Further Experiments on the Cross-breeding of Two Races of the Moth Acidalia virgularia.

By W. B. Alexander, B.A., late Vintner Exhibitioner of King's College, Cambridge.

(Communicated by Prof. E. B. Poulton, F.R.S. Received January 3,—Read February 22, 1912.)

A communication on the heredity of the two forms of this species was read to the Royal Society on February 25, 1909. In that paper Messrs. Prout and Bacot gave an account of a large number of broods of this moth reared by them for nine generations.

At the end of the paper they mentioned that they had handed to Mr. W. Bateson ova produced by pairings in generation F_{10}. It was the larvæ derived from these ova which Mr. Bateson gave into my charge in February, 1909.

Of the five broods which I thus obtained I only managed to continue two, as the individuals of the others emerged at such long intervals that I never had a male and female alive at the same time. Fortunately, however, the two I reared were the most interesting.

Brood 2.—This brood had been labelled DI*, gen. xi, by Mr. Bacot. Eleven moths emerged (4 ♂, 7 ♀) and from them I obtained two lots of ova —Broods 6 and 7. From Brood 6 I reared 35 moths (16 ♂, 19 ♀) and obtained three more lots of ova—Broods 9, 11, and 12. Brood 11 was liberated in the larval stage; from Brood 9 I reared 64 moths and from Brood 12, 38 moths (23 ♂, 15 ♀). From Brood 7 I reared 30 moths (14 ♂, 16 ♀) and obtained one lot of ova—Brood 13; from which I reared 92 moths (45 ♂, 47 ♀).

Thus of this strain I reared no less than 270 individuals, distributed in six families and three generations, and the striking feature was that they showed no appreciable variation. They were all of a yellowish colour with a slight amount of dark speckling on the wings.

I had not at this time seen any specimens of the original light form of the species, var. *canteneraria*, from Hyères, but when, through the kindness of Prof. Poulton, I was enabled to examine Messrs. Prout and Bacot's specimens in the Hope Collection at Oxford, I saw at once that all the descendants of my Brood 2 should certainly be classed as *canteneraria*. They did not show any more speckling of black than typical forms of that variety, but their yellow ground-colour was much darker than that of typical *canteneraria*,

though certain males, even reared from Hyères eggs direct, were as yellow as my moths.

The history of this race appears to be as follows:—Some light forms appeared among the dark ones in generation 6 in a box which was supposed to contain the pure dark strain. A number of the descendants of this brood were reared and in general gave a mixture of pure light forms and forms intermediate between light and dark. In some cases where two of these light individuals were mated they yielded nothing but lights, in others a mixture of lights and intermediates. On the other hand the darkest forms continued to throw light individuals when mated together. The actual parents of my Brood 2 appear not to be known, but there is no doubt that they were members of the generation 4 in descent from the original aberrant individuals. We thus see that it took five generations to establish a pure light brood from the original light individuals whose origin was quite inexplicable.

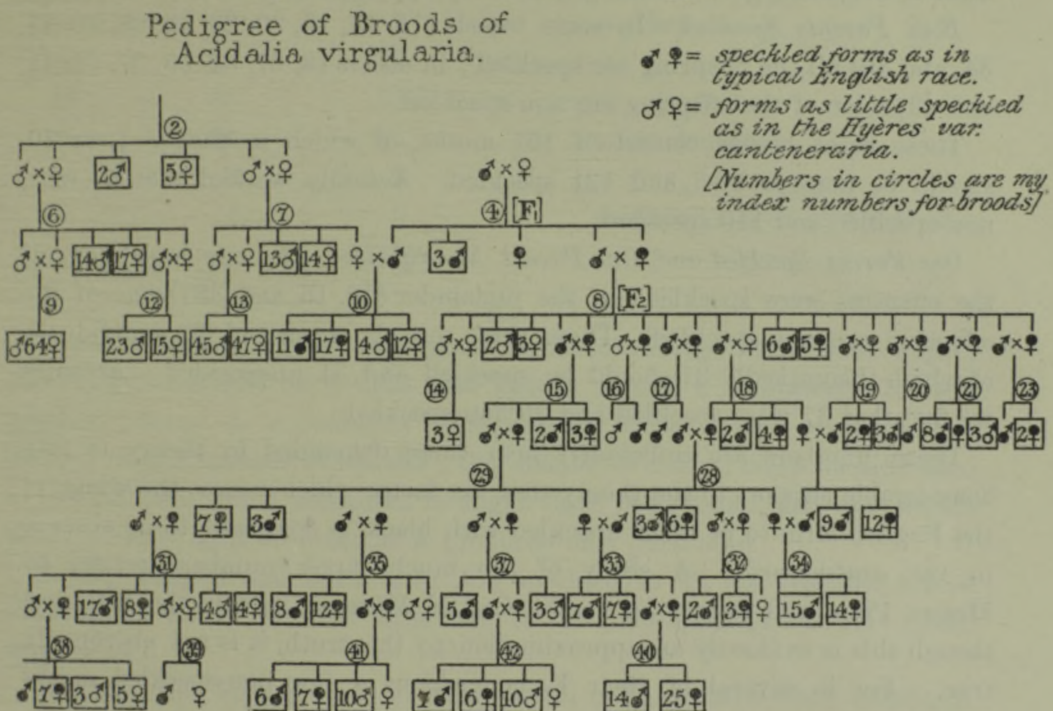
Brood 4.—This was the brood labelled DxLMxi by Mr. Bacot. They were the result of mating a pure dark male with a pure light female, both of whose ancestors had been reared in captivity for 11 generations. It was thus what is ordinarily known as an F_1 brood, though readers of Messrs. Prout and Bacot's paper will note that, though they claim to have numbered their broods according to "the well-known Bateson method," they have not confined F_1, F_2, etc., to the first, second, etc., generations of a hybrid strain, but have given these numbers also to broods of the pure strain, dating arbitrarily from the broods which were first reared in confinement.

My F_1 brood consisted of seven moths (5 ♂, 2 ♀), all of them distinctly speckled with black to a greater extent than in the light form, whilst their ground colour was a dirty white, not nearly so yellow as in my Brood 2.

From this brood I obtained one lot of ova—Brood 8, both of whose parents were of the type just described, and another lot of ova from a male of this brood mated with a female of Brood 7 (one of the pure yellow *cantencraria* broods descended from Brood 4). This I labelled Brood 10.

Brood 10.—This brood, consisting of 44 moths, possessed the yellow ground colour of its mother throughout, but 28 of the moths were distinctly speckled like their father, whilst 16 were exactly like their mother. This points to the ratio of 2 speckled : 1 unspeckled, but I think the numbers in this brood must be distrusted, since for some reason there were also 29 females and 15 males, which again suggests a ratio of 2 to 1, though of the total number of moths I have examined 305 were males and 309 females.

Brood 8.—This was the F_2 brood of 34 individuals, and exhibited a very considerable amount of variation, much more than that in F_1. I found, however, that it was quite easy to distinguish 9 moths as having no more speckling than pure *canteneraria*, whilst the remaining 25 were distinctly more speckled. Of the nine unspeckled (4♂, 5♀), three males were as yellow as the individuals of Brood 4, one distinctly paler, the females were all paler, two of them as white as the lightest of the pure Hyères race. It thus appeared that about one quarter (9 out of 34) of the F_2 brood were referable to the pure light race, though several of the males were yellower than any but extreme examples of it. The numbers suggested at once that the



unspeckled Hyères race was recessive to the speckled English form. I reared a number of further broods from pairs of the F_2 moths, and with one exception (Brood 39) this supposition would account for the composition of all the succeeding broods (see Pedigree).

I do not think it is necessary to deal with the subsequent broods in so much detail, as their mutual relationships will be visible from the pedigree.

The last generation emerged at irregular intervals in the early months of 1911, and only 3 females laid eggs. From one set of ova a few larvæ emerged, but only lived for a few days. Thus the race became extinct, perhaps owing to complete inbreeding for six generations.

The descendants of Brood 8 may be classified as follows :—

Both Parents Unspeckled (Broods 14 and 39).—Offspring, 4 unspeckled, 1 speckled. The one speckled moth resulting from this type of mating in Brood 39 is the only serious difficulty in the way of accepting a normal Mendelian relationship between speckled and unspeckled types. If it stood entirely alone I should have to regard it as due to some sort of error, though I did my utmost to guard against mistakes. But in the history of Messrs. Prout and Bacot's aberrant strain already mentioned, and in some of their other broods, there are definite cases of speckled forms originating from two non-speckled parents. Possibly one of the parents in these cases is a heterozygote indistinguishable in appearance from a recessive.

Both Parents Speckled.—In some broods (15, 17, 19, 20, 23, 29, 28, 30, 33, 34, and 40) all the offspring are speckled; in others (8, 21, 31, 35, 37, 32, 41, and 42) some of the offspring are non-speckled.

These eight broods consist of 161 moths, of which in theory $\frac{1}{4}$, or 40, should be non-speckled, and 121 speckled. Actually we find that 51 were non-speckled and 110 speckled.

One Parent Speckled and One Parent Non-Speckled.—In one brood (18) all the offspring were speckled; in the remainder (10, 16, and 38) some of the offspring are non-speckled. These three broods consist of 62 individuals, of which theoretically 31 should be speckled and 31 unspeckled. Actually we find that 37 were speckled and 25 non-speckled.

These numbers are sufficiently near those demanded by theory to give considerable support to the theory that the factor which causes the wings of the English form to be much speckled with black is dominant to its absence in var. *canteneraria*. A study of the much larger numbers reared by Messrs. Prout and Bacot, now in the Oxford Museum, reveals the fact that though this is evidently an approximation to the truth, it is not universally true. For in several of their F_1 generations a few non-speckled moths occur, the numbers being as follows:—

A	58	speckled,	2	non-speckled
C	3	„	0	„
c	7	„	0	„
h	12	„	0	„
H	56	„	0	„
G	68	„	1	„
*g**	20	„	3	„
7 F_1 broods,	224	„	6	„

* Several much rubbed not included.

These figures again suggest that heterozygotes may occasionally be indistinguishable from recessives. This, as already mentioned, would account for all the anomalies met with so far.

I have studied also the F_2 families at Oxford, grouping them also into speckled and non-speckled. I find that they are :—

A	65 speckled,	0 non-speckled.		
E	32 „	12	„	
e	38 „	10	„	
C	42 „	12	„	+ several much rubbed.
c	27 „	3	„	+ a few „
h	22 „	12	„	+ „ „
H	33 „	11	„	+ „ „
F	94 „	56	„	a few doubtful.
g	2 „	2	„	
G	4 „	0	„	doubtful, a good deal rubbed.
10 F_2 broods,	359 „	118	„	

These numbers appear very good, as theoretically we should expect 358 : 119, but it will be noticed that Broods A and F compensate for one another. Brood A is definitely exceptional; Brood F is a composite one derived from a number of females; this should, of course, make no difference to the numbers, but if separated it might have been found that one female produced only light offspring as A produced only dark ones.

Taking away A and F we have 200 speckled to 62 non-speckled, where expectation would be 197 speckled to 65 non-speckled—a very close agreement.

It would thus appear that speckling is an ordinary Mendelian dominant to the absence of speckling, but that whilst in most cases the heterozygous individuals resemble the dominant they may occasionally be indistinguishable from the recessive. Perhaps it would be more accurate to say that speckling is usually dominant to non-speckling, but that occasionally non-speckling is dominant to speckling.

The speckled individuals vary from moths whose wings are only slightly more speckled than in var. *canteneraria* to moths whose other markings are almost obscured by black scales. I believe that this variation is met with among the specimens taken wild in England, but the dark ancestors of my moths were of the most thickly speckled type found in the neighbourhood of London. This type occurred at intervals among the descendants of my Brood 4, and I think it is probably the homozygous speckled type, especially

as Broods 33 and 40, consisting of 16 and 39 individuals respectively, maintained this type with practically no variation for two generations. It is impossible, however, to draw a line between this type and less speckled forms, as in some broods there is a perfect gradation down to the (so-called) non-speckled type.

The original London moths differed from any of my rearing in being suffused with a brown colour, which was, however, approached by some three of the most speckled individuals in my Brood 8, which were all males. Among the Oxford specimens there are some of this brown colour without speckling, so that it is evidently due to a factor independent of the speckling. I cannot account for the lack of inheritance of this colour nor for the various yellow and whitish ground-colours among my own moths. As already mentioned, the descendants of Brood 2 all had a uniform yellow ground-colour. Amongst the unspeckled descendants of Brood 4 the males were mostly of this same yellow colour and the females invariably lighter; the same is true of the pure *canteneraria* from Hyères, in which, however, only occasionally are the males yellow like my Brood 2, the majority being much paler. I have found it impossible to classify the speckled moths according to ground-colour, as, when they are much speckled, this is difficult to estimate. In general, however, the females are lighter than the males.

There is another respect in which these moths vary to a considerable extent, and that is in size. The following table shows the numbers of moths among the descendants of Brood 4, of various breadths across the wings:—

Numbers of	Millimetres. 13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.
Males............	—	1	6	29	57	66	36	8	—	—	—
Females.........	—	—	—	5	11	24	49	66	35	12	—
Totals	—	1	6	34	68	90	85	74	35	12	—

It will be seen that these figures form a normal curve, which is due to the combination of two curves, one of males with an average of about 17·5 mm., and one of females with an average of about 19·5 mm.

Now, some of the individual broods depart markedly from these averages, *e.g.* :—

Numbers of individuals of		Millimetres. 13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	Average. mm.
Brood 40	♂ ...	—	—	—	—	1	4	7	2	—	—	—	18·4
	♀ ...	—	—	—	—	—	—	1	7	12	4	—	20·7
Brood 34	♂ ...	—	1	3	7	4	—	—	—	—	—	—	15·9
	♀ ...	—	—	—	2	8	2	1	—	—	—	—	17·2

It would be natural to suppose that such a great difference in size between two broods would be due to heredity or to some great difference in the conditions in the larval stage, but I can find nothing of the sort to account for it.

The parents of Brood 40 measured ♂ 18·0, ♀ 20·3, whilst those of Brood 34 measured ♂ 17·9, ♀ 19·6.

The parents of 40 were both members of 33, which averaged ♂'s 17·7, ♀'s 19·7; the ♂ parent of 34 belonged to 30, which averaged ♂'s 17·0, ♀'s 18·7, whilst the ♀ parent of 34 belonged to 28, which averaged ♂'s 17·5, ♀'s 18·2. It is true that the ancestors of Brood 34 were distinctly smaller than those of Brood 40, but they did not differ from the normal nearly so widely as does Brood 34 itself; and, taking into account all broods containing over 10 moths, I find that the average spread of the brood shows no correlation with that of its parent nor with those of the broods of which the parents were members. I conclude, therefore, that breadth of wing is not hereditary.

It is, of course, impossible to prove that these variations in size are not due to differences of conditions, though most of the conditions have been kept very uniform. The moths were reared throughout in similar boxes and the larvæ never fed on anything but dandelions. The main difference of conditions between the broods was due to the different temperatures of different periods of the year. This has a marked effect on the length of time required for development. Eggs laid from April to August only take about two months to reach the imago stage, whilst eggs laid from September to November take about four months if the larvæ continue feeding, or from seven to eight months if they definitely hibernate and cease feeding.

In spite of these great differences, moths of the broods which have hibernated are, on the average, exactly the same size as those which have completed their development in two months.

If the differences were due to different temperatures at the moment when the moths emerged, those broods which emerged from July to October must have experienced warmer conditions for emergence than those which emerged from December to June, yet on the average there is no difference between the two groups. I am therefore entirely at a loss to explain the considerable variations in size which occur.

Summary.

It will be seen that I agree with Messrs. Prout and Bacot's conclusion that *Acidalia virgularia* and its variety *canteneraria* are not two Mendelian forms of the species, though I believe I have shown that one of the

differences between the two forms, namely, the speckling of *virgularia*, behaves in most cases as a Mendelian dominant to the absence of this speckling in *canteneraria*. Even to this rule I am bound to admit there are certain exceptions. To account for this I have to assume that, whilst the heterozygote is usually more or less like *virgularia*, it may sometimes be indistinguishable from *canteneraria*.

The other difference between the species and its variety is that *virgularia* is often, and in the London form always, much browner or yellower in ground-colour than *canteneraria*. Of the various colours of ground seen in the hybrid race I have been able to suggest no explanation, though, as already noted, a certain amount of order is discernible, especially in the fact that the males are almost invariably darker than the females.

In regard to size it has been shown that, taken as a whole, the individuals fall into a normal curve of error, but that some of the broods show considerable departures from the normal, for which no explanation in heredity or environment is forthcoming.

It should be mentioned also that both speckled and non-speckled, yellow and white individuals occurred in broods which hibernated as larvæ as well as in broods which passed through their whole development in a couple of months in the summer, so that these differences are not likely to be due to environment, nor are they seasonal forms.

The specimens have been placed, with Messrs. Prout and Bacot's material, in the Hope Department of the Oxford Museum.