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THE MANIFOLD EFFECT OF SELECTION

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PART II

4. OTHER CORRELATED CHARACTERS

(*a*) *Spermathecæ*

WHEN dissecting females for mating tests it was observed in S–79 that some of them were abnormal in their numbers of spermathecæ. In *Drosophila melanogaster*, as indeed in all species of the genus and in most genera of the group *Drosophilinæ*, the normal number of spermathecæ in a female is 2 (plate I (*a*)). Occasional cases of 3 spermathecæ, or very rarely 4, have been reported in wild-type females of various species. In our own examination of various wild-type strains, obtained from elsewhere or begun from wild flies and maintained in this laboratory for several years, one strain has been found to show as many as 7 per cent. of females with other than 2 spermathecæ, and another strain has 3 per cent. (table 15). In the remainder of the wild strains abnormalities were either absent or so rare as to be found only when large numbers of flies are dissected. Single examples with 3 spermathecæ have been found in the Or and Sk stocks, out of 852 and 1382 females respectively.

In our strains, as in others reported earlier by Sturtevant (1926), Wexelsen (1928), and Nonidez (1920), the abnormalities lay in the possession of 1 or 2 spermathecæ in excess of the normal 2, while occasionally one of the usual 2 spermathecæ was abnormally large, this being described as 2+ in our notation. Sturtevant and Wexelsen were able to establish lines with a greater frequency of these abnormally high spermatheca numbers, Wexelsen's being indeed a line breeding almost true for 3 spermathecæ, a condition which Hadorn and Graber (1944) state to be normal in many Diptera.

In Wexelsen's flies the extra spermathecæ were due to the action of genes in at least three of the four chromosomes, but Hadorn and Graber describe a case of extra spermathecæ, the precise kind and frequency of which depended on the temperature at which the flies

were raised, ascribable to a single recessive gene in chromosome II. Most allelomorphs of the sex-linked gene *lz* have the effect of reducing the number of spermathecæ, the extent of reduction again being sensitive to temperature changes (Anderson, 1945).

TABLE 15

Frequency of flies with more than two spermathecæ in wild-type strains and crosses

Strain	Number of females	Percentage of females with abnormal spermathecæ	
		2+	3
Coombe Hill	139	...	...
Hampton Hill	193	...	...
Merton Park	69	...	...
Ockley	132	...	...
Sutton Bonnington	195	...	...
Teddington	147	1·4	2·0
Amherst	182	...	0·5
Florida	106	...	...
Ealing	234	0·4	6·4
Crimea	97	...	...
Ealing × Crimea F_1	528	...	...
,, ,, F_2	560	...	...

On discovering the extra spermathecæ at S–79 all the lines of the selection experiment were examined for the occurrence of this anomaly. It was quickly found that each had its own characteristic frequency of abnormality, and even its own characteristic types of abnormality (table 17). The spermathecal ducts were sometimes branched and they ranged in number from 1 to 4, while the spermathecæ themselves ranged from 0 to 5, their size also varying. A fly could, for example, have 2 spermathecæ but still be abnormal in that one of them was abnormally large or small, conditions designated as 2+ and $1\frac{1}{2}$ respectively. Some examples of abnormal spermathecæ are shown in plate I. The frequencies of the various types are given separately in the table. The pooled frequencies of those with less than the normal 2 (<2), and those with more than the normal 2 (>2), are also given ; and finally the pooled frequencies of all abnormalities are shown.

External conditions appear to influence the frequency and types of abnormality. Lowering the temperature from 26·6° to 15·6° reduced the frequency of abnormalities from 20 to 10 per cent. in one test. Our normal practice is to mix up the cultures of the various lines in the incubator (which like all incubators has its temperature gradients), so that there is little chance of a consistent difference being due to consistency in placing. It was nevertheless thought desirable to test the lines in randomised blocks within the incubator to remove all possibility of the differences being due to other than genetic causes.

Two such tests were made, one including the Or and Sk parents together with lines 3, 7 and 10, and the other the Sk parent and lines 1, 2, 4, 5, 6 and 9. Generally speaking the agreement was good of the frequencies of abnormality in these tests with those in the lines as maintained normally. Where the difference was above a few per cent. it could be seen that the line in question was showing some heterogeneity within the randomised test itself, which was of course replicated. Evidence of genetic heterogeneity will be seen later in the results from the lines as normally kept. The test left, in fact, no doubt of the general reliability of the counts of abnormality in females from the regular lines.

One important precaution, however, soon proved necessary. The females emerging early in a culture were found to have fewer abnormalities than those emerging later. The result of a test made at S–81 is shown in table 16. The percentage of abnormal females

TABLE 16

Spermatheca abnormalities and time of emergence in the culture

Day of emergence . .	1	2	3	4	5	6	7-9
Females emerging . .	78	101	98	63	77	49	44
Percentage abnormal .	*7·7*	*7·9*	*30·6*	*38·1*	*35·1*	*42·8*	*27·3*

is much the same for the first two days' emergence. It rises sharply on the third day, stays at a more or less stable maximum during the fourth, fifth and sixth days, and then falls slightly. After this test all the females emerging in a culture were dissected where it was desired to have information on the spermathecal abnormalities, and all data in table 17 are from such complete surveys.

The parent stocks Or and Sk show only rare abnormalities of the spermathecæ. In an F_1 made for the purpose no abnormal female was seen out of 893 examined. An F_2 was bred from this F_1 and it proved to contain 1·5 per cent. of females with 3 spermathecæ, out of 2898 dissected. No other abnormality was found. Thus not only do the genotypes of the parents give virtually no abnormalities whether individually or jointly in the F_1, but even their first segregation in F_2 leads only infrequently to disturbances, and then only of one kind. The genic balance is not so precise within each chromosome as it is when the whole genotype of a parent is taken as a unit, but it must nevertheless be good.

The mass culture from the low selection (line 1) was not examined extensively. Of the 57 females dissected all had the normal 2 spermathecæ. The mass from the high selection (line 3) on the other hand proved to contain 29·6 per cent. of females with abnormalities, nearly all having 3 spermathecæ but two of them being in the 2+ class and

two more in the 4 class (table 17). No females with less than 2 spermathecæ were found. Evidently despite the return of this line to a chaeta number little different from that of its Or parent, the genic content of the chromosomes was not fully like that of Or. The

TABLE 17

Spermatheca abnormalities in the selection experiment

Line	Generation	Percentage of females with spermathecæ												No. of females
		2 (normal)	0	½	1	1½	All <2	2+	3	4	5	All >2	All abnormalities	
Or	...	99·9	...	...	...	...	...	...	0·1	...	...	0·1	0·1	852
Sk	...	99·9	...	...	...	...	...	...	0·1	...	...	0·1	0·1	1382
F_1	...	100·0	...	...	...	...	...	...	...	...	...	...	0·0	893
F_2	...	98·5	...	...	...	...	...	...	1·5	...	...	1·5	1·5	2898
1	...	100·0	...	...	...	...	...	...	...	...	...	...	0·0	57
2	81- 90	86·3	...	0·1	0·2	0·1	0·4	0·1	13·0	0·2	...	13·3	13·7	1895
	91-100	99·5	...	...	...	0·2	0·2	...	0·3	...	...	0·3	0·5	1461
	101-109	100·0	...	...	...	...	...	...	...	...	...	...	0·0	565
3	...	70·4	...	...	...	...	...	0·2	29·2	0·2	...	29·6	29·6	1233
4	...	94·6	...	...	...	...	...	0·5	4·6	0·3	...	5·4	5·4	387
5	83- 90	64·9	...	...	0·4	0·1	0·5	0·1	33·9	0·5	0·1	34·6	35·1	1646
	91-100	64·7	...	...	2·3	...	2·3	0·2	32·6	0·2	...	33·0	35·3	1532
	101-110	79·6	...	...	0·8	...	0·8	0·2	19·2	0·2	...	19·6	20·4	475
6	81- 90	84·4	0·1	...	0·8	...	0·9	0·1	14·6	...	...	14·7	15·6	1551
	91-100	84·8	...	...	0·4	...	0·4	0·2	14·3	0·3	...	14·8	15·2	1440
	101-110	95·8	...	...	0·6	...	0·6	...	3·6	...	...	3·6	4·2	617
7	...	92·5	...	...	...	...	...	...	7·2	0·3	...	7·5	7·5	362
8	81- 90	92·5	...	...	...	0·2	0·2	0·4	6·6	0·3	...	7·3	7·5	933
	91-100	93·8	...	0·2	0·2	...	0·4	0·2	5·1	0·5	...	5·8	6·2	660
	101-110	97·3	...	...	...	...	...	...	2·6	0·1	...	2·7	2·7	862
	111-120	97·4	...	...	0·5	...	0·5	...	2·1	...	...	2·1	2·6	387
	121-130	94·1	...	...	0·3	...	0·3	0·4	4·9	0·3	...	5·6	5·9	709
	131-134	94·3	...	...	...	...	...	...	5·1	0·6	...	5·7	5·7	349
	80- 90	81·4	0·4	0·3	4·0	0·8	5·5	0·1	12·9	0·1	...	13·1	18·6	1118
	91-100	84·6	0·9	0·1	2·0	0·2	3·2	...	12·2	...	...	12·2	15·4	877
	101-110	87·5	0·2	...	4·0	0·6	4·8	...	7·3	0·4	...	7·7	12·5	543
	111-120	93·5	1·0	0·2	0·8	0·7	2·7	...	3·8	...	...	3·8	6·5	402
	121-131	95·5	0·2	...	1·8	1·4	3·4	0·2	0·7	0·2	...	1·1	4·5	438
	131-134	93·4	0·6	...	2·1	1·7	4·4	...	2·2	...	...	2·2	6·6	181
10	80- 94	82·5	0·3	0·1	2·1	0·6	3·1	0·2	14·0	0·2	...	14·4	17·5	1432

original selection for chaeta number must have resulted in a correlated readjustment of the polygenic system governing the spermathecæ, and this cannot have been eliminated when fertility took charge and brought about the return to the parental chaeta number between S–20 and S–24.

The first reselection from line 3, later maintained as the mass line 7, also shows only the same limited range of abnormalities in spermathecæ as does line 3 itself. Furthermore it shows them less often. The reselection for high chaeta number has reduced the frequency of the abnormalities which appeared as a correlated response to the original selection; though of course we do not know that this reduction is due to a return to the parental genotypes. In fact the results from chaeta number and fertility make this very unlikely. Line 8, the continuation under selection of this first reselection from line 3, shows nearly the same frequency of abnormalities as its mass counterpart, but they cover a somewhat wider range of types. A few of them are even <2. Since, however, the observations on spermathecæ were made after the delayed response at S–81 this widening of the range of types may have come about at that time, in which case it would be expected to mark a difference from line 7. When separated out by generations the records suggest that the frequency of abnormalities in line 8 may have fluctuated a little.

The reselection which rose to the middle level of chaeta numbers and was then mass cultured (line 4) has a percentage of abnormalities slightly lower than that of line 7, but the difference is not significant. It also includes two flies with 2+ spermathecæ, a type which was not found in line 7. This difference too could easily be due to chance. There is thus no indication of a genetic difference in spermathecæ content between lines 4 and 7, and it must therefore be inferred that chromosome II, which distinguishes these lines (table 12) was uniform or very nearly so in respect of its effect on this character.

Line 6 was the counterpart of line 4 in which selection had been continued, and as we have already seen in section 3*b* this selection resulted in a slow but ultimately substantial advance in chaeta number subsequent to the rapid attainment of the middle level. The difference in chaeta number between lines 4 and 6 is accompanied by an even more striking difference in spermatheca content. Line 6 had at one time more than twice the percentage of abnormalities that line 4 showed, and the range included classes with both 1 and 0 spermathecæ. There is an indication, however, that after S–100 line 6 tended to settle down towards a lower frequency of abnormal females (table 17).

Neither line 7 nor line 3 has given flies with <2 spermathecæ. Admittedly the number of females dissected has been only 749 for the two lines together; but these data would make it unlikely that 2 per cent. or even 1 per cent. of females could fall into the <2 class. Line 9 and 10 were both developed by selection for increased chaeta number from crosses between lines 7 and 3, and both gave evidence that the selection had been effective in raising chaeta number by building up new polygenic combinations rather than by recovering the original constitution of line 7. The data on spermathecæ vindicate this view. Up to S–110 2538 females were dissected from line 9 and of these 411 were abnormal, 116 showing <2. Out of 1432 females

from line 10 (which shows signs of some change in spermatheca abnormality during the 14 generations of observation), 250 females were abnormal and of them 44 had <2. In regard to the overall frequency of all abnormalities line 9 (up to S–110) and 10 are homogeneous, but line 9 has a proportion (4·6 per cent.) of females with <2 significantly higher than that of line 10 (3·1 per cent.) at about the 0·02 level of probability. Nevertheless it seems, as the chaeta numbers also suggest, that substantially the same new polygenic combinations were selected in the two lines ; a result which emphasises the determinacy of the response to selection in the same way as did the findings of Sismanides (1942) with extra scutellar bristles. We may therefore pool the data from line 9 (up to S–110) and line 10 for comparison with line 7, their ancestor. They have a joint frequency of all abnormalities of 16 per cent. against 7·5 per cent. in line 7, and a joint frequency of <2 abnormalities of 4·0 per cent. against 0·0 per cent. in line 7. Both differences are significant. Not only do the combinations newly built up by selection in lines 9 and 10 exceed their parental combinations of line 7 in total percentage of abnormalities, but they give types not seen in either that parent or the other, line 3. About 1 female in 200 had no spermathecæ at all, a type which was otherwise seen only in line 6, and even there in only 2 females out of 3608.

Before leaving line 9 a further point requires notice. At S–112 there occurred a delayed response of chaeta number to selection. It was accompanied by a significant fall in percentage of females with abnormal spermathecæ. Comparison of the spermathecæ data of line 9 up to and after S–110 shows, however, that the change in the <2 class was not significant. This new correlated response thus predominately affected the >2 class. The history of line 9 brings out very clearly the complexity of correlated response. Whenever selection was practised in the ancestry of this line it was for increased chaeta number. The first selection gave rise to line 3 with 29·6 per cent. abnormalities, all >2. The second selection from line 3, which gave rise to line 7, reduced the frequency to 7·5 per cent., again all >2. The third selection from line 3 × line 7 gave line 9 (up to S–110) with 16·2 per cent. abnormalities including 4·6 per cent. of the new type <2. The final delayed response to selection reduced the overall figure to 5·7 per cent. but chiefly by affecting the class >2. Selection of chaeta number in one direction can thus both raise and lower the percentage of spermatheca abnormalities, and can affect one class of abnormalities while leaving another without significant change. Such results expose in all its nakedness the inadequacy of simple pleiotropy to account for correlated responses.

Lines 5 and 2 originated in a back selection from line 7. They were already separated before observations on spermatheca abnormalities began, line 2 being the successful continuation of the back

selection while line 5 was the unsuccessful attempt at upward reselection. Up to S–100 line 5 with 35·2 per cent. gave the highest proportion of abnormalities seen in the whole experiment, some of the females being <2. After S–100 the frequency fell to 20·4 per cent. though the <2 class persisted. The fall in chaeta number of line 2 from the level of line 5 was accompanied by a rapid fall in spermatheca abnormalities. Only 0·5 per cent. were seen after S–90, the <2 class being nevertheless still present, and none at all after S–100.

The chromosome assays described in section 3*c* were made after the discovery of the spermatheca abnormalities, and the females they produced were therefore dissected in order to discover which chromosomes were responsible for the changes in spermathecæ. The assays of the parents and of lines 1, 2, 6, 7 and 8 gave no flies with abnormalities, so that no analysis was possible. Such a result is not, of course, surprising in view of the fact that generally only some 300 females were available from each assay, and that of these only about 40 would be of the parental type. The remaining assays gave the results shown in table 18. The data were analysed in the way

TABLE 18

Results of the assays for spermatheca abnormalities

Class	Line 3	4	5	9-1	9-2
B Pm Sb	9·5 (42)	3·3 (30)	4·8 (42)	3·2 (63)	3·8 (53)
B Pm	27·6 (29)	10·5 (38)	18·6 (43)	8·2 (49)	2·1 (47)
B Sb	17·5 (40)	5·0 (40)	10·0 (40)	6·3 (64)	0·0 (63)
B	36·1 (36)	7·7 (39)	27·9 (39)	7·4 (68)	3·5 (57)
Pm Sb	6·1 (33)	0·0 (33)	2·4 (41)	1·9 (54)	1·8 (56)
Pm	20·6 (34)	0·0 (37)	5·7 (35)	8·0 (50)	2·3 (43)
Sb	10·5 (38)	0·0 (36)	4·7 (43)	1·5 (68)	0·0 (61)
+	12·8 (39)	0·0 (39)	9·8 (41)	7·9 (57)	1·8 (57)

The figures are percentages of females with abnormal spermathecæ, followed by (in brackets) the number of females examined.

described earlier, the percentages being, however, first converted into angles (Fisher and Yates, 1943 ; Mather, 1946), in order to make their variances more nearly equal. As might be expected the assays, undertaken primarily for chaeta number, permit only general conclusions about the control of spermathecæ. They are not, therefore, presented in detail.

There was no evidence of interaction between any of the chromosomes. Furthermore no variation, whether among the lines or between them and the tester, could be traced to chromosome II, which we earlier found by comparison of line 7 and 4 to have been unaffected in its determination of spermathecæ by the first selection. Chromosome X and III differ between the lines on the one hand and the tester on the other, though no significant differences were

demonstrable between the lines themselves. There can, however, be little doubt that a larger experiment would reveal such differences. Chromosome III from the lines results in more spermathecal abnormalities than chromosome III from the tester, but surprisingly enough the reverse is true of the X. Evidently the *ClB* chromosome itself is unbalanced in regard to spermatheca control. Whether the high frequency of spermatheca abnormalities in *ClB*/+ flies is a form of heterosis or not cannot be determined, because of course *ClB* cannot be made homozygous. We may note, however, that no flies heterozygous for *ClB* and the Or or Sk X chromosomes were found with extra spermathecæ. Thus the selected X's, though giving fewer abnormalities when homozygous than when heterozygous with *ClB*, must still have a greater abnormality producing power than the parental X's. The same applies *a fortiori* to chromosome III.

One final point about the assays requires comment. The + class recovered in each of them has the same chromosomes, apart from recombination as the parental lines. It has, however, only half (or even less) the frequency of abnormalities of the corresponding parent line. Now the chaeta results have already shown us that the genetical difference between the parents and the recovered + classes due to recombination with the tester chromosomes can only be very small. We cannot, therefore, attribute the difference in spermathecæ to this cause. It could be due to a maternal effect, part of the genes determining spermatheca number acting primarily in the mother, and their effect being transmitted through the cytoplasm of the egg to the daughter where it becomes manifest. On this view reciprocal crosses between lines differing in frequency of spermatheca abnormality should themselves differ. A cross between lines 3 (with 18·2 per cent. abnormalities in the generation in question) and line 8 (with 3·8 per cent.) gave :—

(i)	8×3	. . .	2·7	per cent.	abnormalities
(ii)	8×3	. . .	10·7	,, ,,	,,
(iii)	3×8	. . .	9·2	,, ,,	,,
(iv)	3×8	. . .	20·7	,, ,,	,,

F_2's from (ii), (iv) and their intercrosses were much alike in proportions of abnormalities. This is in general agreement with expectation, but the differences between the pairs of like crosses in F_1 must leave the result inconclusive. Special and more extensive tests must be undertaken to establish the detailed control of spermatheca number, and the possible part played in it by a maternal effect.

(*b*) *Discriminative Mating*

Flies of the two parental stocks, Or and Sk, are not alike in their mating propensities. When females of the two kinds are placed simultaneously with a male from one or other stock mating does not take place at random. Furthermore the discrimination which is

exercised changes with circumstances of which the ages of the flies appears to be one.

These conclusions are based on experiments undertaken some years ago in which five Or females and five Sk females were placed simultaneously with a single male of either Or or Sk for from eighteen to twenty-four hours, at the end of which the females were dissected in order to determine which had successfully mated. The presence of moving sperm in spermathecæ or ventral tube was taken as the criterion of mating. The time allowed for mating was varied in order to avoid the extreme, and uninformative, result of either complete mating of all females or complete failure of them all to mate. One type of female in each test was marked by a small notch in one wing in order that the types could be distinguished. The tests were always made in pairs, one with the Or and the other with the Sk females so marked. As will be seen later the results fail to reveal any effect of marking on the frequency of mating.

Four ages of fly were used, designated as one, four, seven and ten days old. Flies emerging as adults during any one day were pooled and used on the next day if required as one day old, on the fourth day later for four days old and so on. All flies, both male and female, were kept at 20° C., separated from individuals of the opposite sex from collection until use in a test, which was carried out at 27° C. Any necessary etherisation, as for marking, was done at least twenty-four hours before the test began.

Such tests yield results which consist of the frequencies of four classes, gravid (or mated) and virgin (or unmated) females of each of the two kinds Or and Sk. Since the numbers of Or and Sk females were made equal at the start, two quantities are necessary to specify completely the four frequencies. One of these may conveniently be taken as p, the proportion of gravid females out of the total of ten of the two kinds. The other must represent the distribution of the gravid females between the two stocks, and so must measure the discrimination, if any, exercised in mating. A quantity, which we may call D can be found as

$$D = \sqrt{\frac{q}{p}}a_{go} - \sqrt{\frac{p}{q}}a_{vo} - \sqrt{\frac{q}{p}}a_{gs} + \sqrt{\frac{p}{q}}a_{vs}$$

where $q = 1-p$, a_{go} is the number of gravid Or, a_{vo}, a_{gs} and a_{vs} the numbers of virgin Or, gravid Sk and virgin Sk females respectively. Now V_D can easily be shown to be n, the total number of females in the test, and so is independent of the value of D itself. Thus D^2/n will be distributed as a χ^2, at least in the theory of large samples; and in any case D will offer the best statistic for subjection to an analysis of variance, which apart from difficulties introduced by the small size of sample can be converted into an analysis of χ^2 by dividing the sums of squares by n, *i.e.* 10 in the present case.

The calculation of D can be simplified for

$$D = \sqrt{\frac{\bar{q}}{p}}a_{go} - \sqrt{\frac{\bar{p}}{q}}a_{vo} - \sqrt{\frac{\bar{q}}{p}}a_{gs} + \sqrt{\frac{\bar{p}}{q}}a_{vs}$$

$$= \sqrt{\frac{\bar{q}}{p}}(a_{go} - a_{gs}) + \sqrt{\frac{\bar{p}}{q}}(a_{vs} - a_{vo})$$

$$= d\left(\sqrt{\frac{\bar{q}}{p}} + \sqrt{\frac{\bar{p}}{q}}\right) = \frac{d}{\sqrt{pq}}$$

where $d = a_{go} - a_{gs} = a_{vs} - a_{vo}$.

Then where n is 10 we can calculate the table of values which D can take for all possible values of pn and d (table 19). Where d is negative D will also of course be negative. Given the values of p and D the original results of a test can be completely reconstructed.

TABLE 19

The values of D *for all possible values of* pn *and* d *in tests where* n = *10*

pn	d 1	2	3	4	5
9 or 1	3·333	...	...	...	...
8 or 2	...	5·000	...	...	...
7 or 3	2·182	...	6·547	...	...
6 or 4	...	4·082	...	8·165	...
5	2·000	...	6·000	...	10·000

D cannot be found when pn is 0 or 10

The quantity D essentially affords a convenient test of significance of departures from randomness of mating, and for this purpose it is efficient. It does not, however, give an equally valuable measure of any discrimination which be found, for it will not be efficient as a statistic at all levels of discrimination, nor is its value independent of p. Nevertheless as most of the tests to be described are of significance, D will be retained for the few estimates that will be given of the magnitude of discrimination. More desirable methods of estimation, such as the use of a product formula, are made impracticable by the infinite values to which the distribution of frequencies must lead fairly often in experiments where n is so small as 10.

Table 20 gives the values of pn and D obtained in tests of both Or and Sk males with mixtures of the two kinds of females. All combinations of females and males of the four ages, one, four, seven and ten days, were used, and each age combination was tested in duplicate with the markings reversed between duplicates as already explained. The values of p require little comment. They can be converted into angles by Fisher and Yates' (1943) transformation if analysis is desired. In brief, p rises steadily with age of male, whether Or or Sk ; older males mate more readily. There appears to be a

TABLE 20

Discriminative mating between Or *and* Sk *stocks. In all tests* 5 Or *and* 5 Sk *females were used with one male for twenty-four hours*

Or males

Age of females	Test	Age of Male 1 pn	1 D	4 pn	4 D	7 pn	7 D	10 pn	10 D	Total pn	Total D
1	1	6	0·000	6	−4·082	4	8·165	6	−4·082	40	−9·081
	2	2	−5·000	5	−2·000	5	2·000	6	−4·082		
4	1	1	3·333	5	−10·000	6	−8·165	5	6·000	36	−17·746
	2	1	3·333	6	−8·165	6	−4·082	6	0·000		
7	1	1	−3·333	5	−6·000	7	−6·547	5	−2·000	37	−36·392
	2	3	−2·182	4	−8·165	6	−8·165	6	0·000		
10	1	5	6·000	7	−6·547	5	−6·000	7	2·182	43	−15·365
	2	4	0·000	5	−10·000	5	−6·000	5	5·000		
Total . .		23	2·151	43	−54·959	44	−28·794	46	3·018	156	−78·584

Or females marked in test 1 and Sk females in test 2.

A positive value of D indicates an excess of Or females and a negative one an excess of Sk females mated.

Sk males

Age of females	Test	Age of Male 1 pn	1 D	4 pn	4 D	7 pn	7 D	10 pn	10 D	Total pn	Total D
1	1	5	10·000	7	−2·182	5	−6·000	6	0·000	46	
	2	5	2·000	8	5·000	3	2·182	7	2·182		11·364 *
4	1	4	−4·082	6	8·165	8	5·000	8	0·000	53	
	2	4	−4·082	7	6·547	9	−3·333	7	−2·182		−3·050 *
7	1	0	...	6	8·165	6	4·082	1	−3·333	35	
	2	3	6·547	3	−6·547	7	6·547	9	−3·333		3·214 *
10	1	5	10·000	7	6·547	10	...	10	...	62	
	2	5	6·000	7	2·182	9	3·333	9	3·333		14·848 *
Total . .		31	10·465 *	51	7·182 *	57	8·729 *	57	0·000 *	196	26·376 *
											49·525 †

* Totals of second tests only. † Total of all tests excluding the three in boxes.

Or females marked in test 1 and Sk females in test 2.

A positive value of D indicates an excess of Sk females and a negative one an excess of Or females mated.

little variation in p between the age classes of females, but it is quite irregular and most probably does not represent a genuine effect of age. The time of exposure to mating was held constant through the series of tests. With the Or males no difficulty resulted from pn becoming 0 or 10 in any test ; but three of the tests with Sk males gave this worthless result. Fortunately in no case did both duplicates of any age combination behave in this way, so that all combinations are represented by at least one test in table 20.

The analyses of variance of D are set out in table 21. With the data from Or males a single full analysis is possible. There are 32 tests in all and consequently 32 degrees of freedom (N) in the analysis, one coming from each test as D is expected to have a value of 0 in the absence of discrimination. The grand sum of D when squared and divided by 32 gives the sum of squares (S.S.) testing the overall deviation from this expectation of 0. The four totals for ages of male when squared, summed and divided by 8 give an S.S., which, after deduction of the S.S. for overall discrimination, tests the effect of age of male on discrimination, and corresponds to $N = 3$. An S.S. for age of females can be obtained similarly and it too corresponds to $N = 3$. If the pair of tests in each age combination is summed, the totals squared, summed over all age combinations and divided by 2, an S.S. is obtained from which the three S.S. already found can be subtracted to leave an S.S. corresponding to $N = 9$, and testing interaction in effects of ages of male and females, or testing residual heterogeneity if we prefer so to describe it.

The differences in D between corresponding tests in each age combination measures the effect of marking. The difference summed over all age combinations is squared and divided by 32 to give the S.S. testing the overall effect of marking. The sixteen single differences in D, one from each age combination, are squared, summed and divided by 2 to give an S.S. which, after deducting the S.S. for overall effect, leaves an S.S. corresponding to $N = 15$ and testing heterogeneity in the effect of marking.

All these S.S. are divided by $n = 10$ to reduce them to χ^2 (table 21*a*). The effect of marking is clearly insignificant, and there is equally clearly an overall discrimination in mating, Sk females being mated more often than Or. The discrimination appears to vary with age of male, but not, or at least not so much, with age of female. There is, however, an interaction in effect of age in the sexes, or if we prefer so to style it, a residual heterogeneity unaccountable by age changes alone. The effect of age of males is less obviously significant when compared with this interaction or residual heterogeneity by means of a variance ratio, and so it should perhaps not be over-emphasised. Granted that it is real, however, there would appear to be little discrimination with males of one and ten days ($\overline{D} = -0{\cdot}27$ and $-0{\cdot}38$ respectively) marked discrimination with four-day males ($\overline{D} = -6{\cdot}87$) and fairly strong discrimination with seven-

day males ($\overline{D} = 3{\cdot}60$). There is a suggestion, for what it is worth, of the same type of change with age of female. The two chief points of the analysis are, however, clear: that mating is not at random, and that the marking used has no detectable effect on mating.

TABLE 21

Analyses of variance of D *from the* Or *and* Sk *mating tests*

(*a*) Or males

Item	Sum of squares	N	χ^2	P
Overall discrimination	192·983	1	19·298	very small
Age of male (M)	289·932	3	28·993	very small
Age of female (F)	51·748	3	5·175	0·2-0·1
Interaction (M × F)	339·962	9	33·996	very small
Overall effect of marking	8·438	1	0·844	0·5-0·3
Heterogeneity of marking	87·499	15	8·750	0·9-0·8
Total	970·562	32		

(*b*) Sk males—excluding three age combinations as shown in table 20

Item	Sum of squares	N	χ^2	P
Overall discrimination	94·336	1	9·434	0·01-0·001
Heterogeneity of discrimination	382·733	12	38·273	very small
Overall effect of marking	20·700	1	2·070	0·20-0·10
Heterogeneity of marking	240·139	12	24·014	0·05-0·02
Total	737·908	26		

(*c*) Sk males—excluding all first tests

Item	Sum of squares	N	χ^2	P
Overall discrimination	43·481	1	4·348	0·05-0·02
Age of male (M)	15·842	3	1·584	0·70-0·50
Age of female (F)	48·828	3	4·883	0·30-0·20
Interaction (M × F)	208·444	9	20·844	0·02-0·01
Total	316·595	16		

No similarly complete analysis is possible of the data from the Sk males, owing to the failure of one of the duplicate tests in each of the age combinations 7 × 1, 10 × 7, and 10 × 10. Two partial analyses have therefore been made (table 21*b* and *c*). In making the first of them the three incomplete age combinations were omitted, so that the effects of age of male and females cannot be properly disentangled. The analysis, made in a manner comparable with that of the data from Or males, except that the marginal totals are not used to remove the age effects, is divided into four parts; overall discrimination,

obtained from the grand sum of D using a divisor of 26 ; heterogeneity of discrimination, from the totals of pairs of corresponding tests, using a divisor of 2 and subtracting the S.S. for overall discrimination ; overall effect of marking, from the grand difference of D from corresponding pairs, using a divisor of 26 ; and heterogeneity of effect of marking, obtained as in the Or analysis.

There is no significant overall effect of marking, but a suggestion appears of heterogeneity between the age combinations in this respect. When taken in conjunction with the Or results, however, little importance can be attached to this item. Marking has no clear effect. The overall discrimination is significant, though not so strongly as with Or males, and there are, in conformity with the Or results, differences between the age combinations in discrimination.

The second analysis was made using only the second of the pair of tests in each age combination. This rejection of all the first tests permits male and female age effects to be isolated, but prohibits any analysis of the effect of marking. The results of the analysis, which is made like any other simple analysis of variance into orthogonal items except that the item usually employed as the correction term is here retained as the item for overall discrimination, are given in table 21*c*. The results are less conclusive than might have been hoped. There is evidence of an overall discrimination and of some heterogeneity in discrimination, but the effects of age are insignificant. It is, however, possible to say that Sk males, like Or males, mate more often with Sk females than with Or females when the two kinds are present simultaneously, and that this discrimination is not constant, though the factors influencing its variation are not clear.

Similar tests of discriminative mating were made using various of the mass lines from the selection experiment in conjunction with the parents. With 8 exceptions the 154 tests discussed below were made with seven-day-old males and females. The remaining 8, 2 from each compartment of the fourth column of table 22, were made with the age combination 4×4. Thus, in so far as the evidence of age effect is trustworthy from the data on the parents, the age combinations used should increase the chance of detecting discriminative mating and its variation. All tests were made with a mating time of eighteen hours, and despite the varied lines used only 14 tests had to be rejected owing to either complete mating or complete failure to mate. The mass lines used were 3, 4 and 7, and flies were taken from them at various generations between S–73 and S–124. A few additional tests were made in combinations other than those reported in table 22, but these were too few to be informative and are therefore omitted from the account.

The results are summarised in the total and average values of D given in table 22. In no case was the group of tests in a given combination significantly heterogeneous by itself, but when they were pooled a slight but significant heterogeneity was detectable ; the χ^2

for heterogeneity, based on 142 degrees of freedom, was 1·376 times its expected value of 142. The χ^2 tests applied to the data of table 22 must therefore be rather more stringent than the data really warrant; significance is liable to be over-estimated by them. The tests cannot in any cases be regarded as giving anything but a general indication, as the numbers of them in the different cells of the table vary from 4 to 18, so making proper orthogonal analysis impossible.

TABLE 22

Discriminative mating of lines 3, 4 and 7 with the parents

Male	Female combinations			
	Or+Sk	Or+line 3	Sk+line 3	Sk+line 4
Or	−27·111 15 −1·81	−0·667 4 −0·17	−41·794 18 −2·32	−50·821 13 −3·91
Sk	−23·843 15 −1·59	−11·264 4 −2·82	−16·264 15 −1·08	−14·364 8 −1·80
Line 3	−15·983 4 −4·00	0·182 4 0·05	−59·680 17 −3·51	−36·605 13 −2·82
Line 7	−1·001 9 −0·11	−1·667 4 −0·42	−9·818 5 −1·96	−14·164 6 −2·36

In each compartment the upper left figure is the sum of D, the upper right figure the number of tests, and the lower figure the average of D.

For consistency's sake a positive sign has been given to D where in a combination including Or females these females were mated more often than expected on the basis of equal chances, or where in a combination including Sk these females were mated less often than expected. It will be seen that, with the exception of the single combination of Or and line 3 females with line 3 males of which only 4 tests were made, −ve discrimination was always realised. The combinations of females from Or and line 3 showed little evidence of discrimination no matter which type of male was used, and indeed none of the values of D, even that from Sk males, is significant. Nor are they significant when pooled. It would appear that line 3 females are so like Or females in mating behaviour as not to be distinguishable by these 16 tests. This combination of females will therefore be omitted as uninformative from the further discussion of these data, attention being confined to the Or+Sk, Sk+line 3, and Sk+line 4 combinations of females.

It is clear from these three female combinations that line 3 and 4 females show a similarity to Or, in that when mixed with Sk females they have less than an equal chance of mating. Furthermore the tests fail to reveal any significant difference in discrimination between

the Sk+line 3 and Sk+line 4 combinations of females. The females of lines 3 and 4 may be regarded as quite alike so far as these tests go. There is, however, some indication that they jointly differ from Or. With both Or and line 7 males the average value of D is markedly lower in the Or+Sk combination than in the Sk+line 3 and Sk+line 4 combinations taken together. Pooling the data from these two types of males we obtain a $\chi^2_{[1]}$ of 3·933 for this difference; a result which is at least suggestive even when the slight heterogeneity noted earlier is taken into account. Sk males give very similar average values of D for these opposing combinations of females, and line 3 males even seem to contradict the Or and line 7 males. But even when we bring the data from these apparently contradictory line 3 males into the test with the Or and line 7 males, $\chi^2_{[1]}$ is still 3·850 and the evidence for the difference between the combinations with Sk of the Or females on the one hand and the line 3 and line 4 females on the other is hardly affected. It would thus appear that there is some indication, though not final evidence, that line 3 and line 4 females are discriminated against more than are Or females in competition with Sk. In other words there is a suggestion that the females from the two selected lines, though more like Or than Sk in mating, have mating properties differing from those of both parent lines; that in fact a correlated response to selection from chaeta number is to be seen in mating behaviour. The 16 direct tests of Or and line 3 females would hardly be expected to reveal a difference in discrimination of the size suggested.

Turning to the males, we can see as in the earlier tests of the parental lines that the Or males show stronger discrimination than the Sk, though both show discrimination in the same direction. This direction is in fact also common to the line 3 and line 7 males. The line 3 males seem approximately to match the Or males in discrimination (overall averages of D of −3·3 and −2·6 respectively), and the line 7 males to match the Sk (overall averages of D of −1·2 and −1·4 respectively). The difference in strength of discrimination between Or and line 3 males taken together on the one hand and Sk and line 7 males taken together on the other hand is highly significant, even when allowance is made for heterogeneity of the size found, for $\chi^2_{[1]}$ is 7·871. Thus of the two lines, 3 and 7, one resembles each parent in male behaviour. Line 3 females may well, however, not be quite like Or females as we have already seen. Line 7 females have not been tested so that it is impossible to say whether they show the resemblance to Sk females that might be expected from the comparison of the males. However this may be, it is worthy of note that both lines 7 and 4 were derived from selection from line 3. Line 4 resembles line 3 on the evidence of females, while line 7 differs from line 3 on the evidence of males. Lines 7 and 4 of course also fall into different categories. Now lines 4 and 7 both differ from line 3 in the X and III chromosomes (section 3*c*) while

they differ from one another only in chromosome II. Line 7 differs from line 3 in chromosome II also, but lines 3 and 4 are alike in this chromosome. It would thus appear that at least one of the main determinants of the difference in mating behaviour between Or, which lines 3 and 4 resemble in the broad sense, and Sk, which line 7 resembles, is to be found in chromosome II.

These data on mating behaviour cannot be regarded as affording more than a suggestion of the origin of mating behaviour differing from those of both parent stocks. There is therefore only a suggestion that the difference in mating behaviour between Or and Sk depends on more than one gene, and that a correlated response to selection for chaeta number has occurred in this character. But that there is such a suggestion is itself of some interest. It would have been more satisfying to have extended the tests to the point where the question was settled one way or the other ; but tests of this kind are prodigal of time and labour, and in view of the many other observations which it seemed necessary or desirable to make on the products of selection for chaeta number the idea of extending the tests of mating discrimination had to be abandoned.

(c) *Miscellaneous Characters*

While most attention was devoted to the effects of selection for number of abdominal chaetæ on fertility, spermathecal content and mating behaviour, changes have also been observed during the course of the experiment in a number of other characters, to which we will now turn.

(i) *Sternopleural and coxal chaetæ.*—The number of sternopleural chaetæ is known to be a character which shows continuous variation (so far as a meristic character can) in wild-type flies and to respond to selection in much the same way as does the number of abdominal chaetæ itself (Mather and Wigan, 1942 ; Wigan and Mather, 1942). No attention was paid to this character before S–99 in the present experiment. The number of sternopleural chaetæ was then found for each line, some being counted in S–99, some in S–103, some in S–104, some in S–105 and some in S–107. The sternopleurals were also counted for the parent stocks at the same time, and a second count was made in line 9 at S–129 after this line had showed its delayed response to selection between S–112 and S–116. In conformity with the assays the count on this line at S–107 is designated as 9-1 and that at S–129 as 9-2. Finally a cross was made between lines 8 and 9 at S–124 and a high selection was made from it for abdominal chaetæ. This new line, termed 8×9, soon became stable with a number of abdominal chaetæ just above that of line 9. Its number of sternopleurals was counted at S–138. All sternopleural counts were made on females only, as were the counts of the abdominal chaetæ with which they are compared. The abdominal averages are

thus higher than those given for these same lines in earlier sections, where the means of the sexes were used.

These various average sternopleural numbers are given together with the corresponding abdominal averages in table 23*a*. Where

TABLE 23

Mean numbers of sternopleural and abdominal chaetæ in (a) *the lines obtained from the experiment and* (b) *thirteen wild stocks. All data are from counts on females only*

(*a*)			(*b*)		
Line	Sternopleurals	Abdominals	Wild stock	Sternopleurals	Abdominals
Or	19·93	47·07	Samarkand	21·53	41·87
Sk	20·00	37·27	Ealing	23·53	45·87
	20·00	38·00	Wellington	20·33	47·07
1	21·60	34·20	Hampton Hill	18·80	47·20
	22·60	34·40	S. Bonnington	20·27	47·53
2	22·46	36·46	Rothamsted	20·00	47·87
	21·60	35·95	Florida	24·07	48·27
3	17·13	42·67	Ockley	21·60	49·40
4	17·33	49·47	Coombe Hill	21·07	51·53
5	17·87	47·73	Merton Park	21·47	51·53
6	18·40	53·80	Amherst	22·13	54·13
7	16·73	54·26	Crimea	16·27	55·00
8	19·60	62·53	Teddington	22·87	56·87
	20·30	61·20			
9-1	18·42	59·00			
9-2	20·55	69·10			
8×9	23·10	71·70			

Duplicate counts are joined by brackets

duplicate counts were made it will be seen that they show good agreement. The sternopleural averages are plotted against the

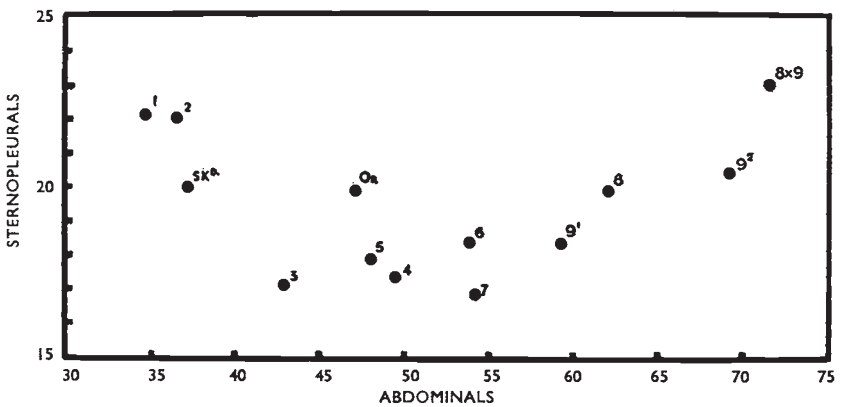

FIG. 9.—The relation of average abdominal and sternopleural chaeta numbers in females of the parents and eleven of the lines.

abdominals in fig. 9. It may be added that the differences in sternopleural numbers between the Or parent and lines 2, 3, 4, 6, 7, 8, 9-2 and 8×9 were tested in an experiment of two randomised blocks

and were found to be significant with P just below 0·001. Males were counted as well as females in this experiment and there was a suggestion too of variation between the lines in the size of the sex difference in sternopleural number. This variation, however, was not quite significant at the 0·05 level and will not be discussed further. The male data were put to no further use.

Thus the lines separated by selection for abdominal chaetæ also differ in their numbers of sternopleural chaetæ. Selection for the one has also altered the other. A glance at fig. 9 shows, however, that there is a regularity in the relation between these two chaeta characters which is not shared by the relations between, for example, spermathecal content and abdominal chaetæ. The points on fig. 9 fall approximately on what seems to be a parabolic curve, the lowest sternopleural counts being associated with the mid-range of abdominal numbers. Nor is this relation like that found so often with fertility, where selection lines depart from the parental fertility in the same way, no matter in which direction chaeta number was selected. In the present case the parents are both well to the same side of the turning point in the curve relating the two chaeta characters, and Sk is in fact near to one extreme.

It would thus appear that there is some basic relation between the sternopleural and abdominal chaeta numbers which can fairly be represented by a parabola in this material. The relation is not a simple positive correlation, such as would appear if selection for one set of chaetæ cause a change in the capacity for general chaetæ production. Nor is it a simple negative correlation, such as would appear if there were a limited capacity for general chaeta production with consequent competition between the various groups of chaetæ. The regularity of the results suggests, nevertheless, that the relation seen is physiological and that this correlated response to selection is therefore to be attributed largely to pleiotropy of gene action ; it does not show the irregularity expected of correlated response due to linkage.

That linkage may, however, still be playing a part, though a lesser one, is suggested by two considerations. First, some of the points in fig. 9, especially that from the Or parent, appear to fall further away from the best fitting parabola than would be expected from a sampling error of the size indicated by the duplicate observations of table 23*a*. This would indicate that smaller irregularities, of the type depending on breakable linkage, are superimposed on the regularity of pleiotropy. Secondly, counts made on females of thirteen different wild-type stocks maintained in the laboratory fail to show the parabolic relation between sternopleural and abdominal chaeta numbers (table 23*b*). In fact they show no obvious relation at all, so that something other than a relatively simple physiological relation must be determining their differences. Their gene differences, in respect of these two characters, appear to be at least in part

recombinable in the way demanded by correlated response due to linkage.

A second group of chaetæ, those borne on the two posterior coxæ jointly, were also counted in S–139 on 20 females of six of the selected lines and also of the parents. The eight points relating coxal and abdominal chaeta numbers are plotted in fig. 10. As in the case of the sternopleurals, only females were used in these counts. The parents and the six lines clearly differ in coxal number, so that selection for abdominal chaetæ has affected coxal chaetæ. The coxal number is, however, related to the abdominal number in a way which suggests a simple physiological relation, as though both groups of chaetæ reflected some general overall capacity for chaeta production. It is impossible to say, from these data, whether such a minimal assumption

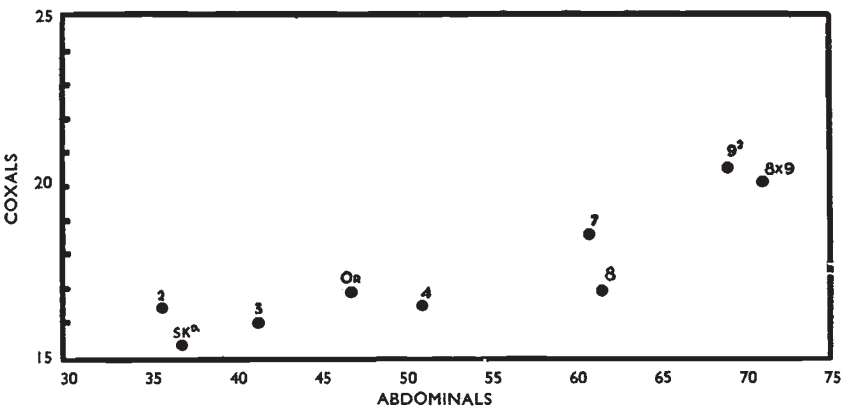

FIG. 10.—The relation of average abdominal and coxal chaeta numbers in females of the parents and seven of the lines.

would be sufficient in itself; but we may observe that in any case the physiological relations that are indicated between sternopleurals and abdominals on the one hand, and coxals and abdominals on the other, are not the same. Whatever pleiotropic effects there may be of genes on different groups of chaetæ, they are not all alike.

(ii) *Abdominal pigmentation.*—Two variants of the normal pattern of abdominal pigmentation have been found in the later generations of the experiment. Both affect the regularity of the dark areas at the posterior end. In females these appear, of course, as a series of transverse bands, but in males there is a solid dark area (fig. 11*a*). The variants in question are not confined to males in their expression, but the normal alternation of dark and light bands makes them more difficult to see and record in females than in males, where they appear as alterations of the solid dark area. Records have therefore been taken from males only.

The first variant is the appearance of patches, whose colour is lighter than that of any other part of the body, on the left or right

sides of the normally dark area. These pale patches are somewhat irregular in shape and vary in size and position. They are, however, usually about quarter the size of the dark area and they have never been seen to occur centrally on the fly. Some examples are illustrated in fig. 11.

This variant has been seen only in line 9 and in its derivative, line 8×9. It was first seen in line 9 at S–116. Since that time it has appeared in a proportion of males in every generation except two, S–127 and S–128, where the numbers of males available were only

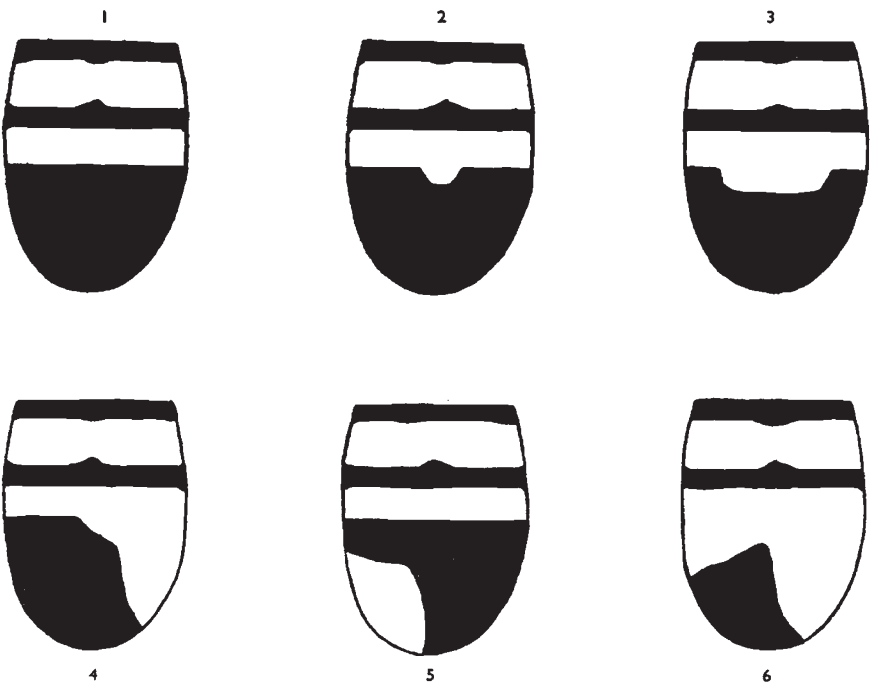

FIG. 11.—The posterior darkly-pigmented area of the male abdomen, seen dorsally :— (1) Normal. (2) Small patch from crosses of line 2 and Or. (3) Large patch from line 2. (4)-(6) Various examples of patching from lines 9 and 8×9.

47 and 48 respectively. Since the total frequency of appearance, up to and including S–138, was 113 patchy males out of 1951 the failure to appear in these two generations may fairly be ascribed to chance.

There is, however, evidence that the frequency of patchy males has fluctuated significantly in line 9 since its first appearance. Generations S–116 to S–124 gave 7·53 per cent., S–125 to S-131 gave 3·9 per cent. and S–132 to S–138 gave 6·56 per cent. Data are available from line 8×9 for S–125 onwards, and they show that S–125 to S–131 gave 3·03 per cent. and S–132 to S–138 gave 7·19 per cent. Since the corresponding generations of the two lines were

raised in the incubator at the same time it appears that although each line gave significantly different percentages at different times, the two lines gave percentages which did not differ significantly when raised at the same time. In other words the change in percentage seems to have depended immediately on external circumstances. It will be observed too that the patchy variant lost nothing of its percentage manifestation upon transfer from line 9 to the new line 8×9.

It is of importance to note that patchiness was first observed in line 9 at S–116, the generation at which the delayed response to selection for abdominal chaeta number was completed (section 3*f*). Patchiness appears therefore to have been a correlated response to this selective change in the chaetæ. Its subsequent behaviour can be reasonably interpreted on this view. Once the delayed rise of chaeta number under selection was completed there was no reason why the correlated character should change further, except in so far as such changes reflected differences in external conditions. Furthermore, on the assumption that the delayed response to selection was due to the release of variability by the occurrence of crossing-over in an unusual place, any change to which this crossing-over gave rise should be inherited as a unit except when it was broken down by further, and presumably equally rare, crossing-over in the same unusual position.

Chromosome III of line 9, to which the delayed change in chaetæ could be traced by the assays (section 3*c*) had a higher chaeta producing power than its homologue from line 8. It would be expected therefore that line 8×9 would, as a result of the selection practised on it, have chromosome III from line 9. If patchiness was due to the change that occurred in this chromosome between S–112 and S–116, line 8×9 should have the same patchiness as line 9 : as indeed it does. With the further assumption that the low percentage manifestation of patchiness was due at least in part to low penetrance—a not improbable postulate—all the facts would fit the view that it originated as a correlated response, depending on linkage ; but further work will clearly be necessary to prove the case.

The second variant in abdominal pigmentation is the occurrence of a large nick in the anterior edge of the area of dark pigment (fig. 11). This has been seen only in line 2 and was first observed at S–118. All males of this line (and one must presume all females too) show this patch. Its size appeared to have increased at about S–133, until it affected nearly the whole anterior edge of the dark area. Whether this was due to a further genetic change, or to an alteration in the environment, it is impossible to say, though it might be suspected that the latter was the more likely.

The inheritance of this patching was tested by crosses of line 2 to the Or parent. The reciprocal F_1's agreed in showing a patch on all males, though a distinctly smaller one than that of line 2 flies.

F_2's and backcrosses to both line 2 and Or were then raised. Again reciprocals were alike and the segregations were :—

Generation \ Patch	Large	Small	Absent
F_2	67	107	90
Backcross to line 2 . .	111	110	...
,, ,, Or . .	...	93	137

The indication is clearly of inheritance controlled by a single gene difference, with a small amount of misclassification between the Small (like F_1) and Absent (like Or) classes. Further crosses of males and females of F_2 with one another and with Or flies agreed with this view, except for five anomalous cultures out of the total of 51. Two of these five could reasonably be ascribed to misclassification of males as falling into the Absent class when they were genetically in the Small class. Such misclassification was in any case presumed to occur in F_2 and the backcrosses. The other three families comprised the whole of one group of like matings and all were anomalous in the same way, the anomaly being in fact one which could not easily be explained by the assumption of more than one gene. These three cultures might well therefore be due to a common mistake in recording the matings and their type. Thus in the absence of further tests the evidence strongly favours the postulation of a single gene of incomplete dominance for the patching in line 2.

Line 2 was also crossed with the *Cy/Pm ; H/Sb* tester stock. All F_1 males had small patches. Males of the *Pm Sb* type from this F_1 were crossed back to line 2. In their offspring all the + and *Pm* flies had large patches while all the *Sb* and *Pm Sb* flies had small ones. The gene is therefore in chromosome III.

This gene for patching could of course be regarded as having arisen by a chance natural mutation, though such mutations are seldom incompletely dominant in the way observed. It could, however, have been a correlated response to the selection which was effective in giving the delayed change in line 2 between S–82 and S–90. This change is known to have involved chromosome III, and as we have just seen the correlated response could be inherited as a unit even though it arose by crossing-over. It is an obvious weakness of this interpretation that it requires at least 21 generations to have elapsed between the origin of the patching and its being recorded. This particular patching is not, however, so easy to see as that of line 9 because it occurs in a place normally covered by the intersecting edges of the wings when at rest. It might well, therefore, have escaped detection until the discovery of patching in line 9 at S–116 led to a search for patching on the other lines. The evidence as to the nature and origin of the line 2 patching is ambiguous, but with an ambiguity differing from that of the patchiness in line 9.

(iii) *Eye form.*—Flies in which one eye is abnormally small, sometimes to the point of being absent, are known as rarities in many stocks, even apart from the effects of genes such as *ey*. It was noticed, however, at S–100 that flies with such eye abnormalities were more common than usual in certain of the selection lines. The abnormalities were equally common in the two sexes. No cases were observed where both eyes were reduced in size, but the abnormal eye might itself be either the left or the right.

Abnormal eyes were found only in lines 8, 9 and 8×9. No examples were seen in any of the other lines either before or after the search began at S–100. It cannot be said that the eye abnormalities were not present in lines 8 and 9 before S–100, since the percentage of abnormal individuals must have been so small as to be fairly easily overlooked.

Out of 6204 individuals recorded in line 9 between S–100 and S–138, 88 or 1·42 per cent. had the abnormality. There was no detectable change in frequency at the time of the delayed response to selection for abdominal chaetæ between S–112 and S–116. In line 8, 6188 individuals recorded over the same range of generations included 4, or 0·065 per cent., with the abnormality. Line 8×9 gave 9, or 0·34 per cent., out of 2669 individuals between S–125 and S–138.

The abnormality occurs in three lines and with its special frequency in each. It cannot therefore be due to chance mutation nor to a single gene. It seems fairly clearly ascribable to polygenic correlated response, though the time at which the response first appeared cannot be established from these data. The three lines in question are the most highly selected for chaeta number, but the abnormality cannot be due to a simple pleiotropic effect of high chaeta number, for two reasons. First, the number of chaetæ is slightly higher in line 8×9 than in 9, and higher in 9 after S–116 than in 8. Yet the order of frequency of eye abnormality is 9, 8×9, 8. Secondly, the chaeta number of 9 increased between S–112 and S–116 to an extent greater than the difference which had previously existed between 8 and 9. Yet the frequency of eye abnormality was unchanged. This shows us too that the part of chromosome III changing in line 9 between S–112 and S–116 was neutral in its gross effect on the eye abnormality.

(iv) *Chromosome behaviour.*—It has already been recorded that the salivary gland chromosomes of the F_1 and F_2 between Or and Sk were examined by Dr M. Westergaard in 1946, without any abnormal behaviour being found. Dr Westergaard also examined the salivary chromosomes of line 1 at the same time, in order to discover whether any structural differences existed which could account for the balanced sterility displayed by this line (section 3*a*). No gross structural changes were in fact found, but it was observed that in flies of line 1 the polytene chromosomes showed a non-specific incompleteness of the pairing between homologues. Quite long segments in any or all of

the chromosomes would be seen to be unpaired in most nuclei. This was in sharp contrast to the F_1 and F_2 where pairing was regular and complete. It would thus appear that chromosome behaviour also reveals the changed genetic constitution of line 1. No other lines have been examined for this character.

5. VARIABILITY, CORRELATED RESPONSE AND EFFECTIVE FACTORS

Three general points emerge as worthy of comment from this experiment. The first of these is the immense storage capacity of the genotype for variability. Once started from the cross between the Or and Sk stocks, the lines were closed to new genetic material. Yet 20 generations of selection served to raise the chaeta number by 50 per cent. in the high line and 35 to lower it by 30 per cent. in the low line. Nor was the limit set in these lines by the available variability in chaeta number. Both lines were still advancing under selection when sterility applied the closure to them.

Not all the variability was so rapidly available to selection. Delayed responses occurred in one line or another throughout the whole course of the experiment as variability became available to selection ; and we have no reason to suppose that the limits eventually attained, wide though they were, represented the maximum effect that selection could exercise on chaeta number, using the differences available from only this one cross. With the larger numbers and longer time available to natural selection, responses compared with which those obtained in the experiment would be small could undoubtedly be brought about, provided of course that the phenotypes favoured by selection were sufficiently extreme. The variability available in the genotypes of one species, and disguised under the cloak of a much smaller range of phenotypic variation, must be great enough to permit the establishment of differences even greater than those observable between related species.

The size of the reservoir of potential variability is shown perhaps still more strikingly by the changes in spermathecæ. Phenotypically the parent lines were nearly uniform for 2 spermathecæ, and even their F_2 contained only 1·5 per cent. of individuals with the relatively minor abnormality of 3. Yet combinations of genic materials were possible which gave frequencies of abnormality as high as 35 per cent., the abnormalities themselves transcending the limits shown by the species of the whole group of these flies in ranging from 0 to 5 spermathecæ. It is worth observing, too, that although we refer to these novel numbers of spermathecæ as abnormal (which, in relation to the common range of variation within the species, they clearly are) there is nothing to indicate that they are also necessarily deleterious in themselves. Rather the evidence from some of the mass lines, such as 3, indicates that they are capable of maintaining themselves

even under conditions of unrestricted competition with the normal number of 2.

In respect of fertility also the initial cross contained genetic differences which, when recombined during the course of the experiment, could lead to a sterility comparable with that produced by the relational unbalance characteristic of the genic complements of hybrids between distinct species. Taking all these characters together we can see little reason to doubt that the variability available beneath the surface in a single species is great enough of itself, without the immediate aid of further mutation, to permit the establishment by natural selection of the specific differences observed in the wild. And the observations which we have made on delayed responses in the experiment suggest that the variability within a species may be made more readily available to natural selection by those very changes in environment which impose the demand for readjustment of the phenotype.

Mention of spermathecæ and fertility brings us to the second general point which emerges from the experiment, *viz.* the power of correlated response to bring about changes in characters for which no direct selection is practised. The magnitude of the changes produced by correlated response requires no emphasis. Furthermore these changes can hardly have been actively advantageous in their initial stages, and indeed the lowering of fertility was clearly disadvantageous. In general, therefore, correlated responses will tend to impose a disadvantage which must be carried along by the main selection.

In considering the effects of selection we must never forget that the partition which we make of the phenotype into characters is not matched by a corresponding physical partition of the genotype into genic combinations, and *vice versa*. The correspondence of phenotype and genotype is a relation of wholes which does not extend to parts (Mather, 1949*a*). It is the overall advantage or disadvantage of the phenotype, its Darwinian fitness so far as natural selection is concerned, which determines its fate, and with this fate that of the genotype by which the phenotype was engendered. Thus not only does the organisation of the genetic materials into genically heterogeneous linkage groups ensure that change by recombination of the genic combinations affecting one character will be accompanied, at least initially, by changes in the combinations affecting others ; but the association of characters in the phenotypes also permits the resulting disadvantage in the selectively subordinate character to be outweighed by, and carried along with, the advantage gained by the change in the selectively capital character. The partial phenotype, or character as we call it, may change against the partial selective force acting on it, provided that the total phenotype is changing with the total selective force. And the non-correspondence of partial phenotype and partial genotype will generally ensure that this is in fact what happens.

We can have, therefore, no such thing as a neutral character or

neutral gene in selection ; for the properties in selection of a gene, and with them those of the character or characters which the differences in that gene affect, will depend, at least in the short run, not merely, and indeed not so much, on its capacity for change by mutation, but on its mechanical relations with other genes, by which are governed its reassociations through recombination, itself so much more common a source of change than is mutation. The variability stored in the species' reservoir can be released only by recombination, which is not confined in its effects to the genes of a system affecting one character. In fact, only slowly, as the necessary set of complex recombinations accumulates, can one character be readjusted while another is achieving its best expression, whether at the old level or a new. It has been claimed that genes, and with them character expressions, may under some circumstances drift even against selection. Whatever drift there may be, and under whatever restricted circumstances it may occur, linkage of the genes must always be causing the characters to push one another about, the trend in any one, relative to its optimum level of expression, depending on the strength of the selection under which it finds itself relative to the strength of selection acting on the others.

It thus appears that in attempting to understand response to selection the individual gene is not a sufficient unit. We must also be concerned with the mechanical organisation of the genes. This brings us to the third general point, the relation of the observations we can make on polygenic variation to the familiar concepts of mendelian genetics.

We are bound to suppose that the members of a polygenic system have all the mechanical properties of mendelian genes, that they are transmitted on the chromosomes, segregate and recombine. The evidence on this point is conclusive (Mather, 1949*a* and *b*). Such genes cannot, however, be followed as individuals, for they have no individual actions by which each is uniquely recognisable. Thus although the polygenes can be shown to segregate and to recombine, we cannot count them. At best we can say that some demonstrable minimum number is involved in the system ; we must always make the reservation that each of the units we count may itself be composite.

Now the action of any such composite unit, or effective factor as it may be called (Mather, 1949*a*), may be multiple, not because any single polygene is pleiotropic, but because the chromosome segment, which is the physical basis of the factor, carries polygenes of disparate systems. Indeed general experience would lead us to expect that this would be the case, and the observations of correlated response, described above, vindicate this expectation.

In the same way the properties of a composite factor may change either by mutation of the individual genes, or by their recombination. The relation of the rate of change to degree of hybridity shows that recombination must in fact be responsible for most of it. But mutation

seems also to occur (Mather and Wigan, 1942). Whether this polygenic mutation is comparable with that of major genes is, however, still another question into which we need not enter here. Our main concern must be to see that both the multiple action, and the change of the factors we can recognise, are attributable in the main to their composite nature ; that pleiotropy and mutation of the individual and indivisible ultimate genes, though always theoretically possible, are in the practical sense residual postulates, which must always be difficult and often impossible to substantiate. To ascribe the multiple effects and the changes we observe in selection experiments to pleiotropy and mutation is to deny both the theoretical expectation and the practical evidence that such effects and changes could and must come about by the linkage and recombination of simple elements into composite factors : it is to extend the unanalysable residue to include the analysable whole.

This technical inability to recognise the single polygene affects our inference and conception not only of pleiotropy and mutation, but also of dominance and interaction. The evidence from the level crosses (section 3*d*) is of an absence of dominance from the factors with which we were dealing (which there were whole chromosomes), since the F_1 was always intermediate between the parent lines. This does not, and indeed cannot, rule out the possibility of dominance of the individual genes. Those within one factor could show balanced dominance, some in one direction and some in the other, so that the factor as a whole showed no preponderance one way or the other. Potence of an effective factor, as we would follow Wigan (1944) in calling it, must indicate some dominance of its constituent genes ; but lack of potence does not of necessity indicate lack of dominance. And we may note further that genic interaction within the effective factor will appear as an effect on its total action and on its potence. Such interaction will not be distinguishable from individual action and dominance. Indeed, changes of scale may of themselves convert what appeared as interaction to the appearance of dominance in a polygenic system (Mather, 1949*a*).

So in recognising that in polygenic systems we must deal with effective factors of a composite nature rather than with single genes, we recognise that the familiar concepts, developed for the understanding of the single and individually traceable genes with which mendelian genetics is concerned, must be used with due reservation. These concepts will apply to the single polygenes, of which we must suppose the systems to be composed, but the properties of the effective factors will not allow them to be applied in a simple way. Some of the distinctions which we must draw when considering single genes are no longer useful or even possible, and new distinctions and concepts may become necessary.

Finally, before leaving the effective factors, we should note that being composite they can speedily be built up by recombination and

selection. We have seen how this resulted in much more rapid responses when selection was relaxed and restarted than in the initial stages of our experiment. Change was more rapid because the effects of the factors, the units of segregation, were bigger. It only remains to add that, should such a synthetic factor become associated with an inversion, a new gene would virtually be created. The production of new variation by recombination (Wigan, Misro and Thompson, 1949) suggests further that such a synthetic gene could have effects transcending the limits expected from simple addition of those of its parts. The study of such genic evolution is, however, only beginning, and while we may canvass possibilities (Mather, 1949*a* ; Darlington and Mather, 1949) much more experimental evidence will be needed before we can outline the processes with any confidence.

6. SUMMARY

A cross was made between the inbred Oregon (Or) and the Samarkand (Sk) stocks of wild-type *Drosophila melanogaster*, and selection was practised for both high and low numbers of abdominal chaetæ in separate lines from F_2 (= generation S–0) onwards.

Chaeta number fell erratically for 35 generations in the low selection line, at which time sterility caused the line to die out. A mass culture, begun at generation S–20 maintained its chaeta number with fair consistency. Further selection, whether for high or low chaeta number, from this mass became increasingly difficult in that sterility put an end increasingly early to the lines so selected. Evidently the low mass was building up a balanced sterility system within itself. Flies with the more extreme chaeta numbers were the less fertile. The mechanism of infertility was not elucidated, though in males it seems to have depended partly on inability to inseminate females.

The high selection line showed a progressive increase in chaeta number and decrease in fertility until S–20, when the number of flies produced was so small that selection was abandoned and resort made to mass culture. The chaeta number of this mass line (line 3) fell back 80 per cent. of the way to the S–0 mean in 5 generations. Reselection begun at S–24 resulted in recovery to the level of S–20 in 4 generations, but without the extreme loss of fertility observed earlier. The selection line (line 8) was maintained over 100 generations, and a mass line (line 7) made from it showed no fall in chaeta number over a similar length of time. Evidently prior to S–20 selection for chaeta number led, by correlated response, to a fall in fertility, and between S–20 and S–24 natural selection for fertility led by a corresponding correlated response to a fall in chaeta number ; but after S–24 the relation of high chaeta number and low fertility was partly broken in line 8 by further recombination of the polygenic systems governing them. The rapid fall of chaeta

number in line 3 and its rapid rise in line 8 showed that the selection before S–20 had replaced the balanced polygenic combinations of the parental chromosomes by coupled combinations of polygenes having more extreme total effects, which behaved as virtual units in segregation and selection. Later reselections suggest that the three major chromosomes were in fact acting each as a large unit of this kind.

A technique is described for assaying the effects of the three major chromosomes on chaeta number, and the efficiency of this technique is shown to be about 90 per cent. The assays confirm that selection before S–20 had built up combinations of large total effect within each chromosome, and that the later results could be understood in terms of these combinations acting each virtually as a unit. Dominance of the genes within each chromosome was either absent or balanced, so that the units as wholes showed no overall dominance (or potence as it is better termed). Selection seems also to have favoured interaction between chromosomes X and III in the way that might be expected.

Lines were established by the high reselections, whose average chaeta numbers were stabilised at three levels. The line at the upper level differed from that at the middle in chromosome II only, while that at the lower level differed from the middle in chromosomes X and III only. The results of crosses between the levels were in reasonable accord with the view that the three chromosomes behaved as units of segregation. Selection from the F_2's of these crosses restored the parental chaeta number with the speed to be expected on this view, except in the case of high selection from the cross between the upper and lower levels. In this, on two occasions (lines 9 and 10), progress was so slow as to suggest that it depended not on mere recovery of the parental genotype of the upper level, but on building up a new genotype giving high chaeta number. This interpretation was vindicated by the transgression of the parental level by the new lines, and also by new correlated responses in fertility and other characters.

The behaviour of fertility in these lines selected for high chaeta number, and in back selections (including line 2) from them, was in accordance with the expectation of correlated responses to selection arising from the linkage of the member genes of the systems governing chaeta number and fertility. The assumption of pleiotropic action of the single genes does not offer a satisfactory explanation of the results.

Delayed responses to selection occurred in lines 2, 8 and 9 after long periods of stability under selection. The evidence from these and similar responses observed by Sismanides, and from the correlated responses accompanying them, indicates a determinacy in time and relation between characters for which mutation could not account. The delayed responses seem more likely to be due to release of variability by recombination following crossing-over in new positions

along the chromosomes, the changes in position of crossing-over possibly resulting from temporarily changed environmental conditions, on the lines of the known effects of temperature alterations.

Normally a female has 2 spermathecæ and only rarely are wild-type flies abnormal in having 3 spermathecæ. Only 2 abnormal females were found out of 2234 examined from the Or and Sk stocks. No abnormals were found in F_1, and 1·5 per cent. in F_2. In the selection lines the proportion of abnormals ranged up to 35 per cent. and the number of spermathecæ varied from 0 to 5. The appearance of these variations must have resulted from correlated responses to selection for chaeta number. The changes in proportion and type of abnormality served to confirm that the selection of recombinant chromosomes was concerned in the delayed responses of lines 2, 8 and 9, and in the unexpected behaviour under selection of lines 9 and 10. Genes affecting the spermathecæ were located in chromosomes X and III.

Flies of the parent stocks and of selection lines 3, 4 and 7 were tested for discrimination in mating. A statistical technique is described for analysing data from such tests. Discrimination in mixtures of Or and Sk flies varies with age of male and possibly of female too. It is at a maximum in flies of four to seven days age after emergence. Sk females mated more often than did Or females whether the male was Or or Sk. There was a suggestion that the properties in discrimination of flies from some selection lines differed, as a result of correlated response, from those of both parent stocks.

Correlated responses to the selection for abdominal chaeta number were also observed in sternopleural and coxal chaeta numbers, where they may be due largely to a physiological relation (though not a simple one) of the chaeta characters, and in body pigmentation, eye form and possibly chromosome behaviour.

In conclusion are stressed :—

(i) the large capacity of the reservoir of hidden variability within a species ;

(ii) the power of correlated response, due to linkage, to change characters even against the trend of natural advantage ;

(iii) the importance, for the application of the familiar concepts of mendelian genetics, of the composite nature of the effective units we can recognise in polygenic variation.

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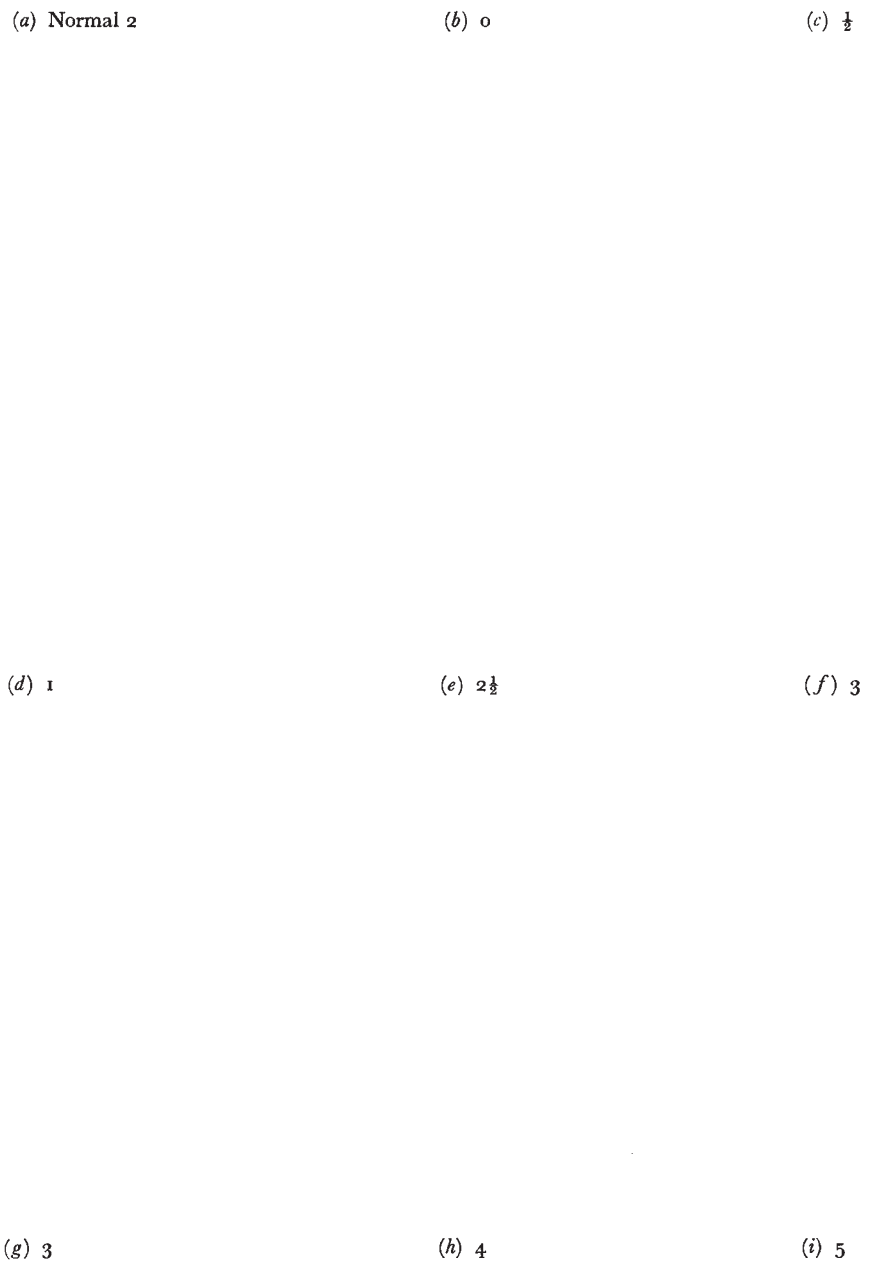

Plate I.—Normal, (*a*), and abnormal, (*b*)-(*i*), spermathecæ. The types shown in (*b*)-(*d*) are included in the group <2, and (*f*)-(*i*) in >2. All approximately $\times 100$, except (*f*) which is approximately $\times 150$.