AN EXPERIMENTAL APPROACH TO THE STUDY OF MULTIPLE PEAK EPISTASIS IN *DROSOPHILA MELANOGASTER**

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Summary

In an attempt to demonstrate an existence of multiple peak epistasis in scutellar bristles of *D. melanogaster*, mass selection on total number of scutellar bristles was practised in 1 large and 10 small lines in each of three crosses, F_1 and two backcrosses, between the two unrelated isogenic lines, Oregon-R and Sevelen.

Results of 22 generations of selection showed that in all lines the genes modifying scutellar expression were located on the second chromosomes. The magnitude of the response was the same in the large lines, but less and varied in the small lines. These results were discussed in relation to the "multiple peak" hypothesis.

I. INTRODUCTION

It is well recognized that in many situations a gene replacement does not contribute to the selective value in the same sense in all possible combinations, a phenomenon referred to as epistasis or gene interaction. The potential mode of epistasis varies tremendously. It is possible, however, to classify all systems of epistasis into "single peak" or "multiple peak" where peak has the meaning coined by Wright (1932). That distinction is important in natural and artificial selection. In single peak systems initial gene frequency has no bearing (other than that exerted by drift) on the ultimate array of gene frequencies that a population approaches as a consequence of recurrent selection. In other words, the fate of a population under selection is not affected by the epistasis involved. In multiple peak systems, however, the peak most likely to be attained as a result of selection may differ depending on initial gene frequency. For discussions of the role multiple peak epistasis plays in evolution the reader is referred to Wright's articles in 1932 and 1959.

The purpose of this study was to determine whether multiple peaks could be demonstrated when the genetic segregation and recombination was of the magnitude represented in populations commonly employed in selection programmes in experimental quantitative genetics or in plant and animal breeding.

II. MATERIALS AND METHODS

Fraser *et al.* (1965) selected for increase in scutellar bristles of *Drosophila melanogaster* in small populations (size of several pairs) initiated from single females. Two features of those results significant to the present study were: (1) lines varied in total response to selection, and among those reaching the highest level there were sharp variations in rate of response; and (2) evidence

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was obtained that lines reached a high level of response by different mechanisms (either 1st or 3rd chromosomes). Those results may indicate that the genetic system on hand might be that of multiple peak. Any clear interpretation, however, is complicated by the fact that populations were very small, where drift was expected to play a major role, and no attempt was made to maintain a constant or near constant selection intensity.

In an attempt to demonstrate multiple peaks, two isogenic lines of *D. melanogaster* (Oregon-R and Sevelen) were used. At the start of the experiment Sevelen had been maintained for 118 generations by brother–sister mating and Oregon-R for 44 generations. Oregon-R originated in Oregon and Sevelen originated in Switzerland, indicating a probable wide divergence in their genotypic arrays and a cross between them likely to produce a high level of genetic variability.

TABLE 1

HARMONIC MEAN EFFECTIVE POPULATION NUMBERS (N_e) AND SELECTION INTENSITIES (k) FOR EACH OF *OR-SV*, *SV-BC*, AND *OR-BC* LINES

Generation numbers are given in parenthesis

Line	*OR-SV*			*SV-BC*			*OR-BC*		
	k(1–8)	k(9–22)	N_e	k(1–8)	k(9–22)	N_e	k(1–8)	k(9–22)	N_e
Large	1·24	1·62	75·9	1·24	1·61	71·7	1·24	1·63	79·3
Small									
S_1	1·20	1·57	12·18	1·20	1·57	11·10	1·18	1·59	9·84
S_2	1·17	1·57	11·44	1·20	1·57	11·73	1·19	1·58	11·87
S_3	1·21	1·57	11·05	1·17	1·57	10·16	1·19	1·57	11·32
S_4	1·20	1·58	11·01	1·19	1·56	9·79	1·18	1·55	10·71
S_5	1·20	1·58	10·48	1·19	1·58	11·72	1·18	1·60	11·39
S_6	1·20	1·58	11·55	1·17	1·58	10·98	1·21	1·51	6·39
S_7	1·19	1·57	11·55	1·20	1·59	11·46	1·20	1·58	12·66
S_8	1·19	1·60	12·06	1·19	1·58	12·21	1·20	1·60	10·04
S_9	1·19	1·59	9·68	1·19	1·60	11·61	1·21	1·56	12·03
S_{10}	1·18	1·59	10·38	1·21	1·58	10·05	1·21	1·58	11·74

Oregon-R and Sevelen were crossed to give an F_1 base population (*OR-SV*), then the F_1 was backcrossed separately to each of the parents to give rise to Oregon-backcross (*OR-BC*) and Sevelen-backcross (*SV-BC*) base populations. In each of the three base populations two control lines, one large (L), and 10 small lines (S_i) were initiated. A large population consisted of 40 random-pair matings (40-1-8) with four male and four female progeny scored per mating. Mass selection was practised among the 320 progenies for increase in total numbers of scutellar bristles; 40 males and 40 females were selected as parents of the next generation. The same was true of a small population which had six-pair matings (6-1-8) and four male and four female progeny per mating. As a result of the mating scheme the top 25% of males and females were selected in each generation. However, in the 9th generation selection was raised to 12·5% by scoring eight males and eight females per mating and keeping the total number of pair matings at 40 for a large population and 6 for a small population. Pair matings were performed in vials. The control lines were maintained in bottles under mass mating. 100 males and 100 females were scored and transferred each generation.

Table 1 presents the harmonic mean effective population number (N_e) and average selection intensities (k) for each population over generations. Due to the failures of some matings in each generation, N_e and k could not be kept constant. The variation, however, was at random (no evidence exists to indicate at this stage that a mating failure is correlated with the scutellar number of the pair) and of the same general magnitude for each population. The effective

population number (N_e) for each generation was calculated from the equation of Kimura and Crow (1963):

$$N_e = (N_{t-1}-1)\bar{k}/(1+V_k/\bar{k}),$$

where $\bar{k}$ = the mean progeny number per parent = 2, and V_k = the variance in progeny number.

The effective population number as defined is for genes under no selection. It is realized that N_e would probably decrease, but not greatly, with directional selection. The selection intensity (k) was calculated from the properties of the normal curve for a large population and from table 20 of Fisher and Yates (1938), based on ordered statistics for small populations. Scutellar bristles were scored according to Fraser (1963) into anterior right (*ar*), anterior left (*al*), posterior right (*pr*), and posterior left (*pl*). The extra scutellars were all *ar* or *al* and a χ^2 test showed no significant deviation from a 1:1 ratio indicating that each site develops a bristle at random with a probability of a half. Consequently the analysis was done on the total number ($ar+al$) of bristles for each fly.

From the foregoing it is clear that three large populations (F_1 and two backcrosses), where the effect of drift due to small population size is minimal, were started at three different initial gene frequencies, namely 0·5, 0·25, and 0·75. Also 10 small lines where drift is a major factor were initiated from each of the three base populations. The selection intensity was maintained at near constant for all populations under selection. Comparisons were made in rate and magnitude of response among the large populations of different base and among the 10 small populations and large population of the same base. Chromosomal analysis was performed at the end of 16 and 19 generations for the large populations and at the 19th generation for the large and small populations of each base. Of the small populations only lines that showed considerable response were analysed. These included S_7, S_2, S_5, S_9, and S_{10} for *SV-OR* lines; S_3, S_2, S_{10}, S_7, and S_1 for *SV-BC* lines, and S_2, S_9, S_5, and S_6 for *OR-BC* lines.

TABLE 2

COMPARISONS OF MAIN CHROMOSOMAL EFFECTS AND THEIR INTERACTIONS

Chromosomal Effect	+/+; +/+; +/+	+/+; +/+; *Ubx*/+	*ClB*/+; +/+; +/+	*ClB*/+; +/+; *Ubx*/+	+/+; *Cy*/+; +/+	+/+; *Cy*/+; *Ubx*/+	*ClB*/+; *Cy*/+; +/+	*ClB*/+; *Cy*/+; *Ubx*/+
1	0	0	0	0	0	1	0	−1
2	0	0	0	1	0	0	0	−1
3	0	0	0	0	0	0	1	−1
1×2	0	1	0	−1	0	−1	0	1
1×3	0	0	0	0	1	−1	−1	1
2×3	0	0	1	−1	0	0	−1	1
1×2×3	1	−1	−1	1	−1	1	1	−1

The chromosomal analysis was the same as that used by Fraser *et al.* (1965) and described in greater detail by Scowcroft (1966). Inversion-marked chromosomes used in the analysis were *ClB* for the 1st chromosome, *In* (2*L*+2*R*) *Cy* for the 2nd, and Ubx^{130} for the 3rd chromosome. 25 *ClB*/+; *Cy*/+; *Ubx*/+ virgin females were crossed to 60 +; +/+; +/+ randomly chosen males of a selected population. From that, 25 *ClB*/+; *Cy*/+; *Ubx*/+ virgin females were backcrossed to 100 males of the same population. There were two replications (two bottles) of the latter cross per population. Each bottle was transferred at the end of 3 days for a total of four or five transfers. Female progeny in each bottle were segregated into the eight classes presented in Table 2 and 20 flies were scored within each class. An analysis of variance (Table 3) with a fixed model was performed on the logarithm of the means of total bristle number. Each cell mean was based on the two replications and 20 scores per replication. The distribution of scutellar bristles is not normal when the mean is close to the threshold of four bristles. It can be viewed as a truncated normal where the truncation is at the threshold. Thus it is obvious that the variance and mean are positively correlated, which makes the data suitable for a logarithmic transformation. The analysis of variance on the means is justified, however, by the fact that the means are likely to be normally distributed.

As in previous experiments on scutellar bristles the genes contributing to selection advance appeared to be recessive. Therefore, the triple heterozygote *ClB*/+ ; *Cy*/+ ; *Ubx*/+ was considered as a control for assessing the magnitude of response on separate chromosomes or combination of chromosomes. Coefficients for the comparisons of main chromosomal effects and interactions are presented in Table 2. Each effect has one degree of freedom and can be tested against its error variance (second-order interaction) in the analysis of variance (Table 3).

TABLE 3

ANALYSIS OF VARIANCE FOR THE THREE LARGE LINES IN GENERATIONS 16 AND 19 AND FOR LARGE AND SMALL LINES WITHIN *OR-SV*, *SV-BC*, AND *OR-BC* BASES IN GENERATION 19

The experimental unit was the logarithm of the total scutellar number. Generation numbers are given in parenthesis

Source of Variation	Large Lines (16)		Large Lines (19)		*OR-SV* (19)		*SV-BC* (19)		*OR-BC* (19)	
	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.	D.F.	M.S.
Total	119		96		191		191		159	
Class	7	0·01724**	7	0·01298**	7	0·03029**	7	0·02655**	7	0·01299**
Line	2	0·0009**	2	0·00484	5	0·00328**	5	0·00578**	4	0·01347**
Transfer	4	0·0002	3	0·00052	3	0·00226**	3	0·00178**	3	0·002856**
Class × line	14	0·00025*	14	0·00045	35	0·00065**	35	0·00022	28	0·00089**
Class × transfer	28	0·00026**	21	0·00012	21	0·00035*	21	0·00020	21	0·000305**
Line × transfer	8	0·00036**	6	0·00103*	15	0·00024	15	0·00043**	12	0·000305*
Line × transfer × class (error)	56	0·000118	42	0·000343	105	0·00020	105	0·000165	84	0·000138

* Significant at the 5% level. ** Significant at the 1% level.

III. RESULTS

For each generation the mean scutellar number of each line was calculated and its deviation from the average for the two replications of the control was observed. Tables 4, 5, and 6 list the deviations from control for 22 generations of selection. A linear regression of deviation on generation number was calculated, and the regression coefficients tabulated. It is seen that the selection response was remarkably similar in the large lines of *OR-BC*, *OR-SV*, and *SV-BC* (Tables 4, 5, 6, and 7). In *OR-BC* and *SV-BC*, selection response of the small lines was invariably less than that of the large lines. This is evident by the fact that the regression coefficients for the small lines were significantly less than that for the large lines (Table 7). For *OR-SV*, three small lines (S_2, S_7, and S_{10}) were equivalent in response to the large line, whilst the rest were significantly less. As expected, the variability in response over generations was in general larger for the small lines than for the large line.

In the analysis of variance (Table 3) the interest was in class and class × line interaction. For the larger populations the class mean square was highly significant in generations 16 and 19. The class × line mean square, however, was only significant at the 5% level in generation 16. For the lines of *OR-SV*, *SV-BC*, and *OR-BC* in generation 19 (Table 3), the class mean square was again highly significant. The class × line mean square was highly significant for *OR-BC* and *OR-SV*, but not for *SV-BC*. The class × line interaction was further examined by testing for significance of class comparisons (Table 2) within lines. A comparison within each line was based on the total over replications for each class. Results of those comparisons (Table 8)

showed that, for the large lines, chromosome 2 main effect was highly significant both in generations 16 and 19. Other significant comparisons were not consistent in generations 16 and 19 and not much importance was attached to them. Class

TABLE 4

DEVIATIONS FROM CONTROL OF MEAN SCUTELLAR BRISTLES IN EACH GENERATION FOR *OR-BC* LINES

Generation	Control	Large Line	Small Lines									
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
1	4·06	−0·02	0	0	0	−0·02	−0·02	−0·06	−0·06	−0·06	−0·02	−0·02
2	4·14	−0·04	0·24	0·12	0·03	0·03	0·08	−0·14	0·03	0·15	−0·14	0·03
3	4·07	0·01	0·14	0·14	0·15	0·30	0·01	−0·03	0·18	0·10	−0·03	−0·07
4	4·05	0·10	0·43	0·25	0·13	0·08	0·16	0·08	0·12	0·12	0·12	0·03
5	4·03	0·15	0·07	0·16	0·30	0·17	0·06	0·05	0·18	0·01	0·10	0·01
6	4·11	0·06	0·15	0·19	0·15	0·09	0·23	0·15	0·17	0·16	0·13	−0·02
7	4·04	0·09	0·12	0·22	0·06	−0·02	0·05	0·35	0·06	0·55	0·10	−0·07
8	4·07	0·30	0·14	0·54	0·13	0·09	0·57	0·48	0·0	0·46	0·33	0·04
9	4·07	0·24	0·38	0·49	0·21	0·06	0·31	0·03	0·0	0·55	0·09	0·02
10	4·07	0·22	0·40	0·41	0·27	0·05	0·32	0·09	0·06	0·60	0·25	0·12
11	4·12	0·54	0·49	1·10	0·44	−0·10	0·21	0·27	0·0	0·43	0·04	−0·10
12	4·08	0·90	0·52	0·77	0·33	−0·02	0·67	0·30	−0·03	0·45	0·09	−0·06
13	4·13	0·97	0·40	0·64	0·23	0·0	0·64	0·14	0·05	0·73	0·05	0·02
14	4·14	0·89	0·29	0·55	0·46	0·04	0·39	0·14	0·01	0·71	0·05	0·01
15	4·14	1·08	0·61	0·90	0·60	0·27	0·50	0·34	0·03	0·49	0·08	0·18
16	4·09	1·34	0·75	0·97	0·77	0·17	0·81	0·58	0·41	0·93	0·41	0·04
17	4·11	1·16	0·68	0·80	0·46	0·37	0·79	0·93	0·44	0·49	1·0	0·06
18	4·12	1·26	0·86	0·65	0·15	0·31	0·64	0·66	0·18	0·81	0·95	0·08
19	4·14	1·25	1·04	0·86	0·47	0·43	1·21	0·69	0·37	0·77	1·03	0·48
20	4·06	1·54	1·11	0·82	0·64	0·64	1·03	0·77	0·54	0·85	0·55	0·43
21	4·04	1·60	1·04	1·0	1·13	0·47	1·42	1·17	0·87	0·73	1·02	1·04
22	4·14	1·37	0·92	0·13	0·75	0·23	1·15	0·47	0·47	0·21	0·31	0·24

TABLE 5

DEVIATIONS FROM CONTROL OF MEAN SCUTELLAR BRISTLES IN EACH GENERATION FOR *SV-BC* LINES

Generation	Control	Large Line	Small Lines									
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
1	4·18	0·16	0·28	0·61	0·17	0·08	0·17	0·28	0·23	0·18	0·07	0·30
2	4·10	0·12	−0·01	0·51	−0·01	0·08	0·13	0·40	0·03	−0·02	0·16	0·28
3	4·04	0·09	0·04	0·37	0·14	0·34	0·05	0·36	0·04	0·21	0·17	0·28
4	4·14	0·18	0·11	0·36	0·12	0·59	0·15	0·19	0·07	−0·09	0·19	0·34
5	4·08	0·35	0·35	0·22	0·42	0·65	0·32	0·34	0·32	0·17	0·63	0·71
6	4·09	0·33	0·30	0·26	0·49	0·24	0·16	0·41	0·33	0·29	0·26	0·54
7	4·17	0·07	0·37	0·16	0·01	0·83	0·13	0·15	0·18	0·09	0·38	0·54
8	4·11	0·31	0·35	0·15	0·29	0·67	0·02	0·06	0·07	0·18	0·18	0·72
9	4·15	0·28	0·29	0·44	0·70	0·32	0·22	0·21	0·20	0·56	0·18	0·63
10	4·10	0·61	0·43	0·40	0·90	0·57	0·58	0·18	0·45	0·66	0·41	0·81
11	4·05	0·30	0·35	0·20	0·20	0·11	0·30	0·20	0·66	0·34	0·24	0·78
12	4·09	0·49	0·53	0·44	0·47	0·42	0·15	0·24	0·44	0·29	0·38	0·74
13	4·05	0·42	1·05	0·58	0·35	0·62	0·48	0·40	0·72	0·46	0·50	0·87
14	4·12	0·47	0·79	0·36	0·46	0·62	0·19	0·26	0·34	0·48	0·12	0·82
15	4·08	0·94	1·27	0·78	0·71	0·38	0·52	0·60	0·80	0·72	0·77	0·69
16	4·07	1·24	0·42	1·10	1·11	0·37	0·47	0·19	0·62	0·51	0·59	0·93
17	4·06	1·33	0·42	1·10	0·64	0·74	0·70	0·72	0·77	0·67	0·70	1·04
18	4·04	1·50	0·47	1·05	0·85	1·22	0·53	0·39	0·30	1·12	0·75	0·66
19	4·06	1·65	0·72	1·27	0·86	1·53	1·04	0·41	0·39	1·34	0·63	0·94
20	4·01	1·85	0·50	0·63	1·45	0·94	0·96	0·69	1·06	0·77	1·67	0·86
21	4·06	1·60	0·45	1·13	0·45	1·45	0·68	0·33	0·11	0·63	0·92	0·62
22	4·02	1·56	0·55	1·05	0·54	1·04	1·01	0·25	0·48	0·50	0·90	1·05

comparisons of lines within *OR-SV*, *SV-BC*, and *OR-BC* showed that chromosome 2 main effect was highly significant. Chromosomes 1 and 2 interaction effect was negative and significant in S_{10} of *SV-BC*, in S_6 of *OR-BC*, and in S_9 of *OR-SV*.

TABLE 6

DEVIATIONS FROM CONTROL OF MEAN SCUTELLAR BRISTLES IN EACH GENERATION FOR *OR-SV* LINES

Generation	Control	Large Line	Small Lines									
			S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
1	4·10	0·04	−0·02	−0·06	−0·02	−0·02	0·15	0·03	−0·06	0·07	−0·01	0·08
2	4·17	0·06	−0·13	−0·17	0·09	−0·09	0·16	−0·01	0·08	0·09	−0·08	−0·11
3	4·03	0·16	0·05	0·10	0·10	0·14	0·36	0·10	0·22	0·02	0·06	−0·03
4	4·14	0·08	−0·05	0·02	−0·09	0·19	0·22	0·07	0·16	−0·04	−0·10	−0·10
5	4·11	0·08	−0·03	0·10	−0·11	0·02	0·43	−0·06	0·02	0·14	−0·01	−0·11
6	4·13	0·12	0·0	−0·09	0·20	−0·13	0·12	−0·13	0·05	−0·09	−0·13	−0·04
7	4·21	0·13	−0·13	0·06	0·40	−0·16	0·36	−0·07	0·12	0·01	−0·04	−0·17
8	4·05	0·35	0·03	0·22	0·12	−0·01	0·25	0·53	0·37	0·03	0·16	0·04
9	4·14	0·33	−0·08	0·15	0·38	0·01	0·60	0·32	0·37	0·13	0·62	0·05
10	4·06	0·59	0·13	0·11	0·19	0·03	0·92	0·17	0·57	0·21	0·66	0·26
11	4·06	0·50	0·08	0·14	0·22	0·08	0·60	0·23	0·64	0·19	0·60	0·54
12	4·10	0·71	0·16	0·24	0·20	−0·04	1·11	0·50	1·15	0·27	0·90	0·74
13	4·10	0·67	0·04	0·12	0·03	0·15	1·0	0·50	1·19	0·28	0·61	0·82
14	4·11	1·02	−0·08	0·38	0·16	0·28	0·65	0·34	1·54	0·15	0·59	0·42
15	4·09	0·85	0·03	0·52	0·19	0·35	0·87	0·37	1·29	0·08	0·58	0·85
16	4·09	1·07	0·12	1·16	0·29	0·52	1·18	0·73	1·37	0·68	0·93	1·29
17	4·10	1·12	0·10	0·83	0·21	0·26	1·32	0·74	1·21	0·70	0·63	1·42
18	4·06	1·26	0·89	0·90	0·26	0·39	1·07	0·51	0·61	0·56	0·56	1·29
19	4·14	1·25	0·53	0·94	0·13	0·17	0·70	0·44	0·42	0·44	0·76	0·74
20	4·07	1·57	1·18	1·14	0·26	0·40	1·14	0·77	1·33	0·10	1·37	1·27
21	4·08	1·61	0·66	0·87	0·46	0·40	1·34	1·10	1·18	0·13	1·01	1·12
22	4·08	1·40	0·50	0·94	0·24	0·18	1·17	0·50	1·04	0·38	0·59	0·76

TABLE 7

LINEAR REGRESSION COEFFICIENTS OF DEVIATIONS OF MEAN SCUTELLAR BRISTLES ON GENERATION NUMBER

Line	*OR-SV*	*OR-BC*	*SV-BC*
Large lines	0·080±0·0043	0·086±0·0054	0·085 ±0·0086
Small lines			
S_1	0·039±0·0082	0·048±0·005	0·026 ±0·0084
S_2	0·060±0·0066	0·035±0·0086	0·041 ±0·0084
S_3	0·012±0·0042	0·035±0·0058	0·0086±0·031
S_4	0·021±0·0046	0·018±0·0051	0·045 ±0·0099
S_5	0·055±0·0069	0·059±0·0059	0·040 ±0·0059
S_6	0·041±0·0062	0·043±0·0068	0·0089±0·0054
S_7	0·064±0·0115	0·024±0·0060	0·025 ±0·0079
S_8	0·021±0·0060	0·033±0·0069	0·041 ±0·0076
S_9	0·054±0·0079	0·042±0·0088	0·043 ±0·0085
S_{10}	0·073±0·0093	0·024±0·0068	0·028 ±0·0049

Chromosome 1 main effect was negative and significant in S_2 of *SV-BC* and S_7 of *OR-SV*. Also a significant interaction between chromosomes 1, 2, and 3 was shown in lines S_2, S_5, and S_9 of *OR-SV* (Table 8).

TABLE 8

COMPARISONS (Q) OF MAIN CHROMOSOMAL AND INTERACTION EFFECTS IN GENERATION 19 BASED ON THE COEFFICIENTS OF TABLE 2 FOR LARGE (L) AND SMALL (S) LINES IN *OR-BC*, *OR-SV*, AND *SV-BC*, AND FOR THE THREE LARGE LINES IN GENERATIONS 16 AND 19

Chromosomal Effects	*OR-BC*					*OR-SV*					
	Q(L_1)	Q(S_2)	Q(S_9)	Q(S_5)	Q(S_6)	Q(L_2)	Q(S_7)	Q(S_2)	Q(S_5)	Q(S_9)	Q(S_{10})
1	0·003	−0·064	−0·044	−0·045	0·006	0·011	−0·084*	0·021	0·076	0·005	0·01
2	0·258**	0·252**	0·04	0·178**	0·146**	0·389**	0·241**	0·257**	0·233**	0·303**	0·159**
3	0·036	0·067	0·033	0·046	0·028	0·04	0·036	0·004	0·072	0·031	0·019
1×2	−0·058	−0·071	0·019	−0·047	−0·141**	−0·065	0·087	−0·112	0·071	−0·197**	−0·046
1×3	0·048	−0·083	0·020	−0·046	−0·033	−0·02	0·019	0·026	0·004	−0·062	−0·041
2×3	−0·022	−0·034	−0·027	0·071	0·0	0·005	0·079	0·001	0·084	−0·062	0·071
1×2×3	0·033	0·135	0·025	0·091	0·098	0·008	−0·144	0·160*	−0·212**	0·175*	−0·027

Chromosomal Effects	*SV-BC*						Generation 16			Generation 19		
	Q(L_3)	Q(S_3)	Q(S_2)	Q(S_{10})	Q(S_7)	Q(S_1)	Q(L_1)	Q(L_2)	Q(L_3)	Q(L_1)	Q(L_2)	Q(L_3)
1	0·01	0·0	−0·084*	−0·04	−0·014	−0·041	−0·082*	0·001	−0·029	−0·003	0·015	0·01
2	0·266**	0·266**	0·264**	0·295**	0·249**	0·263**	0·273**	0·259**	0·240**	0·258**	0·269**	0·266**
3	0·07	0·025	0·054	−0·026	0·006	0·042	−0·022	0·037	0·029	0·036	0·111*	0·070
1×2	−0·111*	−0·055	0·017	−0·108*	−0·03	−0·077	0·003	0·027	−0·004	−0·058	−0·069	−0·109
1×3	−0·072	−0·058	0·055	0·005	0·014	−0·036	0·046	−0·023	−0·022	0·048	−0·135	−0·072
2×3	0·016	−0·003	−0·016	0·005	0·043	−0·096	0·073	0·079	0·140**	−0·022	−0·066	0·016
1×2×3	0·126	0·036	−0·048	0·038	−0·061	0·121	−0·041	−0·062	0·053	0·033	0·107	0·124

* Significant at the 5% level. ** Significant at the 1% level.

IV. Discussion

It seems reasonable that any differential response to selection in *OR-BC*, *OR-SV*, and *SV-BC* large lines ought to be a result of the different initial gene frequency in these lines. In the three large lines the selection intensities for generations 1–8 and 9–22 were very nearly the same; and the effective population numbers were of the same order of magnitude and probably large enough to make insignificant any drift effect due to sampling. Also, as a result of initiating the three lines from the F_1 and two backcrosses between two isogenic lines, the array of genes for scutellar bristles in all lines was expected to be the same with two alleles per segregating locus. Linkage disequilibrium might have been larger at the outset in the F_1 than in the two backcrosses. However, this difference would be expected to diminish after some generations of recombination and would probably not be significant. Thus, the only significant factor differentiating the three large lines was probably the gene frequency at all segregating loci. Each segregating locus can be assumed to have an expected favourable allelic frequency of 0·5 for *OR-SV*, and 0·75 or 0·25 for *OR-BC* and *SV-BC*. At the extreme the favourable allelic frequency at all loci would have an expected value of 0·75 in one backcross and 0·25 in the other backcross. However, from the similarity of response in the three large lines it would seem that the frequency of a favourable allele was about 0·75 for roughly half of the loci and 0·25 for the other half in each backcross. This would occur if Oregon and Sevelen isogenics were fixed for favourable alleles at different halves of the loci. Also, the similarity and smooth trend of response was probably an indication that the genes controlling scutellar bristles were of small and roughly equal effects.

Within each of the F_1 and two backcrosses small lines are expected, as a result of random genetic drift due to small population number, to deviate in gene frequency from that of the large line. Thus, under the multiple peak hypothesis, some of the small lines are likely to manifest a different mechanism of response than the large line, depending on the initial gene frequency of the latter.

It is clear from Tables 7 and 8 that in the 22 generations of selection the evidence is strongly against the presence of a multiple peak system in those lines. This evidence stems from the fact that the mechanism of response was very similar in all large and small lines in that it was located on the second chromosome. Furthermore, the magnitude of response in the small lines was invariably less than that of the large lines; and the large lines exhibited the same magnitude of response. Not much weight can be attached to the seemingly significant chromosomal interactions in the small lines of *OR-SV*. This can arise from linkage disequilibrium, since these lines were started from an F_1 between two isogenic lines; and with their small size and intermediate gene frequency one would expect the linkage disequilibrium not to decrease rapidly and perhaps to increase as was found in simulation studies by Nassar and Comstock (unpublished data). These interactions are probably transient as was found with 2×3 chromosomal interaction in the *SV-BC* large line.

The magnitude of response of the small lines within any one cross varied, but more so for the *OR-SV* lines. This was as expected since the range of variability in gene frequency as a result of drift would be most pronounced at intermediate gene frequency. The variability in response among the small lines, plus the fact that only

three lines showed near equal response to the large lines, might be indicative of having few modifier genes affecting scutellar bristles.

Rendel, Sheldon, and Finlay (1965) hypothesized that the *scute* locus determines the number of scutellar bristles with modifier genes regulating its action. This system would lead to a single peak and would be in line with the present evidence of this work. Miller and Fraser (1968) and Fraser, Erway, and Brenton (1968) presented evidence to show that scutellar bristle number might be controlled by two major loci (sc^+ and *x-vert*) with each of these having a specific system of modifier genes (α and β) which are incompatible in that the presence of one system suppresses the other. Results of the present experiment do not support nor disprove the above hypothesis, since it is not known whether the modifier genes on the second chromosome are of the α or β system or both. This will be tested by substituting *scute* or "*extra-verticals*" in the selected lines. It is likely, however, that the modifier genes on the second chromosome belong to the α-system, since the *x-vert* gene does not appear to be present in these lines. If this is the case, then introducing *x-vert* in an α-background ought to suppress the expression of these genes provided that the *scute–x-vert* hypothesis is correct.

It is worth noting that in the previous work of Fraser, Erway, and Brenton (1968) and Miller and Fraser (1968) the gene modifiers appeared either on the first or third chromosome. In the present work all genes appeared to be on the second chromosome. There can be two explanations. The first is that in the cross between the two isogenic lines there was no genetic variability affecting scutellar bristles on the first and third chromosomes. The second is that gene expression on the second chromosome could have interfered to prevent its expression on other chromosomes. The latter explanation can be tested by introducing into the selected lines a *Cy*-marked second chromosome inversion and selecting for an increase in scutellar bristles in the structural heterozygote genotype.

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