BRISTLE INHERITANCE IN DROSOPHILA

I. EXTRA BRISTLES

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SIX FIGURES

The four bristles normally found on the dorsal surface of the thorax of *Drosophila ampelophila* form a rectangle. The extra bristles studied in the following experiments occur in the two longitudinal rows of the normal bristles, or just mediad or laterad to these rows. Figure 1, showing some random patterns of bristles, gives some idea of the different arrangements possible for the most common numbers of extra bristles. These patterns were drawn free hand, since the overlapping of the bristles prevented the use of a camera. The drawings are not exact, but the relative positions in the two rows are sufficiently accurate to indicate that it would be practically impossible to make more than a roughly approximate classification of flies according to patterns, even if a very large number of standard patterns were established. There appears to be no position in the two rows that may not be occupied by an extra bristle, thus making it impossible to develop any satisfactory homologies. This is especially true in the higher grades. For these reasons the best method seems to be recording merely the numbers of extra bristles. As will be seen, the results based on the data of bristle numbers shows very plainly that any attempt to obtain and use data of patterns of extra bristles would be futile with the present cultural methods.

All matings described below were made in pairs. The virginity of the females mated was determined by the pale pigmentation which deepens when the cuticula hardens a few hours after hatching. Accurate pedigrees and daily records of the sex and bristle number of each fly observed are on file. The results presented in this paper are based on 350 pedigreed matings and bristle counts of over 54,000 flies.

Grateful acknowledgment must be made of the assistance rendered by Prof. T. H. Morgan in suggesting that the extra bristles found at times among his flies might form suitable material for the problem the author had in mind, and also in supplying numerous stocks of flies for observing the frequency of the occurrence of the extra bristles.

Fig. 1 Random bristle patterns showing some of the arrangements possible for the most common numbers of extra bristles. The numbers indicate the numbers of extra bristles in the patterns following.

THE ESTABLISHMENT OF THE RACE WITH EXTRA BRISTLES

The flies which gave rise to a race of *Drosophila ampelophila* having extra bristles on the thorax were found in a stock of wild flies that had been caught at Woods Hole in 1912 and bred in mass cultures for a year in Professor Morgan's laboratory. This stock had never been mixed by crossing, and in working

with the flies there has been found no reason to suspect accidental contamination. Flies with extra bristles were occasionally found in this stock. Their frequency is shown in table 1. Various other stocks were examined and extra bristles were found in smaller numbers.

TABLE 1
Extra bristles found in various stocks

STOCK	EXTRA BRISTLES					TOTAL EXTRA BRISTLES	PER CENT EX- TRA
	0	1	2	3	4		
Woods Hole wild.....	891	17	20	4	2	434.8	
Falmouth wild.....	442	3				30.6	
Edgewater wild.....	474	2				20.4	
Cherry.....	266	2			1	31.1	
Vermilion.....	163	2				21.2	
Black.....	80					0	
Orange.....	183					0	
Peach.....	263					0	
Bow.....	123					0	
$\frac{1}{2}$ Balloon.....	189					0	
White head.....	142					0	
New York '12.....	3892	46	3			491.2	

TABLE 2
Percentages of normals in successive inbred generations, selected for increase in bristles.

GENERA- TION	NUMBER COUNTED	PER CENT NORMAL
1st	249	44.9
2nd	7139	10.0
3rd	2380	3.1
4th	1503	2.1
5th	1819	2.8
6th	2780	0.9
7th	5833	2.6
8th	2343	0.8
9th	1690	1.4
10th	2479	1.9
11th	2859	3.4

From the Woods Hole 1912 stock a male with one extra bristle was mated to a female with two extra bristles. Of their offspring 55.1 per cent had extra bristles. Thirty-three matings of first generation flies with extra bristles resulted in an F_2 with 90 per cent extra bristles. The subsequent generations produced by inbreeding brothers and sisters in pairs gave higher proportions of *extras* (table 2). One might suppose that the occurrence of normals in the later generations indicated impurity; but in spite of being normal in regard to the group of bristles selected for observation, these flies frequently show extra bristles on other parts of the thorax, and, further, there will be presented evidence indicating that such normal appearing flies may produce all *extra* children; in other words, that other than genetic causes may prevent the development of potential extra bristles.

To test the stability of the *extra* race further, three mass cultures were started from the 4th and 6th inbred generations and have been carried along from bottle to bottle with no further selection. Occasional counts have been made of these cultures and it has been found that the extra bristles have been retained. Table 3 shows the per cent normals in mass counts. It is evident that there are more normals in some cases in these mass cultures than in the inbred lines, but these normals are more than likely potential extras whose greater frequency is due to the fact that in mass cultures there are larger numbers of flies which are smaller on account of the crowding. It will be demonstrated later that there is a relation between the size of the fly and the number of extra bristles. (See the discussion on the influence of environment).

INHERITANCE

1. THE FACT OF INHERITANCE

That a race of flies constantly bearing extra bristles has been established, indicates in itself that the extra bristles are inherited. To show that this is not pseudo-inheritance, due to certain constant environmental conditions, a stock of wild flies caught in New York has been carried along under the same conditions as the mass cultures of the extra stock, and for a few generations this wild stock was bred in separate pairs as were the inbred selected lines. A small percentage of *extras* was found in this wild stock (tables 1 and 4) but this did not increase in time, nor did the progeny of separate pairs show any higher proportions of *extras*. Conclusive evidence that extra bristles are inherited and not primarily due to environment is afforded by crosses, which show also the mode of their inheritance.

2. THE KIND OF INHERITANCE

The wild stock New York 1912 was found to have fewer *extras* than any other wild stock examined and so from this stock normal flies were selected for several generations to attempt to reduce the percentage of *extras*. A preliminary set of four crosses

TABLE 3

Occurrence of normals in three mass cultures

MASS CULTURE	MONTH COUNTED	NUMBER COUNTED	PER CENT NORMAL	MASS CULTURE	BOTTLE NUMBER	NUMBER COUNTED	PER CENT NORMAL
105B	Oct.	67	0	176	10.18	150	2.6
	Nov.	107	0.9		12.50	432	4.4
	Dec.	391	2.5		12.12	95	2.1
	Jan.	251	5.1		12.28	152	1.3
	Feb.	84	3.5		1.13	193	8.8
	Mar.	138	4.3		1.22	90	2.2
	May	233	2.5		4.14	180	1.1
105B	Total	1304	3.6	105B ₁	12.90	156	0.6
176	Total	1375	3.1		1.12	56	7.1
105B ₁	Total	394	2.5		4.14	167	1.8

TABLE 4

Three generations of selection by pairs of normal New York stock to reduce the numbers of extra bristles and test the influence of mating by pairs on the numbers of bristles.

GENERATION	BOTTLE NUMBER	FLIES COUNTED		
		Nos. extra bristles		
		0	1	2
1st	198	227	3	
2nd	214	265	2	
2nd	218	584	9	
2nd	219	290	5	
2nd	220	262	9	
3rd	241	220	1	
3rd	242	245	7	1
3rd	255	11		

between *extra* females from the first inbred generation, and normal males from the unselected New York 1912 stock, showed in general a dominance of the normal, although less than .5 per cent *extras* were found. These *extras* were always of very low grade, +1 (one extra bristle), while the corresponding inbred generation of *extras* showed a range up to +7 (seven extra bristles) and a mode at +2. If extra bristles were supposed to be due to environmental conditions it would be difficult to account for their general disappearance in this generation. In the following generation, F₂, the *extras* appear in proportions approximating the simple Mendelian ratio, although the normals are somewhat more numerous than expected. The *extras* that appear in F₂ include higher grades (table 5A).

A second set of crosses between *extras* and normals, after each race had been selected for a number of generations, was made. The *extras* used were from the 9th and 10th generations of inbred selection. In every cross the dominance of normal was

clear, although a few *extra* flies appeared in F₁. Among 1419 F₁ flies, 12 had one extra, 3 had two extra bristles. Sixty-five pairs of normal F₁ flies were mated. The total counts for their children are 8421 normal, 2499 extra, which give a ratio of 3.3:1. (table 5B). As in the previous crosses there is a practical incomplete dominance in F₁, whatever theoretical explanation may be given, and in F₂ the numbers of extras are a little too low, yet there can be no question that this is clear evidence of the existence of a Mendelian factor that influences the number of bristles.

To test for sex linkage, five of the above crosses were made with *extra* males and three were made with *extra* females. In

TABLE 5

Giving the results of crosses between extra bristled parents and normals from wild stock (New York '12)

A—Extra bristled parents from the second inbred generation

BOTTLE NO.	PARENTS ♂: ♀	F ₁				F ₂											RATIO
		Normal	Extra		No. of matings	Normal	Number of extra bristles									Normal: extra	
			1	2			1	2	3	4	5	6	7	8	9		
20	0:2	173	3	—	2	172	5	10	1								10.7:1
21	0:2	210	—	—	4	463	47	60	11	2							3.8:1
22	0:2	255	1	—	2	466	63	46	14								3.8:1
IX	0:3	197	—	—	3	423	58	49	11	2							3.5:1
Total		835	4	—	11	1524	173	165	37	4							4.0:1

B—Extra bristled parents from the eighth inbred generation

316	3:0	156	3														
311	3:0	301	2	—	8	994	89	86	52	23	10	2	1				3.5:1
314	2:0	40	—	—	5	762	67	61	46	34	27	3	2		1		3.1:1
312	6:0	93	2	—	15	1383	169	102	93	51	18	8	2				2.9:1
313	6:0	189	2	1	15	1487	138	121	90	38	20	5	4				3.5:1
304	0:3	5	—	—	1	195	22	14	8	3	2	1					3.9:1
305	0:2	432	3	2	9	1646	166	145	111	45	31	9	4				3.1:1
307	0:6	203	—	—	11	1954	192	166	109	64	33	6	5				3.3:1
Total		1419	12	3	64	8421	843	695	509	258	141	34	18		1		3.3:1
Totals of all crosses		2254	16	3	75	9945	1016	860	546	262	141	34	18		1		3.4:1

both types of crosses the children showed dominance of the normal, and all grandchildren gave the same general ratios of normal to *extra*. Moreover, the distributions of these F_2 *extras* were similar in the two types of crosses (table 9 and figure 4). This is enough to indicate that there is no sex linkage involved.

3. MODIFICATION OF THE DISTRIBUTION OF EXTRACTED EXTRAS

In comparing the distribution of the extra bristles in the F_2 of the above crosses with that of the extra bristles in the selected race of the corresponding generations, a marked difference is observed. This is shown in figure 2. In these curves the males and females have been put together and the normals occurring in the inbred have been omitted, since corresponding normal appearing flies in the F_2 of the crosses could not be separated from the genetically normal flies. The data have been plotted on the basis of 500 in each curve, to facilitate comparison. In general the distribution of the *extras* appearing in the F_2 of a cross is lower than the distribution of the corresponding generation of uncrossed *extras*. The mode of the extracted extra bristles from the first cross is at +1, although the frequency of +2 is nearly as great; the mode of the second selected generation is at +2. In the later crosses, the mode of the extracted extra bristles is at +1, and the mode of the tenth selected generation is at +3.

With these curves in mind it will be well to compare the extracted *extras* from flies crossed before continued selection, with the extracted *extras* from flies crossed after selection. It is readily seen that the extracted curve from the later crosses is strikingly higher than that from the first crosses. The range is much higher; but the mode is still at +1, even more prominently so than in the first case. The standard deviation in the first set of extracted *extras* is $.695 \pm .017$, that of the corresponding inbred generation is $.863 \pm .005$. In the later crosses standard deviations are as follows: extracted *extra*, $1.329 \pm .018$, inbred, $1.402 \pm .021$ (table 6). It appears to be a very definite fact that the modification of the distribution of extra bristles by

Fig. 2 Comparisons of the distributions of inbred and extracted extra bristles in corresponding generations. Upper curves, selected inbreds of the second generation (broken line) and extracted *extras* before selection (solid line); lower curves, selected inbreds of the tenth generation (broken line), and extracted *extras* of the corresponding generation after selection (solid line).

crossing becomes more marked when parents are selected for several generations before being crossed, yet this is not accompanied by any increase in variability.

4. SUMMARY

It has been shown that the normal number of four bristles is dominant to the extra bristled condition; that the simple Mendelian ratio of 3 : 1 is closely approximated; that no sex linkage is involved. Further, it has been shown that the flies having extra bristles in the F_2 of a cross have a distribution lower than that of the selected flies of the corresponding generation, and that this difference is more marked when extra bristled flies are crossed that have been selected for nine generations

TABLE 6

To compare the means and the variability of extra bristles that appear after a cross with those in corresponding generations of the extra race.

	MEANS	NUMBERS	STANDARD DEVIATION
Extracted Extras (Gen. 2).....	1.665	377	0.695±0.017
Inbred Extras (Gen. 2).....	1.921	6418	0.863±0.005
Extracted Extras (Gen. 10).....	2.335	2499	1.329±0.018
Inbred Extras (Gen. 10).....	3.229	981	1.402±0.021

than when flies of the first selected generation are crossed. The variability of the extracted extra bristles is slightly less than that of the corresponding inbred generation.

5. DISCUSSION

The interpretation that seems most simple to apply to the above facts is that there is a positive restricting factor present in the wild fly that prevents more than four bristles from developing. Although the *extra* race has something added somatically to it, genetically it has lost a restricting factor. Now this condition of extra bristles seems not to be phylogenetically new, as studies on the chaetotaxy of related flies suggest. The mechanism that is most concerned must have appeared when *Drosophila ampelophila* arose, or before, so the extra bristles are not formed by the origin of a new mechanism, but rather by the removal of the restriction of the more recent mechanism.

THE SELECTION PROBLEM

The main problem to which the preceding experiments have been contributory, has been the attempt to throw some light on the question of selection as a formative process in evolution. The attempt has been made to increase the numbers of thoracic bristles as much as possible by selecting and inbreeding. Accordingly, after the first pair that came from wild stock, high grade brothers have been mated to high grade sisters. In the following account the first eleven generations of this process will be described.

1. DO HIGHER GRADE PARENTS PRODUCE HIGHER GRADE CHILDREN?

To show what basis there may be for selection to progress, comparisons have been made of children from high and low parents from the same grandparents. All the matings in F_1 can be used for this purpose, as all the parents were sibs. Curves showing the 35 F_2 fraternities were plotted, but they were too numerous for publication. The children from parents of like grades have been grouped together. Table 7 shows the averages of the males and females, the numbers of flies and of families involved in the various types of matings. No increase in the means is found that directly corresponds to the increase in parental values. However, in grouping together children from parents with one or two extra, and comparing them with children from parents with three or four extra, the higher group has, in general, higher means. The low means of the one 4:4 mating involves too few individuals to make a serious exception. In the second generation there seems to be a basis for some effective selection.

Similar comparisons can be made in the later generations. Seven families in F_4 came from the same grandparents. Thirteen of the parents were + 5 and one, + 6. Here is a case where the parents are closely alike and one would expect the children in the different families to be closely alike. The curves and the

TABLE 7

To show the relationship between the grades of the parents and the means of their children in the second selected generation

GRADES OF PARENTS	NUMBER OF MATINGS	MEANS		NUMBERS	
		Male	Female	Male	Female
1:1	4	1.70	2.01	397	418
1:2	3	1.45	1.88	367	375
2:2	17	1.42	1.84	2061	2204
3:3	3	2.05	2.46	171	229
3:4	2	1.94	2.39	207	220
4:4	1	1.61	1.98	33	41

averages (fig. 3 and table 8) show that this is not the case. The averages for females range from +2.83 to +4.05. This set of families gives some idea as to how much variation is possible when the grandparents are brother and sister and the parents all sibs of the same grade.

In F_6 are two sets of cousins. In one group are five families (fig. 3B), grandchildren of 115, with parents of various grades. Considering the female averages (table 8B) it is seen that the parental sums of 7, 8a, and 12 give similar filial averages, whereas the parental sums of 8b and 14 give higher averages. Considering the males, parental values of 7, 8b, and 12 give similar

TABLE 8

Six groups of cousins. In each group the parents are sibs; grades of parents and means of offspring arranged according to the sums of the parents' grades. See figure 3

BOT. NO.	PAR- ENTS		SUM OF PAR- ENTS	MEANS OF CHILDREN		NOS. OF CHIL- DREN		BOT. NO.	PAR- ENTS		SUM OF PAR- ENTS	MEANS OF CHILDREN		NOS. OF CHIL- DREN	
	♂	♀		♂	♀	♂	♀		♂	♀		♂	♀	♂	♀
A children in F ₄								D children in F ₇							
71	5	5	10	2.29	3.53	58	68	177	4	4	8	2.61	3.90	278	216
72	5	5	10	2.46	2.83	115	105	166	5	6	11	3.19	5.16	41	30
77	5	5	10	3.15	4.05	60	55	178	5	9	14a	3.00	4.98	73	75
87	5	5	10	2.81	3.37	37	32	180	6	8	14b	2.85	5.19	27	31
88	5	5	10	2.66	3.91	63	71								
89	5	5	10	1.98	2.74	58	58	E children in F ₁₀							
92	6	5	11	2.87	3.13	16	22	289	5	5	10	3.28	4.40	68	77
B children in F ₆								284	6	5	11	2.75	3.88	122	120
149	2	5	7	2.91	4.17	91	81	281	5	7	12a	3.25	4.57	31	19
135	4	4	8a	2.36	4.15	90	89	290	6	6	12b	3.40	5.46	15	13
134	4	4	8b	3.01	4.66	75	93	F children in F ₁₁							
148	3	9	12	2.95	4.23	39	38	413	5	5	10	3.41	4.61	48	42
132	6	8	14	2.78	4.97	50	35	411	6	5	11	3.98	5.73	46	38
C children in F ₆								329	6	6	12a	1.76	2.72	102	80
141	3	4	7	3.27	4.54	77	90	412	6	6	12b	3.20	4.54	29	37
155	4	4	8	3.00	4.63	41	11								
146	2	7	9	2.22	3.90	66	52								
144	5	6	11	2.67	3.71	80	98								
142	6	8	14a	2.60	4.73	41	69								
153	5	9	14b	2.97	4.76	37	26

Fig. 3 Six groups of cousins; in each group the parents are sibs. Solid lines are females, broken lines males. The parents are indicated by squares (males) and triangles (females) above the curves of their children. The fractions following the numbers of the mating indicate the grades of the parents. The numbers of males and females involved in the curves are given at one side. A, grandchildren of no. 56, in the 4th generation; B, grandchildren of 115, in the 6th generation; C, grandchildren of 116, in the 6th generation; D, grandchildren of 143, in the 7th generation; E, grandchildren of 246, in the 10th generation; F, grandchildren of 274, in the 11th generation.

averages, 14 a little lower, and 8a much lower. In the second group of families in F_6 (fig. 3C and table 8C) female averages from parental sums 7, 8, 14a, and 14b are similar, whereas those from sums 9 and 11 are a whole bristle lower. The male averages for parental sums 7, 8 and 14b are similar while those from 11 and 14a are lower, and from sum 9 a whole bristle lower.

In F_7 is a set of cousins consisting of four families (table 8D and figure 3D). In this group the low parental sum has the low male and female averages, whereas sums 11 and 14a and 14b are much alike. In F_{10} there is a group of cousins in four families (table 8E and fig. 3E). The female average for parental sum 12b is considerably larger than that for the sum 10, yet the female average for the sum 12a is about equal to that for the sum 10. Parental sum 11 has the lowest male and female averages. In F_{11} a group of four families (table 8F and fig. 3F) shows averages for parental sum 11 that are above averages for 10 and 12b. Parental sum 12a has averages much below any of the other families. This study of averages must be followed by careful observation of the figures referred to above, which show the frequency distributions of each of the families that has been considered. These curves have been plotted so as to enclose in each polygon similar areas, to facilitate comparisons. The actual numbers of flies are given both in the tables and the figures.

Further data bearing on the question of the relation between the grades of parents and children is to be found in the series of curves representing inbred lines through single matings (table 11 and fig. 6). One case must be especially emphasized. The last generation, No. 216, in the series beginning with No. 18 (fig. 6A) was produced by flies with no extra bristles. This generation which follows seven selections has a higher distribution than the preceding ones which were from high grade parents. There are no normals from these normal parents, and no females with less than two extra bristles. This extreme case is in accord with all that has been found in regard to the independence of the parental and filial grades. This is further borne out by these inbred lines in the comparison of parents and children in successive generations.

So the general conclusion seems to stand, that after the first few generations there is no close relationship between the grades of the parents chosen and the grades of the children. This appears to mean that in regard to extra bristles the flies in later generations do not differ genetically from each other; that in the later generations the variability in the number of extra bristles is due mainly, if not entirely, to conditions outside the germplasm. A further test of this would be to cross with normals flies with many extra and flies with few extra bristles and compare the results in the second generations. If the difference between the many and the few extra bristles be due to genetic factors, this would be shown in the F_2 of the crosses. Data is given that shows the distributions of extracted extra bristled flies from crosses of high and low *extras* with normals of a different race. To avoid any complication that might arise from not considering sexual differences, and at the same time to find evidence in regard to sex linkage, four types of crosses with wild flies have been made: high and low males, high and low females. In table 9 the data are arranged to facilitate comparisons between males and females of like grades, but by comparing alternate lines the relations between high and low grades are clear. The ratios and the distributions are practically alike in all cases (fig. 4). In order to make the curve for high males, including over 800 individuals, more easily comparable with the others, these data were plotted on the basis of 600.

In conclusion, comparisons of families in the second generation of inbreeding show a tendency for higher parents to produce higher children, but this is not found in the sixth or subsequent generations; in single inbred lines there is found singular independence of the parental and filial grades, seemingly normal parents being able to produce all *extra* children; by the analysis of crosses it is found in the ninth and tenth generations that, as should be expected from the foregoing statements, high and low bristle grades are genetically indistinguishable; that the variability found in the late generations is apparently not due to genetic factors.

TABLE 9

Showing that low and high grades of extra bristles give the same results when crossed with wild normals; also that reciprocal crosses show that no sex linkage is involved

P ₁	F ₁			F ₂										RATIO NORMAL TO EXTRA
	normal	1	2	normal	1	2	3	4	5	6	7	8	9	
Low ♂♂ × Wild ♀♀	341	2		1756	156	147	98	57	37	5	3		1	3.4:1
Low ♀♀ × Wild ♂♂	437	3	2	1841	188	159	119	48	33	10	4			2.8:1
High ♂♂ × Wild ♀♀	282	4	1	2870	307	223	183	89	38	13	4			3.3:1
High ♀♀ × Wild ♂♂	203			1954	192	166	109	64	33	6	5			3.3:1

Fig. 4 Extracted extra bristles appearing in F₂ of reciprocal crosses of high and low grades of extra bristles with normal wild, showing that there is no factorial difference between the high and low grade *extras* crossed.

2. RESULTS OF SELECTION

a. Total generations

On the basis of the conclusions just reached, we should expect to find selection effective in the first few generations and later, ineffective. Data will be presented to show the results of selection in the form of curves for total generations and for single lines. Owing to the difficulty of obtaining high grade females that were unquestionably virgin, the most rigid selection was not always possible, so that even in the later generations some matings were made of low parents. In order to avoid the possibility of hiding a real increase in the offspring from high grade parents by including the offspring from low grade parents,

the children having either parent below certain grades were omitted. Along with these were excluded all flies whose grandparents or more remote ancestors had been excluded. This reduced the numbers in the total generations very considerably, and this is especially so in the latest generations. In these resulting curves the parents of each generation are included in the curve of the preceding generation. In figure 5 the curves have been plotted in such a way as to include similar areas, although representing different numbers of individuals. For this reason the numbers of males and females included are put down beside the curves. The solid line represents females; the broken line, males. The small curves between the larger ones in each case represent the parents selected from the generation above, to produce the generation below.

Discussion of the curves for total generations. The original parents selected from wild stock were a male with one extra bristle (+1) and a female with two extra bristles (+2). Their children are represented in the first pair of curves. The large number of normal flies in this generation 44.90 per cent is due to the fact that the mother was not virgin. Disregarding these, the male and female modes are at +2. In the following generation the modes remain at +2 but the upper limit of the range goes up from +4 to +7. The flies chosen as parents from this generation, range from +2 to +7. In the third generation the modes are still at +2 but the male curve has moved a little higher and the female curve is markedly above the male. This is clearly seen by comparing the areas of the polygons above the vertical line drawn at +2. The flies chosen from this generation range from +4 to +7. In the fourth generation the female mode is raised to +3 and a clear advance is shown by the whole curve. Although the male mode is still at +2, the proportion of +1 males has decreased and the proportions of +3 and +4 have strongly increased. In the fifth generation the male curve has increased its range over the preceding generation but as a whole has fallen back a very little. The female curve has a much increased range, and a slight sag above the mode, otherwise no change. In the sixth generation

the male mode still holds to +2 but the proportion of +3 flies has grown to nearly equal the mode. The proportions of +4 and +5 flies have also increased although +5 is the limit. The female mode is actually at +5 but the expected mode is easily seen to be at +4. So far the progress and increase has been steady and unquestionable. The following five generations do not show any such advance. In generations seven, eight, nine, and ten the male mode remains at +2 and the proportion of +3 flies is very slightly less. In the eleventh generation the +2 and +3 males are equal, however the proportion of +1 flies has greatly increased in this generation, so this does not mean any general advance. The female curves have modes at +3 in the 7th and 8th generations, with +3 nearly as high. In the last 3 generations the female mode is at 4 and the curves are in general of the same kind.

To show what parents were used in the different generations more clearly than can be done by the curves, their distributions are shown in table 10. The averages of the parents are given in two columns at the left and the averages of their children are in two columns towards the right. This table gives a clear summary of the progress of selection. The averages of the children, males and females, show increase up to the sixth generation, when minor fluctuations appear seemingly without any significant change.

If the variability in the last five generations be amenable to selection, a gradual decrease in the standard deviation would be expected, even though some hypothetical physiological limit prevented the means and modes from advancing as before.

Fig. 5 Eleven generations of selection. Original parents indicated by a square (father) and triangle (mother) above F_1 broken lines, males; solid lines females. The flies chosen as parents are arranged in small curves above the curves of their children. In generations 8 and 9 the male parents were all grade 5 and are represented by a square the proper distance from the base line. A finely dotted line means males and females together. The curves are plotted in such a way as to include like areas, so the numbers of individuals included are given at the side of each curve. The upper limits are in some cases drawn on the base line to show that such extremes appeared, although in too small numbers to form a whole unit in plotting.

But the standard deviations show a gradual increase while selection is effective according to the curves and the means, and when selection has seemingly become ineffective, the standard deviations fluctuate, but do not show a gradual decrease.

b. Single lines

The most immediate facts are presented in the form of the history of five inbred lines through single matings, as shown by curves in figure 6, A, B, C, D, E. The grades of the parents selected from each generation are indicated below the curve of their own fraternity and above the curves representing their offspring. Squares are the males; triangles are the females. Four of the lines start from the same pair of first generation flies. The first two generations which are common ancestors of all four lines, are represented in only one set of curves. The parents of families Nos. 87, 71 and 88 are all sibs in family No. 56. The parents of family No. 116 come from family No. 88. The line headed by family No. 17 starts with a different pair of first generation flies. As the history told by these curves is clear it does not seem necessary to discuss each set of curves in detail as has been done for the total generations. In Table 11, A, B, C, D, E, the means for these curves are given. These curves give the clearest picture of the basic facts. Clear progress is shown in the first five or six generations. In each line are differences counter-balanced in the total generations, yet in no case do these differences modify the general conclusion drawn from the study of total generations.

3. SUMMARY

In brief, selection appears to have made advances for six generations. When large enough numbers are observed to afford a counter-balancing of the fluctuations of individual families, the advance is steady. That this increase is really genetic is shown by the increase in the distribution of *extras* extracted from a cross of long selected flies, over the distribution of *extras* extracted from a cross of unselected flies. In later generations

Fig. 6 Five inbred lines. The first parents of lines B and C (nos. 71 and 87) are both from no. 56 in line A; the first parents in line D are from 71 in line B; line E is from a different pair of F_1 flies. Males, broken lines; females, solid lines. The parents selected to produce the following generation are shown below the curves of their fraternity; males, squares; females, triangles. Curves are plotted to form polygons of equal areas; the numbers of flies involved are given alongside each curve. In E no. 114 there were too few individuals to plot on the same basis as the others.

TABLE 10

Showing the distributions of the parents selected and the averages and variability of their children in the eleven generations of inbreeding and selection.

PARENTS										OFFSPRING										
Generation	Distribution according to no. of extra bristles									Means		Generation	Nos. of individuals		Modes		Means		Standard deviations	
	1	2	3	4	5	6	7	8	9	Males	Females		Males	Females	Males	Females	Males	Females	Males	Females
F ₁	12	38	10	4	—	—	—	—	—	1.96	2.21	F ₂	3415	3703	2	2	1.494±.010	1.453±.009	0.912±.007	0.829±.006
F ₂	—	14	7	4	5	1	1	—	—	3.25	3.18	F ₃	1090	1185	2	2	1.916±.021	2.708±.022	1.035±.014	1.201±.017
F ₃	—	—	—	2	14	1	1	—	—	5.00	5.11	F ₄	769	776	2	2	2.219±.027	2.925±.031	1.122±.019	1.284±.022
F ₄	—	—	—	5	12	2	1	—	—	4.80	5.10	F ₅	662	724	2	3	2.300±.030	3.480±.035	1.147±.021	1.415±.025
F ₅	—	—	—	9	4	7	—	3	14	75	6.16	F ₆	934	1007	2	5	2.703±.026	4.055±.036	1.164±.018	1.729±.026
F ₆	—	—	—	6	27	10	2	2	14	96	5.79	F ₇	2105	2064	2	3	2.507±.015	3.694±.023	1.025±.010	1.530±.016
F ₇	—	—	—	—	11	4	2	1	—	5.00	6.22	F ₈	668	668	2	4	2.682±.031	3.878±.037	1.186±.023	1.407±.027
F ₈	—	—	—	—	6	2	—	—	—	5.00	5.50	F ₉	152	149	2	3	3.065±.069	4.382±.083	1.276±.049	1.504±.059
F ₉	—	—	—	—	10	4	5	1	—	5.30	6.40	F ₁₀	517	477	2	4	2.615±.035	3.800±.045	1.172±.025	1.469±.032
F ₁₀	—	—	—	—	6	13	1	—	—	5.70	5.80	F ₁₁	592	526	2	4	2.050±.044	3.831±.053	1.589±.031	1.801±.037

TABLE 11

Grades of parents and means of children in five inbred lines (see figure 6)

GEN.	BOT. NO.	PAR-ENTS		MEANS OF CHILDREN		NOS. OF CHILDREN		GEN.	BOT. NO.	PAR-ENTS		MEANS OF CHILDREN		NOS. OF CHILDREN				
		♂	♀	♂	♀	♂	♀			♂	♀	♂	♀	♂	♀			
A	2	17	2	2	1.53	2.03	170	192	C	4*	71	5	5	2.29	3.53	58	68	
	3	53	4	3	1.57	2.57	67	69		5	115	4	5	2.27	3.35	52	91	
	4	67	4	4	2.10	2.76	101	110		6	135	4	4	2.36	4.15	90	89	
	5	98	5	4	1.87	3.14	64	80		7	175	3	9	2.18	3.93	38	44	
	6	114	4	6	2.42	3.16	33	13		8	205	5	5	2.62	4.27	108	118	
	7	124	4	5	2.51	3.57	119	157		9	239	6	6	2.84	4.25	38	70	
	8	151	4	4	3.32	4.98	94	119		10	276	5	6	2.44	3.75	105	108	
	9	207	5	5	2.73	3.98	93	104		11	334	7	5	3.07	4.60	46	45	
	10	232	3	3	3.41	5.06	37	18		D	4*	87	5	5	2.81	3.37	37	32
	11	267	5	8	2.49	3.41	196	191			5	110	5	5	2.44	3.68	104	108
12	377	5	6	2.95	4.26	149	194	6	137		5	8	2.95	4.45	46	55		
B	2	18	1	1	2.23	2.59	129	117	7	173	5	5	2.83	4.59	109	97		
	3	56	7	6	2.50	3.44	116	125	8	206	5	6	2.91	4.26	96	87		
	4	88	5	5	2.67	3.91	63	71	9	250	4	7	2.67	3.73	134	127		
	5	106	5	5	2.47	3.57	124	94	10	287	6	6	2.47	3.36	119	131		
	6	131	4	4	2.50	3.20	187	203	11	325	5	5	1.65	2.28	105	123		
	7	187	6	6	2.01	2.42	170	172	E	5†	116	4	4	2.57	4.00	99	120	
	8	216	0	0	2.98	4.56	48	50		6	146	2	7	2.22	3.90	66	52	
										7	191	4	5	2.25	3.86	63	57	
								8	226	3	13	3.51	4.60	37	52			
								9	268	5	5	3.10	4.00	59	70			

* Preceding generations are those given under B.

† Preceding generations are those given under C.

(after the 6th) as far as the eleventh, no further advance has been detected. This is what the comparisons of different families from the same grandparents, or successive generations of inbred lines, had led one to expect. In the second generation there is a tendency for parents of higher grades to produce children of higher grade, while this is not the case in the later generations where high and low variates seem to have the same genetic potentiality, and in so far as this is true, the variability must be explained by extra germinal causes.

THE ROLE OF THE ENVIRONMENT

1. INFLUENCE OF FOOD

In seeking causes for variability in the number of extra bristles, studies have been made on certain factors of the environment, namely, food and temperature. It was early observed that if any normal flies came from a bottle of inbred selected flies they were apt not to appear among the first flies to hatch in that bottle. Also it became evident that more of the high grades did appear among the first flies hatched, than among the last. To make this clear the data was arranged to show the distributions of the flies counted on successive days from individual bottles. In many cases it was clearly shown that successive days showed lower and lower distributions of extra bristles. In other cases there was no such decline. However, in all cases the highest flies appeared among the first flies drawn off. That the falling off in bristle numbers is not due to any differences in the fertilized eggs is shown by the fact that whenever there is found such a decline in bristle numbers at the end of a bottle, the first flies hatched from the next bottle into which the same parents had been placed, show bristle numbers as high as those found at first in the preceding bottle. This was found to hold good for all cases, even though the parents were moved into three or more successive bottles. When the changes from bottle to bottle were frequent there was less falling off at the end of a bottle. Since the facts are so clear it seems needless to publish a large number of examples. Table 12 gives 3 ex-

amples from the 50 families so arranged. In mating No. 78, there was no falling off at the end of first bottle, but the second bottle shows very clear falling off. There is found, then, a certain relation between the time of hatching and the number of extra bristles, due to conditions external to the germplasm, namely, that the last flies from a bottle may be of lower grade

TABLE 12

Daily counts of progeny from the same parents in successive bottles, showing that when the bristle numbers are lower at the end of a bottle, the high grades are regained at the beginning of the next bottle.

MAT- ING NO.	BOT- TLE	MO.	DAY	0	1	2	3	4	5	6	7	8	MAT- ING NO.	BOT- TLE	MO.	DAY	0	1	2	3	4	5	6	7	8	
131	1st	10	14		1	1	2	4	5				145	1st	10	22			2	5	3	3	1			
			15		4	18	9	7	3							23		1	6	5	5	3	1			
			16	1	1	6	3	2	1							24		1	4	9	9	2	1			
			17		4	6	7	3								27	2	8	14	8	3	2				
			20	5	9	12	2									28	2	3	7	4	2					
			22		1		1									30		10	5	4						
	2d	22		8	8	26	14	15	7	2	1			2d			31		2	3			1			
		23	1	2	3	3						30						2	2	4	1	1				
		24		4	7	6	3					31						2	2	5	4	3	2			
		27	4	16	29	15	2					11					1		3	1	1	3				
		3d	24			3	5	5	8	8							3	1	4	14	9		1			
			27		2	9	20	21	6	1	1							6		1	4	1	1	1	1	
	28			4	2	1																				
	106	1st	9	23		1	4	10	2	2	1	1			78	1st	9	3		3	26	15	18	3		
				25	1	3	15	8	4									4	1	5	12	11	5			
				26		3	7	3										5		6	16	4	1			
				30	2	1	5	2										6		3	6	5	2			
				26		2	4	2	4	6	5	3		2				7		2	19	12	2	1		
28						7	15	13	12	1	1		8					2	9	2	2					
			30		3	3	2	3					2d				9		10	8	9	6				
			4	2	4	17	7					9						5	11	1	2					
			5	2	1	6	1					10							6	3	3					
			3d	4		3			1		1						11			4	2	5	2			
																	12		1	2	2	2				
																	15	1	3	7	3	1				
																	18		2	1	1					
												19						1	2							

than the first ones, and when this is true, the higher grades reappear in the next bottle

It is common knowledge that the last flies from a bottle are apt to be smaller than the first ones hatched. This decrease in the sizes of the flies depends probably entirely upon the amount of food the flies eat while larvae; this amount in turn depends

upon the condition of the food and the environment. The similarity in the occurrence of decreased bristles and decreased size at the last of a bottle led to the idea that there might be some relation between the two phenomena. To see if smaller flies are apt to have fewer bristles, the total body lengths of 1400 random flies have been measured and their bristles counted. The data have been arranged in correlation tables. From table 13 one finds that up to a certain body size there appears a sort of correlation, in that the smaller the fly, the fewer the extra bristles it is apt to show. Above this size (grade 500) there

TABLE 13

Males and females. Correlation between size and numbers of extra bristles

SIZE GRADES	NOS. OF EXTRA BRISTLES								
	0	1	2	3	4	5	6	7	8
300	1								
325		1							
350	2	5		1	1				
375	2	3	7	1	1				
400	2	3	13	2	1				
425	2	10	24	6	9	1			
450	4	35	73	52	20	3		1	
475	5	39	94	76	39	14	6	1	
500	2	15	76	90	74	41	19	4	1
525			11	18	32	38	17	5	1
550		1	7	13	20	16	15	1	2
575		1	5	3	6	5	5	1	
600			1	1	1	3	3		

appears to be no constant relation between the increase in size and the numbers of extra bristles. However, it will be noted that above this size there are no normals. It has been concluded that the factor controlling extra bristles is not sex linked; however, nearly all the curves of extra bristled flies that have been presented, have shown that the males have fewer bristles than the females. Correlation tables were made for males and females separately (tables 14, 15). These show strikingly enough, that the males have fewer bristles than the females and are smaller, no male being above grade 500. This does not mean

that a male may never be larger than this, but the comparison with the females is accurate since males and females were both used in each group of flies that was measured.

In the mass cultures of extra bristled flies the bristle numbers are somewhat lower and the number of normals higher than in the inbred lines, but the flies themselves being raised in larger numbers and so cramped in close together are much smaller

TABLE 14

Females. Correlation between size and number of extra bristles

SIZE GRADE	NO. EXTRA BRISTLES									
	0	1	2	3	4	5	6	7	8	
350	1									
375										
400										
425			3							
450		3	5	1	2					
475		2	7	6	3	2	2			
500		4	19	20	16	5	4	1		
525			5	1	9	9	4	1		
550		1	5	2	5	5	2	1	1	
575		1	2	1	3	1	1	1		
600			1			2	1			

TABLE 15

Males. Correction between size and numbers of extra bristles.

SIZE GRADE	NO. EXTRA BRISTLES									
	0	1	2	3	4	5	6	7	8	
325		1								
350		1								
375			1	1						
400			1	2						
425		3	3	4	2					
450	1	10	29	15	5	2				
475	1	14	29	15	11	7	1	1		
500	2	3	7	6	4	1				

TABLE 16

To compare children from low grade parents taken from mass cultures and raised in pairs, with children from high grade parents that have come from continuously inbred lines, selected for increase in bristle numbers

BOTTLE NO.	GRADE OF PAR- ENTS	OFFSPRING—NOS. EXTRA BRISTLES										MEANS
		0	1	2	3	4	5	6	7	8	14	
Parents from Mass cultures												
554	2:2	1	15	36	32	25	18	3				3.00
557	2:2	3	15	26	12	16	9	3	2			2.84
561	1:2	1	1	3	5	5	3	1	1		1	4.00
Parents from 16th generation of inbreeding and selecting up												
555	5:5		1	11	26	14	4	4	1			3.55
556	5:5	4	32	61	45	24	9					2.47
559	5:5	6	33	39	42	31	17	6			1	2.87

than the inbred flies mated in pairs. It is also found that the first flies that hatch from a new culture bottle are larger and have increased bristle numbers and low grade pairs separated from mass cultures give children of as high grades as are found in the inbred lines raised at the same time (table 16). The mass cultures, from which the parents in this table were taken, were started from the 5th and 6th selected generations and had been running eight months. The three inbred families shown from the 16th generation are the only ones mated very nearly at the same time as the three pairs taken from the mass cultures. This table has a deep significance in relation to the accomplishments of selection in the ten generations between the 6th to 16th.

2. DISCUSSION

Thus there is evidence to show that flies below a certain size are apt to have fewer extra bristles than larger flies and that the size is largely dependent upon the condition and amount of food, or, more generally, on the environment; further, that males are shorter and have fewer extra bristles than females, and that the differences between mass cultures and the inbred lines disappear when the flies from the mass cultures are bred in pairs. It seems as though the small underfed flies do not have enough material to develop as many extra bristles as the larger flies can.

The measurements used in the correlation tables do not make very satisfactory data, as the distention of the abdomen and so, the length of the fly, varies with the amount of food contained, but, however little light the tables may give as to the actual amount of correlation and influence of the environment, it is believed that the errors in measurement due to the varying abdominal contents are not great enough to prevent the conclusion that such an influence of environment does exist. The fact of most importance at present, whatever its explanation may be, is that the numbers of extra bristles are influenced in some direct or indirect way by the conditions in the bottles.

With these facts in mind it will be well to return to the generally low percentage of extras in the F_2 of crosses with normals of other races. It has previously been shown that apparent normals may none-the-less be homozygous for extra bristles; a plausible explanation of this fact seems to be the influence of food.

3. TEMPERATURE

The regularity with which a new bottle produces an increase in bristle numbers, if it follows a falling off at the end of the preceding bottle, shows that these variations are not mainly due to temperature. This does not say that temperature may not have a real, however slight, influence on bristle development.

A series of preliminary experiments were performed to test the influence of temperature. Sister matings were raised in various temperatures, constant to a small fraction of a degree centigrade. In one experiment different sets of eggs from the same parents were raised in various temperatures and compared with controls in room temperatures. Two main questions were to be answered: Can the number of extra bristles be increased by using higher or lower temperatures? Do constant temperatures, irrespective of the degrees, influence the variability of bristles and thus indicate an influence of temperature? In the nine matings employed, 1860 flies were observed. The data were all arranged in curves, males and females separated and together, but no clear evidence was found to show that temperature had any influence, either in producing higher or lower numbers of extra bristles or by changing their variability. It is very clear that temperature influences the bacterial and fungal floras of the fermenting banana, and due to the chemical activity of the food, the environment of the developing flies goes through wide variations, as is witnessed by the multitude of different shades of odors from a single bottle during its productivity. Should any relation between temperature and bristle number be found in later investigations, it would be extremely difficult to show that this supposed relation was not an indirect effect, act-

ing through the influence on the food. There can be no question that extra bristles are not influenced by temperature in the way that Miss M. Hoge found applied to extra legs.

DISCUSSION

The question in relation to which these experiments have most interest is whether Mendelian units can be modified by selection, and whether selection can accomplish anything more than a sifting and sorting of hereditary elements whose origin is still unknown. The tendency is either to hold that the hereditary elements can never be modified by simply propagating certain ones, or to hold that there is no integrity of such seeming elements or factors, and that almost anything can be accomplished by sufficiently long and painstaking selection.

Some of the observations will admit both interpretations. According to the selectionist's view, the modification of the extra bristles appearing in a cross with normal, indicates that a factor has been modified. The restricting factor of the normal gamete may have transferred some of its restrictive properties to its allelomorphic mate in the *extra* germplasm, so that all the children formed by the union of two so modified *extra* gametes, would have fewer extra bristles than if the gametes had been unmodified. Selection made steady advances at first, showing that the gametes of flies of higher grades must be somewhat different from those of the lower grades, and therefore, the factor for extra bristles must be a variable thing, however truly a Mendelian unit. That the progress of selection does not continue after the sixth generation may only mean that the conditions have been so irregular or unfavorable that the real phenomena of the germplasm were entirely veiled.

On the other hand, these same facts may be used to form the basis of an interpretation involving one or more smaller, or accessory, restricting factors, which are found in many low bristled flies (some flies may lack all restricting factors, and still be low grade on account of their small size). The more and

more complete removal of these in successive generations by selection will permit the numbers of bristles to gradually increase. However, when all the flies become homozygous for the absence of the accessory restricting factors, no further increase by selection could be expected. Since there were no greater irregularities in the environment apparent after, than before, the sixth generation, and since flies from the fifth, sixth and seventh generations were being raised on the same lot of food and at the same time, the other interpretation of the failure of selection becomes very weak. And when the evidence showing that there is apparently no genetic difference in the later generations between flies of different extra bristle grades, is reviewed, including as it does the weighty finding that seemingly normal parents may produce higher grade *extra* children than preceding high selected ancestors and the conclusion that after nine selections, high and low bristle grades give the same result in crosses, when all these facts are born in mind, it will be realized that the interpretation of the results by accessory factors seems in closer agreement with the facts than does the alternative hypothesis.

The hypothesis of accessory factors needs no elaboration or change to explain the phenomena that accompany crosses with a normal race. The main factor for restriction keeps the number of bristles down to four whenever it is present, so the accessory factors can only be detected when the main one is absent. For this reason a simple Mendelian ratio may be found. From the selected flies some if not all the accessory factors have been removed. These would be present in the normal race used in the cross, and, due to the segregation of these independent restrictors, one would find in F_2 among the flies lacking the main restrictor, all combinations of the accessory factors, forming groups with various restrictive powers from strong to weak. The strongly restrictive groups would make the bristle numbers lower than in the uncrossed flies under the same conditions, while the weakly restrictive groups would make slight or no modification. This would result in an increase in the proportions of flies with few extra bristles, yet the high bristle grades would still be found. This is shown to be the case in figure 2,

where the inbred and extracted distributions are compared. The first set of curves show this fairly well, but in the second set, in which the extra parents had been selected much longer, the phenomenon is very clear. On the other hypothesis, if the factor for extra bristles were modified in F_1 , one would expect to find the whole curve of the F_2 *extras* lowered, and another supposition would have to be made to explain the occurrence of high grades. Such an added supposition would involve the modification of the factor, sometimes in various degrees, and other times not at all—a supposition easy to make (Castle '14) but difficult to explain.

That there is a greater modification in the F_2 extracted *extras* when the *extra* parents had been selected for several generations, than when they had not been selected, is the result expected if the selection had accomplished no more than to drop out certain accessory restricting factors, which being present in the wild parent would produce their effect in the second generation at the normal end of the distribution just as strongly as they did in the crosses before selection. To interpret this without using accessory factors one would have to suppose that a factor for extra bristles, that had been made more extra by selection, was more susceptible to contamination, just as squeezing a sponge will make it take up more water; but as has been shown, this kind of a mechanism will not explain the occurrence of the high extremes in this modified distribution. When one considers that the extra bristled condition is due, not to a single factor of which anything is known, but to the nucleus of determiners that carry the main heritage, such a modification hypothesis becomes vague and confused.

The occurrence of extras in the F_1 of the crosses may be explained by incomplete dominance, in which case the proportion of F_2 extracted *extras* should be too high instead of too low. Their occurrence may be explained by a heterozygous condition of the restricting factors in the wild New York 1912 race, which had been escaped being weeded out by selection. The occurrence of *extras* in the New York 1912 race suggests such a heterozygous condition. It seems probable that the slightly low propor-

tions of *extras* in F_2 is connected with the modification of their distribution. A strongly restrictive group of accessory restricting factors, even in the absence of the main restrictor may produce a normal fly, especially if the fly happens to be small.

Much of the recent genetics work supports the principle of accessory or multiple factors. According to this principle more than one independent factor may influence a single character. Besides the cases which prove unquestionably the existence of such multiple factors by means of definite ratios, as the ligula in oats and the red grain in wheat (Nilsson-Ehle '09), yellow endosperm in maize, (East and Hayes, '11) and the triangular form of capsule in shepherds purse (Shull '14) there are a large number of investigations in which the presence of multiple factors is strongly indicated. Since the author has already discussed such cases (MacDowell '14 a and b) it will be necessary only to add references to the recent work of Wichler ('13), Ikeno ('14), Hayes ('14), Davenport ('13), Lotsy ('13), Phillips ('14) and Punnett ('14), and to call attention to the critical discussion of Shull ('14). Investigations showing transgressing segregation, in which the F_2 distributions form continuous gradations but with modes dividing the individuals into groups corresponding to 3:1, or 1:2:1, (Balls '07, Leake '11, Biffen '05) have been interpreted by an hypothesis involving one main factor and accessory factors, similar to the one employed above. Still closer resemblance to the work herein described is that of Castle and Phillips ('14) with piebald rats. They dealt with a variable character that proved to be influenced by a Mendelian factor. The distribution of the variations of this character was modified by crosses. No hypothesis other than that of accessory factors could be found to explain all the results. However, Castle still holds that the continued success of selection goes to prove the modifiability of a Mendelian factor. The main difference between the investigations on the piebald rats and the extra bristled flies, aside from the fact that no minus race of flies has been established, lies in the fact that in the case of the flies the progress of selection does not seem to continue after the sixth generation, while in the rats it appears to continue as far as the

selections have been made. Another difference that becomes illuminating in the light of these facts is that the flies have been inbred absolutely, while in the rats the inbreeding was of a fairly low degree. Return selection proved equally effective in the piebald rats. If the interpretation of accessory factors be accepted for the results of crossing the rats, it is evident that at least part of the success of selection was due to the sorting out of these accessory factors. Now the fact of a successful return selection can not be sighted in this case to prove the theory that a factor has been modified, since as long as selection was showing steady progress, it should be possible to start a successful return selection. As long as the original selection was progressing there still is evidence of some heterozygosis of the accessory factors. This would afford a basis for the return selection. At this time any positive statement would be premature, but the results given by an attempted return selection of the extra bristles after the upward progress had seemed to stop, appear to indicate that this return selection is ineffective. Finally the more complicated hypothesis of Pearl ('12) supported by his extensive investigations on fecundity in fowls, must be sighted as one based fundamentally on the conception of multiple factors.

Taken then on their own merits, the results presented in this paper do not give critical evidence in support of either the hypothesis of modification or of accessory factors. However, the failure of selection after success for six generations and the probable genetic equality of the various bristle grades in the later generations, seem to bear the balance strongly towards the hypothesis of accessory factors. Taken in the light of much of the recent genetics investigations, many of which have close theoretical similarities, it is almost impossible to avoid the conclusion that the interpretation of accessory factors is the more probable. Besides, this hypothesis affords a more thinkable mechanism and is more readily understood and tested. For these reasons this interpretation has been adopted at least as a working hypothesis upon which to base further investigations. Experiments are already under way to attempt the isolation of accessory factors and, by crossing, to prove unquestionably their existence.

CONCLUSIONS

From a pair of wild flies a race of *Drosophila ampelophila* has been established which has regularly more than the normal four thoracic bristles.

By selecting high grade parents and inbreeding brother to sister, the number of extra bristles was gradually increased for six generations.

From the seventh to the eleventh generations fluctuations were found showing no further increase.

The maintenance of the high grades of extra bristles does not depend upon selection as, low grade parents from mass cultures started from the fifth and sixth generations that have run eight months, when raised in single pairs, give as high grade offspring as inbred and selected parents mated at the same time.

A Mendelian factor is involved in the inheritance of extra bristles, and as *normal* dominates *extra*, this may be regarded as a dominant factor that restricts the number of bristles to four.

This factor is not sex linked, although males are apt to have fewer extra bristles than females.

The extracted extra bristled flies have a lower distribution than that of the inbred flies of the corresponding generation, although the high extremes of the inbred race are also found among the extracted *extras*.

There is a greater difference between the inbred and extracted distributions when the cross is made after eight selections than when made after only one selection.

Environment influences the number of extra bristles, and since small flies are not apt to have as many extra bristles as large ones, it appears that the amount of food eaten is an important factor.

From the above statement an explanation may be found for the fact that an apparently normal fly may be genetically homozygous for extra bristles.

The hypothesis of accessory factors will explain all the facts, and that of modification of a Mendelian factor may be employed to interpret most of them.

The similarity with much recent work that has given more or less positive evidence of multiple and duplicate factors, persuades the author that the hypothesis of accessory factors is probably the best one for the facts.

The following hypothesis is adopted upon which to base further work: the extra bristles in *Drosophila ampelophila* are due to the absence of one main restricting factor, and their number is also influenced by accessory restricting factors, which, in the absence of the main one, produce flies with reduced numbers of extra bristles.

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