VARIATION OF SCUTELLAR BRISTLES IN *DROSOPHILA*

V. COMPONENTS OF SELECTION ADVANCE*

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Summary

A study of the "inversion marked" chromosome technique for separation of components of selection advance is described and the accuracy of this method is discussed, for the character, number of extrascutellar bristles in *D. melanogaster*.

Analysis of variance of mean scutellar number showed no significant effect of replicates or transfers at the 1% level. Calculation of the number of replicates required to detect certain differences between genotype means at the 5% level indicated that three replicates with two transfers were required. The mean for the triple heterozygote was within the error of that of the original base stock, confirming that most of the selection response has been recessive.

Analysis of chromosome means for scutellar number showed significant effects of the first and third chromosomes as well as a large component from the I–III interaction term, which was reduced but not completely eliminated by application of the probit transformation.

The data provided a means of separating chromosomal components of scutellar-producing ability from those of survival. Trends relating genotype to survival were present but the components were non-significant at the 5% level. No indication of density-dependent survival of certain scutellar phenotypes was found.

I. INTRODUCTION

Mather (1942), Sismanidis (1942), and Reeve and Robertson (1953) have used "inversion marked" chromosomes in *Drosophila melanogaster* to separate the components of selection advances on the basis of their chromosomal location. Fraser *et al.* (1965) have used a similar method to dissect the advances produced by selection for extrascutellars and for missing scutellars. They found that interchromosomal interactions were a major component of selection advances in some lines, and they suggested that the interactions could be an artefact due to the canalization of scutellar number at four. Rendel (1959), Rendel and Sheldon (1960), and Latter (1964) have shown that probit analysis is extremely useful in the transformation of data, and it has been applied to this data in an attempt to resolve the nature of chromosomal interactions, since if these were due wholly to the threshold effect then the probit transformation would remove them.

Fraser *et al.* (1965) have suggested from their data that there are complex relationships between scutellar number and reproductive fitness. The extensive data of our analyses make it possible to compare the relative survivals of the different genotypic classes, and to correlate survival with scutellar number.

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II. Experimental Materials

The experimental stocks used in the analysis were taken from the B11.1 selection line at generation 21. The B set of selection lines, of which B11.1 is a member, originated from base stock 70 in 1962. Each line was derived from a single inseminated female and selection has been for high scutellar number, selecting 2–5 females from 30–60 females scored each generation. B11 showed no marked advance under selection until generation 8 when it was split into three replicate sublines. These all remained at the former level until generation 15 when replicate 1 began to climb rapidly and by generation 20 had reached a mean of 6·8 bristles. It has remained at this level since generation 21 (see Fig. 1). This level is equivalent to the third stasis level described by Fraser *et al.* (1965).

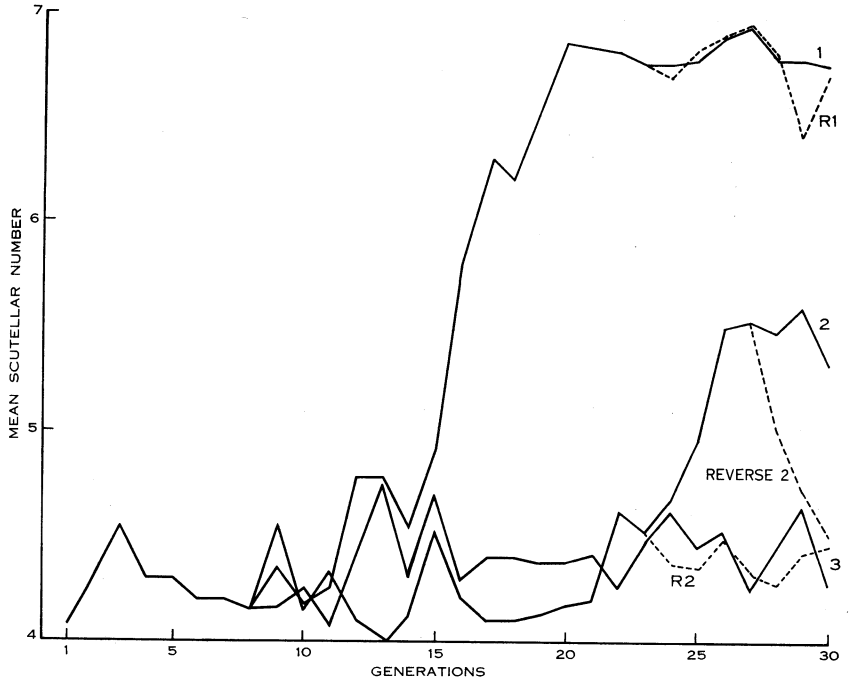

Fig. 1.—Selection history of the B11 set of selection lines. 1, 2, and 3 are replicates. R1 and R2 designate relaxed selection of replicates 1 and 2 respectively. Reverse 2 designates reverse selection of replicate 2.

III. Experimental Design

The mating procedure and conditions to which these matings were exposed were similar to those employed by Fraser *et al.* (1965) in their chromosomal analyses. All matings were carried out in half-pint cream jars at a constant temperature of 23°C. Six sets of matings were set up each consisting of 10 *C1B*/+; *Cy*/+; *Ubx*/+ virgin females and five random males from generation 21 of the B11.1 selection line (see Table 1). In this paper "+" designates any stock chromosome while "+*s*" indicates that the chromosome is from the B11.1 line. A further sample of 80 males from generation 21 were kept in vials and transferred to fresh medium every 3 days. These

males were mated to the *C1B/+s*; *Cy/+s*; *Ubx/+s* virgin female progeny of the first cross. Seven cultures of this type were set up. Each culture comprised 10 of these females selected at random and 10 B11.1 males. The term "replicate" is used here to designate cultures from different sets of parents.

TABLE 1

MATING SCHEME FOR THE ANALYSIS

Females		Males	
C1B/+; *Cy/+*; *Ubx/+*	×	*+s/Y*; *+s/+s*; *+s/+s* (B11.1)	Six replicates
C1B/+s; *Cy/+s*; *Ubx/+s*	×	*+s/Y*; *+s/+s*; *+s/+s* (B11.1)	Seven replicates, four transfers
C1B/+s; *Cy/+s*; *Ubx/+s*		*+s/+s*; *Cy/+s*; *Ubx/+s*	
C1B/+s; *Cy/+s*; *+s/+s*		*+s/+s*; *Cy/+s*; *+s/+s*	
C1B/+s; *+s/+s*; *Ubx/+s*		*+s/+s*; *+s/+s*; *Ubx/+s*	
C1B/+s; *+s/+s*; *+s/+s*		*+s/+s*; *+s/+s*; *+s/+s*	

The males were mated to their final mates for 2 days before transfer to half-pint cream jars. The object of this procedure was to ensure that all females were inseminated before the first culture was set up. The parents were transferred to new cream jars at 4-day intervals to give four transfers per replicate. The term "transfer" is used here to designate successive cultures from the same set of parents.

Fifteen days after setting up each transfer, the progeny were collected and transferred to clean medium. Female progeny were scored as soon as possible for both genotype and scutellar bristle number.

TABLE 2

ANALYSIS OF VARIANCE OF MEAN SCUTELLAR NUMBER FOR REPLICATES C, D, E, F, AND G OVER TRANSFERS 1, 2, AND 3

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	*F*
Genotypes	92·576	7	13·225	500·947**
Transfers	0·117	2	0·058	2·197
Replicates	0·195	4	0·049	1·856
Genotype × replicate	0·551	28	0·019	0·746
Error	2·034	77	0·026	

** $P<0·01$.

In the later transfers of the final cross, some male parents in certain replicates were lost. However, all of the female parents survived in all replicates through to the fourth transfer. Replicates A and B were excluded from the final analysis due to loss of some progeny in moist culture medium. Transfer 4 was excluded due to abnormally low numbers of progeny in most replicates. There were, therefore, five replicates, each with three transfers in the final analysis.

IV. Results and Discussion

An analysis of variance of mean scutellar number showed that the mean square for genotypes was significant at the 1% level. None of the other variance components were significant at 5% (see Table 2). A test for number of replications required to obtain an accuracy of $\pm 0{\cdot}05$ scutellars, with probability $0{\cdot}05$, showed that six replicates were required if two or three transfers were used. Since previous analyses by Scowcroft (see Fraser *et al.* 1965) were based on three replicates and three transfers, it appears that their results have a high degree of accuracy, and considerable weight can be given to their conclusions.

Table 3

SCUTELLAR MEANS FOR EACH GENOTYPE AND MEAN SCUTELLAR ANALYSIS OF CHROMOSOMAL COMPONENTS (AFTER METHOD OF FRASER *et al.* 1965)

Genotype	Mean Scutellar Number*	Chromosomal Component		
		No.	This Paper†	Fraser *et al.* (1965)
C1B/+*s*; *Cy*/+*s*; *Ubx*/+*s*	4·02 (0·03)	—	—	—
+*s*/+*s*; *Cy*/+*s*; *Ubx*/+*s*	4·41 (0·12)	I	0·38 (0·26)	0·44
C1B/+*s*; +*s*/+*s*; *Ubx*/+*s*	4·03 (0·04)	II	0·01 (0·10)	0·03
C1B/+*s*; *Cy*/+*s*; +*s*/+*s*	4·35 (0·11)	III	0·33 (0·28)	0·39
+*s*/+*s*; +*s*/+*s*; *Ubx*/+*s*	4·48 (0·12)	I–II	0·06 (0·30)	0·15
+*s*/+*s*; *Cy*/+*s*; +*s*/+*s*	6·02 (0·19)	I–III	1·29 (0·40)	1·00
C1B/+*s*; +*s*/+*s*; +*s*/+*s*	4·57 (0·13)	II–III	0·11 (0·30)	0·09
+*s*/+*s*; +*s*/+*s*; +*s*/+*s*	6·47 (0·19)	I–II–III	0·27 (0·40)	0·23

* Values in parentheses are standard errors calculated from the five replicates over three transfers.

† Values in parentheses are standard deviations calculated from the separate estimates of the 15 replicates without regard to transfers.

The analysis of mean scutellar number between genotypes, over transfers 1, 2, and 3, and replicates C, D, E, F, and G (see Table 3) shows that the mean for the triple heterozygote (*C1B*/+*s*; *Cy*/+*s*; *Ubx*/+*s*) was within error of the mean of the unselected base population. This fact shows that selection has been acting almost entirely upon genes which are recessive to their alleles in the "inversion" chromosome. Estimates of chromosome effects were made by comparison of the effects of one and two doses of the selected chromosomes on the bristle mean. The mean for the triple homozygote for the selected chromosomes (+*s*/+*s*; +*s*/+*s*; +*s*/+*s*) was $6{\cdot}47 \pm 0{\cdot}19$ bristles as compared to a mean of 6·80 bristles for the selection line in generation 21. This bristle difference of 0·3 is significant at the 10% level and suggests that either the fourth chromosome is involved in bristle number or that the "inversion" chromosomes do not completely prevent crossing over in the female parents of the second cross. The chromosome components were calculated by subtraction of the mean for the control genotype (*C1B*/+*s*; *Cy*/+*s*; *Ubx*/+*s*) from that of the genoytpe for the respective component, e.g. the chromosome I component = (+*s*/+*s*; *Cy*/+*s*; *Ubx*/+*s*)−(*C1B*/+*s*; *Cy*/+*s*; *Ubx*/+*s*). The chromosome II component =

(*C1B*/+*s*; +*s*/+*s*; *Ubx*/+*s*)−(*C1B*/+*s*; *Cy*/+*s*; *Ubx*/+*s*). In the case of the interaction components, the values for each of the single chromosome components contained in the genotype were also subtracted, e.g. the I–II interaction component = (+*s*/+*s*; +*s*/+*s*; *Ubx*/+*s*)−(*C1B*/+*s*; *Cy*/+*s*; *Ubx*/+*s*)−(I+II).

The analysis showed that the major component of selection advance was due to an interaction between the chromosomes I and III which was of the order of $1{\cdot}29 \pm 0{\cdot}40$ bristles, while smaller but still significant effects of $0{\cdot}38 \pm 0{\cdot}26$ and $0{\cdot}33 \pm 0{\cdot}28$ bristles were contributed by chromosomes I and III respectively. Other components were of considerably less magnitude and were not significantly different from zero at the 10% level. These results are within their own standard error of the

TABLE 4

PROBIT WIDTHS FOR THE FIVE- AND SIX-BRISTLE CLASSES*

Values in parentheses are standard deviations

Genotype	Width of Five-Bristle Class (probits)	Width of Six-Bristle Class (probits)
C1B/+*s*; *Cy*/+*s*; *Ubx*/+*s*	0·79 (0·30)	—
+*s*/+*s*; *Cy*/+*s*; *Ubx*/+*s*	1·34 (0·10)	—
C1B/+*s*; +*s*/+*s*; *Ubx*/+*s*	—	—
C1B/+*s*; *Cy*/+*s*; +*s*/+*s*	1·12 (0·09)	—
+*s*/+*s*; +*s*/+*s*; *Ubx*/+*s*	1·25 (0·08)	0·97 (0·17)
+*s*/+*s*; *Cy*/+*s*; +*s*/+*s*	1·26 (0·11)	1·07 (0·07)
C1B/+*s*; +*s*/+*s*; +*s*/+*s*	1·27 (0·08)	1·08 (0·18)
+*s*/+*s*; +*s*/+*s*; +*s*/+*s*	1·29 (0·17)	1·01 (0·07)
Mean	1·19	1·06

* See text for explanation of method.

results obtained in a similar but less extensive analysis of the same line performed in generation 18 [see Fraser *et al.* (1965) and Table 3]. The large positive I–III chromosomal interaction is a feature of two of the selection lines which have reached the third stasis level of selection progress (lines A1 and A18).

The occurrence of such a strong interchromosomal interaction is worthy of special analysis since it is so commonly found in lines in this group of selection experiments. It appears to become important in the progress from the second to the third stasis level and is not found in the analysis of lines which have not reached this level. The inherent difficulty in the analysis of scutellar bristle data caused by the threshold at four bristles warrants the use of the probit method of analysis to correct the distribution for the existence of this threshold. The use of the probit method and its application to scutellar data has been described in detail by Rendel (1959). The probit transformation converts a sigmoid dosage curve into a linear relationship. The threshold effect which is a characteristic of the scutellar trait may be due to either developmental scaling or to non-additive gene action when the phenotype is in the neighbourhood of four bristles. It was suggested by Fraser *et al.* (1965) that the probit method might reduce the interaction between chromosomes by eliminating

the threshold effect. Analysis of the present data showed that the width of the 5- and 6-bristle classes remained fairly constant over all genotypes (see Table 4). This information was used to estimate the width of the 5- and 6-bristle classes which were absent in some replicates and thereby enable the use of the probit analysis for detection of chromosomal components.

The analysis of chromosomal components in terms of the distance in probits from the 4–5 cut-off is shown in Table 5. In several replicates and genotypes it was not possible to calculate the distance of the 4–5 cut-off from the mean of the group due to absence of females with five bristles. For these groups, estimates were made using the average value from those groups where the data was available.

TABLE 5

CHROMOSOMAL COMPONENTS CALCULATED ACCORDING TO THE PROBIT METHOD AS DESCRIBED IN THE TEXT

Values in parentheses are standard deviations

Genotype	Flies with more than Four Bristles (%)	Probits − 5	Chromosomal Component	
C1B/+*s*; *Cy*/+*s*; *Ubx*/+*s*	1·97	−2·06 (0·14)	—	
+*s*/+*s*; *Cy*/+*s*; *Ubx*/+*s*	36·08	−0·36 (0·06)	I:	1·71 (0·28)
C1B/+*s*; +*s*/+*s*; *Ubx*/+*s*	3·29	−1·84 (0·10)	II:	0·22 (0·17)
C1B/+*s*; *Cy*/+*s*; +*s*/+*s*	29·70	−0·53 (0·06)	III:	1·53 (0·28)
+*s*/+*s*; +*s*/+*s*; *Ubx*/+*s*	40·14	−0·25 (0·05)	I–II:	−0·11 (0·30)
+*s*/+*s*; *Cy*/+*s*; +*s*/+*s*	96·32	1·79 (0·12)	I–III:	0·61 (0·28)
C1B/+*s*; +*s*/+*s*; +*s*/+*s*	47·00	−0·07 (0·06)	II–III:	0·26 (0·28)
+*s*/+*s*; +*s*/+*s*; +*s*/+*s*	98·94	2·30 (0·17)	I–II–III:	0·17 (0·17)

A comparison of Tables 3 and 5 shows that the probit method does reduce the importance of the interaction components, particularly the I–III term. That a large amount of this I–III component obtained from the mean scutellar analysis is due to threshold effects at four bristles is obvious when Figures 2(*a*) and 2(*b*) are compared. The interaction can be seen in the plots for chromosomes I and III by the difference in slope of the graphs for "balancer" and "+*s*" on the step between "+*s*"—"*Ubx*" and "+*s*"—"*C1B*" respectively [see Fig. 2(*a*)]. In Figure 2(*b*), however, the graphs run almost parallel at this step, showing clearly that the mean scutellar analysis is confounded by a threshold effect and that the effect of this is removed by the probit method. Probits reduce the I–III terms from 52·7% of the total scutellar advance to 14·2%. The other interaction terms and the single chromosome effects are mostly of the same relative order of magnitude on both probit and mean scutellar analysis. The other interactions are non-significantly different from zero and may be disregarded.

Fraser *et al.* (1965) suggested that natural selection operates at the third stasis level to neutralize the effect of selection for high or low bristle number. The present experiment provided data for examination of the survival of individuals with scutellar numbers ranging from 4·0 to 6·5. Analysis of variance of number of individuals in

each genotypic class showed highly significant differences for genotypes and replicates ($P<0{\cdot}01$) (see Table 6). The data should then allow detection of differences of survival of individuals with different scutellar numbers. No correlation was found between numbers of progeny and scutellar mean. Also, since no significant differences were found for replicates and transfers in the mean scutellar analysis of variance it is obvious that density-dependent survival is absent. Hence, an individual's scutellar phenotype does not determine its chances of survival in this analysis. We would then expect that the genotype other than the scutellar-producing genotype is involved in fitness. This explanation would satisfy the fact that there is sometimes a positive and sometimes a negative correlation between scutellar phenotype and fitness.

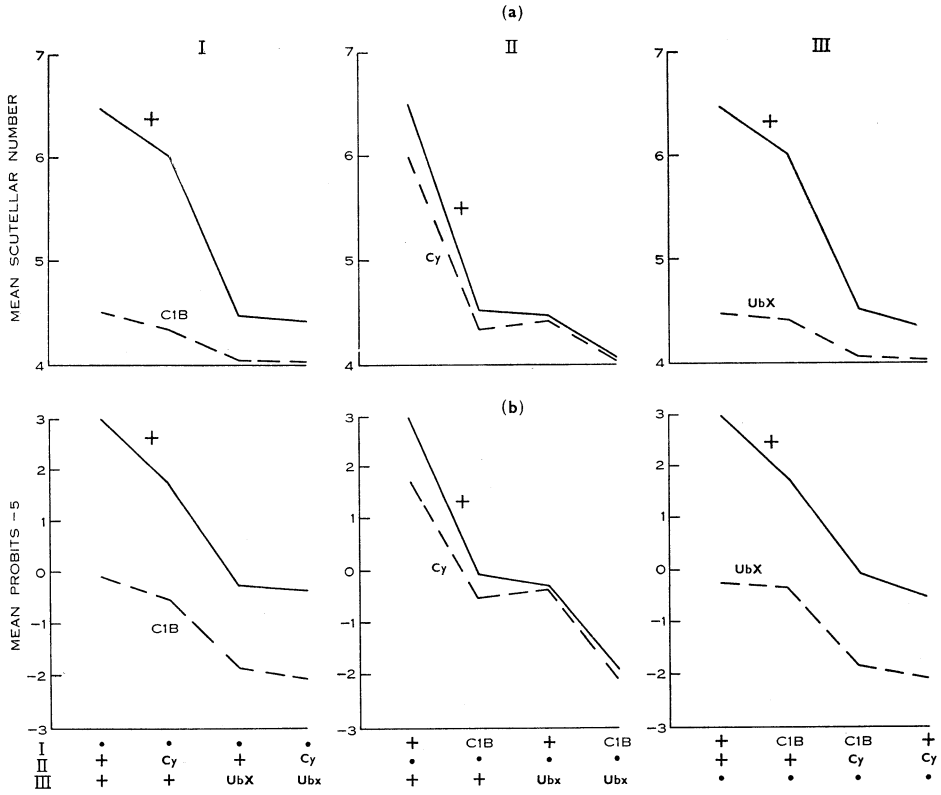

Fig. 2.—(*a*) Plots of mean scutellar number by genotypes. Each point is calculated from the average of scutellar means over five replicates and three transfers. (*b*) Plots of distances in probits of means from the 4–5 bristle cut-off by genotype. Each plot is calculated from the mean probit value over five replicates and three transfers. The scale is then adjusted by subtracting 5 from the probit mean thus obtained.

Analysis of chromosomal components on the basis of number of individuals was performed to ascertain if there was a relationship between each genotype and survival (Table 7). The data showed that genotypes with the highest mean scutellar number were not necessarily the most or the least fit. "+; *Cy*; +" which had a scutellar mean of 6·02 had the least number of individuals while "+; +; *Ubx*" which had a low bristle mean (4·48) was intermediate in survival. The chromosomal components of

survival were all non-significant but certain trends were noted which prove interesting. The selected third chromosome appeared to reduce the chances of survival when homozygous and was always less frequent than the *Ubx* chromosome except when the *C1B* and *Cy* chromosomes were present. There is evidence of an interchromosomal inter-

TABLE 6

ANALYSIS OF VARIANCE ON NUMBER OF INDIVIDUALS IN EACH GENOTYPIC CLASS ON THE BASIS OF POOLED RESULTS FOR TRANSFERS 1, 2, AND 3

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	*F*	*P*
Genotypes	3999·38	7	571·34	5·39	0·01
Replicates	1871·60	4	467·90	4·42	0·01
Error	2861·00	27	105·96		

action of chromosomes I and III which tends to increase chances of survival. Such a result would partly explain previous selection results and also the work of Nassar (unpublished data) who obtained rapid selection advance for scutellars when selecting in lines in which only one of the three major chromosomes were variable.

TABLE 7

CHROMOSOMAL ANALYSIS OF NUMBER OF INDIVIDUAL FEMALES IN EACH GENOTYPE CLASS

Values in parentheses are standard errors calculated from the 15 estimates without regard to transfers since differences between transfer were not significant

Genotype	Total Number of Individuals	Chromosomal Contribution	
C1B/+*s*; *Cy*/+*s*; *Ubx*/+*s*	456		—
+*s*/+*s*; *Cy*/+*s*; *Ubx*/+*s*	510	I:	−3·9 (3·4)
C1B/+*s*; +*s*/+*s*; *Ubx*/+*s*	547	II:	15·5 (5·4)
C1B/+*s*; *Cy*/+*s*; +*s*/+*s*	505	III:	−12·8 (4·9)
+*s*/+*s*; +*s*/+*s*; *Ubx*/+*s*	578	I–II:	−5·8 (3·6)
+*s*/+*s*; *Cy*/+*s*; +*s*/+*s*	408	I–III:	8·3 (2·6)
C1B/+*s*; +*s*/+*s*; +*s*/+*s*	517	II–III:	−0·9 (2·5)
+*s*/+*s*; +*s*/+*s*; +*s*/+*s*	470	I–II–III:	−1·3 (4·3)

The survival data may explain some of the compensative interactions found by Fraser *et al.* (1965) and one could easily visualize a system whereby two chromosomes which reduce fitness when alone, but would increase fitness when in combination. It is possible that conditional lethals dependent upon the genetic background are present. Such systems were reported in selection lines analysed by Reeve and Robertson (1953). However, since there are many factors causing variability for number of individuals in each replicate and genotype and since we do not know the absolute effect of the

balancer chromosomes on survival, we must treat the results obtained from this experiment with caution when studying fitness and its relation to scutellar number. In the future, more stress needs to be given to the study of the relation of the various components of fitness to scutellar number.

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