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Organization of polygenic systems: Cell death modifiers from natural populations of *Drosophila melanogaster*

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SUMMARY

Expression of three wing-cell death mutants in *Drosophila* melanogaster was used to survey cryptic polygenic modifiers of cell death in wild type strains. Females carrying the X-linked mutants Beadex-3, notchoid and scalloped were crossed to males from each of 20 isofemale strains. Phenotypic variation in the amount of cell death was measured in F_1 mutant males that were heterozygous for polygenic loci segregating in the wild strains. As expected, each mutant uncovered a broad range of polygenic variation among strains. Yet, when cluster analyses were used to evaluate the degree of correlation among the expressions of Bx^3, *sd* and *nd*, the isofemale strains could be partitioned into a small number of groups that were similar in the effects they had upon the severity of cell death. Chromosome mapping of one cell death suppressor strain demonstrated that different polygenic loci could produce the same phenotype in different mutant backgrounds.

INTRODUCTION

Essentially every aspect of development can be influenced by polygenic modifiers. In this way, polygenes provide a major reservoir of genetic variation that can respond to selection for one or a few adapted phenotypes (*cf.* Wright, 1980). Yet, with the exception of biometrical analyses of strain differences (Mather & Jinks, 1971), only a few studies have attempted more than a superficial analysis of the types of polygenic effects that are segregating in natural populations (Milkman, 1970*a*, *b*; Boyer, Parris & Milkman, 1973; Parsons, 1973, 1980). The reason for this is not hard to understand. Polygenic alleles have small, somewhat interchangeable effects upon development and are often difficult to separate from environmental influences.

In spite of the difficulties, there are several ways to analyse the polygenic structure of a population and to begin building a picture of the type of genetic potential it offers. One of the most broadly applicable approaches is to compare the polygenic expression of different isofemale strains (Parsons, 1979, 1980). Isofemale strains originate from single wild-inseminated females, and each typically carries only a small sample of the genetic variation in the base population. Consistent phenotypic differences among strains for a quantitative character provide a simple measure of the effects of whole polygenic genotypes.

The identification of whole chromosome segregations within an isofemale line (Thompson & Hellack, 1982) brings one a little closer to categorizing the types of genes that influence quantitative traits and the frequencies of specific polygenic units in the population. The information provided by isofemale strains can also be refined by assaying several related traits using the same set of strains. In this way one can evaluate the degree to which the polygenes affecting one aspect of development overlap with the sets that affect separate, but phenotypically related, developmental processes. We have used both of these approaches to look at polygenic influences upon cell growth and death in the *Drosophila* wing. In particular, we wished to investigate the idea (*e.g.*, Milkman, 1970*a*; Carson, 1982) that, in spite of a potentially large amount of polygenic segregation, a comparatively small number of polygenic combinations or modifier phenotypes will be common in a natural population.

2. MATERIALS AND METHODS

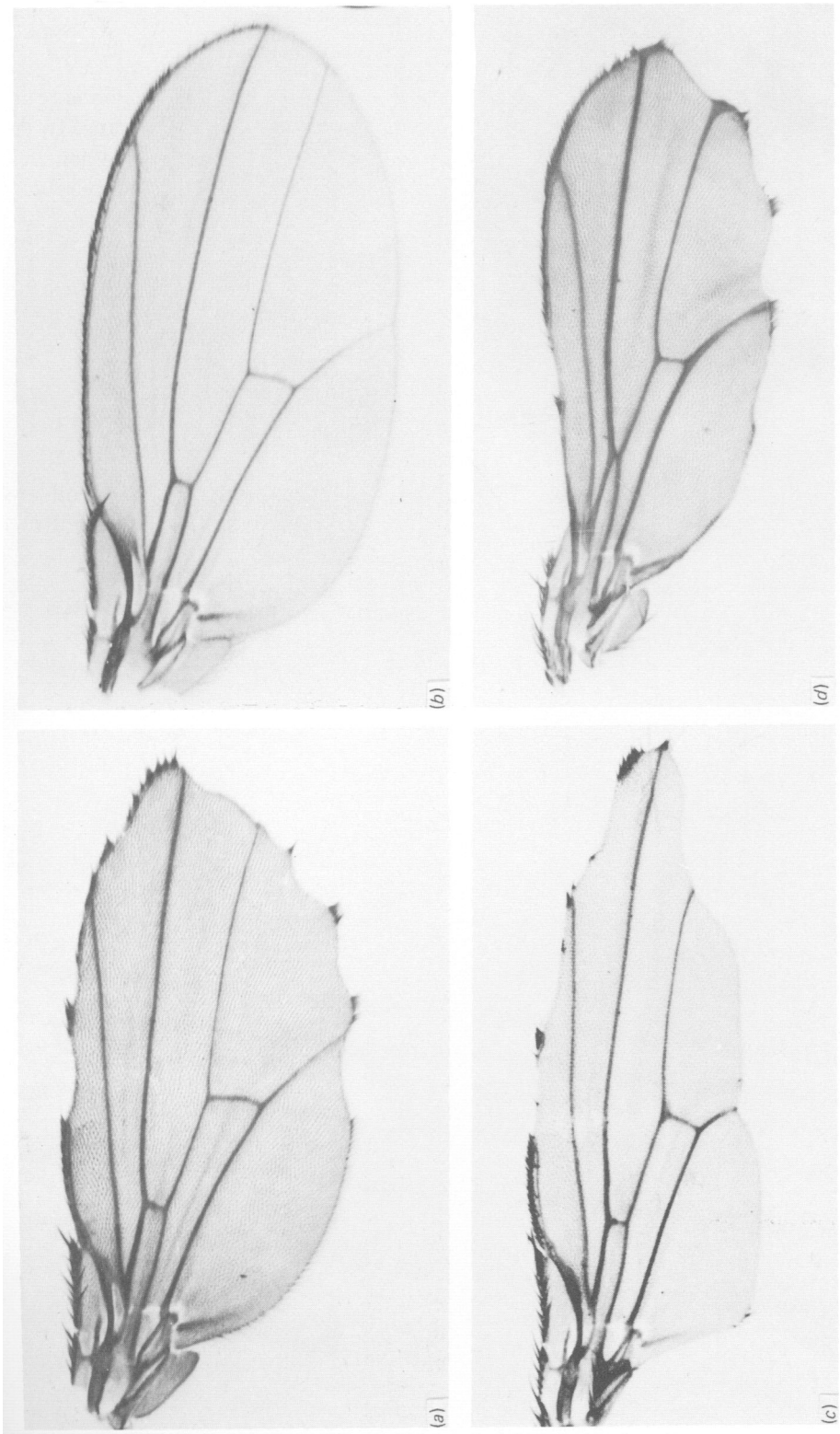
Polygenic modifiers of wing size were measured in 20 isofemale strains collected from natural populations in Australia, New Guinea and the United States. In the Results, these are referred to as wild type strains nos. 1 to 20 and are identified by place of origin in Table 1. Each isofemale strain originated from a single wild inseminated female, and the strains have been maintained by bulk transfer each generation to minimize the effects of drift.

In a wild type fly, segregation of polygenic loci that influence rates of cell growth and death is not easily detected, since relative organ sizes are well-regulated in a normal individual. Thus, we have used variation in the phenotypic expression of three different X-linked notching mutants to give added sensitivity and to help focus upon specific aspects of the dynamics of cell populations in the wing. Notching is due to wing-cell death in Beadex-3 (Bx^3, 1–59·4), notchoid (*nd*, 1–3·0), and scalloped (*sd*, 1–51·5). Each has a distinctively different pattern of notches and thus presumably is losing wing cells due to defects that differ from each other in cause or timing (Fristrom, 1969). Typical wings are shown in Plate I. Bx^3 is characterized by the greatest amount of scalloping, with notches located mainly along the anterior and posterior margins. The recessive *nd* has its largest effect upon the distal anterior and posterior edges, and *sd* has small notches at scattered points along the entire margin. There is little change in wing length associated with any of these.

All strains were maintained at $25 \pm 0{\cdot}5$ °C on a standard cornmeal, molasses, yeast, agar medium seeded with live yeast.

(i) *Experimental design and wing measurements*

Males from each strain were outcrossed to females homozygous for a particular X-linked mutant, and each F_1 male therefore carries the mutant and a second and third chromosome from the tested wild line. By averaging F_1 male phenotypes, one can estimate the action of the polygenic factors carried by each line. If, for example, a major cell death mutant is caused by unstable lysosomal membranes



Wings of *Drosophila melanogaster*. (*a*) scalloped; (*b*) scalloped on the Sarnia-2 polygenic background, extreme example of suppression; (*c*) Beadex female; (*d*) notchoid.

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(*cf.* Hochschild, 1971), 'relevant' polygenic loci might suppress scalloping by stabilizing membranes (a modifier effect specific to this class of mutant lesions) or by increasing the replacement of cells through more rapid cell division in that region of the wing (a more general modifier effect).

One intact wing was removed from each male or female and mounted on a microscope slide with DePex mountant. Wing areas were then measured with a Bausch and Lomb Omniscan Image Analyser, which electronically scans areas of defined contrast, giving extremely accurate measurements of irregular structures. Three separate estimates of each wing were averaged to minimize error due to instrument variation. Individual measurements typically varied by less than 0·1 %. Average area was scaled by dividing it by the total wing length (L3 vein; see Thompson, 1974) to reduce the effect of variation in fly size.

(ii) *Cluster analysis*

The 20 isofemale strains were ranked according to average areas, from small to large, for each of the three mutant phenotypes. Pearson Product-Moment Correlation Coefficients were calculated between all pairs of strains based upon the three character expressions. Cluster analysis is a multivariate statistical technique which can be used to define groups of observations which share similar multi-dimensional properties. Cluster analyses were carried out by the Unweighted Pair Group Method with Arithmetic Averages (UPGMA; Sneath & Sokal, 1973) using NT-SYS, a package of multivariate statistical programmes (Rohlf, Kishpaugh & Kirk, 1979).

(iii) *Whole chromosome assays*

Phenotypic effects were measured in second and third chromosomes isolated from tested isofemale strains. Males from an isofemale strain were mated to an inbred standard carrying the recessive eye colour mutants brown (*bw*, 2–104·5) and scarlet (*st*, 3–44·0), in addition to the appropriate X-linked cell death mutation. F_1 males were test-crossed singly to the inbred standard strain. The F_2 includes four different genotypes, each distinguishable by eye colour: white-eyed flies (*bw/bw*; *st/st*) serve as an internal control. Scarlet-eyed flies (*+/bw*; *st/st*) carry only a single tested second chromosome, brown-eyed flies (*bw/bw*; *+/st*) carry a third, while red-eyed flies (*+/bw*; *+/st*) allow interchromosomal interactions to be measured. Results of replicated assays were analysed using nested analyses of variance. A modification of this programme enabled us to substitute a tested chromosome from one mutant background into another. This modification is described in the Results.

3. RESULTS

Average male relative wing areas of Bx^3, *sd* and *nd* on each of 20 genetic backgrounds are summarized in Table 1. Strains are numbered from 1 to 20, based upon their phenotypic effect on Beadex. Wing area phenotypes are ranked separately for Bx^3, *sd* and *nd* in Fig. 1. Based upon a sample of 20 F_1 flies per isofemale strain, there are highly significant differences among strains (for Bx^3,

Table 1. *Average relative wing areas of three scalloping mutants measured on different isofemale strain genetic backgrounds*

Strain	Collection locality	Mean area ± S.E.		
		Bx^3	*sd*	*nd*
1	Chateau Tahbilk 116	0·663 ± 0·007	0·840 ± 0·006	0·870 ± 0·006
2	Fairfield 4 Feb	0·671 ± 0·006	0·841 ± 0·008	0·902 ± 0·007
3	Noble 1504	0·682 ± 0·007	0·821 ± 0·005	0·764 ± 0·009
4	Lake Boga 22	0·695 ± 0·006	0·857 ± 0·005	0·859 ± 0·007
5	Lake Boga 6	0·696 ± 0·006	0·848 ± 0·008	0·806 ± 0·009
6	Fairfield 9 Feb	0·702 ± 0·007	0·846 ± 0·006	0·855 ± 0·009
7	Chateau Tahbilk 111	0·704 ± 0·012	0·867 ± 0·007	0·832 ± 0·010
8	Melbourne 5 Jul	0·705 ± 0·006	0·859 ± 0·006	0·896 ± 0·011
9	New Guinea 6	0·707 ± 0·005	0·844 ± 0·006	0·809 ± 0·007
10	Chateau Tahbilk 124	0·727 ± 0·008	0·821 ± 0·007	0·818 ± 0·007
11	Chateau Tahbilk 104	0·736 ± 0·007	0·848 ± 0·006	0·815 ± 0·007
12	Chateau Tahbilk 106	0·736 ± 0·010	0·867 ± 0·007	0·846 ± 0·007
13	Chateau Tahbilk 118	0·745 ± 0·009	0·838 ± 0·008	0·882 ± 0·013
14	Fairfield 8 Feb	0·745 ± 0·012	0·827 ± 0·007	0·863 ± 0·008
15	Chateau Tahbilk 102	0·750 ± 0·007	0·821 ± 0·007	0·827 ± 0·010
16	Best's 2	0·751 ± 0·005	0·869 ± 0·006	0·877 ± 0·006
17	Chateau Tahbilk 122	0·753 ± 0·007	0·852 ± 0·006	0·795 ± 0·010
18	Fairfield 2 Jan	0·760 ± 0·005	0·859 ± 0·013	0·819 ± 0·008
19	Lake Boga 18	0·762 ± 0·008	0·855 ± 0·008	0·802 ± 0·012
20	Sarnia 2	0·762 ± 0·009	0·859 ± 0·011	0·810 ± 0·008

$F = 10{\cdot}93$; for *sd*, $F = 3{\cdot}93$; for *nd*, $F = 9{\cdot}35$; all $P < 0{\cdot}001$) and highly significant differences in the effects of polygenic loci in strains when compared across mutants ($F_{38,\,1135} = 7{\cdot}89$; $P < 0{\cdot}001$, for the analysis of variance of the strain by mutant interaction).

Although the polygenic backgrounds of these strains produced a fairly broad spectrum of phenotypic effects, cluster analysis appears to identify subsets of strains that are phenotypically similar in their suppression or enhancement of scalloping. An analysis of Bx^3 expression gave the clearest evidence of high, intermediate, and low scalloping strains. Strains nos. 1, 2 and perhaps 3 carry more effective enhancers of Bx^3 wing notching, strains 4 through 9 are intermediate, and 10 through 20 carry suppressors of cell loss. These groups are significantly different from each other (for 'enhancers' versus 'intermediate' strains, $t_{178} = 4{\cdot}74$, $P < 0{\cdot}001$; for 'intermediate' versus 'suppressors', $t_{358} = 10{\cdot}92$, $P < 0{\cdot}001$). Although this is not a very large sample of genomes from natural populations, it is interesting to note that the largest class involves strains that suppress cell loss, as might be expected for a polygenic system affecting a cell fitness trait.

The NT-SYS program allowed us to evaluate the degree of correlation among the expressions of Bx^3, *sd* and *nd* shown on different isofemale strain backgrounds, as summarized in the dendrogram in Fig. 2. There are four major clusters of strains. Within a cluster, the critical value is the length of the horizontal distance separating forks between pairs of strains. The vertical sequence is somewhat arbitrary. The first five strains (nos. 20, 19, 18, 17 and 3), for example, suppress scalloping most in Bx^3 wings and produce intermediate wing areas in *sd* and smaller

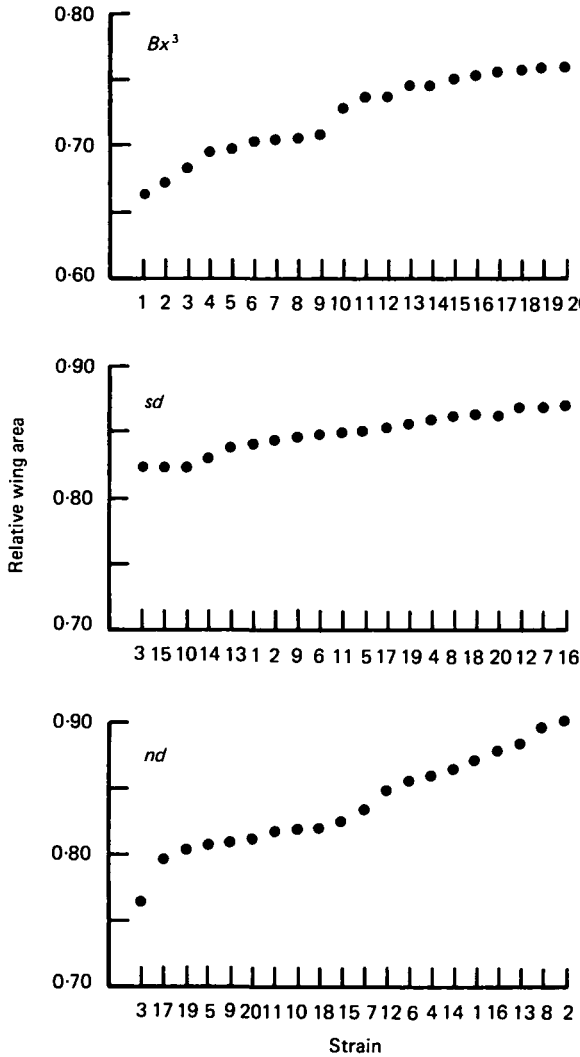

Fig. 1. Relative wing areas of Bx^3, *sd* and *nd* on 20 different isofemale strain backgrounds. Strains have been ranked from smallest to largest for each mutant; standard errors are given in Table 1.

wings in *nd*. The second cluster includes strains 5, 7, 9, 11 and 12, which have intermediate effects upon all three major mutants, though nos. 7 and 12 are similar in having their greatest effects upon *sd*. The third cluster (nos. 2, 13 and 14) tended to have its greatest influence in *nd*, though strains differed in their effects upon Bx^3 expression. Finally, the remaining strains were essentially similar in producing smaller wings in Bx^3 and larger wings in *nd*, the inverse of the pattern seen in the first cluster.

It therefore appears that there is a relatively small number of recognizable patterns in the way individual genomes influence phenotypic expression of this quantitative trait. Polygenic modifiers of cell longevity and replacement may be limited in number or have individually large effects in these strains.

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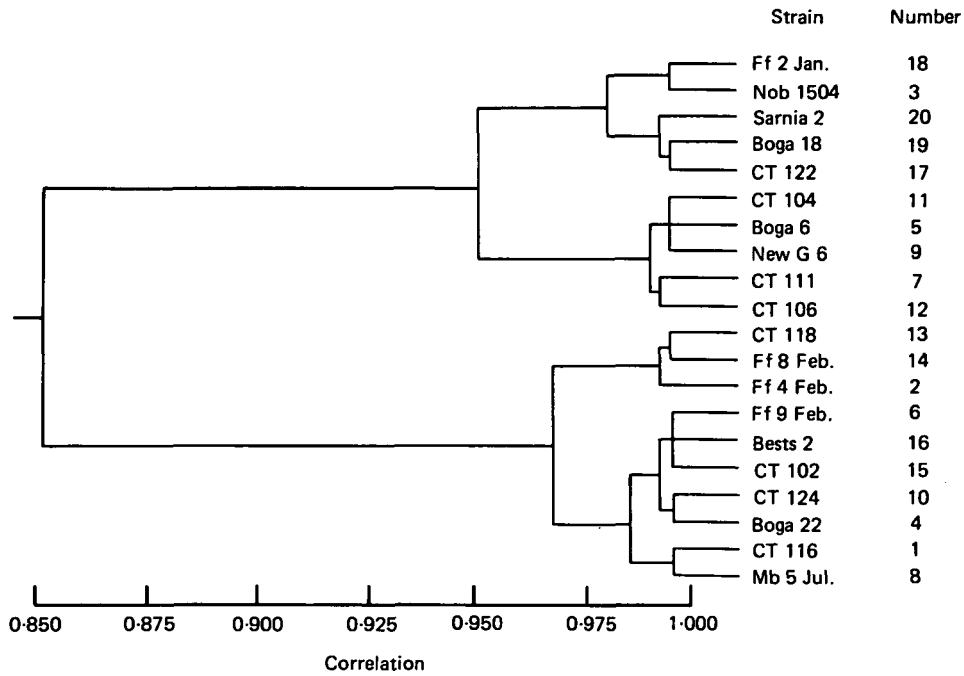

Fig. 2. Dendrogram of similarities among isofemale strains in their correlated expressions in Bx^3, *sd* and *nd*.

Table 2. *Magnitude and significance of the change in relative wing areas associated with heterozygous whole chromosomes from Sarnia-2 (significance of each effect was determined in an analysis of variance)*

Mutant	N†	chromosome 2	chromosome 3	2 × 3 interaction
Bx^3	240	0·0086	0·0749***	−0·0009
sd	320	0·0255***	0·0308***	−0·0024
sd (substitution)	110/130	0·0230**	0·0550*	

† N is given for the separate substitutions of chromosomes 2 and 3 as described in the text; the programme did not allow measurement of the interchromosomal interaction.
*$0·05 > P > 0·01$; **$0·01 > P > 0·001$; ***$P < 0·001$.

Phenotypic contributions of isolated chromosomes give a more precise idea of polygene action. Scalloping is reduced in both Bx^3 and *sd* on the Sarnia-2 polygenic background. In order to determine whether this is due to similar polygenic expression in both mutants, we assayed replicated whole autosomes from Sarnia-2 (Table 2). Heterozygous effects were measured because the initial isofemale strain survey detected only dominant or semi-dominant polygenic alleles, and because completely recessive polygenic loci would contribute little to phenotypic variability.

Chromosome 3 accounted for all of the increase in relative area of Bx^3 wings (Table 2). In an independent assay of *sd*, however, the contributions of Sarnia-2 second and third chromosomes were both highly significant, though chromosome

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3 had a slightly larger effect. This suggests that chromosome 2 carried one or more polygenic loci (as defined by Thompson & Thoday, 1974) that act upon processes inhibiting the death of cells normally caused by the *sd* mutation, an example of a mutant-specific polygenic effect. Chromosome 3, on the other hand, might carry general modifiers of cell growth or survival that show a different level of expression in these two mutant backgrounds, or it might carry separate mutant-specific modifiers like those on chromosome 2. These possibilities, or some combination of them, can only be tested by intrachromosomal mapping of polygenic loci (Thompson & Thoday, 1979).

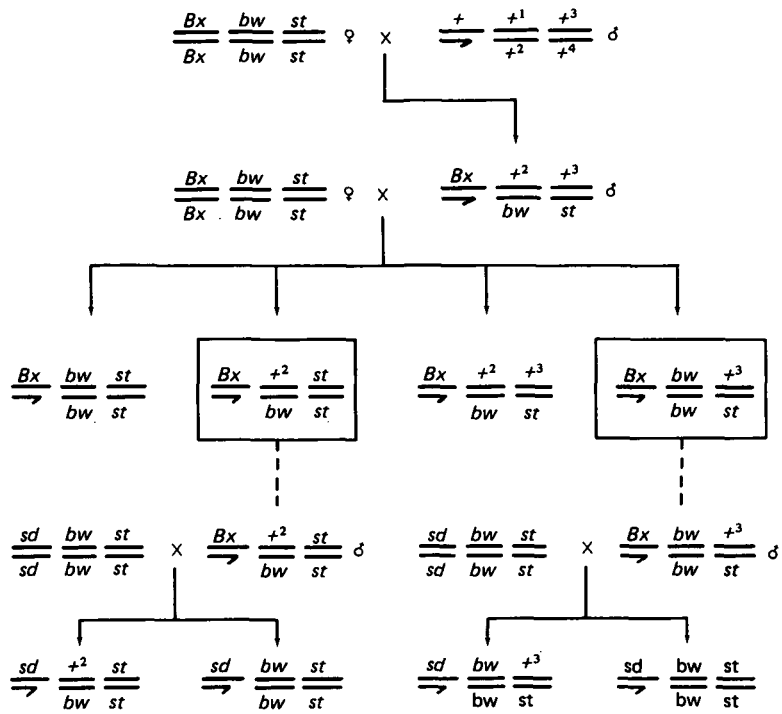

Fig. 3. Breeding programme used to substitute a tested autosome from the Beadex-3 whole chromosome assay into the genetic background of *sd*.

Alternatively, the apparent mutant-specific effects might be due to a trivial cause – testing genetically different chromosomes from the Sarnia-2 line in the independent assays of Bx^3 and *sd*. To eliminate this possibility, we substituted the replicated second and third chromosomes directly from the Bx^3 assay and repeated the measurement in *sd* using the modified whole chromosome assay shown in Fig. 3. The results (Table 2) confirm that the polygenic modifiers of *sd* cell death are distributed between chromosomes 2 and 3, with chromosome 2 showing mutant-specific effects. Furthermore, the sum of their effects (approximately +0·0780) is similar to that produced by the third chromosome alone in Bx^3 (+0·0749), accounting for the phenotypic similarity of the Sarnia-2 isofemale line when outcrossed to these two mutants.

4. DISCUSSION

The manipulation of environmental variables or the presence of a major gene mutation has frequently been used to uncover cryptic polygenic variation. Such variation can be traced to the segregation of alleles that have effects not usually expressed overtly in the adult phenotype (Thompson, 1979). Polygenic expression is generally observed to be normally distributed within a strain or population and is commonly interpreted as the joint effects of segregation of a large number of polygenes, each with small effects, and the random influences of the environment. There is, therefore, no simple correspondence between phenotypic expression and the genotype or genotypes that produce it. Our study of cell death modifiers has shown, however, that only a small number of genotypic categories need exist in order to account for the range of polygenic effects we detected upon cell growth and survival in the wing.

Our view of the polygenic structure of natural populations is very similar to that hypothesized in various forms by Wright (1980), Carson (1982), and others. From his studies of *cve* polygenes affecting development of the crossveins in *Drosophila* wings, for example, Milkman (1970*b*) found that natural populations carry a large number of *cve* modifiers, but certain combinations occur at a measurable frequency and persist over time in a sampled population. This is in sharp contrast to the more generally held expectation that polygenic alleles assort in an array of essentially unique combinations. Milkman concluded that selection was acting to maintain constellations of polygenes that produced a particular level of phenotypic effect, though the specific polygenic loci that contribute to those phenotypes could vary from one individual to another.

Milkman's assays were aided by the fact that some of his loci responded to temperature shifts in unique ways, allowing him to determine allelic makeup fairly directly. In our assay of cell death polygenes, we were unable to distinguish among loci. Instead, we surveyed genotypes by comparing their effects upon the development of different major mutants. Since each mutation has a slightly different developmental defect, each would be expected to be modifiable by a slightly different subset of the polygenic influences upon cell growth and death in each isofemale strain.

The chromosome manipulation of Sarnia-2 is a first step towards distinguishing between general and specific polygenic action in this system. The second chromosome from Sarnia-2 carries one or more polygenic loci that appeared to have effects limited to *sd*. There was little evidence of segregation within this line, which is perhaps not too surprising considering the fact that it was one of the most extreme isofemale strains and might consequently be homozygous for polygenic loci of comparatively large effect (see Parsons, 1975). Loci having relatively specific actions in development allow much greater flexibility in the response to selection than might occur if all loci had significant pleiotropic effects.

In conclusion, in spite of the wide range of polygenic influences that can be seen in quantitative traits, selection in natural populations may favour the maintenance of a more limited array of balanced polygenic combinations. Phenotypic expression of major mutants offers a powerful tool for focusing upon those loci and

combinations that help buffer or regulate development of complex characters in natural populations.

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