose their power. Müller has confirmed these observations in the case of individuals isolated by Burri's method. A single non-rhamnose-splitting individual gives rise in the presence of this substance to offspring which are partly like itself and partly able to ferment rhamnose.

In the case of certain paratyphoid organisms (*paratyphosus B.*, Schottmüller) Müller has found that rhamnose and lactose do not produce any corresponding change. But the organisms behave towards raffinose exactly as *mutabile* behaves towards lactose, or *typhosus* behaves towards rhamnose. In other words, *paratyphosus B.* grown in raffinose-containing media produces daughter-colonies which can ferment this sugar—though the mother-colony cannot do so. These raffinose-fermenting organisms never revert to the non-raffinose-fermenting type.

[1] No less than 120 races of *B. typhosus* were studied.

A case of a similar nature has been recorded by Jacobsen (1910). From a typhoid epidemic he obtained a bacillus (*A*) which differed from a typical *B. typhosus* in that it refused to grow properly on the Drigalski-Conradi medium[1] used in the laboratory. Finally, however, nodules appeared in these weakly colonies, and were found to consist of individuals which grew strongly in the orthodox manner. On cultivation the latter organisms were proved to be typical *B. typhosus*. Experiments showed that the Drigalski-Conradi medium had been altered in some way by repeated autoclaving[2]. The altered medium retarded the growth of the strain *A*[3], which was able, however, to undergo a partial mutation into strains which could grow easily in the medium (*B. typhosus*). Jacobsen—on analogy with Massini's results—proposes to call his original strain *A* by the name *B. typhi mutabile*. The mutated form—*B. typhosus*—never reverted to the original type (*A*). Jacobsen's results have been in the main confirmed by R. Müller (1911).

Some other cognate facts may be briefly considered here. Schröter and Gutjahr (1911) record the following observations. A race *Y* of *coli-typhosus*-group organisms cannot ferment maltose. After cultivation, however, it partially acquires the power—thus coming to resemble Flexner bacilli. The *Y* organisms appear to behave towards maltose as *B. coli mutabile* behaves towards lactose. They found similar changes in other related organisms. For example, Shiga-Kruse bacilli may acquire the power of splitting both maltose and saccharose when grown in media containing these sugars. The change appears to be permanent—that is, once the property is acquired by the race, it is never lost.

Sobernheim and Seligmann (1911) have isolated an organism which behaves exactly like Massini's *B. coli mutabile*. They have confirmed their results by isolating individuals by Burri's method. (See p. 330, footnote.) In addition, they record that they have obtained four different pure races from one pure original race. From a *coli-typhosus*-group organism (Haustedt) they have obtained in pure culture (1) a true Gärtner strain, (2) a similar strain, but differing in agglutinating power, (3) a typical *typhosus*, and (4) a strain almost identical with *B. coli mutabile* (Massini).

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[1] See p. 328 footnote.

[2] An autoclave is an apparatus used for sterilizing culture media, etc. Sterilization is effected by steam under pressure.

[3] But not, of course, of the typical strains of *B. typhosus*.

In a preliminary note Thaysen (1911) announces that he has isolated and studied eight different races of *coli-typhosus*-group organisms. These have the following properties: Four races ferment dextrose, maltose and lactose, but not saccharose. By cultivation, however, they acquire the power of splitting this sugar also. One race splits dextrose and maltose, but not saccharose or lactose. But it can acquire the power of splitting the last named sugar. It appears to be identical with *B. coli mutabile*[1]. Two races are similar to the preceding, but can acquire the power of splitting saccharose. They appear to be similar to *B. imperfectum*[2] of Burri. Finally, one race splits dextrose, maltose, and saccharose, but not lactose. It can, however, acquire the power of fermenting this substance [3].

Up to this point I have mentioned only those results which are essentially similar to Massini's. I have purposely avoided referring to the work of Burri and his collaborators. The work so far is at the same stage, but Burri's work represents a step forward. Let us now consider it.

The papers of Burri and Düggeli (1909) and Burri and Andrejew (1910) may be considered—for the present purposes—as parts of the admirable work of Burri (1910), which I shall now endeavour to chronicle.

Burri has isolated[4] a race of organisms of the *coli-typhosus* group which are unable to ferment saccharose and lactose. He calls this race *Bacterium imperfectum*. It never acquired the power of splitting lactose: but on the other hand, when grown in media containing saccharose, some colonies acquired the power of splitting this sugar. The saccharose-splitting mutant Burri names *B. perfectum*[5]. (Both organisms belong to the *paratyphosus* sub-division of *coli-typhosus* organisms.)

Burri's observations were originally made upon organisms grown in "shake-cultures[6]"—not on Endo's medium. The cultures were made

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[1] The lactose-splitting mutant from *B. coli mutabile* is, according to Thaysen, unable to produce indol. It is therefore not a typical *B. coli*.

[2] *Vide infra.*

[3] Thaysen's full paper has appeared whilst this article is in the press. See *C. B. Bakt.* I. Abt. (Orig.), Vol. LXVII. 1912, p. 1.

[4] From fermenting grass.

[5] On analogy with this, he proposes to call the lactose-fermenting mutant derived by Massini from the non-lactose-fermenting *B. coli mutabile* by the name *B. coli mutatum.*

[6] In a shake-culture the organisms are distributed, by shaking, through a liquefied jelly—in this case containing saccharose. When the jelly has set, the isolated organisms produce colonies. Those which ferment the sugar produce bubbles of gas in the jelly and can therefore be readily distinguished.

both in the ordinary way and also from individuals isolated by his Indian ink method (p. 330, footnote). Each non-saccharose-splitting initial organism was found to give rise to only "a very low percentage" of saccharose-splitting colonies. The latter, however, had acquired the power permanently, and always bred true to this character—after "any number" of sub-cultures in many different media.

Burri then obtained Massini's strain from Neisser, and tested it by shake cultures. He made, conversely, test cultures of *B. imperfectum* on a modified Endo medium (containing saccharose instead of lactose). He concluded from all his experiments that *B. imperfectum* behaves towards saccharose exactly as *B. coli mutabile* behaves towards lactose[1].

Experiments were then made to determine what percentage of the offspring of *B. imperfectum* (non-saccharose-splitting) underwent mutation into *B. perfectum* (saccharose-splitting). The results of some ingenious work were surprising. An initial culture begun with some 10,000,000 germs of *imperfectum*, produced about 50 colonies of *perfectum*, but the majority were of the *imperfectum* type. Beginning with about 100,000 *imperfectum* (only $\frac{1}{100}$ of the first number), however, he again obtained about 50 colonies of *perfectum*. And by beginning with a very few germs, he found that practically every colony was of the mutated form (*perfectum*). "In a series of cultures with a diminishing number of initial germs, of which the greatest is a million times greater than the least, each member of the series shows approximately the same number of mutated colonies[2]." Burri was thus able to demonstrate that *all* the individuals of the *imperfectum* race are—under suitable cultural conditions—able to mutate completely into *perfectum* forms. In other words, all *imperfectum* individuals are, as regards their power of mutation into *perfectum* races, equipotential. The apparent partial mutation of the race is dependent upon the different environmental conditions to which the different individuals of a large colony are subjected. When the individuals are sufficiently separated so that every one is offered the same favourable opportunities, they all behave in the same way—that is, all mutate. To borrow a term from Driesch, every individual has the same prospective potency.

[1] Massini, p. 328 *supra*.

[2] In other words, suppose we begin by sowing 100 million individuals—we get then nearly all the colonies like the initial germs, and only x colonies with the new character. If we sow only x individuals of the initial germs, however, we obtain x colonies with the new character. Similarly, any number of initial germs between 100 million and x, always produced x mutations.

By a further set of nice experiments which cannot here be considered in detail, Burri has tried to shew that the power to split saccharose does not appear all at once[1]. Between the non-saccharose-splitting *imperfectum* and the saccharose-splitting *perfectum* intervene many generations of individuals shewing every transitional stage. The power of fermenting saccharose is gradually acquired in small successive steps, until it manifests its full development in the actively fermenting form *B. perfectum*. The power once acquired is never lost—it always persists in the offspring. Moreover, individuals which are in a transitional stage do not lose such partial activity as they have acquired. Individuals which have only "half-acquired" the power of splitting saccharose[2] may be transplanted to a saccharose-free medium. If their offspring subsequently come in contact with the sugar they then acquire complete power of fermenting it in half the time necessary for an ordinary *imperfectum* race to do so.

Burri supposes that the power to ferment saccharose is latent in every *imperfectum* individual, probably in the form of a zymogen or pro-ferment of some sort. The enzyme is produced gradually by the constant action of the sugar on successive generations. He thinks it probable that the newly acquired power of attacking saccharose does not represent a "regeneration" of a power originally present in the race but temporarily lost[3].

All workers whose records we have so far considered have been unanimous on one point—namely, that when a race has once acquired the power of fermenting a certain sugar, it remains constant in this respect (i.e. "breeds true"). A few observations have been made, however, which shew that a reversion to the original state may occur in some races—that is, the acquired power may be subsequently lost under certain conditions. An instance of this sort is to be found in the work of Bernhardt and Markoff (1912). They isolated[4] a *coli-typhosus* organism ("No. 459") which behaved exactly like *B. coli mutabile*. It grew as a blue colony on Drigalski-Conradi agar[5], and constantly gave rise to red nodules in the mother-colony. The organisms

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[1] The change is said to be "relatively quick, but not sudden."

[2] That they have partially acquired the power is inferred from this and other experiments. There is no outward and visible sign (gas production, etc.) of this "half-acquisition."

[3] Burri will not call the change a "mutation," because it is a gradually acquired adaptation. According to him the change is really not the acquirement of a new character, but the realization of a faculty already existing potentially. This appears to me, however, to be applicable to all variations—of whatever sort—in all organisms.

[4] From a patient suffering from an intestinal complaint.

[5] See p. 328, footnote.

from these nodules were found to breed true (i.e. as regards power of fermenting lactose). Attempts to obtain blue colonies from the "red" individuals almost always gave negative results. However, by passing the organisms from the red nodules through mice and rabbits they succeeded in obtaining a race like the original—one, that is, which produced blue colonies with red nodules on Drigalski-Conradi agar[1]. It seems, therefore, that the acquired power of fermenting lactose—which usually breeds true—may, under certain conditions, be lost.

Results somewhat like those of Bernhardt and Markoff, but different from those of most other investigators, have been just recorded by Baerthlein (1912). He has studied 13 different races of *coli-typhosus* organisms from the guts of healthy and diseased persons. All these races behave like Massini's *B. coli mutabile*; producing blue colonies on Drigalski-Conradi agar, colourless colonies on Endo agar, but shewing subsequently the characteristic mutations in the form of red nodules. These lactose-splitting organisms were found to retain this character after cultivation for a long time on Drigalski-Conradi and various lactose-free media. Nevertheless, Baerthlein claims that if the lactose-splitting organisms are cultivated continuously on ordinary agar, they revert in part to the non-lactose-splitting form. After only 6–7 days on agar, organisms transplanted back on to Drigalski-Conradi medium develop into both red and blue colonies. It follows, therefore, that after even so short an interval, the organisms may go back to their original form. These observations do not seem to square with those of most other workers. Perhaps the explanation is to be sought in the fact that different observers have studied different races, which do not all behave alike. Baerthlein, it may be noted, has supplemented his work by a morphological, cultural, and serological study of his races[2]. Confirmation of his results—by experiments on isolated individuals and on related races—is much to be desired.

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[1] The original "red" race was first tested and found to be pure. Bernhardt and Markoff state that they succeeded "often" in performing this experiment, but "not always." With the original strain of Massini, however, they obtained only negative results.

[2] From Baerthlein's paper it is to be gathered that the behaviour of *B. coli mutabile* is even more complicated than at first appeared. He says that races of this organism (and also of ordinary *B. coli*) undergo mutation when grown on ordinary agar. The original race splits up into two *constant* daughter-races—(1) forming transparent colonies consisting of long, slender, individuals ; (2) forming opaque yellowish colonies of shorter and stouter individuals. Both these races when transplanted to a lactose-containing medium mutate into a lactose-splitting and a non-lactose-splitting race—though both these retain the morphological characters of their originals. Four different races are thus produced from the initial race. All may " revert " under suitable conditions.

The changes undergone by organisms of the *coli-typhosus* group appear to be in all cases of the same nature—so far as they have been considered up to this point. All the changes appear to be direct *adaptations*. An organism comes in contact with a new sugar which it is unable to use for its own growth. It then changes itself so that it can split the sugar—the change being thus definite, towards a definite end, and apparently purposive. There appears to be a definite relation between the sugar and the mutation. In some other cases, however, this relation is not obvious. Two instances of this may be briefly mentioned. *B. typhosus* when grown on glycerin-agar produces acid. A closely related form (*B. metatyphi*), however, according to Mandelbaum (1912) produces alkali instead. But if *metatyphi* is grown for a long time on this medium, it forms nodules in the parent colonies—exactly like those produced in colonies of *B. coli mutabile*. The individuals in these nodules are typical acid-producing forms indistinguishable from ordinary *B. typhosus*. They do not revert to the alkali-producing form. In other words, *metatyphi* mutates partially into *typhosus*[1].

Revis (1911) has studied certain *coli-typhosus* organisms which produce both acid and gas when grown in peptone broth containing certain sugars or polyhydric alcohols (e.g. lactose). He was able gradually to acclimatize these organisms to a medium containing $0.1 °/_{\circ}$ of malachite green. The organisms after this, however, had permanently lost the power of producing gas in the original media, though they could still form acid. The dye appears therefore to have made a lasting change in their method of attacking certain food-substances[2].

It will be seen, I think, that in these two cases just quoted the changes produced are not obviously of an adaptive nature—though possibly a greater knowledge of the chemistry of the matter might bring them into line with the preceding observations. But in the cases which we are now about to consider the changes produced seem to be quite definitely *not* adaptive. The mutations concern pigment-production—not ferment-production[3].

[1] Mandelbaum calls it a "remutation" or "atavistic throw-back" because he believes that *metatyphi* has arisen by mutation from *typhosus*.

[2] Revis (1911) recorded only a single instance in which this change was observable. But in a subsequent paper (Revis, 1912) he states that he has been able to effect the same change in two other races of similar organisms.

[3] For earlier work on both these subjects consult Pringsheim (1910). Here also will be found an account of similar changes in ferment-production among Fungi.

There is one important paper to be considered in this connexion. It is a record of some extensive experiments performed by Franz Wolf (1909)[1], who has studied *Bacillus prodigiosus, Staphylococcus pyogenes, Sarcina lutea,* and numerous Myxobacteria. The aim of the experiments was to induce mutations, in respect of colour, by chemical or physical means. It may be stated at once that *S. lutea* gave only negative results, and that most of the changes observed in the other species were modifications—that is, transitory changes, not mutations (cf. p. 326). Many interesting observations were made concerning the effects of temperature changes and of a large number of chemical substances. But as we are now concerned with the mutations, further mention of the modifications will be omitted[2]. It should also be mentioned that all the observations were carefully controlled by means of plate cultures[3].

The first series of experiments was made with the historically interesting *B. prodigiosus*[4]. The initial colony on gelatine was coloured bright red[5]. Its purity was carefully tested, and as a further control the organisms were propagated under "normal" conditions for a long time. The strain was many times transplanted (altogether more than 50 times), being tested by a series of plate cultures each

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[1] It should be noted that Wolf's results are not always easy to comprehend from his account of them. Certain statements, for example, in his summary contradict others in the body of the paper. Dr Wolf has, however, kindly elucidated (in correspondence) all the doubtful passages, so that I trust my statements in the following lines coincide accurately with the facts. The discrepancies arose chiefly in the following way. The paper originally contained a summary—in graphic form—of *all* his experiments. This was eliminated by the editor, as he thought it superfluous. Without it, however, the paper is not always easy to understand, as in the text details are not given of all the cultures.

[2] These modifications may, however, have some significance for the interpretation of the mutations. The original should be consulted.

[3] No cultures were made from isolated individuals, as the organisms were found to be too small for this to be done. The plating out was done so carefully and frequently, however, that this is probably a matter of small moment.

[4] This is Ehrenberg's *Monas prodigiosa*—the organism which is supposed to have given rise to the legend of the Bloody Host. It occasionally appears in large masses on bread, etc.

[5] The bright blood-red pigment has been named "prodigiosin." Though it has been much studied, its chemical composition is still practically unknown. It possesses several remarkable properties. It is formed only in presence of oxygen. It is bleached by sunlight—both from colonies of the bacteria and from solutions. Like most similar pigments, it is not present in the organisms themselves, but is excreted into the surrounding medium—thus giving the colonies their characteristic colour. It is not known what part it plays in the economy of the bacteria.

time. As some of the colonies isolated in this way were found to be slightly paler or darker than the original, attempts were made to obtain new varieties by selecting these. All such attempts gave negative results. It seems certain therefore that Wolf was not dealing with mixed cultures or with a race which was spontaneously mutating.

By growing *B. prodigiosus* in media containing minute quantities of various salts, Wolf succeeded in obtaining a few races with permanently altered colour. He obtained one white race and four dark red races. The white race appeared after 14 successive transplantations on a medium containing $0.01 \, ^{\circ}/_{\circ}$ of corrosive sublimate (at 37.5° C.). The dark red races arose in a similar manner, after a varying number of transplantations, on media containing minute quantities of potassium permanganate, cadmium nitrate, corrosive sublimate, and potassium bichromate[1]. All these changes were absolutely permanent—the colonies never reverting to the original colour when grown for a long period in normal media[2].

In some other experiments the mutations were of a different type. They occurred in four cases in organisms grown upon media containing potassium bichromate, copper acetate, cadmium nitrate, and nickel nitrate. All these mutated races were white, but they proved to be inconstant. When plated out, the white races gave rise both to white colonies and to colonies of the original red colour. The white colonies behaved again in the same way—giving when plated out some red and some white colonies. A white race could be constantly maintained in this manner by selection, but it was, obviously, differently constituted from the permanent white race obtained by the action of $HgCl_2$. These impermanent white forms Wolf calls "reverting mutants."

One of the most striking features in these experiments is, I think, the fact that the same chemical substance may produce several quite different mutations. Thus, $HgCl_2$ produced a pure dark red race, and a pure white race—that is, mutations in two different directions (intensification of colour and loss of colour). Similarly, $Cd(NO_3)_2$ produced a constant dark red mutation and a reverting white. The action of $K_2Cr_2O_7$ is even more remarkable. Under the influence of this salt,

[1] These also were in cultures kept at 37.5° C. But with $K_2Cr_2O_7$ the mutation was obtained at room temperature also.

[2] In Wolf's experiments raising the temperature produced only a white *modification* in colonies grown on ordinary media. But it is stated by Migula (*Syst. d. Bact.*, Vol. II., p. 845) that *permanent* white races can be obtained by this procedure.

the original bright red colony gave rise to a *dark red mutation*, and a *white reverting mutation*. In addition to these, it produced a *white modification*. And when the dark red form was subjected to the continued action of the bichromate, it produced in turn another *white reverting mutation*. Certain substances seem, therefore, undoubtedly to cause profound changes in the pigment-metabolism of *B. prodigiosus*. But it seems that the changes themselves are purely a matter of chance: for they may be permanent, partly permanent, or impermanent, and in either of two opposite directions. Another noteworthy point is that two quite different poisons may produce the same sort of mutation. For example, $HgCl_2$ and $K_2Cr_2O_7$ may both give rise to dark red races.

In his investigation of *Staphylococcus pyogenes aureus*, Wolf obtained less definite results[1]. In some cultures temporary modifications of colour appeared, but he was never able to produce a permanent change—or mutation—by chemical means. Only one mutation occurred, and that was in the control series of cultures on ordinary media. On plating out the organisms at the 22nd transplantation, Wolf found three white colonies among the remaining typical dark-yellow *aureus* colonies. These white races subsequently bred true. They never reverted to yellow. Moreover, they were found to answer all the cultural and other tests of the organism known as *Staph. pyogenes albus*[2]. It would seem, therefore, that *albus* may arise under "normal" conditions from *aureus*: though—as the author says—the mutation arises "from unknown causes."

Wolf did not obtain any other colour mutations in the case of true Bacteria. But he made some curious observations upon the Myxobacteria[3], which he also investigated. (He observed a number of transient colour changes (modifications) in *Myxococcus* races, but these need not be considered here.)

It had already been shewn by Quehl that physiological differences exist between different races of Myxobacteria. If two swarms[4]

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[1] Neumann had previously stated that he was able to *select* races of several different colours from this form. Wolf, working upon a *pure* line, was unable to do this.

[2] Several similar *albus* mutations occurred in the course of plating out subsequent sub-cultures of this series.

[3] The Myxobacteria constitute a remarkable group of the Protista. They differ in many ways from ordinary bacteria, and their systematic position is still very doubtful.

[4] The swarms consist of large numbers of separate individuals (like ordinary bacteria in some respects) invested with a common covering of slime. The swarms give rise to fructifications (resembling those of the Mycetozoa) in which spores are formed in a peculiar manner.

belonging to the same race are brought side by side on the same culture medium, they are seen to fuse as soon as their edges come in contact. In the case of different races, however, no fusion occurs between the contiguous edges under such conditions. Wolf endeavoured by various means to obtain from a pure race (in which the swarms readily fused) physiologically different races which would not coalesce when brought in contact. He easily succeeded in doing so in races belonging to two different species—*Myxococcus rubescens* and *M. virescens*. He found that the same race could produce swarms which would not fuse, if they were cultivated for a sufficiently long time. The change occurred even when the organisms were grown on the same medium and under the same conditions (*M. rubescens*). But the change took place more rapidly if the swarms were cultivated at different temperatures, on different media, or on media containing various salts ($K_2Cr_2O_7$, KNO_3, etc.). The peculiar feature of the change was that it was irreversible. When once the swarms had lost the power of fusing, no amount of further cultivation on normal media would induce them to revert to their original condition. It seems obvious, therefore, that under certain ill-defined conditions, a permanent physiological change may occur. But it is by no means obvious what the nature of the change is—its only manifestation being an inability to fuse, which might be due to several different causes. It seems impossible at present to draw any further conclusion from these observations.

A place must here be given to some statements just made by Baerthlein (1912 A), as they concern the colour mutations of *B. prodigiosus* and *Staph. pyogenes*. This worker states that by merely plating out on agar old cultures (in agar or broth) of *B. prodigiosus*, he can obtain numerous mutated colonies. These may be dark red, pink, white, white with red spots, red with white sectors, etc. The individual organisms in these different colonies also differ structurally from one another. These mutated races are said to breed true. They preserve all their characteristics when cultivated further on various media or after passage through animals. "Atavistic phenomena in the form of reversions" occur, however, if the mutated colonies are left for a long time in the same medium, and then transferred to fresh media. Similar changes are said to occur in the case of *B. pyocyaneus*. Baerthlein's brief statements are not easy to reconcile with Wolf's careful work on the same organism. It seems possible that he has not been dealing with pure lines.

Baerthlein states, further, that by a similar procedure he is able to make *Staph. pyogenes aureus* mutate into *Staph. pyogenes albus*[1]. The *albus* race breeds true. But by treating it similarly, it will "mutate back" into *aureus* races. *Albus* and *aureus* races are, moreover, said to differ as regards the structure of the individual organisms.

Baerthlein records similar observations on a number of other bacteria. But until further details of his work are available, it seems useless to attempt to criticize his results or to correlate them with those of Wolf and other workers in the same field.

A case of an altogether different sort may now be mentioned. Every bacteriologist is familiar with the fact that the power to form spores is a very variable character in some races of bacteria. Sporogenic and asporogenic races have been observed in many different species. In a recent paper, Eisenberg (1912) has given some interesting facts concerning this matter in the case of anthrax bacilli. His results are particularly noteworthy: for, although very many similar observations have been made, the interpretation is not excluded that the results were due merely to a selection of certain pure lines from an originally mixed population.

Laboratory cultures of *Bacillus anthracis* may consist—according to Eisenberg—of mixtures of sporogenic and asporogenic races (most frequently), of pure asporogenic races (less often), or of pure sporogenic races (seldom). Pure sporogenic and asporogenic races may be obtained from a mixed culture; the former by the action of heat, the latter by constantly transplanting young cultures on to fresh media. A pure sporogenic race may thus be obtained. And Eisenberg has found that if it is constantly grown (5–20 transplantations) on glycerin-agar, it completely loses its power of forming spores. These asporogenic races bred true for a considerable time. They never reacquired the power of forming spores. The conclusion appears to be justified, therefore, that in *B. anthracis* a sporogenic race may mutate into an asporogenic race under certain conditions (in the present case under the influence of glycerin?).

In his ingenious experiments with bacteria, Barber (1907)[2] endeavoured to obtain asporogenic races by the isolation of individual organisms. A culture of organisms isolated from the juice of the sugar-cane (*B. megatherium*?) was found to contain some individuals

[1] Compare Wolf, p. 340 *supra*.   [2] *Vide infra*, p. 345.

which formed spores and others which did not. Barber isolated a number of individuals of the latter type, and cultivated them further. In only one instance, however, did he succeed in thus obtaining a permanently asporogenic race. In all other cases the isolated individuals gave rise to spore-forming colonies. The mutation in this case may be called spontaneous. There is nothing to indicate that it was due to any particular external conditions.

Some remarkable statements concerning "mutations" in the cholera vibrio have been made recently by Baerthlein (1911), and a further contribution to the same subject has just been made by Eisenberg (1912 A). It is said that pure cultures of this organism when plated out on agar constantly give rise to several different kinds of colonies—produced by "mutations" from the original race. The "mutated" forms are said to be in some cases constant, in others inconstant or capable of undergoing further "mutations" or "reversions." The facts at present available seem so confusing, and sometimes so contradictory, that I think no very satisfactory conclusions can yet be drawn from them. I will therefore merely refer the reader to this work without attempting to discuss it here.

It seems legitimate to conclude from the foregoing facts that some races of bacteria are able permanently to acquire new characters under certain conditions: and also that they may in a similar manner lose these characters subsequently. So far we have considered the behaviour of bacteria under experimental conditions only, but one is naturally led to inquire whether similar changes occur in nature. The answer to this question is obviously of the greatest importance not only to the biologist but also to the medical man: and it has frequently been given both in the negative and in the affirmative. A little reflection on the problems involved in the question will suffice to make this easily explicable. It is, in fact, at present impossible to answer the question in other than a most tentative manner. Nevertheless, a few cases have been recorded which seem to throw some light on the matter. With one of the best and most recent of these I will conclude the present section.

Sørensen (1912) has just recorded the following remarkable facts. A patient suffering from glycosuria, developed in addition pneumaturia. This was found to be caused by a peculiar bacillus (*B. pneumaturiae*) which had gained access to the bladder, and by fermenting the sugar there produced large quantities of gas. The organisms were isolated

and carefully studied in cultures, in which their behaviour was similar to that observed in the bladder. They fermented glucose, lactose, and saccharose with the production of much gas. After about two years, the patient recovered spontaneously from the pneumaturia, but the bacteria were still present in the bladder. Both here and in the cultures, however, they were found to have lost completely the power of forming gas by the fermentation of sugars. The cultures were kept for a long time subsequently, and repeatedly tested to see whether they would regain their gas-forming power. After about a year, these organisms suddenly reacquired the power of forming gas from lactose and glucose; and shortly after, the patient began to suffer once more from pneumaturia. Examination of the bacteria from the bladder, showed that they had—like the organisms in the cultures—reacquired their gas-forming power[1].

The behaviour of the organisms in the patient was therefore closely parallel to their behaviour in the artificial cultures. Moreover, the experiments seem to shew that the same bacteria were present throughout—that is, it was not a case of mixed cultures or a reinfection. It seems justifiable, therefore, to conclude that in this case at least a physiological character was lost and reacquired by the bacilli not only in artificial cultures, but also under "natural" conditions in the living organism.

B. Morphological Mutations.

Concerning structural variation in bacteria very little indeed is known. This is not because structural differences between members of the same species are uncommon, but because the normal structure and life-history of nearly every species is still largely a matter of conjecture. Many species are probably polymorphic, the various forms depending partly upon the particular stage which has been reached in the specific life-cycle and partly upon external conditions. Many bacteria, moreover, continue to exist and multiply after they have assumed a degenerate or abnormal form (so-called involution forms). The modifications dependent upon these various factors are not variations in the ordinary sense of the word. One would be equally justified in classifying a spermatozoon, a foetus, a leper, and a man with both his legs cut off, as structural variations of the human species.

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[1] Both the organisms in the cultures and those in the bladder had also acquired the new power of clotting milk.

There is little to record concerning structural variations which are permanent—mutations, that is, which when they have once appeared breed true in subsequent generations. Only two cases of this sort will be noted here.

First, the work of Barber (1907) on *Bacillus coli*[1] must be mentioned. This observer began with pure cultures of bacteria, and grew all his sub-cultures on the same media and under the same conditions. The environment was therefore alike for all individuals—as far as possible. Barber noticed that there were constantly present, among the typical individuals in his cultures, a small number distinguished by their greater length. By a special method which he devised[2], he was able to select these long individuals and propagate them further. No less than 140 such individuals were so isolated in one series of experiments. With a single exception they all gave rise to colonies consisting of individuals with the normal dimensions[3]. The variations were, in other words, modifications—not permanent changes. In the one exceptional case, however, he succeeded in obtaining a new race of long individuals. This race bred true. It was kept for 32 months—being frequently transplanted—without undergoing any change. Selection of maximal and minimal sized individuals of this race was also without effect: neither the original nor a new race could be obtained. The mutated race had partially lost its motility, and differed also in certain cultural characters from the original race. In another series of experiments, Barber isolated 50 long individuals from another strain of *B. coli*. In this instance he succeeded in obtaining a similar long race which proved to be constant as regards this character. It was found necessary sometimes to make several successive selections from the colonies of mutating organisms in order to attain a pure fixed race. Altogether three new long races were finally established.

Similar experiments were only partially successful in the case of *B. typhosus*. No constant long race was obtained.

The mutations, it will be seen, occurred spontaneously in all these cases. New races were established by the selection of individuals which had already varied. It seems that the long individuals which occurred in the original cultures were of two classes—though outwardly

[1] Barber made a number of similar experiments with yeasts.

[2] The method is fully described in the original. It consists essentially in a direct selection, under the microscope, of a desired individual by means of a very fine glass capillary tube.

[3] That is, in those cases in which successful cultures resulted. In many cases, the isolated individuals grew badly or not at all.

more or less alike. The majority represented merely temporarily modified individuals: only a very few were permanently mutated organisms. There is, of course, nothing to indicate what factors may have been concerned in the production of these forms in the first place.

I will end this section by referring to some work which has just been published by Revis (1912). His observations also concern *B. coli*. A typical strain of this organism was grown in peptone broth to which malachite green had been added[1]. The effect of this dye was to produce a new race of organisms which differed both structurally and culturally from typical *B. coli*. When the organisms were grown subsequently at 20° C. on ordinary gelatin or agar, they formed "large viscous, circular masses," consisting of "a mixture of very long filaments[2] and short bacilli, together with a gummy cementing substance." Presumably the race breeds true to these new characters. The organisms were not propagated by the isolation of individuals, but the cultures were very carefully plated out. The purity of the original culture is guaranteed. Revis therefore claims to have produced—by means of malachite green—from a typical *B. coli* a new race "which is neither physiologically, morphologically, nor culturally a colon bacillus." Further details and confirmation of these observations are to be desired.

Concluding Remarks.

To epitomize in few words the numerous facts given in the foregoing pages is hardly possible, for what has been written is itself one long epitome of facts and their interpretations. A few general remarks may, however, be permissible to a writer who has travelled thus far over very stony ground. They may serve, moreover, to call attention to certain facts which are undoubtedly important—though the magnitude of their importance and their significance may well be appraised in very different terms by different individuals. It must be understood,

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[1] See also p. 337 *supra*.

[2] It was already known that organisms of the *coli-typhosus* group—and others also—assume a remarkable filamentar form when grown on media containing certain dyes. The first observations in this connexion were made in 1904 by Walker and Murray (*British Med. Journ.*, Vol. II. p. 16). Similar results have since been obtained by Vay (*C. B. Bakt.*, I. Orig. 55, 1910, p. 193) and others.

then, that the following remarks embody merely my own conclusions drawn from the facts given in the body of this paper and from many other related facts of which no mention has been made. They lay claim to no finality, for the subject is not one upon which any final judgment can yet be passed.

If it be assumed that the statements made by various workers, whose observations we have been considering, are correct, then the following conclusions are justifiable. First, it seems established that the Bacteria are subject to mutation—that is to say, in a given race individuals may occur which differ from their fellows in their genetic constitution. Individuals frequently occur which possess new structural or functional features; and these features, though often the transient peculiarities of the individual only, are in some cases transmitted to the offspring for many successive generations. There is reason to suppose that this phenomenon occurs in nature as well as in laboratory cultures. The progeny of an organism which varies may thus constitute a new race, in which every individual possesses the new character. We might anticipate this, indeed, by consideration of the fact that the Bacteria are non-sexual organisms. For a change in the genetic constitution of the parent—where there is but one—appears likely to find expression in all its offspring. There is no additional complication—in the transmission of characters—introduced by a second parent. In sexually-producing organisms, the genetic constitution of two parents must always be considered, and there is not, therefore, such an obviously direct relation between any one parent and its offspring as is seen in non-sexual forms.

It seems impossible to gauge the permanency of new races which arise in this fashion. For there are indications that a new race may give rise to other new races or to one indistinguishable from the old race—all races arising in the same way. A race A may produce an abnormal individual, which becomes the ancestor of a new race B. In the same way, the race B may produce abnormal individuals giving rise to races C, D...etc., of which one may be identical with A. There is at present little to indicate the extent to which "reversion" of this sort may occur.

The factors which determine changes in genetic constitution are in most cases obscure. It is impossible to say how most mutations have been "caused." In Barber's experiments, the environment was the same for all individuals—or at least he tried to make it so. The factor which determined the appearance of individuals with an altered

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genetic constitution can therefore hardly be sought—for the moment—anywhere but in the organisms themselves. In Wolf's experiments, on the other hand, there appears to be evidence that the variations depended in some way upon the environment; for they occurred most frequently in organisms subjected to the action of poisons. But the relation between the mutation and the chemical is not apparent. The same substance seems to be able to produce two opposite results; and two different substances seem capable of producing the same result.

The remarkable phenomena so well studied by Massini, Burri, and others seem at first sight more illuminating. It appears that certain bacteria, which cannot ferment a certain substance, can acquire the power of fermenting it if kept in contact with it for a sufficient time. At first the organisms cannot avail themselves of the new food around them, but they then undergo a change which enables them to do so. It seems at first sight that the new power is the result of necessity—the "mutation" being a direct and indispensable adaptation to a definite end. But to necessity alone the change can hardly be ascribed—even by a confirmed Aristotelian. For it is apparent (e.g. from the work of Massini) that the ability to attack a certain substance (in this case lactose) is not a necessary condition for the survival of the race. It is, rather, a luxury. The lactose-splitting individuals arise and flourish in a non-lactose-splitting colony; but the latter can survive for a very long period, and there is nothing to prove that the new race would supplant the old as a result of natural selection. If every individual—as Burri supposes—possesses the power (*in posse* or *in esse*) of fermenting the sugar; and if, under ordinary cultural conditions, only a minority avails itself of this power: then surely it seems absurd to suppose that the splitting of the sugar is necessary for the survival of the race. These considerations do not affect the fact, however, which seems to be established that there is a direct relation of some sort between the sugar and the change which it produces in the organism. The action of the sugar is specific. Lactose, and lactose alone, makes *B. coli mutabile* able to ferment lactose, but does not enable it to ferment saccharose or any other sugar. We are not dealing here with a stimulus which may produce one of two opposite reactions, or with a reaction which may be produced by another stimulus.

Variations of this sort seem to stand in a class by themselves. Pringsheim (1910) calls them "functional adaptations" or "accommodations." It has been maintained that all variations in Protista are

really of this sort: but there is little ground for the foundation of such a hypothesis. Many established facts appear to be flatly contradictory. It should not be forgotten, however, that acquired heritable adaptations of this sort are described not only among the Bacteria, but also among the yeasts and other Fungi; and the observations have been made by many independent and competent workers.

These few remarks by no means exhaust the significant inferences to be drawn from the works which have been under consideration. But it is hoped that they may serve to call more attention to certain facts which have hitherto been left in the obscure by-ways of bacteriology. It will be admitted, I think, that the outcome of the work fragmentarily recorded in the foregoing pages will be not merely of interest, but probably of very great importance, to every student of genetics.

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MATERNAL INHERITANCE AND MENDELISM.

(FIRST CONTRIBUTION.)

By K. TOYAMA.

(Zoological Institute, College of Agriculture,
Tokyo Imperial University.)

(With Plate XX.)

CONTENTS.

In my first contribution to the study of hybridology of insects published in 1906 (10), it was shown that certain colour-characteristics of the egg of the Siamese silk-worm follow Mendel's law of heredity. Thanks to the kind suggestion of Prof. Bateson of London, we again undertook a similar series of experiments with various breeds of the silk-worm. Some of the results obtained during the last five years which seem to us to be not without interest to students of heredity will be described in the following pages.

I. Certain Egg-characteristics of the Silk-worm.

Before going further, we shall first enumerate certain egg-characteristics which are the subject of the present paper.

Colour. The ordinary colour of the Japanese silk-worm eggs is a light greenish white when newly laid. With the formation of the blastoderm, the egg gradually assumes a brownish tint which at last turns into brownish slate shaded with some light pink or purple (Figs. 1, 3, 11). There may be found, however, many variants, some rather deeper, some lighter, and some with different shades. Now and again it happens that some eggs characterized by extraordinary variations of colour are found among normal ones, such as reddish brown (Fig. 2), whitish grey (Fig. 4), blue (Fig. 5), greenish slate (Figs. 6, 10, 12), crimson (Fig. 7), orange, greenish white and many others. Most of the eggs deposited by Japanese green breeds are more or less shaded with green. When newly laid, they are yellowish green and are much deeper in colour than those deposited by ordinary white breeds. Most of the Chinese or European breeds come in a similar category.

Shape. The normal shape of the silk-worm eggs is oval, slightly pointed at one end, where a micropyle is situated (see Figs. 1—7). It is a little flattened and its surface is convex when newly laid but after a few days it becomes depressed in the middle, thus producing the characteristic form which is familiar to us. In this characteristic, as in the case of the colour, we observed many variants, some of them being quite extraordinary, for instance such as spindle-shaped eggs (Fig. 13) or other irregularly shaped ones, etc. which will be discussed minutely afterwards.

II. ORIGIN OF THE CHARACTERISTICS ABOVE ENUMERATED.

The egg consists of the shell, vitelline membrane, serosa and yolk, and each of them is coloured or shaded with certain tints or pigments, except the vitelline membrane which mostly remains colourless in nearly all breeds.

The shell is usually translucent and is slightly tinted with certain colours. In Japanese breeds it is usually white or slightly shaded with brown, flesh-colour, green, or dirty-white or some other tint. That of some Japanese green, Chinese or European breeds is coloured yellowish green or pale green. The colour of the eggs is consequently more or less influenced by the colour of the shell. As to the shape, it is chiefly determined by the characteristics of the shell, which is derived from the epithelium of the oviduct.

The cause of the egg-colour is, however, mostly due to the pigments deposited in the serosa which are seen through the shell.

The colour of the yolk plays a certain part in the production of the egg-colour only in the case where the formation of the dark pigments in the serosa does not take place, i.e. in newly laid eggs or those oviposited by the spring brood of di-, tetra-, or multivoltine breeds.

The object of the present series of experiments is to know what influence, if any, these variants have upon the trend of heredity in their offspring. As to the origin of these variants we are quite ignorant whether they are produced by mutation or by hybrid mutation or some other causes which are yet unknown to us. We only know that they are seldom found among eggs laid by the normal-egged breeds generally reared in Japan.

III. RESULTS OF LINE BREEDING OF CERTAIN VARIANTS.

SERIES 1. *The Reddish-Brown Eggs* (Figs. 1, 2).

In the winter of 1907, Mr K. Ishivata, one of the famous silk-worm breeders in the district of Fukushima in Japan, kindly offered me some normal (Fig. 1) and brown (Fig. 2) egg batches[1] laid by a divoltine white breed called "Shinkawachi," and said that both of them, even when inbred, gave the antagonistic characteristics in the offspring and thus it was very difficult to establish them as constant forms.

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[1] All through this paper, the word "batch" represents the total eggs laid by a moth.

As the colour of the shell and yolk of both variants was the same, we must attribute the chief cause of those characteristics to the pigment of the serosa, a product of the combination of both parental gametes.

We started our breeding experiments in the spring of 1908.

The First Generation.　1908.　Spring.

We reared two batches of eggs from each variant. The normal series gave 72 matings or batches and the brown series 87, all of which were divoltine white in colour and we were unable to distinguish which were normal and which were brown. This characteristic, producing uncoloured eggs, is one of the normal characteristics of di-, tetra- or multivoltine breeds in Japan. In these breeds, the egg laid by the spring brood generally produces no dark pigments in the serosa and consequently it remains whitish until the embryo is completely developed. Tropical multivoltine breeds, such as Siamese white or yellow which produce 8 or 9 broods in a year, never produce any dark pigment in the serosa, the colour of the egg therefore being determined by that of the yolk and the shell. Sometimes it happens that certain eggs of the spring brood of di- or multivoltine breeds turn into the ordinary dark slate-colour, in which case most or all of them become univoltine in character and do not hatch until next spring comes. On the contrary, all the eggs laid by the summer or autumn broods of divoltine or other multivoltine breeds deposit normal dark pigments in the serosa, thus giving various colours characteristic to the respective breeds.

The Second Generation.　1908.　Summer.

Summer broods derived from the whitish eggs of the spring broods from normal and brown series yielded the antagonistic characteristics as shewn below.

1.　*Eggs laid by the Summer Broods of the Brown Series.*

Number of Matings	Normal Batches	Brown Batches	Mixed Batches	Totals
No. 15. 11	0	11	7	18
No. 19. 12	10	19	29	58
Totals　...	10	30	36	76

2. Eggs laid by the Summer Broods of the Normal Series

Number of Matings	Normal Batches	Brown Batches	Mixed Batches	Totals
No. 18. 12	4	4	4	12
,, 14	2	5	12	19
,, 9	1	1	2	4
No. 4. 1	3	0	0	3
Totals ...	10	10	18	38

In the former or brown series, 10 were normal, 30 brown and 7 a mixture of both normal and brown eggs in the same batch, and therefore in this series the normal colour-characteristics remained as recessive. The reverse is the case in the normal series, which produced 10 normals, 10 browns and 18 mixtures, that is to say, the brown is recessive in this series.

The Third Generation of Normal Series.

In the autumn of 1908, three normal-coloured batches derived from summer broods of the normal series were reared. They gave 58 batches, among which there were 28 normals, 14 browns and 16 mixed batches, that is to say, they again produced the antagonistic character.

The third generation of the brown series or brown batches laid by the summer brood in 1908 were reared in the spring of 1909. They gave, as is usual, all divoltine whitish eggs.

The Fourth and Further Generations.

The fourth generation of the normal series derived from normal eggs laid by the normal series in the autumn of 1908 gave divoltine white eggs, a few being univoltine normals and browns. The same is the case in the fifth generation which was reared in the spring of 1910. In the spring of 1911, i.e. in the sixth generation, we noticed for the first time that this series of normal characteristic inbreeding gave all normal batches with a few divoltine white batches, which may be considered to be normal coloured in character, that is to say, they breed true to parents.

On the contrary, the fourth generation of the brown series or summer brood of 1909 yielded, without exception, brown eggs. Since then we have reared them through two generations without producing any antagonistic characteristic. Hence it may be said that this brown form is established as a constant form.

Respective figures obtained by this series of experiments are given in Table I.

TABLE I.

Brown-egged Series.

Number of Matings	Number of Univoltine batches produced			Divoltine batches	Total batches
	Normal	Brown	Mixture	White	
1908 (Spring brood). First generation.					
15*	0	0	0	26	26
19*	0	0	0	61	61
Totals	0	0	0	87	87
1908 (Summer brood). Second generation.					
19. 12*	10	19	29	0	58
15. 11	0	11	7	0	18
Totals	10	30	36	0	76
1909 (Spring brood). Third generation.					
19. 12. 9	0	0	0	32	32
,, 12*	0	0	0	35	35
,, 25	0	0	0	all white	all white
Totals	0	0	0	67	67
1909 (Summer brood). Fourth generation.					
19. 12. 12. 1*	0	3	0	0	3
,, 2	0	2	0	0	2
,, 3	0	18	0	0	18
,, 4	0	8	0	0	8
,, 5*	0	33	0	0	33
Totals	0	64	0	0	64
1910 (Spring brood). Fifth generation.					
19. 12. 12. 1. 2*	0	0	0	26	26
,, 5. 8	0	0	0	18	18
Totals	0	0	0	44	44
1910 (Summer brood). Sixth generation.					
19. 12. 12. 1. 2. 1	0	107	0	0	107
,, ,, 2	0	116	0	0	116
,, ,, 3	0	86	0	0	86
,, ,, 4	0	108	0	0	108
,, ,, 5	0	105	0	0	105
,, ,, 6	0	50	0	0	50
Totals	0	572	0	0	572

Normal-egged Series.

Number of Matings	Number of Univoltine batches produced			Divoltine batches	Total batches
	Normal	Brown	Mixture	White	
4*	0	0	0	30	
18*	0	0	0	42	
Totals	0	0	0	72	
1908 (Summer brood). Second generation.					
4. 1	3	0	0	0	
18. 9	1	1	2	0	
18. 12*	6	4	4	0	
18. 14	5	5	12	0	
Totals	15	10	18	0	
1908 (Autumn brood). Third generation.					
18. 12. 5*	0	9	4	0	
,, 9*	0	5	8	0	
,, 20*	28	0	4	0	
Totals	28	14	16	0	
1909 (Spring brood). Fourth generation.					
18. 12. 5. 17	4	0	0	18	
,, 9. 25	0	1	0	10	
,, 20. 18*	2	0	0	20	
Totals	6	1	0	48	
1910 (Spring brood). Fifth generation.					
18. 12. 20. 18. 19*	6	0	0	20	
18. 12. 20. 18. 26	0	0	0	13	
Totals	6	0	0	33	
1911 (Spring brood). Sixth generation.					
18. 12. 20. 18. 19 (5 matings)	25	0	0	4	

* Eggs laid by the mating marked with an asterisk are used as the parents of the next generation.

To avoid complication, we give below a graphical summary :

B = brown egg batch; N = normal-coloured; W = divoltine white; M = mixture of brown and normal-coloured eggs in a batch.

Common parent ... N

Parent egg ... ...   (B + N)

First generation inbred W (87) W (72)

Second generation inbred B (30) + N (10) + M (36) B (10) + N (15) + M (18)

Third ,, ,, W (67) B (14) + N (28) + M (16)

Fourth ,, ,, B (64) N (6) + B (1) + W (48)

Fifth ,, ,, W (44) N (6) + W (33)

Sixth ,, ,, B (572) N (25) + W (4)
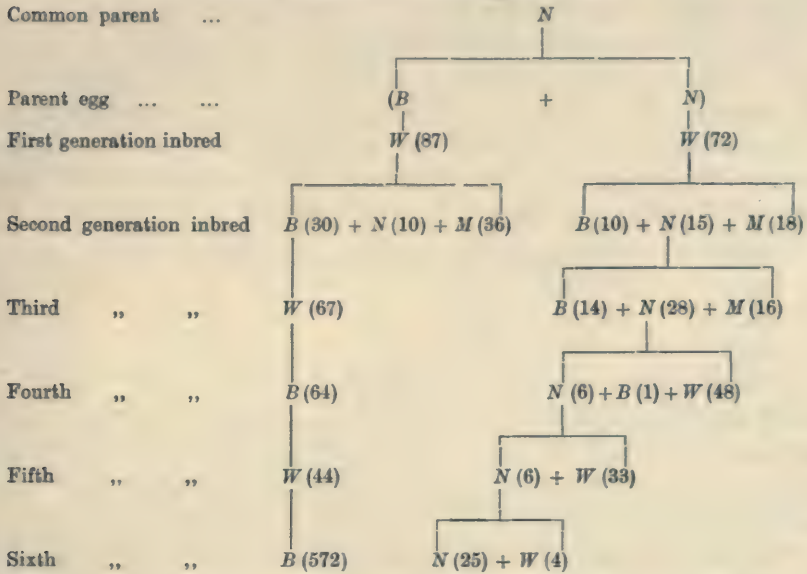
From the results above obtained, we observe that complete segregation between the two characteristics, the brown and the normal-coloured, took place and that each may be established as a constant form from their common stock. It is much easier, however, to establish the brown as a constant form than the normal.

Moreover, we learn that during two or three generations both characteristics even when inbred produce antagonistic characteristics in their offspring, a fact which apparently seems to run counter to Mendelian principles, but which in reality is in perfect accordance with the principles, as will be seen in "General considerations."

Series 2. *The Blue-egged Variant* (Fig. 5).

The phenomena of inheritance, similar to those above described, were observed in the inbreeding of the blue variant. This form is a sport from a divoltine normal-egged breed called "Kuni-nishiki," and is characterized by the special blue colour of the egg.

In the spring of 1910, only one batch (No. 20) was obtained, through the kindness of Mr S. Saito in Ghifu-Ken. The worms, cocoons, and

moths derived from them were all normal in character. They, inbred, gave 30 batches of eggs, of which, 12 batches were divoltine white, and the remaining 18 all normal-coloured, which suggest to us that all the batches in this generation would be all normal-coloured ones.

Three divoltine white batches (Nos. 1*, 3 and 4) which were reared in the summer gave three kinds of eggs, some batches being blue, as in the parental blue, others blue shaded with a brown, which we called "intermediate colour" and the rest normal-coloured batches. There were no batches in which both blue and normal or intermediate forms were found mixed. Respective figures obtained are given below:

Number of Matings	Number of blue batches produced	Number of intermediate batches	Normal-coloured	Totals
20. 1*	21	54	68	143
20. 3	12	65	76	153
20. 4	14	46	60	120
Totals	47	165	204	416

Of 416 batches derived from three parent batches, 47 were blue, 204 normal-coloured and the remainder intermediate. In the blue-coloured eggs, we distinguished both light and dark-shaded ones. The former we called "light blue" and the latter "dark blue."

Five blue batches (Nos. 6, 14*, 24*, 25 and 28) laid by the mating No. 20. 1 were reared in the spring of 1911. They oviposited, without any exception, 251 batches of eggs, all of them being divoltine white.

Eight white divoltine batches were reared in the same summer. Both the light and dark blue series yielded again the antagonistic characteristics as shewn below:

Number of Matings		Light Blue	Dark Blue	Normal	Totals
20. 1 light blue 14. 3		13	0	8	21
	14. 15	21	12	15	48
	14. 20*	4	4	0	8
Totals ...		38	16	23	77
20. 1 dark blue 24. 4		0	0	3	3
	24. 9	1	0	0	1
	24. 14	2	0	2	4
	24. 25	0	1	2	3
	24. 27	0	0	3	3
Totals		3	1	10	14
Grand totals ...		41	17	33	91

Of the eight parent batches, four gave both blue and normal-coloured batches, two all blue batches and the rest only normal-coloured ones, the total number of batches produced by them being 91.

In the spring of 1912, six blue batches (three light and three dark blue) derived from the lineage which produced only blue batches were reared separately as in the former generations. They gave the following egg-batches:

Number of Matings			Number of Univoltine blue batches	Number of Divoltine white batches	Mixed batches	Total number of batches
Dark blue series						
No. 20. 1. 14. 20.		2	0	63	0	63
,,	,,	3	1	18	0	19
,,	,,	4*	22	64	1	87
Light blue series						
No. 20. 1. 14. 20.		1*	5	91	0	96
,,	,,	8	0	52	0	52
,,	,,	10	0	18	0	18
Totals	...		28	306	1	335

Of 335 batches derived from the six blue parent batches, 306 were divoltine white as is usual in the divoltine breed, and 28 were univoltine blue coloured and only one was a mixed batch consisting of both divoltine white and univoltine blue-coloured eggs.

The summer brood derived from four batches of light blue series and four batches of dark blue series gave the following batches:—

Light Blue Series.

Number of parent batch			Number of dark blue batches laid	Number of light blue batches laid	Normal	Totals
No. 20. 1. 14. 20. 4.		4	29	42	—	71
,,	,,	5	22	36	—	58
,,	,,	19	14	16	—	30
,,	,,	22	6	2	1 (?)	9
Totals	...	...	71	96	1 (?)	168

Dark Blue Series.

Number of parent batch			Number of dark blue batches laid	Number of light blue batches laid	Normal	Totals
No. 20. 1. 14. 20. 1.		1	6	33	—	39
,,	,,	12	20	45	—	65
,,	,,	15	25	46	—	71
,,	,,	16	19	49	—	68
,,	,,	25	13	31	2 (?)	46
Totals	...	...	83	204	2 (?)	289

Now we are able to extract the blue-egged characteristic as a constant form. As to the light and dark forms, they seemed to be fluctuations of the same character, blue.

Résumé:

B = light and dark blue batches; I = intermediate; N = normal; W = divoltine white; M = mixed batch consisting of divoltine white and univoltine blue-coloured eggs.

Parent eggs		B
Eggs of the first generation		N (20)
,,	second generation	$\{N\ (204) + I\ (165) + B\ (47)\}$
,,	third ,,	W (251)
,,	fourth ,,	B (9) $\{B\ (49) + N\ (27)\}$ N (6)
,,	fifth ,,	$\{B\ (28)\ +\ W\ (306)\}$
,,	sixth ,,	all B (445)
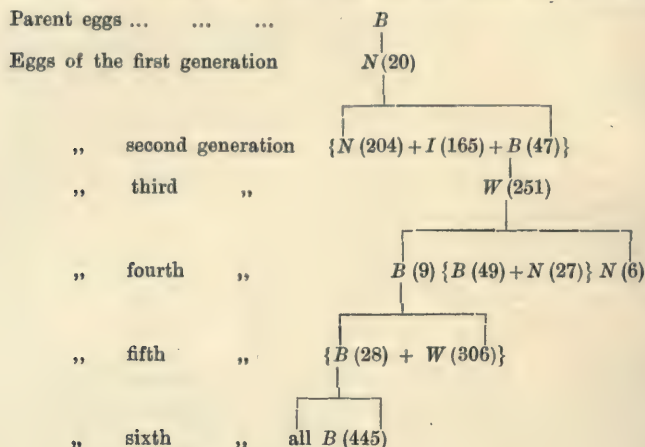
SERIES 3. *The Whitish-Grey egged Variant* (Fig. 4).

This variant from the normal egged breed is characterized by the peculiar structure of the shell. As is well known, the shell of normal breeds is elastic and translucent, its surface being smooth. That of the variant, on the contrary, is rather brittle and opaque, and is milky white in colour, in consequence of which the colour of the serosa can barely be seen through the shell and thus a peculiar whitish grey colour is produced. The surface of the shell is not smooth as in the normal shell, but begins to become irregularly corrugated as soon as the ventral plate is formed. There is no depression in the middle, which is a common characteristic of the egg laid by normal breeds.

In the spring of 1909, we obtained two batches of grey eggs, one being derived from the univoltine white reared in the district of Hyōgŏken, and the other which came from Fŭkŭshimanken is derived from the normal divoltine white, "Aobiki." They were reared separately and each of them gave both normal and grey batches, that is to say, the former deposited one grey and one normal batch (Table III) and the latter 15 normals and 20 greys (Table II); no mixed batches were produced in these cases. (See Tables II and III.)

In the second generation (summer brood of 1909), moths derived from both normal and grey eggs paired *inter se*, yielded again, with no

TABLE II.

Pedigree of the Whitish-Grey Variant, No. 24.

Parent egg ... ... All whitish-grey

First generation (Spring of 1909)   15 normals + 20 grey batches

Normal-egged Series.

	Number of Matings	Number of normal batches	Number of grey batches	Total batches
Second generation	No. 24. 3	4	4	8
(Summer of 1909)	,, 6	4	1	5
	,, 21	2	0	2
	,, 22	3	1	4
	,, 24*	7	0	7
Totals		20	6	26
Third generation	24. N 24. 1	34	0	34
(Spring of 1910)	,, 2*	41	0	41
	,, 2 × 1	2	0	2
	,, 3	21	0	21
	,, 3 × 1	5	0	5
	,, 4	22	0	22
	,, 4 × 1	10	0	10
	,, 5	23	0	23
Totals		158	0	158
Fourth generation	24. N 24. 2. N 1*	142	0	142
(Summer of 1910)	,, 2	24	2 (?)	26
	,, 5	5	0	5
	,, 7	71	0	71
	,, 8	87	0	87
	,, 22	66	0	66
	,, 23	3	0	3
Totals		398	2	400
Fifth generation	24. N 24. 2. No. 1. (N 10, 14, 18, 22, 25)			
	Mixture of 5 batches	38	0	38
	do. No. 12	47	0	47
Totals		85	0	85

Grey-egged Series.

Number of Matings	Number of normal batches	Number of grey batches	Number of B-grey batches	Mixture of G and BG :	T...
24. G 1	2	1	0	—	
,, 3	2	5	0	—	
,, 4*	10	15	0	—	2
,, 16	1	2	0	—	
,, 20	10	10	0	—	2
,, 16 × 1	0	1	0	—	
Totals	25	34	0	—	5
24. G 4. 1*	7	13	1	—	2
,, 4	18	22	4	—	4
,, 25	15	9	0	—	2
,, 26	9	5	3	—	1
,, 28	18	8	1	—	2
Totals	67	57	9	—	13
24. G 4. G 1. G 1*	0	7	36	—	4
,, 7	11	38	7	—	5
,, 8	32	41	24	—	9
,, 11	22	47	27	—	0
,, 21	9	31	11	—	5
,, 28	26	16	0	—	4
Totals	100	173	69	—	34
24. G 4. G 1. G 1. G 6	0	18	14	1	3
,, 7	0	19	17	0	3
,, 16	0	4	9	0	1
,, 22	0	18	18	0	3
,, 24	2†	5	8	6	2
Mixture of 5 batches	2†	56	40	8	10
Totals	4	120	106	15	24

* Those matings marked with an asterisk are the parent of next generation.

† Normal eggs found in these matings are not true normal form, the shell being rather thin when compared with that of normals.

‡ BG = B-greys.

exception, normal and grey batches. Thus five matings of the normal series from the divoltine breed yielded 20 normals and 6 greys; six matings of the grey series from the same breed similarly gave 25 normals and 35 greys. (See Table II.)

The third generation of the normal series which were reared in the spring of 1910 yielded all normal egged batches which when inbred remained true to parents in subsequent generations: i.e. they became homozygous. This was not the case in the grey series. Five matings of the grey series in the spring of 1910 (third generation) gave 67 normals and 67 greys, in addition nine batches of a new variant which we have as yet never observed in our breeds.

This new variant is characterized by the thin translucent shell which has fine wrinkles over it and by a shape a little longer than normal eggs. There is no depression in the middle. We shall call this kind of variant "B-grey," since it more resembles the grey form than the normal ones. In the case of moths laying B-grey eggs the actual number of eggs laid is always much smaller than the number laid by moths laying eggs of normal colour, even though the parents belong to the same batch. The worms which came out from the *BG* are so weak that we can hardly get any moth and consequently we are unable to trace the order of its inheritance.

Of six grey matings of the grey series which were reared in the summer of the same year (fourth generation), five again yielded 100 normals, 173 greys and 69 B-grey batches. One mating, on the contrary, gave no normal eggs except the grey and B-grey, the respective figures obtained being 7 and 36.

The fifth generation derived from the grey mating, which in the last generation yielded no normal batches, gave 245 batches in which 120 were grey, 106 B-greys, 5 mixture of grey and B-grey, and 4 which look like an intermediate form between normals and B-greys.

Details of figures obtained in each mating of each generation will be seen in Tables II and III.

From the results above quoted, we are able to say that, as in the case of first and second series, both normal and grey characteristics segregate from one another, and it is easier to get rid of the antagonistic characters in the normal than in the grey. The appearance of the new form which may probably be due to the new combination of allelomorphs renders the phenomena of inheritance rather complicated. Hence if we consider the *G* and *BG* forms as a single form, the results come in the same category, which was mentioned in the former series of experiments.

TABLE III.

Pedigree of Whitish-grey Eggs derived from the Normal Univoltine White.

Parent egg batch, No. 12   All Grey eggs (laid in the Spring of 1908)

First generation (Spring of 1909)   G (1)   +   N (1)

Second generation (Spring of 1910)   G (2) + N (4)   N (8)

Third generation   ...   ...   N (15)   +   G (16)
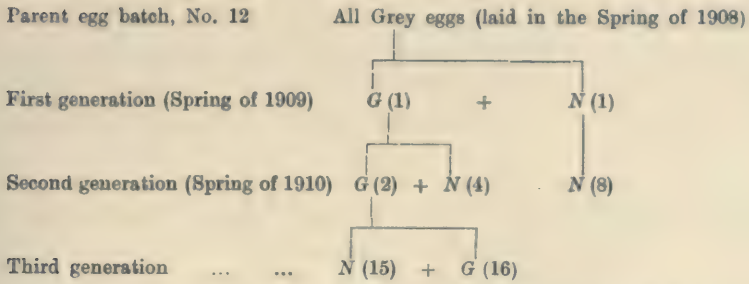
These figures will be again summarized graphically as below :

A.

G = Grey egg batch,   N = Normal,   BG = B-Grey.

1. Parent eggs in 1908 (No. 24)   G

2.   ,,   1909 (Spring)   (N   +   G)

3.   ,,   1909 (Summer)   N   (N + G)   (N + G)

4.   ,,   1910 (Spring)   all N   (N + G + BG)

5.   ,,   1910 (Summer)   all N   (G + BG) (N + G + BG)

6.   ,,   1911 (Spring)   all N   (G   +   BG)

B.

1. Parent eggs 1908   G

2.   ,,   1909 (Spring)   (G   +   N)

3.   ,,   1910 (Spring)   (G   +   N)   all N

4.   ,,   1911 (Spring)   (G   +   N)
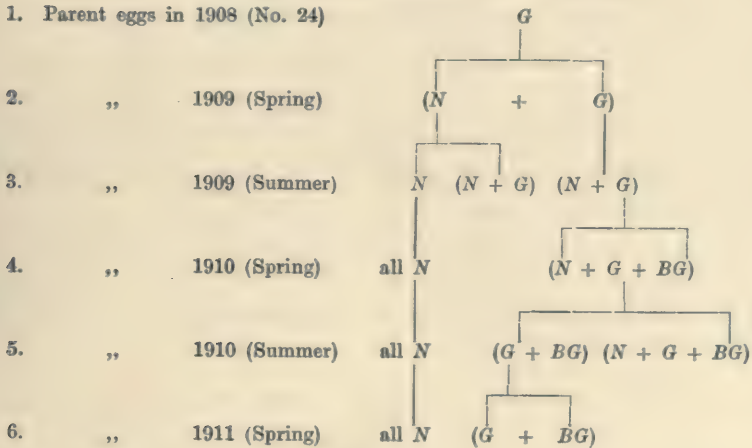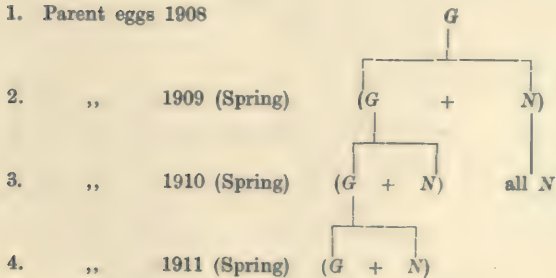
SERIES 4. *The Spindle-shaped Eggs* (Fig. 13).

In the early spring of 1909, we obtained half a batch of the eggs laid by a Japanese normal univoltine white. The egg is long and spindle-shaped, and is slightly pointed at both ends. There is no depression in the middle which is a characteristic common to normal silk-worm eggs.

The first generation which was reared in the spring of 1909 gave eggs which were quite normal in shape and other characteristics. The egg-batches obtained were only six in number.

The second generation derived from the normal eggs yielded moths which paired *inter se* deposited 46 batches of eggs in which we found both normal and spindle-shaped ones, the number found in each mating being as follows:

Number of Matings	Number of normal batches	Number of spindle-shaped batches	Totals
1	18	5	23
6	15	8	23
Totals	33	13	46

Of 46 batches derived from two normal matings, 13 were spindle-shaped and 33 normal-shaped batches, no mixed ones.

In the third generation which was reared in the spring of 1911, both normal and spindle-shaped eggs gave moths which, when inbred, laid two kinds of eggs, normal and spindle-shaped; the respective figures obtained in each mating are shewn below:

Number of Matings	Eggs laid			
	Normal batches	Spindle-shaped batches	Mixture	Totals
Spindle-shaped egg, No. 1. 10				
,, ,, 12	25	2	—	27
,, ,, 24				
Normal eggs, No. 6 (8 batches)	24	3	1	28

Owing to the great havoc made by "flacherie," the mortality of worms was so great that we only obtained a small number of moths, yet we are able to prove that both characteristics even when inbred again produce the antagonistic characteristic. Thus the order of inheritance of these characteristics may be represented as below:

$S =$ spindle-shaped eggs; $N =$ normal-shaped.

```
Parent eggs      ...     ....                    S
                                                 |
Eggs of first generation                         N
                                                 |
                                        ┌────────┴────────┐
Eggs of second generation            (N        +        S)
                                      ┌───┴───┐      ┌───┴───┐
Eggs of third generation           (N   +   S)    (N   +   S)
```
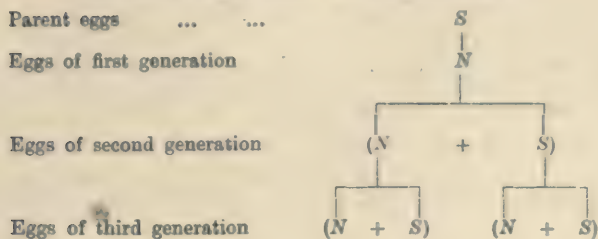
Although we are not yet able to establish this variant as a constant form, we may infer from the above facts that it comes in the same category as the variants just referred to.

Of the various egg-characteristics discussed in the series of experiments above referred to, we know that those in the first and second series are derived from the colour of the serosa, those in the third and fourth series from the shell, whose special structure gave the egg some characteristics different from normal-shelled eggs.

Notwithstanding their origin being different, their order of inheritance is nearly the same.

Let us now compare the results obtained in the third and fourth series, which are represented as below:

1. The Results of the Third Series.

(G and BG are considered to be a single character G.)

```
Parent eggs  ...     ...     ...                      G
                                            ┌─────────┴─────────┐
Eggs of first generation  ...             (N        +        G)
                                        ┌───┴───┐          ┌───┴───┐
   ,,     second   ,,     ...          N   (N + G)       (N   +   G)
                                           |                  ┌───┴───┐
   ,,     third    ,,     ...          N                    (N   +   G)
                                       |                        ┌───┴───┐
   ,,     fourth   ,,     ...          N                     (G + N) +  G
                                       |                              |
   ,,     fifth    ,,     ...          N                              G
```
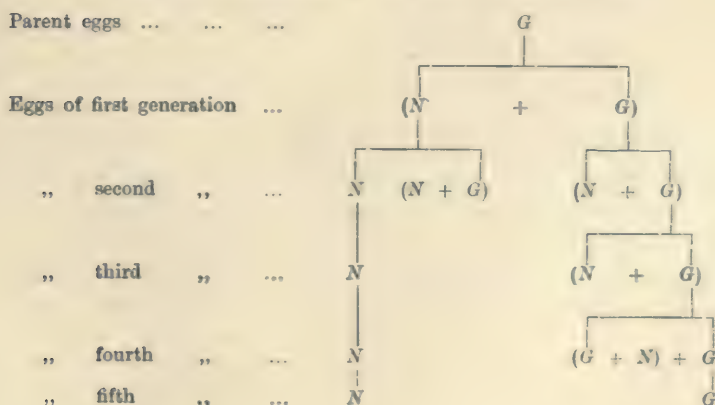
2. *The Results of the Fourth Series.*

```
Parent eggs    ...    ...    ...              S
                                              |
Eggs of first generation    ...               N
                                              |
                                   _____|_____
                                  |                       |
   ,,    second    ,,    ...      (N         +         S)
                                   |                       |
                               ____|____              ____|____
                              |         |            |         |
   ,,    third    ,,    ...   (N   +   S)           (N   +   S)
```
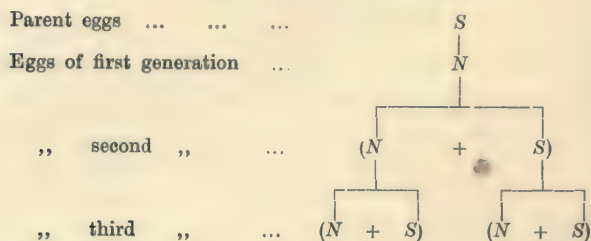
In this case, if we consider the parent egg, *G* in the third series, as the *N* of the first generation of the fourth series, both results come in a single form which may be represented as below:

$$A = G \text{ in the third and } N \text{ in the fourth series};$$
$$B = N \text{ in the third and } S \text{ in the fourth series}.$$

```
Parent eggs ...    ...    ...                 B
                                              |
Eggs of first generation    ...               A
                                              |
                                   _____|_____
                                  |                       |
   ,,    second    ,,    ...      (B         +         A)
                                   |                       |
                              _____|_____             _____|_____
                             |           |           |           |
   ,,    third    ,,   ...   B      (A + B)          (A    +    B)
                             |                             |
                             |                        _____|_____
                             |                       |           |
   ,,    fourth    ,,   ...  B                       (A    +    B)
                             |                             |
                             |                        _____|_____
                             |                       |           |
   ,,    fifth    ,,   ...   B                       A      (A + B)
                             |                       |
   ,,    sixth    ,,   ...   B                       A
```
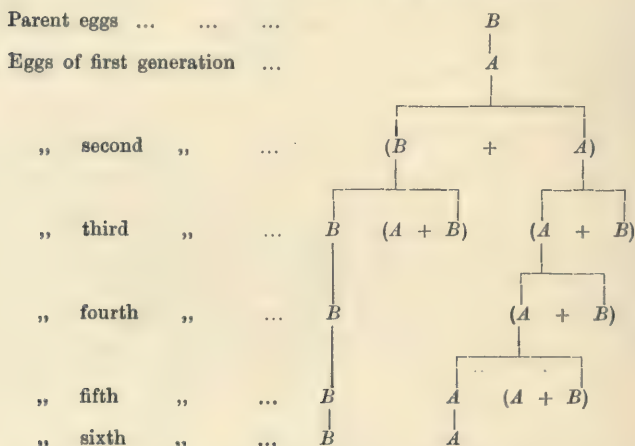
If we compare the results obtained by the first and the second series of experiments, we can easily see that they behaved in inheritance in a similar manner to those above described, certain inconsistent results being due perhaps to the appearance of divoltine white eggs which prevented the elimination of the antagonistic characteristics during one generation.

IV. CROSSING OF VARIOUS BREEDS OR VARIANTS POSSESSING DIFFERENT EGG-CHARACTERISTICS.

SERIES 1. *Crosses between the wild (Theophila mandarina, M.) and the domesticated (Bombyx mori, L.) mulberry silk-worms.*

The egg of the wild mulberry silk-worm (Fig. 10, *a*, *b*) is deposited in a small group on stems or twigs of the mulberry tree. When newly laid, it is a straw yellow (Fig. 10, *b*) which with the formation of the blastoderm gradually assumes a brownish tint and at last turns greenish grey (Fig. 10, *a*). The shape is nearly the same as those of cultivated ones, while the size is little smaller than the latter. The shell is straw yellow and translucent.

The egg of the domesticated silk-worm used in this series of experiments is light greenish white when newly oviposited. It gradually assumes, as in the wild form, a brownish tint and turns brownish slate with some shade of purple or pink, i.e. it assumes the normal colour of Japanese silk-worm eggs (Figs. 1, 3, 11). The shell is nearly white, rarely faintly shaded with a greenish, brownish, or other tint.

In the spring of 1905, five wild female moths were mated with domesticated males (tetravoltine Tōbuhime). They deposited, with no exception, eggs whose characteristics are the same as those of pure wild ones in every respect, such as colour, shape, size and brood character (voltinism). On comparing them with those laid by pure wild parents, we were not able to find any difference at all.

Eleven reversed matings (uni-, di-, and tetravoltine females with wild males) gave, on the contrary, eggs which are similar in shape, colour and voltinism to those of pure domesticated ones (Fig. 11). Even the eye of experienced breeders is not able to distinguish the cross-bred eggs from those laid by maternal pure breeds.

Other five batches derived from divoltine females in the spring brood mated with wild males are all divoltine white in colour, and thus the order of inheritance is entirely maternal, no paternal influence being observed in those reciprocal matings.

The worms which emerged from the reciprocal F_1 eggs were reared in the summer of the same year. Moths derived from the eggs laid by the wild female matings (five matings) gave 56 batches of eggs, all

of them being uniform in their characteristics. When oviposited, they were light greenish yellow and much lighter in colour than the F_1 and gradually assumed a brownish colour which finally turned a greenish slate. Although they resemble the F_1 eggs very much in colour, they are darker and the colours are more decided than the latter, and have no dirty or dull shade which is common in the eggs of *Theophila*. The shell is of clear greenish yellow and deeper than that of the F_1 or pure wild forms. Therefore, we may easily distinguish F_1 eggs from F_2 eggs. There are, however, certain variations of colour in the same batch or between different batches, but no trace of the colour-characteristics of the domesticated parents, and consequently we may say that the colour-characteristic derived from the wild parent dominates over that from the domesticated parent.

In the spring of 1906, we reared worms derived from the F_2 eggs. Owing to the prevalence of grasserie and diarrhoea, all of them died without attaining their mature stage. It will be noted here that, as far as our experiments went, the hybrid form is much more easily injured by those diseases than the pure domesticated form, especially in the case where the male parents were of the wild form. It is, therefore, very difficult to rear a good supply of the hybrid form for experiments. We were therefore compelled to continue our experiments with back-crossed form paired with domesticated one which is healthier than the first cross.

Back-crosses.

In the summer of 1905, cross-bred moths from the F_1 eggs were mated with pure domesticated ones. Reciprocal matings gave, as in the case of F_1, diametrically opposite results. Four F_1 females mated with pure domesticated males gave all greenish slate eggs whose colour is quite the same as that of the F_2 eggs before mentioned, while those laid by 12 domesticated females (tetravoltine white) mated with the cross-bred F_1 males produced, without exception, eggs with characteristics quite maternal.

The worms derived from the former matings all died in consequence of the two diseases above mentioned, while those from the latter, being much more able to resist those diseases, gave some moths in the end of the autumn of 1905, that is to say, the third brood of 1905. 36 batches of eggs resulted from the *inter se* breeding, in which we found many different coloured batches as is shewn below:

Number of Matings	Greenish-slate batches	Japanese normal colour-batches	Mixture of various shaded eggs	Totals
I	0	2	3	5
I b	1	0	0	1
II a	9	8	5	22
II c	0	2	1	3
II e	3	0	2	5
Totals	13	12	11	36

Of 36 batches or matings obtained, 13 were greenish slate as in their parents, 12 Japanese normal colour, the rest being a mixture of both kinds of eggs and some intermediate ones in various proportions.

Owing to certain variations found in a batch, or between various batches, and the scanty number of matings obtained from each parent, we are unable to give the exact numerical proportions of these various coloured batches, but we certainly see that the uniform coloured F_2 characteristic disintegrated into various coloured forms.

In the spring of 1906, we reared worms derived from normal-coloured eggs. Moths paired *inter se* gave all divoltine white eggs. The fourth generation from these divoltine white eggs were reared in the summer of 1906. Three matings gave three kinds of egg batches, as in the former generations, namely:

Number of Matings	Greenish-slate	Japanese normals	Mixture	Totals
IIa, 3	5	7	3	15
,, 4	4	10	5	19
,, 5	2	4	10	16

In 1907, we made similar experiments. The worms which came out from normal-coloured batches gave all divoltine white eggs in the spring. The summer broods gave 30 batches of eggs, all of them being of the normal Japanese colour. Since then they have bred true to parents, never giving any greenish coloured ones.

Résumé :

T = greenish-slate coloured batches like those laid by pure *Theophila*.

N = brownish-slate coloured egg batches, common to normal Japanese breeds.

M = mixture of those two kinds of eggs above-mentioned and some intermediate coloured eggs.

W = divoltine white.

(1)

(♀ Theophila × ♂ Bombyx)

F_1 eggs T

F_2 eggs T

(2)

(♀ Bombyx × ♂ Theophila)

N

T

Back-Crossing.

		(1)	(2)
		($\female$ $F_1 \times$ $\male$ Bombyx)	($\female$ Bombyx $\times$ $\male$ F_1)
Eggs of first back-crossing		T	N
,, second ,,		$(T + N + M)$	
,, third ,,		W	
,, fourth ,,		$(T + N + M)$	
,, fifth ,,		W	
		constant	
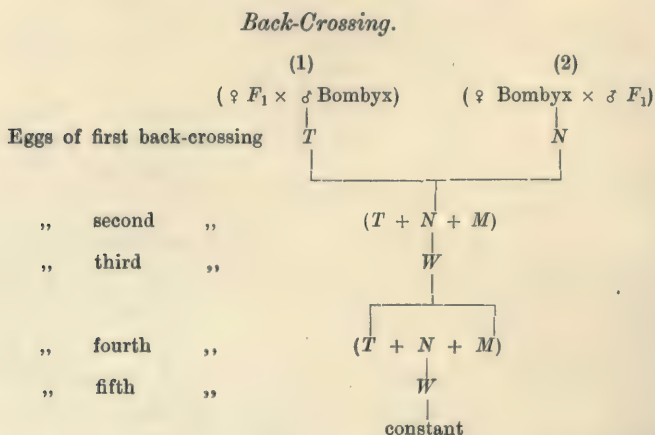
We see that the phenomena of inheritance observed in F_1 eggs and in back-crosses are exactly maternal, no trace of paternal influence, and that both *Theophila* and *Bombyx* coloured characteristics segregate from each other, and may be extracted again in their original form.

SERIES 2. *Crosses between the Whitish grey-egged and Normal-egged forms.*

In the spring of 1909, we made crosses between the whitish grey-egged and the normal-egged breeds. The grey-egged breed used in this series of experiments was the very same breed used in the second series of experiments before mentioned, that is to say, Grey No. 24. Normal-egged breeds were: (1) tetravoltine white (Onodahime), (2) divoltine albino extracted from a cross between divoltine "Chiyodzuru" and "Chūsŭ" albino, (3) divoltine "Shinkawachi," and (4) "H_4 albino" extracted from a cross between Japanese tetravoltine white and the wild mulberry silk-worm.

(*a*) Tetravoltine normal-egged females (Onodahime) mated with males of Grey No. 24.

Three matings were made in the spring of 1909 which gave all normal divoltine white F_1 eggs. Of the summer broods derived from the F_1 eggs, those from two batches yielded both normal and grey (34 normals and 15 greys) F_2 batches and the remainder only normal batches.

Some of the F_2 batches hatched in August, which were the third brood of 1909. Seven batches were reared separately and when

matured gave, without any exception, normal F_3 batches, the number being 461. Their posterity only gave one batch of grey eggs in four successive generations during which they produced 1042 batches. Thus we may safely say that some of the normal-egged form which appeared in the F_2 are homozygous from their first appearance.

Descendants of the F_2 grey batches derived from the same summer brood, on the contrary, produced the antagonistic normal-coloured batches in F_3 (the spring of 1910). Of five matings reared, four yielded grey, normal, and B-grey F_3 batches, while one gave grey and B-grey, no normal batches. In the former, we found 20 normal, 34 grey, and 5 B-grey batches, and in the latter 6 greys and 3 B-greys.

In the summer of 1910 we again reared nine grey batches, one of which was derived from a mating which did not produce any normal eggs in the last generation. Each of them yielded three kinds of F_4 batches, normal, grey, and B-grey, except three matings (Nos. 2. 4. 1; 2. 8. 6; 2. 8. 4) which gave only grey and B-grey batches. The total number of F_4 batches obtained in the former matings was 78 (26·5 °/$_o$) normals, 126 greys, and 90 B-greys, and in the latter 51 greys and 30 B-greys. Two B-grey F_3 batches which were reared in the same season gave only four F_4 batches, one being grey and three B-greys, no normal batches.

The autumn (1910) brood derived from the summer grey brood, which laid only grey and B-grey batches, gave F_5 batches, in which we again found normal batches, but their proportion became gradually lessened as the figures show, namely, of five matings derived from the grey mating No. 6 which did not produce any normal batches in the summer, two gave 28 normals, 71 greys, 39 B-greys, F_5 batches, the remainder 68 greys and 55 B-greys. Of seven derived from No. 1 mating, which as in the former gave no normals in the summer, five gave three kinds of F_5 batches (15 normals, 52 greys and 27 B-greys) and two no normal batches (12 greys, 18 B-greys). In the former lineage, therefore, the proportion of normal batches is 20 °/$_o$ and in the latter 14·4 °/$_o$.

In the spring of 1911, we again reared nine grey batches derived from the brood which produced no normal batches in the last generation. They gave 103 F_6 batches of eggs of which 49 were grey, 49 B-greys and five a mixture of both kinds of batches.

Ten batches of B-grey eggs derived from the same parents as above matings were reared, but all of them died before attaining maturity.

TABLE IV.

Crossing between Tetravoltine Normal-egged and Heterozygous Grey-egged Variant No. 24.

(♀ Normal-egged × ♂ Grey-egged variant)

F₁ eggs (Spring, 1909)   ...   All normals (Nos. 1, 2, 3)

F₂ eggs (Summer, 1909) ...

No. of Matings	No. of normals	No. of greys	Totals
1*	6	0	6
2*	16	14	30
3*	18	1	19
Totals	40	15	55

Normal-egged Series.

	Year	Number of Matings	Normal	Grey	B-grey	Totals
F_3	1909	1. 1*	71	0	0	71
	(August)	1. 2	61	0	0	61
		2. 2*	42	0	0	42
		3. 1	87	0	0	87
		3. 2	7	0	0	7
		3. 3	49	0	0	49
		3. 6	49	0	0	49
		Totals ...	366	0	0	366
F_4	1910	1. 1. 7	39	0	0	39
	(Spring)	,, 10	28	0	0	28
		,, 16	27	0	0	27
		,, 23	28	0	1 (?)	29
		,, 26	50	0	0	50
		2. 2. 2	32	0	0	32
		,, 4	30	0	0	30
		,, 11	28	0	0	28
		,, 7*	39	0	0	39
		,, 18	22	0	0	22
		Totals ...	323	0	1	324
F_5	1910	2. 2. 7. 4	96	0	0	96
	(Summer)	,, 5*	76	0	0	76
		,, 7	24	0	0	24
		,, 11	27	0	0	27
		,, 12	4	0	0	4
		Totals ...	227	0	0	227

Grey-egged Series.

	Number of Matings	Normal	Grey	B-grey	Mixed	Totals
F_3	2. 1*	0	6	3	—	9
	,, 4*	7	10	1	—	18
	,, 5*	11	13	0	—	24
	,, 8*	1	7	0	—	8
	,, 16*	1	4	4	—	9
	Totals	20	34	5	—	59
F_4	2. 1. 5	5	8	4	—	17
	2. 4. 1*	0	27	9	—	36
	2. 16. 8	16	27	13	—	56
	2. 16. 3	29	30	29	—	88
	2. 5. 2	9	34	20	—	63
	2. 5. 6	11	20	12	—	43
	2. 8. 4	0	1	1	—	2
	2. 8. 3	8	7	12	—	27
	2. 8. 6*	0	23	20	—	43
	Totals 3 matings	0	51	30	—	81
	6 ,,	78	126	90	—	294
	No. 2. 1 *BG* 1	0	1	0	—	1
	,, 4. 7	0	0	3	—	3

* Those marked with an asterisk are the parents of next generation.

TABLE IV.—(continued).

Crossing between Tetravoltine Normal-egged and Heterozygous Grey-egged Variant No. 24.

		Normal-egged Series.						Grey-egged Series.				
	Year	Number of Matings	Normal	Grey	B-grey	Totals	Number of Matings	Normal	Grey	B-grey	Mixed	Totals
F_6	1910	2. 2. 7. 5. 4	22	0	0	22	F_5 2. 8. 6. 1	12	39	21	—	72
	(Autumn)	,, 13*	80	0	0	80	,, 4	0	2	2	—	4
		,, 11	39	0	0	39	,, 6	16	32	18	—	66
		,, 17	37	0	0	37	,, 11	0	14	18	—	32
		,, 20	41	0	0	41	,, 44*	0	42	35	—	77
		,, 77	29	0	0	29						
							Totals 2 matings	28	71	39	—	138
		Totals ...	248	0	0	248	3 ,,	0	58	55	—	113
							2. 8. 6. BG 3	0	11	11	—	22
							,, BG 9	0	1	3	—	4
							,, BG 12	0	0	1	—	1
							Totals	0	12	15	—	27
							2. 4. 1. 8	6	15	8	—	29
							,, 9	0	8	12	—	20
							,, 12	11	14	7	—	32
							,, 17	2	13	6	—	21
							,, 20	3	6	3	—	12
							,, 27	0	4	6	—	10
							,, 19	3	4	3	—	10
							Totals 5 matings	25	52	27	—	104
							2 ,,	0	12	18	—	30
F_7	1911	2. 2. 7. 5. 13. 3										
	(Spring)	,, 6					F_6 2. 8. 6. 44. 3	0	5	8	—	13
		,, 17	243	0	0	243	,, 6	0	4	1	—	5
		,, 18					,, 21	0	2	5	—	7
		,, 19					,, 6b	0	3	5	—	8
							,, 9b	0	15	9	3	27
							,, 12b	0	1	2	1	4
							,, 12c	0	7	12	0	19
							,, 16c	0	6	6	1	13
							,, 19c	0	6	1	0	7
							Totals ...	0	49	49	5	103

* Those marked with an asterisk are the parents of next generation.

The respective figures obtained in each mating in every generation are represented in Table IV which will be summarized as below:

(Normal × grey No. 24)

F_1 eggs   N

F_2 eggs   N $(N + G)$

F_3 eggs   N   N $(G + BG)$ $(N + G + BG)$

F_4 eggs   N $(N + G + BG)$ $(G + BG)$ $(G + BG)$ $(N + G + BG)$

F_5 eggs   N $(G + BG)$ $(N + G + BG)$ $(G + BG)$

F_6 eggs   N $(G + BG)$
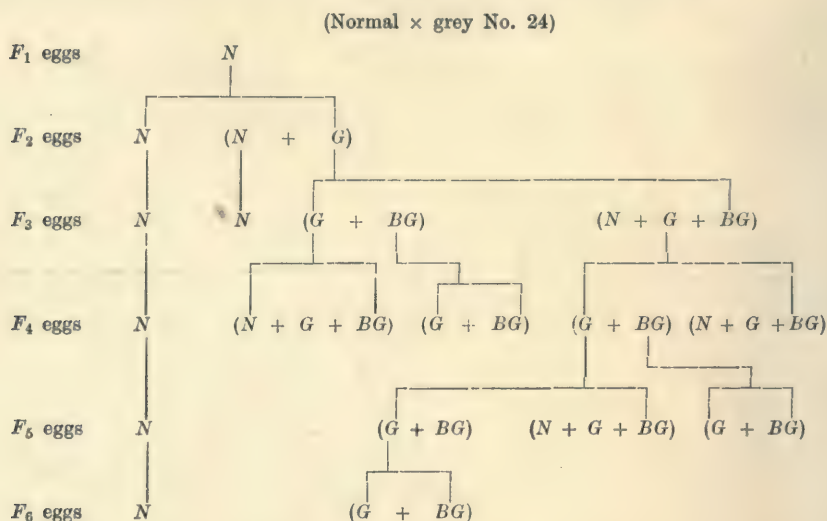
The results are nearly the same as those obtained in the original grey variant which is inbred, except the appearance of the constant N form in F_1.

(*b*) Exactly the same results were obtained when we crossed a normal-egged breed extracted from a cross between divoltine "Chiyodzuru" and the univoltine albino before referred to with the grey-egged variant No. 24. The results of experiments are tabulated below (Table V).

(*c*) In this series we made again similar matings as in the previous two series of experiments. Three females from the divoltine normal-egged white called "Shinkawachi" and two from the normal-egged extracted form called E III were mated with males derived from the very same grey variant used in the preceding experiments. They gave as in former cases, all F_1 normal egg-batches, no grey ones.

Two batches from the former and one from the latter were reared in the next season. The former gave 93 F_2 batches and the latter 13 F_2 batches, all of them being normal batches. Five batches of the former and one batch of the latter were again reared in the next season. The former gave 968 F_3 batches of normal eggs and the latter 10 F_3 normal batches.

TABLE V.

(♀ *Normal* × ♂ *Grey*).

F₁ eggs (Spring, 1909) — Normal divoltine white (one batch)

Egg-batches produced

Number of matings	Normal batches	Grey batches	Total
6*	13	11	24

F₂ eggs (Summer, 1909)

F₃ eggs (Spring, 1910)

Number of Parent	Normal	Grey	Totals
6. 3	5	0	5
6. 7*	10	12	22
6. 21	0	12	21
Totals	24	24	48

Grey series

Number of Parent	Normal	Grey	B-grey	Totals
6. 16*	14	28	5	47
6. 18*	7	11	8	26
6. 19*	7	6	6	19
Totals	28	45	19	92

Grey series

F₄ eggs (Summer, 1910)

Number of Parent	Normal	Grey	B-grey	Totals
6. 7. 3*	0	33	22	55
,, 14	18	12	0	30
,, 8	8	14	0	22
,, 9	17	41	28	86

Grey series

Number of Parent	Normal	Grey	B-grey	Totals
6. 16. 12*	44	49	0	93
,, 13	6	4	0	10
(B-grey series)				
6. 16. 7	0	1	30	31
,, 8	0	11	10	21
,, 18	0	0	4	4
6. 18. 1	0	0	6	6
6. 19. 1	0	2	0	2
,, 8	0	14	3	17

Grey series

F₅ eggs (Spring, 1911)

Number of Parent	Normal	Grey	B-grey	Total
6. 7. 3 (4 and 5)	2	3	6	11

Number of Parent	Normal	Grey	B-grey	Total
6. 16. 12 (5 and 16)	8	16	0	24

* Parents of next generation.

In the next generation paired *inter se*, both series again yielded only normal-egged F_4 batches, the number of batches produced in the former series being 66 and in the latter 123. Now it is quite certain that in these matings there is no grey factor which has lain dormant as in the former matings.

The facts obtained in these three series of experiments and those from the second series of the line breeding suggest to us, *firstly* that among the eggs of grey-batch No. 24, there are two kinds of grey eggs, one having the grey factor in its zygotic composition, while another has no grey factor, in spite of its being grey in colour; that is to say, some grey eggs are heterozygous for the normal factor, some homozygous

normals; *secondly* that the normal and the grey segregate from each other in their succeeding generation as other Mendelian characters do; *thirdly* that it is much easier to free the normal form from the antagonistic character than the grey; and, lastly, that the normal form does not produce any other form when it becomes free from the grey form, while the grey form segregates into another form even after being freed from the normal form. From this fact we may safely infer that the grey is more complicated in its constitution than the normal.

SERIES 3. *Crosses between Yellow and White forms of Japanese Tetravoltine Breed, "Onodahime." (Fig. 9, a and b.)*

Normal Japanese tetravoltine breeds are generally white cocoon-spinners, as far as we are aware. In the year 1905, we obtained a mixed breed consisting of white and yellow cocoon-spinners, the latter being a yellow-blooded form. Each form was reared separately and was established as a constant form. In the spring of 1907 reciprocal crossings between these forms were made.

Yellow females mated with white males gave F_1 eggs which are all yellow when newly laid[1] (Fig. 9, *b*). This is the characteristic egg-colour of the yellow form. The reversed mating gave, on the contrary, all pale white F_1 eggs (Fig. 9, *a*) which is also the characteristic colour of the white form. It will be necessary to note here that the colour of newly laid eggs is determined by that of the shell and the yolk, both of them being maternal in their origin.

The worms which came out from the reciprocal F_1 eggs were reared in the late autumn of the same year. All the worms were yellow-blooded and spun yellow cocoons without any exception. The moths paired *inter se* gave all yellow F_2 eggs which are quite the same as those laid by the pure yellow forms.

In the spring of 1908, the F_2 yellow eggs gave two kinds of worms, the one being yellow-blooded, the other white-blooded; the total figures found in those matings are shown below :—

Number of Group	Number of yellow-blooded worms	Number of white-blooded worms	Totals
I	485	169	654
II	567	160	727
Totals	1,052	329	1,381
Mendelian expectation	1,035	345	1,380

[1] In this series of experiments, we only refer to the colour of the egg when newly laid, i.e. the colour of eggs before the formation of the blastoderm takes place.

The moths derived from these yellow- and white-blooded worms were paired in the following ways:

I	♀	White-blooded moths	×	♂	White-blooded	
II	♀	White	,,	,,	× ♂ Yellow	,,
III	♀	Yellow	,,	,,	× ♂ Yellow	,,
IV	♀	Yellow	,,	,,	× ♂ White	,, ...

They gave the results which are tabulated below :—

Group	Number of Matings		Parents Female	Male	Colour of the eggs laid	Worms which emerged from the egg Yellow	White	Totals
I	I	No. 9	white	white	pale white	0	all white	all white
	II	,, 1a	,,	,,	,,	,,	,,	,,
	,,	,, 10	,,	,,	,,	,,	,,	,,
	,,	,, 13	,,	,,	,,	,,	,, ...	,,
	,,	,, 21	,,	,,	,,	,,	,,	,,
II	II	No. 6	white	yellow	pale white	59	51	110
	,,	,, 13	,,	,,	,,	17	0	17
	,,	,, 14	,,	,,	,,	242	0	242
	,,	,, 23	,,	,,	,,	27	19	46
III	II	No. 9	yellow	yellow	brownish yellow	0	0	0
	,,	,, 3	,,	,,	,, ,,	109	30	139
	,,	,, 4	,,	,,	,, ,,	39	14	53
	,,	,, 5	,,	,,	greenish yellow	0	0	0
	,,	,, 11	,,	,,	brownish yellow	71	17	88
	,,	,, 16	,,	,,	,, ,,	0	0	0
	,,	,, 24	,,	,,	greenish yellow	54	13	67
Totals	...	...	...	...	... ...	273	74	347
IV	I	No. 6	yellow	white	yellow	65	55	120

As the Table shews, if yellow-blooded females (both homozygous and heterozygous) are used, whether they are mated with their own males or other white males, the results are always the production of brownish or greenish yellow eggs which is a characteristic of the yellow-blooded form. In like manner, white-blooded females mated with yellows (homo- and heterozygous) or whites gave all whitish eggs, characteristic of the white form. Thus we may say in this case as in the other cases before cited, the colour characteristics of the egg are not influenced by the zygotic composition of the egg after fertilization, but by their maternal zygotic constitution before fertilization, and therefore there is no sign of male characteristics to be seen in the egg, but in the larval stage their relation is quite Mendelian.

Résumé:

Y=yellow eggs; W=white eggs; (y)=yellow-blooded worms and moths; (w)=white-blooded worms and moths.

	(1)	(2)
	♀ (y) × ♂ (w)	♀ (w) × ♂ (y)
F_1 eggs	Y	W
Worms and moths	(y)	(y)
F_2 eggs	Y	Y
Worms and moths	{3 (y)	:	1 (w)}

{ ♀ (y) × ♂ (w) }   { ♀ (w) × ♂ (y) }

F_3 eggs ... ... Y Y W W

Worms and moths   all (y) . {3 (y) : 1 (w)}   all (y) . {1 (y) : 1 (w)}   all (y) . {1 (w) : 1 (y)} (w)
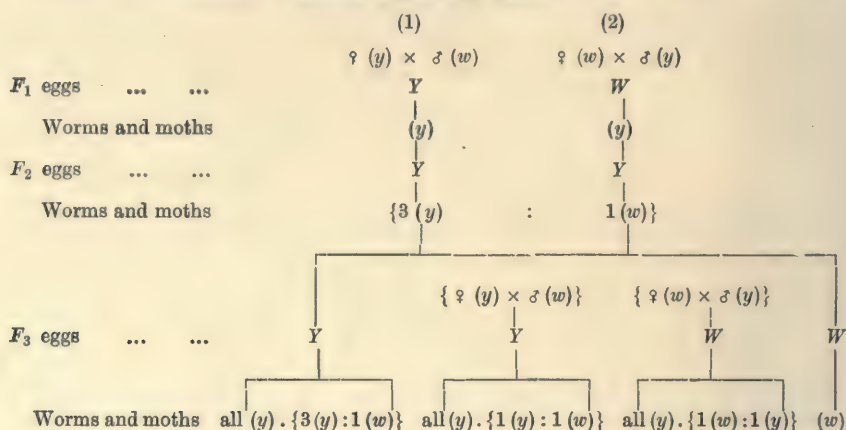
SERIES 4.   *Crosses between various Breeds which lay different coloured Eggs.*

This series of experiments was undertaken with the intention of observing the influence of egg-characteristics belonging to the breed used as male parents towards the characteristics of F_1 eggs.

(1) Reciprocal matings between Chinese whites from the Joken and Kainei districts and Japanese univoltine breeds, "Aojiku" and "Chūsŭ."

Egg-colour of the Chinese breeds: dark-slate, more or less shaded with green in various degrees, sometimes without any green shade. We call this colour Chinese normal colour.

Egg-colour of the Japanese breeds (Figs. 1, 3, and 11): brownish slate, more or less shaded with pink or purple. This is the ordinary egg-colour of Japanese breeds.

Matings					Number of Matings	Colour of F_1 eggs
♀ Chinese Joken	×	♂ Japanese	Aojiku		10	all Chinese colour
♀ „ Kainei	×	♂ „	Chūsŭ		13	„ „
♀ Japanese Aojiku	×	♂ Chinese	Joken		10	Japanese ordinary colour
♀ „ Chūsŭ	×	♂ „	Kainei		15	„ „

(2) Reciprocal crosses between the Japanese green breed (Seihaku) and the extracted normal-egged breed (Ivg), or the univoltine white, "Sekai-ichi."

Egg-colour of the green breed : ordinary brownish slate with various shades of green (Fig. 6), some of them having no green shade. It resembles in colour the Chinese colour but the shades are different, so we are able to distinguish the Chinese colour from that of Japanese green.

Matings	Number of Matings	Colour of F_1 eggs
♀ Green × ♂ "Ivg"	5	one batch normal slate, the rest a mixture of various green shaded eggs, i.e. ordinary colour of green breed
♀ Ivg × ♂ green	6	Japanese normal colour
♀ Sekai-ichi × ♂ green	5	,, ,, ,,

(3) Reciprocal matings between the wild mulberry silk-worm (*Theophila mandarina*) and Japanese normal-egged breeds (Shinyahime), tetravoltine "Onodahime," tetravoltine "Tōbuhime," univoltine "Matamukashi").

Egg-colour of *Theophila*: grey, more or less shaded with green, as has already been described.

Matings	Number of Matings	Colour of F_1 eggs
♀ Shinyahime × ♂ *Theophila*	8	all Japanese normal colour
♀ Onodahime × ♂ ,,	2	,, ,, ,,
♀ Univoltine Matamukashi × ♂ *Theophila*	2	,, ,, ,,
♀ Tōbuhime × ♂ *Theophila*	3	,, ,, ,,
♀ *Theophila* × ♂ Tōbuhime	3	all *Theophila* colour
♀ ,, ♂ Matamukashi	2	,, ,,

(4) Reciprocal matings between the normal (Fig. 1) and the reddish brown egged (Fig. 2) forms found in the divoltine breed, "Shinkawachi."

Matings	Number of Matings	Colour of F_1 eggs
♀ normal × ♂ reddish-brown	20	all normal colour
♀ reddish-brown × ♂ normal	15	all reddish-brown, few being a little darker shaded

(5) Reciprocal crossings between the European yellow, "Papillons noirs" and the Japanese normal-egged breed, "Tatsutahime."

Egg colour of "Papillons noirs": slate grey, more or less shaded with green as in Japanese green; sometimes without any greenish shade; the general colouration, however, differs from that of the Chinese, Japanese or *Theophila* green, each of them having its own characteristics: egg-colour of "Tōbuhime," normal Japanese colour.

Matings	Number of Matings	Colour of F_1 eggs
♀ Papillons noirs × ♂ Tatsutahime	2	European greenish slate
♀ Tatsutahime × ♂ Papillons noirs	2	all divoltine white

(6)   Reciprocal crosses between European breeds (Italian white and Sina blanc) and Japanese normal-coloured breeds (divoltine Shinkawachi, tetravoltine Tatsutahime, and divoltine Asakanishiki).

The egg-colour of these European breeds are practically the same as that of Papillons noirs, i.e. greenish-shaded slate.

Matings	Number of Matings	Colour of F_1 eggs
♀ Italian white × ♂ Shinkawachi	3	slate grey
,, ,, ,,	7	mixture of various greenish-shaded greys and normal slates
♀ Shinkawachi × ♂ Italian white	3	Japanese normal colour
♀ Tatsutahime × ♂ Italian white	25	all divoltine white
♀ Asakanishiki × ♂ Sina blanc	7	,, ,,
♀ Italian white × ♂ Tatsutahime	4	a mixture of various green-shaded greys and normal slates
♀ Sina blanc × ♂ Asakanishiki	6	the same colour as the above

(7)   Reciprocal crossing between albino and colour-egged breeds.

The colour-egged breeds used in this series of experiments were:

1.   European yellow, Papillons noirs.

2.   Japanese white, "Sekai-ichi," "Tako," " Chūsŭ," and the extracted white " Ivg," univoltine green, " Seihaku."

3.   Albino: univoltine Chūsŭ albino (the colour of the egg is a greenish white, sometimes faintly shaded with orange), another is the Chinese orange breed, its eggs being clear orange with certain variations in intensity.

Matings	Number of Matings	Colour of F_1 eggs
♀ Orange × ♂ Papillons noirs	1	light pinkish brown
♀ Orange × ♂ Sekai-ichi	5	mixture of orange to dark-pinkish browns
♀ Orange × ♂ Green	5	4 light pinkish brown with various dark shades 1 dark pinkish grey
♀ Orange × ♂ Ivg	5	2 Orange, slightly shaded with a dark pink 3 a mixture of orange, dark pink, and a few normal eggs
♀ Chūsŭ albino × ♂ Univoltine Chūsŭ		some batches are all white, some white mixed with various shaded eggs
♀ Chūsŭ albino × ♂ Chiyodzuru		the same as the above
♀ Papillons noirs × ♂ Orange	3	European green-shaded eggs, one being little lighter in colour than the others .
♀ Sekai-ichi × ♂ Orange	5	all normal brownish grey
♀ Green × ♂ Orange	5	various green-shaded eggs, characteristic to the green breed
♀ Ivg × ♂ Orange	6	all normal brownish grey
♀ Tako × ♂ Orange	4	the same as the above
♀ Chiyodzuru × ♂ Chūsŭ albino	4	the same as the above

In the crossings above quoted, we compared the reciprocal F_1 eggs with those laid by pure maternal breeds in each case, and came to the conclusion that the colour-characteristics of the egg are governed by those of the female parent. As far as our experiments went, it is very difficult to distinguish between these F_1 eggs and those laid by pure maternal breeds, even to the eye of experienced breeders the line of demarcation was indistinguishable, except in the case of albinotic matings.

As to the albinotic matings, the case is rather different from that of the coloured matings, since the eggs laid by albino females mated with coloured males sometimes were more or less influenced by the male characteristics and sometimes shewed no male influence. In the Chūsŭ albino matings we often obtained pure white F_1 eggs, while in the orange albino these maternally characterized eggs were rarely found.

Now then, we may draw the conclusion that most of the egg-colours which are commonly met with in various breeds are maternal in inheritance, in spite of the origin of these different colours being due to the pigments which are deposited in the serosa. In Chinese, European and in some green breeds the green colour of the shell may help the production of these greenish shades, but it is not the chief cause of the green colour.

Concerning albino breeds which gave rise to more intricate series of results when crossed with other coloured breeds, we shall describe them in the second contribution of this subject.

From the series of experiments above mentioned, we know that all the colour-characteristics of the silk-worm egg are maternal in inheritance with the exception of the albino, in which the recessive uncoloured characteristic of the female parent is sometimes more or less influenced by the dominant coloured male. We must not forget, however, that there are breeds whose egg-characteristics behave exactly as other normal Mendelian characteristics do. We shall now describe a case of this kind studied by us.

SERIES 5. *Inheritance of the "Crimson-egged" Breed.*

In the spring of 1909 we obtained, through the kindness of Mr J. Ariga of the Nagano district, two batches of eggs laid by a divoltine breed. This breed is characterized by the special colour of the egg which is a clear crimson-red. It is one of the most strikingly coloured eggs we have ever had. In one batch (No. 5) we found 30 normal coloured eggs and in another (No. 20) only two normal eggs, all the remainder were crimson-red.

Characteristics of this Breed.

From the results given by rearing through consecutive generations, it was ascertained that all the worms were normal patterned and there was no considerable difference from normal breeds, except in the colour of the head and eyes. In the fourth and fifth stages, we clearly observed that the head of the worm was of a light reddish brown as contrasted with the normal brownish grey of normal-egged breeds. The eyes, both simple and compound, were tinged with crimson. The eggs when newly laid are white as is the case in normal breeds. With the formation of the blastoderm, they gradually changed into a light orange, then into brownish orange and at last into clear crimson. They do not remain the same in colour throughout the year; when the development of the embryo is in its earlier stages they take on some shade of brown which gradually merges into a clear crimson. After the hibernation, when the development of the embryo again takes place in spring, they assume a dark shade of crimson and the pigments are not uniformly distributed in the serosa. The shell is white. The shape is quite normal. The cocoons are white, and their form is cylindrical with the constriction in the middle like that of the normal Japanese breed. The moths are white, with faint markings as in normal light moths. We shall call this breed the "Crimson-egged" breed.

Crossing experiments with Normal Breeds.

Reciprocal matings between the crimson-egged and many normal breeds were made. Female moths derived from the spring brood of the crimson breed were mated with males from tetravoltine white (Onodahime), the extracted normal-egged breed derived from a cross between Japanese white and the Chinese dark-wormed breed, and the divoltine white (Renzoku). These three breeds were all true in their normal egg-colour characteristic. The results of the reciprocal matings are shewn below:

A. F_1 *Eggs.*

1. (♀ normal breed × ♂ crimson breed.)

Female parent	Male parent	Number of Mating	Eggs laid (F_1)	
Tetravoltine white (Onodahime)	Crimson	6	divoltine white	
,, ,, ,,	,,	7	,, ,,	
The extracted normal breed	,,	11	,, ,,	
,, ,, ,,	,,	12	normal dark colour	
,, ,, ,,	,,	13	,, ,,	
,, ,, ,,	,,	14	,, ,,	
Divoltine white (Renzoku)	,,	15	,, ,,	
,, ,,	,,	16	,, ,,	

Of eight matings, five gave all normal dark eggs, while three gave all divoltine white eggs, which is the maternal characteristic of tetra- or divoltine females in their spring brood.

2.   ($♀$ crimson breed $\times$ $♂$ normal breed.)

The reversed matings gave results identical with those represented.

	Female parent	Male parent	Number of Matings	Eggs laid (F_1)
1	{ Crimson	Tetravoltine Onodahime	4	normal dark
	,,	,, ,,	5	,, ,,
2	,,	The extracted normal-egged form from the cross between *Bombyx mori* and *Theophila mandarina*	2 and 3	divoltine white
3	,,	Chinese " Dragon horn "	1	greenish slate

The results of the reciprocal matings shew us that in the F_1 eggs normal coloured or greenish coloured characteristics dominate over the crimson, while the voltine characteristics are, as in other egg-characteristics before enumerated, maternal in inheritance.

B. F_2 Eggs.

The worms and moths derived from the reciprocal normal-coloured F_1 eggs in the summer of 1910 were all dark-eyed, and the moths paired *inter se* gave the following F_2 eggs :

Matings	Number of Matings	Eggs laid by each moth		
		Normal	Crimson	Totals
♀ Tetravoltine " Onodahime " ♂ Crimson	6. 1	342	136	478
	6. 2	294	103	397
	6. 8	369	103	472
	6. 9	374	103	477
	6. 10	314	124	438
	6. 11	192	77	269
	6. 12	334	109	443
	7. 6	300	97	397
	7. 13	272	107	379
	7 × 6. 28	214	88	302
Totals		3001	1047	4048
♀ Extracted normal-egged breed from the cross, Japanese white × Chinese black-wormed breed ♂ Crimson	11. 1	278	96	
	11. 2	176	68	
	11. 4	319	115	
	11. 6	351	123	
	11. 7	330	119	
Totals		1454	521	

Matings	Number of Matings	Eggs laid by each moth		Totals
		Normal	Crimson	
	4. 3	293	93	
	4. 5	301	126	
	9	347	128	
♀ Crimson	10	359	96	
♂ Tetravoltine "Onodahime"	11	332	83	
	13	355	112	
	14	275	89	
	Nos. 1, 2, 4, 8, 15—18 all divoltine whites			
	Totals	2262	727	2989
(♀ Crimson No. 20	2. 1	230	80	310
♂ E 111. 11. 1. 3)	3. 3	82	24	106
	1. 15	319	124	443
(♀ Crimson × ♂ "Dragon-horn")	1. 16	290	105	395
	1. 17	389	110	499
	Totals	1,310	443	1,753
	Grand totals	8,027	2,738	10,765
	Expectation	8,073¾	2,691¼	10,765

Every mating, except those which gave divoltine white eggs, yielded both crimson and normal-coloured eggs in an approximate proportion of $3N : 1R$ respectively; the total number of crimson and normal-coloured eggs obtained from 17 F_1 dark-eyed moth matings being 5026 and 1691 respectively, that is to say, 3 normals : 1 crimson.

C. F_3 Eggs.

The mixed F_2 eggs laid by the cross, Tetravoltine normal egged × Crimson egged breed, were reared in the spring of 1910. Both the normal and crimson-coloured eggs found in each batch were reared separately and gave the following worms and moths:

Number of parent batches		Number of normal and crimson eggs in each batch	Kinds of worms hatched		Moths
			Dark-eyed	Crimson-eyed	
♀ Tetravoltine ♂ Crimson	No. 6. 1	N. 342	281 (82 %)	0	all dark-eyed
		R. 136	0	46 (33 %)	all crimson-eyed
"	No. 6. 8	N. 369	301 (82 %)	0	all dark-eyed
		R. 103	0	28 (27 %)	all crimson-eyed
"	No. 6. 9	N. 374	290 (77 %)	0	all dark-eyed
		R. 103	0	40 (38 %)	all crimson-eyed
Mixed rearing of Nos. 6. 2 and 7. 6		N. 594	465 (78 %)	0	all dark-eyed
		R. 200	0	91 (45 %)	all crimson-eyed
Crimson eggs derived from five batches				89	all crimson-eyed

As the figures show, crimson-coloured eggs always produced crimson-eyed worms and moths and the normal ones gave dark-eyed worms and moths. Both the crimson and normal-eyed moths paired *inter se* gave the following F_3 egg-batches:

(1) *Crimson-eyed moths paired inter se.*

Number of Matings	Colour of the eggs laid
No. 6. 8. 15	all crimson-coloured eggs
No. 6. 9. 14	,, ,, ,,
No. 6. 8. (× pure crimson) 16	,, ,, ,,
No. 6. 1. (× pure crimson) 27	,, ,, ,,
No. 6. 8. (× No. 6. 1) 11	divoltine white
No. 6. 2. (× No. 7. 6) 13	,, ,,
No. 6. 9. 15	,, ,,

Of seven crimson-eyed matings, four gave all crimson-coloured eggs, while the remainder gave all divoltine white eggs which in the next generation gave rise to crimson-eyed worms and moths and laid all crimson-coloured eggs. We may say, therefore, that the crimson-coloured characteristic is segregated from the normal-coloured ones. The lineage of this series gave all crimson-coloured eggs in the succeeding generations.

(2) *Normal-eyed moths inbred.*

Parents	Number of Matings	Eggs laid		Totals
		Normal	Crimson	
No. 6. 1	11	236	79	315
,,	12	all normals		all normals
,,	13	,,		,,
No. 6. 8	15	all divoltine white batches		
No. 6. 9	4	all normals		all normals
,,	5	,,		,,
,,	7	3	:	1
,,	8	all normals		all normals
,,	9	,,		,,
No. 6. 2 × No. 7. 6	6	3	: 1	
,, ,,	8	263	81	344
,, ,,	12	248	83	331
,, ,,	8	312	117	429
,, ,,	15	3	: 1	

Back-crossing of F_1 Dark-eyed Moths with extracted Crimson-eyed Moths.

F_1 dark-eyed moths derived from the mating, ♀ crimson × ♂ tetra-voltine white, were mated with crimson-eyed males derived from the F_2 eggs of the mating, ♀ tetravoltine × ♂ crimson. Each mating, as the

theory demands, gave both normal and crimson-coloured eggs in an approximate proportion of 1 : 1, as the figures quoted below shew :

| Number of Matings | Eggs laid | | Totals |
	Normals	Crimsons	
8	all divoltine white		
9	183	202	385
11	187	188	375
12	211	207	418
13	236	220	456
19	198	187	385
Totals	1015	1004	2019
Expectation	$1009\frac{1}{2}$	$1009\frac{1}{2}$	$2019\frac{1}{2}$

There are certain normal breeds in vogue which often throw off such crimson-coloured eggs. By the kind help of Mr K. Saito in Ghifu, we obtained two breeds reared in the district of Ghifuken which sometimes gave mixed batches consisting of normal and crimson eggs. The number of normal and crimson eggs respectively found in the batches given by him to us are enumerated below :

| Names of breeds | | Number of Batches | Number of normal and crimson eggs found in each batch laid by a parent | | Totals |
			Normals	Crimson	
Univoltine " Ghinpaku "		1	248	84	332
,,	,,	7	413	120	533
,,	,,	8	247	89	336
,,	,,	13	167	73	240
	Totals		1075	366	1441
Divoltine " Tamanashi "		1	404	112	516
,,	,,	3	306	141	447
,,	,,	14	367	104	471
	Totals		1077	357	1434
	Grand totals		2152	723	2875
	Expectation		$2156\frac{1}{4}$	$718\frac{3}{4}$	2875

These figures suggest to us at once that the crimson-coloured characteristic is a Mendelian one, recessive to the normal-coloured as is the former crimson breed. We reared these eggs separately in the spring of 1910. Crimson-coloured eggs from two batches of the second breed gave, without any exception, crimson-eyed worms and moths, while normal-coloured gave all normal-eyed worms and moths. Both

crimson and normal-eyed moths derived from the mixed batches of the above breeds when paired *inter se* oviposited the following batches:

N = normal-eyed ; R = crimson-eyed moths.

Names of breeds	Parent Moths		Eggs laid		
	♀	♂	Normals	Crimsons	Totals
Univoltine "Ghinpaku"					
No. 8. 8	N × N		114	45	159
No. 8. 9	N × N		334	92	426
No. 13. 8	N × N		459	105	564
No. 13. 9	N × N		338	106	444
No. 13. 10	N × N		all normals		
Divoltine "Tamanashi"					
No. 1	R × R		all divoltine white eggs		
No. 3	R × R		,,	,,	,,
No. 14, Nos. 8—14	R × R		,,	,,	,,

As the figures shew, certain normal-eyed matings gave all normal dark-coloured eggs while others gave mixed batches (3 normals : 1 crimson).

The lineage of the crimson-eyed matings which yielded all divoltine white eggs remained true to parents in their successive generations, and gave crimson-coloured eggs without exception in the summer brood.

The order of inheritance of these characteristics is in exact accordance with the F_3 of a monohybrid cross, and the parental mixed eggs given by Mr Saito were undoubtedly F_2 eggs between the crimson and normal-coloured forms. Now we may clearly see that those crimson-coloured characteristics found in the three different breeds are in the same nature and Mendelize in the normal order, the crimson being hypostatic to the normal-coloured one.

Résumé :

R = crimson ; N = normal.

(N × R) (reciprocal)

F_1 eggs $\qquad N$

F_2 ,, $\qquad$ (3N : 1R)

F_3 ,, $\qquad N$ (3N : 1R) $\quad R$

(F_1 × R)

F_2 ,, $\qquad$ (1N : 1R)

Greater Death-rate of the Crimson-coloured Egg.

During our experiments with the crimson breeds, either with the original divoltine or other extracted forms from cross-bred or mixed breeds, it has struck us that the number of worms which came out in spring from these eggs was very small, sometimes over 90 °/₀ were found to be dead; even in the case where the crimson eggs were found mixed with normal ones in the same batch, the mortality is much greater than that of the normal-coloured eggs. We give here certain figures of the death-rate observed in the crimson-coloured eggs laid by some breeds.

Fifteen batches selected at random from 84 crimson-coloured batches laid by the summer brood of the extracted crimson form, from the cross between the divoltine crimson and tetravoltine normal-coloured "Onodahime," gave the following figures:

TABLE VI.

Number of batches	Total number of eggs laid	Number of dead eggs	Death-rate
1	377	341	90·4 °/₀
2	368	269	73 °/₀
3	348	342	98·2 °/₀
4	439	389	88·6 °/₀
5	413	405	97·1 °/₀
6	283	321	88·1 °/₀
7	379	364	96 °/₀
8	277	243	87·7 °/₀
9	438	318	72·6 °/₀
10	365	149	40·8 °/₀
11	254	136	53·5 °/₀
12	304	244	80·2 °/₀
13	319	284	89 °/₀
14	424	403	95·2 °/₀
15	446	360	80·7 °/₀
Totals	5434	4568	84 °/₀

The average death-rate was 84 °/₀, ranging from 40·8 °/₀ to 98 °/₀ in each batch. In 42 batches of eggs derived from the same breed, we have counted 15,194 eggs, of which 12,796 eggs were found dead in the spring; i.e. a death-rate of 84 °/₀.

In the eggs laid by a cross-bred crimson form (a cross between an extracted crimson from the cross, "Divoltine crimson × Divoltine Chiyodzuru," and the crimson derived from the divoltine "Tamanashi") a considerably smaller percentage of dead eggs was noted than in the cross between divoltine crimson and tetravoltine white.

TABLE VII.

Number of batches	Total number of eggs laid	Number of dead eggs	Death-rate
1	481	52	10·8 %
2	487	45	9·2 %
3	481	52	10·8 %
4	466	3	0·6 %
5	471	260	55·2 %
6	476	241	50·6 %
7	514	90	17·6 %
8	543	169	31·1 %
9	515	342	66·9 %
10	490	16	3·2 %
11	529	377	71·2 %
12	471	45	9·5 %
13	459	223	48·5 %
14	554	3	0·5 %
15	477	224	. 47·5 %
Totals	7414	2142	28·8 %

In this crimson form the average death-rate is only 28·8 %; in certain matings nearly all the eggs were hatched, while in some others the death-rate mounted as high as 71·2 %.

In other crimson batches laid by an extracted crimson form derived from a cross between the divoltine crimson and " E III " breed (an extracted breed from a cross between the wild mulberry silk-worm and tetravoltine " Tōbuhime "), the figures quoted below were given :

TABLE VIII.

Number of batches	Total number of eggs laid	Number of dead eggs	Death-rate
1	234	181	77·3 %
2	383	348	90·8 %
3	335	102	30·4 %
4	467	329	70·4 %
5	360	66	18·3 %
6	248	192	79·0 %
7	326	303	92·9 %
8	261	233	89·2 %
9	362	199	54·9 %
10	277	153	55·2 %
11	394	283	71·8 %
12	313	279	89·1 %
13	340	322	94·7 %
14	391	341	87·2 %
15	374	355	94·9 %
Totals	5065	3686	72 %

In this form the death-rate is 72 %, varying, in individual cases, from 18·3 % to 94·9 %.

We have observed moreover that the death of the embryo inside the egg took place in its earlier stage. So we often found some dead eggs in August or September when a white patch appeared in one side of the egg (Fig. 8) which gradually became enlarged in size and at last the clear crimson colour became paler, while others were found dead after the embryo had completely developed. The majority of the deaths seem to occur, however, in the earlier developmental stage of the embryo.

Even in the same breed, the death-rate differed greatly according to the colour of the egg, and the voltine characters. Hence the divoltine white eggs laid by the very same breed which laid the eggs recorded in Table VI yielded a much smaller number of dead eggs than in the univoltine coloured eggs. Ten batches of eggs selected at random from fifty divoltine white egg-batches gave the following figures:

TABLE IX.

Number of batches	Total number of eggs laid	Number of dead eggs	Death-rate
7	191	60	31·4 %
8	361	18	4·9 %
9	111	51	45·9 %
10	329	168	51 %
11	272	196	72 %
12	309	12	3·8 %
13	186	18	9·6 %
13a	272	164	61·4 %
14	413	64	15·5 %
15	381	78	20·8 %
Totals	2825	829	29·3 %

that is to say, the average death-rate is 29·3 %, which varies from 3·8 % to 72 % in individual batches. The average death-rate found in fifty batches of eggs laid by the same breed is 19 %, while in the case of univoltine eggs it is 84 %, as already recorded in Table VI. In certain cases it occurred that some crimson-coloured eggs hatched in the summer. The death-rate of such divoltine crimson-coloured eggs was nearly the same as that of the white divoltine eggs.

Even more striking facts were observed when we examined the number of dead eggs found in the batches in which three different coloured eggs, normal, crimson, and albino, were found. These are the F_2 eggs laid by the cross, " H_4 " albino and the divoltine crimson breed.

TABLE X.

Number of batches	Total number of eggs	Dead eggs	Death-rate	Total	Dead	Death-rate	Total	Dead	Death-rate
	Normal eggs			Crimson eggs			Albino eggs		
1	194	33	17 %	95	77	81 %	91	12	13·1 %
2	209	64	30·5 %	66	49	74·2 %	86	7	8·1 %
3	195	23	13·8 %	99	76	76·7 %	88	30	34 %
4	213	23	10·7 %	75	61	81·3 %	99	22	22·2 %
5	213	79	37 %	94	62	64·8 %	87	5	5·7 %
Totals	1024	222	21·6 %	429	325	75·7 %	451	76	16·8 %

In spite of their being laid by the same parents, the mortality in those eggs whose colour was crimson was much greater than the others. While in normal and white-coloured eggs, the percentage of deaths was only 21·6 % and 16·8 % respectively, that of the crimson eggs was 75·7 %. Other ten similar batches gave the following figures:

	Normal eggs	Crimson eggs	Albino eggs
Total number	2315	956	1076
Number of dead eggs	406	650	288
Death-rate	17·5 %	67·9 %	26·7 %

The facts above enumerated taught us that in every case in every breed which we have studied, those eggs coloured crimson have a greater death-rate than normal-coloured eggs, while divoltine eggs which are crimson in colour did not shew so high a death-rate as univoltine crimson-coloured eggs. These facts led us to conclude that the embryo of crimson-coloured eggs is not so long-lived as that in the normal-coloured eggs.

As to the cause of the early death of the embryo of the crimson-coloured eggs, we are quite ignorant at present, but we are inclined to believe that it may be due to the lack of certain pigments in the serosa which in some way help the respiration of the embryo during its development.

V. General Considerations.

From the results of these series of experiments in both line and cross breedings above quoted, it now becomes clear that (1) those egg-characteristics above enumerated, except in the crimson breed, are determined by the characteristics of the female parent, on account of which the paternal characteristics even when dominant are almost negligible in their influence upon the character of the egg, that is to say, phenomena of inheritance are maternal; (2) gametic segregation of parental characteristics takes place as in normal Mendelian

allelomorphism; and (3) in certain generations, both parental character-
istics even when inbred give rise to the antagonistic characteristics
which at first suggests that there is a departure from the normal rules.

Suppose, now, there are certain Mendelian characteristics which
behave as maternal in inheritance. If they were reciprocally mated,
what would be the result as regards their offspring? Let D represent
a dominant and R a recessive factor, the results of their reciprocal
matings would be diametrically opposite. In the case of a D female
mated with an R male, the resulting F_1 eggs would be all D, while an
R female mated with a D male would give all R F_1 eggs, in spite of
their zygotic constitutions being the same in both matings, namely, DR.
And therefore, all the worms and moths derived from the F_1 D or R eggs
will have the constitution DR, in which the D behaves as an active
factor in determining their characteristics. In the same way the egg-
cell which has the composition of DR during its development in the
parent body is influenced by the D factor only, and consequently
after segregation, when it lost the antagonistic factor and became pure
D or R, it retains the D characteristic before acquired. Thus the results
of fertilization will be the production of all normal-coloured F_2 batches.

Zygotically considered, however, the F_2 D eggs are not the same in
their constitution. As the result of the fertilization, some of them will
be DD, some DR and the rest RR. Consequently, the constitution of
the F_2 moths derived from the F_2 D eggs will be a mixture of DD, DR
and RR. Thus all the F_2 eggs, whether fertilized with D or R sperma-
tozoon, will be all D characterized.

As there is no means in this case of distinguishing a DD worm or
moth from a DR or an RR, random matings between them are expected
to occur. The result will be as below:

			Colour of the F_3 eggs laid	Zygotic composition of the egg
1.	a.	♀ DD × ♂ DD =	D	DD
	b.	♀ DD × ♂ DR =	D	$(DD + DR)$
	c.	♀ DD × ♂ RR =	D	DR
2.	a.	♀ DR × ♂ DD =	D	$(DD + DR)$
	b.	♀ DR × ♂ DR =	D	$(DD + DR + RR)$
	c.	♀ DR × ♂ RR =	D	$(DR + RR)$
3.	a.	♀ RR × ♂ DD =	R	DR
	b.	♀ RR × ♂ DR =	R	$(DR + RR)$
	c.	♀ RR × ♂ RR =	R	RR

In some cases female DD moths will mate with DD, DR or RR
males (series 1), in some others DR females will mate with the same
three kinds of males (series 2). The same holds good in the case of RR

females (series 3). The F_3 eggs laid by the first and the second series of matings will be all D batches, since all females are DR or DD. For the same reason, the third series of matings will give all R batches. *The F_3 eggs* derived from the F_2 moths paired *inter se* will be, therefore, a *mixture of D and R* batches in certain proportions. If we assume that the number of males and females found in each batch is nearly the same, the proportion of D and R batches laid by *inter se* moths derived from an F_2 D batch would be $6D : 3R$ or $2D : 1R$.

As in the case of the F_2, the zygotic constitution of F_3 D and R batches is not simple D or R. As the formulae quoted just above shew, the constitution of certain F_3 D batches is DD (series 1 a), some batches DR (series 1 c), some a mixture of DD and DR (series 1 b and series 2 a), or DR and RR (series 2 c), and the rest DD, DR and RR (series 2 b). We get similar results in the case of the F_3 R eggs, some batches being DR (series 3 a), some (series 3 c) RR, and the rest (series 3 b) a mixture of DR and RR.

If moths derived from F_3 D or R batches were inbred, what will be the result in the dominant series?

In F_3 D batches, as we have already observed, there are five different kinds of batches whose zygotic compositions are respectively : (1) DD, (2) $(DR + DD)$, (3) DR, (4) $(DD + DR + RR)$, (5) $(DR + RR)$. The moths derived from each kind paired *inter se* will produce the following F_4 batches :

Mating		Zygotic composition	Outward appearance of F_4 eggs
1. DD inter se $= DD \times DD =$		DD	D
2. $(DR+DD)$ inter se $=$	1. $\female\, DR \times \male\, DR = (DD+DR+RR)$		D
	2. $\female\, DR \times \male\, DD = (DD+DR)$		D
	3. $\female\, DD \times \male\, DD = DD$		D
	4. $\female\, DD \times \male\, DR = (DD+DR)$		D
3. DR inter se $=$		$\female\, DR \times \male\, DR = (DD+DR+RR)$	D
4. $(DD+DR+RR)$ inter se $=$	1. $\female\, DD \times \male\, DD = DD$		D
	2. $\female\, DD \times \male\, DR = (DD+DR)$		D
	3. $\female\, DD \times \male\, RR = DR$		D
	4. $\female\, DR \times \male\, DD = (DD+DR)$		D
	5. $\female\, DR \times \male\, DR = (DD+DR+RR)$		D
	6. $\female\, DR \times \male\, RR = (DR+RR)$		D
	7. $\female\, RR \times \male\, DD = DR$		R
	8. $\female\, RR \times \male\, DR = (DR+RR)$		R
	9. $\female\, RR \times \male\, RR = RR$		R
5. $(DR+RR)$ inter se $=$	1. $\female\, DR \times \male\, DR = (DD+DR+RR)$		D
	2. $\female\, DR \times \male\, RR = (DR+RR)$		D
	3. $\female\, RR \times \male\, DR = (RR+DR)$		R
	4. $\female\, RR \times \male\, RR = RR$		R

Thus the F_4 eggs laid by the moths of the dominant series will be all D in certain lineages while in others they will be a mixture of both D and R batches in different proportions.

In like manner, F_3 moths derived from the F_3 R batches which consist of (1) DR, (2) $(DR + RR)$, and (3) RR batches, when inbred will produce the following F_4 eggs:

Mating		Zygotic composition of egg-batches	Outward appearance of egg-batch
1. *DR inter se*		♀ DR × ♂ $DR = (DD + 2DR + RR)$	D
2. *(DR + RR) inter se*	1.	♀ DR × ♂ $DR = (DD + DR + RR)$	D
	2.	♀ DR × ♂ $RR = (DR + RR)$	D
	3.	♀ RR × ♂ $DR = (DR + RR)$	R
	4.	♀ RR × ♂ $RR = RR$	R
3. *RR inter se*		♀ R × ♂ $R = RR$	R

The F_4 eggs laid by the moths derived from F_3 R eggs will be all D batches in certain lineages, while there will be a mixture of D and R batches in some other lineages, and all R batches in the remainder.

If we continually eliminate the lineage which produced the antagonistic characterized batches in this way in both series, each of them may be established as a constant or homozygous form. In the dominant series, the lineages which give all F_4 D batches are three, namely: No. 1 or DD series, No. 2 or $(DD + DR)$ and No. 3 or DR. If they are inbred, Series No. 3 would give mixed F_5 batches which are to be discarded from the dominant series, while the other two would give all F_5 D batches. In F_6, those from No. 2 will disintegrate into their components, D and R, and those which produced all F_6 D batches will be the descendants of Series No. 1, or a lineage DD, which is a homozygous dominant from their first appearance.

To extract a homozygous dominant form from these series of crosses the elimination of six consecutive generations will be required, except when we luckily happen to pick out the lineage of DD series, in which case we are able to get it in a constant form in a much less number of generations.

In the recessive series, however, if we select the lineage which produced all R batches in F_4, the R form is easily established as a constant form, since the zygotic composition of this series is RR.

This is what we might expect to find according to Mendelian principles if the characteristic behaved as maternal in inheritance. If the D form consists of two or more factors, disintegration of factors will

Graphical résumé of the phenomena of inheritance above described.
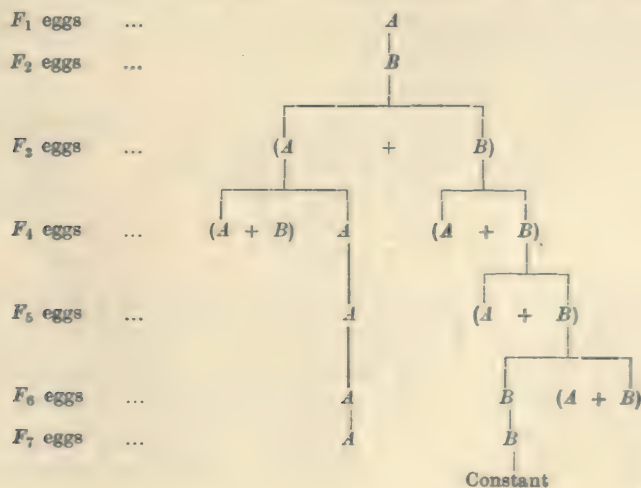
```
                                    (1)                    (2)
Parents      ...           ( ♀ D × ♂ R)            ( ♀ R × ♂ D)
                                    |                      |
F₁ eggs      ...                    D                      R
                                    |                      |
F₂ eggs      ...                    D                      D
                                    |_____|
                                               |
                                    |_____|
                                    |                       |
F₃ eggs      ...                   (D          +           R)
                                    |                       |
                              _____|_____           _____|_____
                             |             |          |            |
F₄ eggs      ...             D         (D + R)        D    (D + R)   R
                        _____|____                                   |
                       |         |                                   |
F₅ eggs      ...       D      (D + R)                                R
                  _____|____                                         |
                 |         |                                         |
F₆ eggs      ... D      (D + R)                                      R
                 |                                                   |
F₇ eggs      ... D                                                   R
```
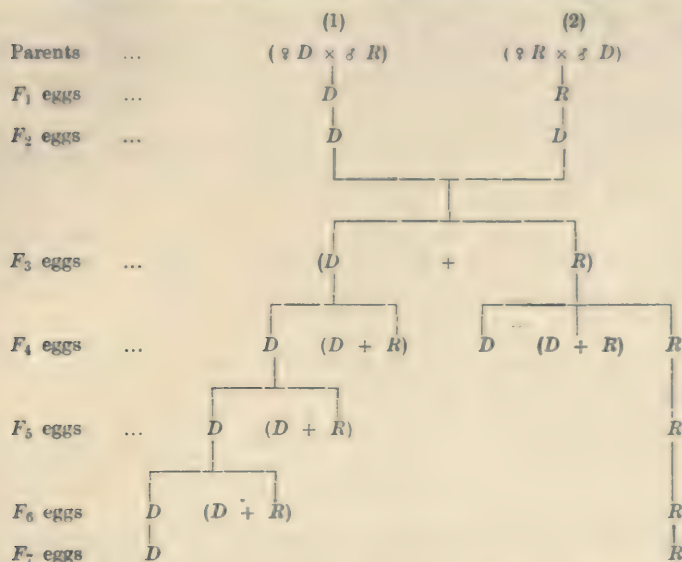
take place even after it is freed from the antagonistic recessive factor. We shall now try to compare the results obtained by us with those demanded by the theory.

First we shall quote here the results of inbreedings mentioned in the first part of this paper which are represented in the following scheme:

```
F₁ eggs    ...                        A
                                      |
F₂ eggs    ...                        B
                                      |
                        _____|_____
                       |                             |
F₃ eggs    ...        (A             +               B)
                       |                             |
                  _____|_____                   _____|_____
                 |          |                  |          |
F₄ eggs    ...  (A + B)     A                 (A    +    B)
                            |                             |
                            |                        _____|_____
                            |                       |          |
F₅ eggs    ...              A                      (A    +    B)
                            |                             |
                            |                        _____|_____
                            |                       |          |
F₆ eggs    ...              A                       B      (A + B)
                            |                       |
F₇ eggs    ...              A                       B
                                                    |
                                                Constant
```

In this scheme, if we replace A by R and B by D we shall see that the results actually obtained and those calculated come in the same category, the whitish grey being dominant to the normal, and the brown and the spindle-shaped characteristics being recessive to normals. As to the yellow and white colours of newly laid eggs, the former is dominant to the latter.

Crosses between *Bombyx mori*, L. and *Theophila mandarina*, M., which gave the result mentioned below:

		(1)	(2)
		(Theophila × Bombyx)	(Bombyx × Theophila)
F_1 eggs	...	all *Theophila* colour	all *Bombyx* colour
F_2 eggs	...	*Theophila* type	*Theophila* type

may come in the same category, the *Theophila* colour being dominant towards that of the *Bombyx*.

Back-crossing.

If females derived from the F_1 D eggs were mated with the parental recessive form, we should expect to have the combination ♀ $DR \times$ ♂ RR, which gives all D eggs, while in the reversed mating we should expect all R eggs, in spite of the zygotic composition being a mixture of DR and RR in an equal proportion in both cases, since the maternal characteristic predetermines the egg-character. Of moths derived from the D eggs, some will be, therefore, DR in their constitution and the others RR. As we have however no means of distinguishing DR males or females from RR males or females, we must expect to have random matings between these two forms which will result in the following combination:
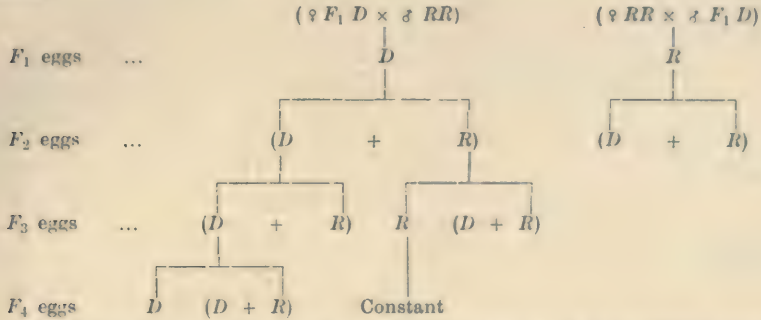
		Zygotic composition of egg-batches	Outward appearance of egg-batches
1.	{ a.	♀ $DR \times$ ♂ $DR = (DD + DR + RR)$	D
	{ b.	♀ $DR \times$ ♂ $RR = (DR + RR)$	D
2.	{ a.	♀ $RR \times$ ♂ $DR = (DR + RR)$	R
	{ b.	♀ $RR \times$ ♂ $RR = RR$	R

Hence in this generation, certain matings will give all D batches, others will give all R batches.

As the formulae above mentioned shew, the D and R eggs are heterozygous except No. 2 b which is homozygous, and therefore the moths which came out from them even when paired *inter se* among those from the same batch would produce antagonistic eggs again.

The further posterity of the lineages above mentioned will follow exactly the same course mentioned in the case of matings Nos. 4 and 5 (see page 393).

Thus the result of back-crossing may be summarized in the following scheme :
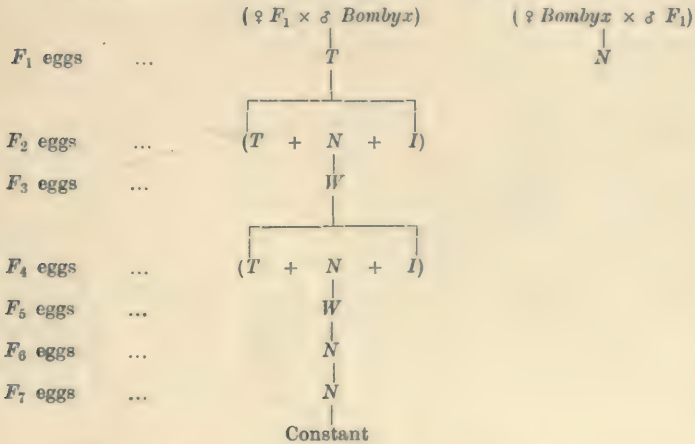
	(♀ F_1 D × ♂ RR)	(♀ RR × ♂ F_1 D)
F_1 eggs ...	D	R
F_2 eggs ...	(D + R)	(D + R)
F_3 eggs ...	(D + R) R (D + R)	
F_4 eggs	D (D + R) Constant	

This expectation was realized by the results of back-crosses between the *Theophila-Bombyx* F_1 moths and pure *Bombyx* ones which are quoted below :

T = green-shaded eggs like those laid by *Theophila-Bombyx* F_1 moths.
W = divoltine white eggs ; N = normal *Bombyx* eggs.
I = intermediate forms between *Theophila* and *Bombyx* or mixture.

|	(♀ F_1 × ♂ *Bombyx*)	(♀ *Bombyx* × ♂ F_1)
F_1 eggs ...	T	N
F_2 eggs ...	(T + N + I)	
F_3 eggs ...	W	
F_4 eggs ...	(T + N + I)	
F_5 eggs ...	W	
F_6 eggs ...	N	
F_7 eggs ...	N	
	Constant	

As the figures shew the result actually obtained by experiments is in perfect accordance with that demanded by the theory except for the appearance of the antagonistic characteristics in the F_4 of *Bombyx* typed or recessive series. This is due, I think, to the presence of divoltine white character which prevents the elimination of the antagonistic character in F_3 eggs.

By similar reasoning, we can explain the results obtained by crosses between males of the grey variant No. 24 and females of the normal-egged breed.

In these crosses, as we have already seen, certain matings gave no grey eggs, while some gave both normal and grey batches in the order mentioned below :

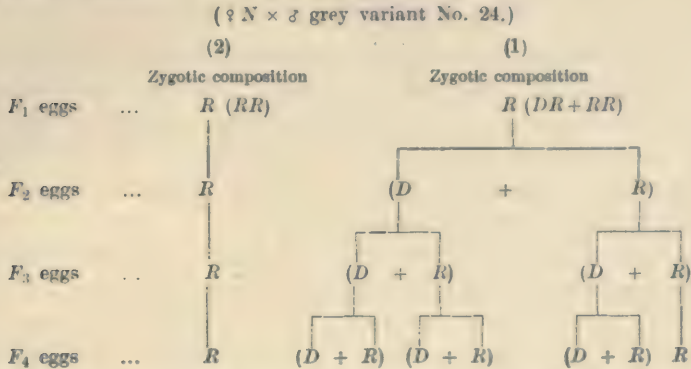N = normal-egged batch ; G = grey-egged batch ; BG = B-grey.
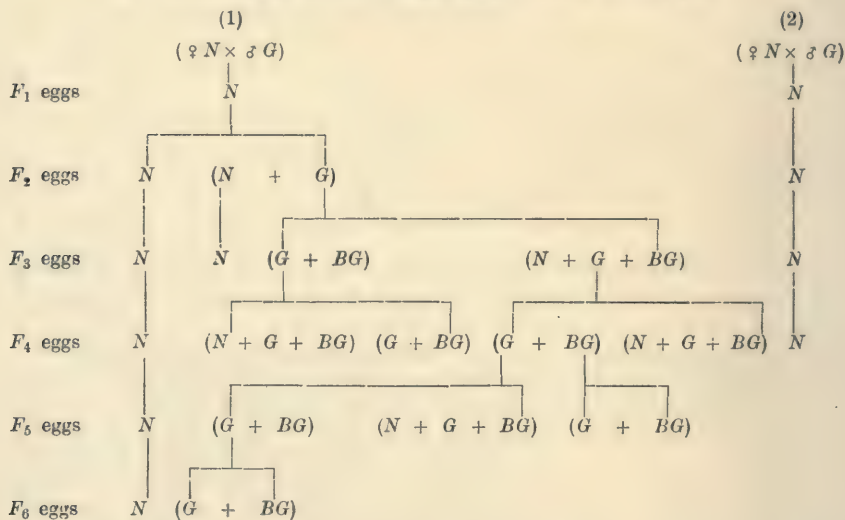
Before attempting to explain this result it will be necessary to note here that the grey variant No. 24 was picked out from certain normal-egged breeds in which a few batches of this variant sometimes appear, and consequently it is proper to consider it to be a heterozygous form. Let us suppose that the grey No. 24 batch is derived from such matings as ($♀ DR \times ♂ RR$), which give all D eggs. In this case the constitution of the egg batch D will be $(DR + RR)$. If normal-egged females which are recessive to the grey were mated with males derived from this D batch, the following gametic combinations will be expected :—

1. $♀ RR \times ♂ DR = F_1 R$ eggs $= (DR + RR)$.

2. $♀ RR \times ♂ RR = F_1 R$ eggs $= (RR)$.

F_1 eggs laid by those matings are all R, but their zygotic constitutions are different from each other, as we easily see from the formulae above shewn. The resulting F_2 eggs will, therefore, be in some matings (No. 1) a mixture of D and R batches and in others (No. 2) all R. The lineage

which gave all F_2 R batches will remain true to parents in the succeeding generations, while another lineage which gave D and R batches in F_2 will again disintegrate into its components in the same way as we have already described in previous pages. The order of their inheritance will be as follows:

$(♀ N \times ♂$ grey variant No. 24.$)$

		(2)						(1)	
		Zygotic composition					Zygotic composition		
F_1 eggs	...	$R\ (RR)$					$R\ (DR + RR)$		
F_2 eggs	...	R		$(D$	$+$			$R)$	
F_3 eggs	..	R	$(D$	$+$	$R)$		$(D$	$+$	$R)$
F_4 eggs	...	R	$(D + R)$	$(D + R)$		$(D + R)$		R

Here both actual and theoretical results, as in the former cases, agree perfectly in every respect.

Similar phenomena of maternal inheritance had already been observed in certain seed-characteristics of wheat by Biffen, of peas and maize by Bateson, Correns, Lock, Tschermak, etc. For instance, certain indented peas reciprocally crossed with the round gave the following results:

		$(♀$ Indent $\times ♂$ Round$)$	$(♀$ Round $\times ♂$ Indent$)$
F_1 seeds	...	Indent	Round
F_2 seeds	...	Indent	Indent
F_3 seeds	...	(Indent + Round)	(Indent + Round)

In certain cases, however, strict segregation took place or an intermediate form was produced in F_2. It was, moreover, mentioned that in certain varieties those characteristics behaved quite normally in inheritance, namely, F_1 seeds are all indents which in F_2 segregate into three indents to one round.

Hence we are led to say that certain characteristics of the eggs of animals and of the seeds of plants behave in inheritance in a similar way.

We shall discuss in our next paper the appearance of intermediate or mixed batches in certain line- or cross-breedings, a fact which seems to be inconsistent with maternal inheritance. We are at present collating the facts gathered from certain experiments which we have just concluded, and from the trend of our results up to the present, we

will, we think, be able to put forward a satisfactory explanation of this phenomenon. We merely, at present, say that this appearance is not really contradictory to the maternal inheritance.

Causes of Maternal Inheritance.

Concerning certain characteristics such as the whitish grey, spindle-shaped, or yellow and white colour of newly laid eggs whose origin is due to the shell or yolk which are entirely derived from the maternal body, the maternal inheritance is the natural consequence, and may be compared with the inheritance of certain characteristics of the seed-coat of plants which are of purely maternal origin.

Concerning the colour of the egg, whose origin is due to the special pigments deposited in the serosa, the case is quite different. As the serosa is formed of cells derived from the conjugation of paternal and maternal nuclei, the egg-colour ought to be influenced by the paternal characteristics if they are dominant, but, as we see, it is entirely maternal in certain colours, such as the reddish brown, blue, normal colour, etc.

Nothing is as yet known as to why these serosa characteristics behaved as maternal in inheritance. We are now waiting the results of the further series of experiments which we have been engaged upon concerning this question.

There are other characteristics of the silk-worm which behave maternally in inheritance. They are the brood characters such as uni-, di-, or multivoltine, or "voltinism" of the silk-worm. The fact of maternal inheritance of these characteristics was first observed by me (1906) and was proved by McCracken (1909), who was led to the conclusion that the order of inheritance was non-Mendelian, while Castle (1910), upholding the fact that they are maternal in inheritance, says that univoltinism is a Mendelian dominant to divoltinism.

Voltinism.

McCracken's results may be compared with those obtained by us in the series of experiments above referred to, but in the case of voltinism, as there are many causes disturbing the proper elimination of parental characteristics which are entirely neglected by her, it is rather premature to consider the phenomena of inheritance displayed by the character "voltinism" as non-Mendelian. We enumerate here those disturbing causes: (1) divoltine character may easily be changed by the influence

of temperature during the incubation of the egg, more strictly, during the embryonal stage after sexual cells are liberated from the mesodermal tissue. If we expose eggs at this stage to a temperature of about 60—65° F. or lower until hatching, all the moths derived from them will lay divoltine whitish eggs; on the contrary, if we subject them to a temperature of 80° F. or more, all the eggs will become univoltine coloured ones. This is a well-known fact among Japanese breeders and has been made use of for industrial purposes for the last twenty years. (2) The eggs laid by the second brood of the divoltine breed are identical in appearance with the univoltine eggs and hibernate without hatching. In the case of crossing, we are, therefore, unable to eliminate divoltine characterized eggs from the univoltine in every alternate generation. (3) The maternal inheritance referred to above, which also prevents the proper elimination of antagonistic characters.

These are the causes why, I think, the character " voltinism " behaved so irregularly that McCracken considered it to be non-Mendelian. Generally speaking, I believe, the order of inheritance of the " voltinism " of the silk-worm will follow the course before mentioned in our scheme.

In a later paper I propose to give a fuller account of the phenomena connected with voltinism.

Before concluding this paper, I wish to express my sincere thanks to Prof. W. Bateson who has kindly assisted me in many ways when preparing this paper for press. Thanks are also due to Mr S. Hashimoto, assistant in our laboratory, who has helped me in rearing the worms used in our experiments since 1906.

VI. Summary.

1. In the egg of the silk-worm there are certain special characteristics of shape, colour, etc. which differ in different breeds or even in the same breed. Japanese green breeds generally lay green-shaded eggs varying in depth of colour, often mixed with normal coloured ones. Most of the Chinese and European breeds lay similar green-shaded eggs, both of them, however, being distinguishable from each other by special lustres and shades. Eggs of the Japanese normal breeds are, however, brownish slate shaded with some light pink or purple.

Among the eggs laid by Japanese normal-egged breeds we often find many variants in shape and colour, a smaller number of the variant being sometimes found in a batch, frequently in Mendelian proportions,

while in other cases the whole of a batch will be found to consist of a variant.

    2. Breeding experiments were made on the following egg-characteristics:

    1. Greenish-shaded *Theophila* colour (Fig. 10).

    2. Various green colours of Japanese green and some Chinese or European breeds (Fig. 6).

    3. The reddish brown variant derived from the normal-egged breed (Fig. 2).

    4. The blue variant from the normal breed (Fig. 5).

    5. The whitish grey variant from the normal breed (Fig. 4).

    6. The spindle-shaped variant from the normal breed (Fig. 13).

    7. The crimson variant from the normal breed (Figs. 7 and 8).

    8. Yellow, brownish-shaded yellow and white colours of newly laid eggs of the yellow and white cocoon breeds (Fig. 9).

    9. The brownish slate colour of Japanese normal breeds (Figs. 1, 3, and 11).

    3. Some of these characteristics such as the normal brownish-slate, reddish brown, blue, crimson, etc., arose from the special pigments deposited in the serosa, which is produced by the conjugation of parental nuclei, while others, such as whitish grey, spindle-shaped or the colour of newly laid eggs are due to the shell or yolk which are of purely maternal origin. The colour of greenish-shaded eggs, such as are found in Japanese green breeds, and some Chinese or European breeds, is mostly derived from the serosa, but it is more or less influenced by the colour of the shell which is slightly tinted with green, or some other colours.
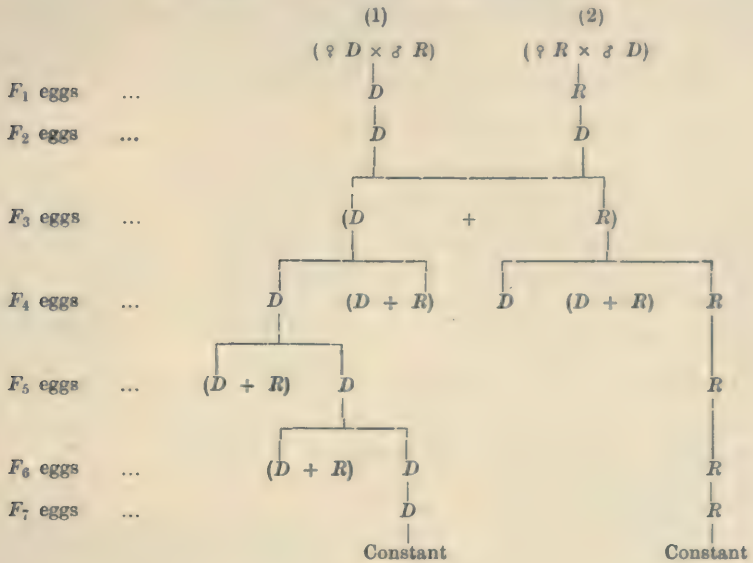
    4. Those characteristics, in spite of the fact that their origin is different, behave in the same way as regards inheritance, except the crimson-coloured variant, which Mendelize in the normal order. The order of inheritance is represented in the following schemes (page 403).

    The order of inheritance represented by the first scheme seems to be non-Mendelian, but really it is Mendelian, the cause of the disturbance of the proper order being due to the fact of maternal inheritance, in which paternal characteristics remain dormant, even dominant ones, in the egg stage.

(1)

The case where characteristics behaved maternally in inheritance, such as normal, reddish brown, grey, etc.

D = dominant ; R = recessive characteristics.

		(1)	(2)
		(♀ D × ♂ R)	(♀ R × ♂ D)
F_1 eggs	...	D	R
F_2 eggs	...	D	D
F_3 eggs	...	(D	+	R)
F_4 eggs	...	D ($D + R$)	D ($D + R$) R
F_5 eggs	...	($D + R$) D	R
F_6 eggs	...	($D + R$) D	R
F_7 eggs	...	D	R
		Constant	Constant

(2)

The case where characteristics behaved in normal Mendelian order, such as the crimson colour.

		(1)	(2)
		♀ D × ♂ R	♀ R × ♂ D
F_1 eggs	...	D	
F_2 eggs	...	($3D$	:	$1R$)
F_3 eggs	...	D ($3D : 1R$) R	
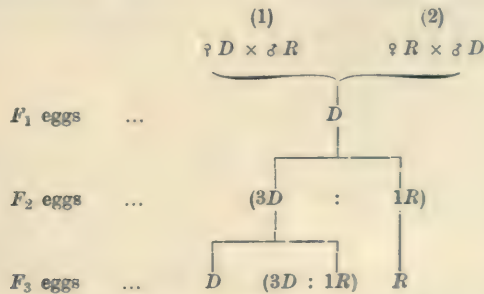
In the former scheme, the D represents dominant coloured batches, the R recessive and $(D + R)$ a mixture of both D and R batches, while in the latter the D and R represent a single batch of D and R eggs and the $(3D : 1R)$ a mixed batch consisting of D and R in the proportion of 3 : 1.

5.　The hereditary relations of those characteristics above enumerated: as to the shape, the whitish grey is dominant towards the Japanese normal which is in turn dominant to the spindle-shaped characteristic. As regards the colour, the greenish slate stands first in dominancy, next comes the Japanese normal brownish slate; hypostatic to it comes perhaps the blue and then reddish brown and lastly the crimson. In the colours of newly laid eggs, the white is hypostatic to yellow or brownish yellow. The relation between the normal and the crimson is the ordinary Mendelian one, the former being epistatic to the latter.

6.　In the crimson-coloured eggs extracted from various breeds or crosses, the death-rate is always much greater than that of eggs from the normal, or albino breeds. Even when those three kinds of eggs are laid by the same parent, the same is the case. In divoltine crimson breeds, the divoltine white eggs laid by the spring or first brood are much healthier than those crimson-coloured eggs laid by the summer or second brood.

7.　The phenomena of inheritance observed in the eggs of the silk-worm may be well compared with those observed in certain seed-characteristics of plants, such as maize, peas, wheat, etc.

EXPLANATION OF PLATE XX.

Fig.　1.　Normal-coloured egg of divoltine "Shinkawachi."
Fig.　2.　Reddish-brown variant from divoltine "Shinkawachi."
Fig.　3.　Normal-coloured egg of the original breed of the whitish-grey variant.
Fig.　4.　Whitish-grey variant.
Fig.　5.　Blue variant derived from divoltine "Kuni-nishiki."
Fig.　6.　Green-shaded egg from the Japanese green breed.
Fig.　7.　Crimson-coloured variant.
Fig.　8.　Dead egg of the crimson variant.
Fig.　9a.　Newly laid egg of tetravoltine white.
Fig.　9b.　Newly laid egg of tetravoltine yellow.
Fig.　10.　Egg of *Theophila mandarina*.
Fig.　10a.　Matured egg.
Fig.　10b.　Newly laid egg.
Fig.　11.　F_1 eggs between female divoltine "Shinkawachi" and male *Theophila mandarina*.
Fig.　12.　F_2 eggs of the above mating.
Fig.　13.　Batch of spindle-shaped eggs laid by a moth.

Every figure except No. 13 is very much magnified, actual size being about 1·15 mm. in length and 0·95 mm. in breadth.

PLATE XX

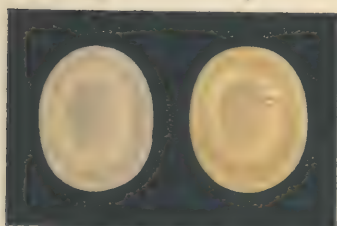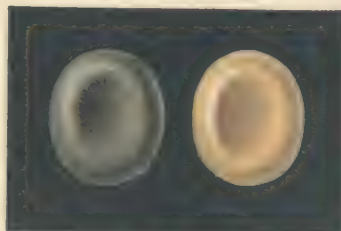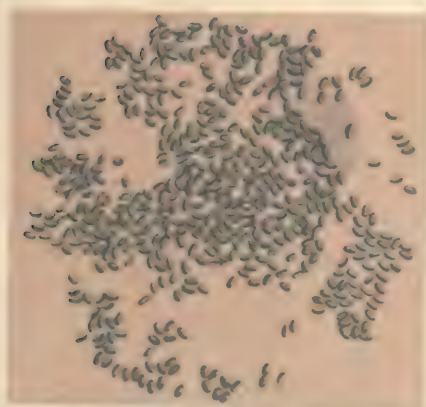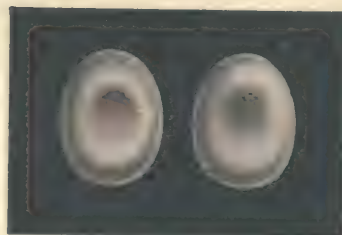## LITERATURE CITED.

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