Inversion polymorphism and accumulation of lethals in selected lines of *Drosophila melanogaster*

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Five long-term selected lines of *Drosophila melanogaster* were monitored for the presence of lethals and inversions on the third chromosome for a period of nearly 100 generations after the cessation of selection. The results provide an example of the action of inversions as a trap for genes with homozygous detrimental effects in small populations. Lines polymorphic for cosmopolitan inversions *In(3R)C* and *In(3L)P* maintain linked lethal and detrimental genes in a near-balanced system. Consideration of the high selection response attained despite inversion polymorphism leads to the conclusion that extreme responses to selection can be obtained without exploiting the potential from large genomic regions.

Keywords: artificial selection, *Drosophila melanogaster*, inversion polymorphism, lethal genes, Müller's ratchet, population genetics.

Introduction

Accumulation of detrimental alleles in artificially selected lines has been mainly studied from the point of view of the limits of response to selection. A limit of selection can be reached in spite of genetic variation for the selected trait when artificial selection is opposed by natural selection (Nicholas & Robertson, 1980).

An extreme example of this occurs when artificial selection favours the heterozygous form of a recessive lethal. The frequency of such a lethal can then increase to values approaching a maximum of one-half in the selected individuals. As a consequence the probability of losing recessive detrimental alleles at other loci linked in disequilibrium to the first and therefore protected from natural selection, is reduced. This will occur whether or not these alleles affect the selected trait (Madalena & Robertson, 1975). A progressive accumulation of detrimentals in coupling or in repulsion with the first will then ensue.

Experimental data on the accumulation of lethals in artificially selected lines of *Drosophila* have been documented on several occasions. Most work on this topic shows that one or more lethals have an effect on the selected trait (Clayton & Robertson, 1957; Frankham *et al.*, 1968; Madalena & Robertson, 1975; Yoo, 1980;

García-Dorado & López-Fanjul, 1983), even though in some cases their frequency was too high to be explained by their effect on the selected trait. On the other hand, there are some examples of accumulation of lethals (Skibinski, 1986; Domínguez *et al.*, 1987; García-Dorado and López-Fanjul, 1987) that could not be accounted for by the action of artificial selection.

Most of the above-mentioned works do not take into account other phenomena of importance in finite populations such as meiotic drive or the suppression of recombination by chromosomal abnormalities. One of the effects of inbreeding is gene fixation in the homozygous state, but another effect may be, paradoxically, to permit the establishment of fixed heterozygosity. In small populations, inversions or other rearrangements suppressing recombination can act as a trap for detrimental alleles (Müller, 1964; Felsenstein, 1974; James, 1992). Chance fixation of detrimental mutations in haplotypes closed to recombination and subjected to genetic drift ('Müller's ratchet mechanism') can result in an increasing superiority of heterokaryotypes. Accordingly, heterozygosity in such regions becomes increasingly obligatory, and the return to a homozygous state becomes increasingly difficult, leading to a balanced system. This process appears to be responsible for a number of cases of permanent heterozygosity in a variety of species (Carson, 1967).

In previous experiments Domínguez *et al.* (1987) have found high frequencies of chromosome II and III

lethals in long-term selected lines. Accumulation of lethals and detrimentals on chromosome II has been explained by its association with the *Segregation Distorter* complex (*SD*, Lyttle, 1991) together with drift and restricted recombination (Domínguez *et al.*, 1993b). This paper describes the evolution of chromosome III lethals during a period of relaxation of nearly 100 generations, and provides evidence that they are kept stably in the lines because of their association with polymorphic inversions.

Materials and methods

Ac-27P, Ac-27S, S-27P, S-27S and N-21 are five long-term selected lines derived from a population caught in a wine cellar in 1976. They were selected for increased dorsocentral bristle number for 110 generations. At first, three lines were established: N-21, Ac-27 and S-27. Lines Ac-27S and Ac-27P were derived from the single selected line Ac-27 at generation 16. In the same way, lines S-27S and S-27P were derived from S-27 at generation 17 (for more details see Domínguez *et al.*, 1987, 1993a). Since generation 110, the lines have been kept without selection in three bottles per line with 10 pairs per bottle per generation.

Before selection was stopped, Domínguez *et al.* (1987) found three different chromosome III lethals in samples of selected males at generation 107. Lethal III_I was detected in line Ac-27S, lethal III_2 in lines S-27S and S-27P and lethal III_3 in line Ac-27P. One representative lethal chromosome from each allelic group was kept in stock balanced with the TM3 chromosome (for a full description of this and other special stocks see Lindsley & Zimm, 1992).

Figure 1 shows the general mating procedure practised to test chromosomes III from the lines for lethals, inversions, fertility and viability. Lethals were scored by individual crosses between a random sample of males from every line and a $TM3/Pr$ balanced stock.

One *Pr* male was picked up from each progeny and tested for lethals previously found by crossing with the appropriate lethal representative stock. Lethality of chromosomes lacking III_I, III_2 or III_3 was tested in the offspring of $TM3 \times TM3$ crosses (G2 in Fig. 1). In every case a chromosome was classified as lethal when no wild-type individuals were recovered among at least 50 progeny of the appropriate mating. This arbitrary number yields a probability less than or equal to 0.08 of misclassifying as lethal a chromosome with a homozygous viability greater than or equal to 0.1.

Inversions were scored by crossing *TM3* males from G2 with Canton S females. Salivary glands from third-instar larvae were dissected, squashed and orcein-stained according to a standard protocol (Ashburner, 1989).

Meiotic drive was tested for by comparing the segregation ratio (k) of lethal versus non-lethal chromosomes in heterozygous males and females. In essence, eight chromosomes were extracted from each individual in a sample of males and females and classified as lethal or non-lethal by progeny tasting. Hence individual genotypes were ascertained and k values calculated for each heterozygote. This was accomplished as follows: a random sample of 30 males and 30 females from each line were individually crossed to the $TM3/Pr$ stock and eight $TM3$ males, taken from each progeny, were crossed with the representative balanced stock of the lethal III_I or III_2. When at least one of these last matings did not yield wild-type progeny, the tested specimen was classified as lethal heterozygote (III_i/III_i^+). The k value for each heterozygous G0 specimen was scored as the proportion of $III_i/TM3$ males in his progeny. The mean k value of III_i/III_i^+ males compared to that of females, provided a test of distortion of segregation within the line.

Lethals were located by using the *rucuca* (*ru, h, th, st, cu, sr, e^s, ca*) and *ruPrica* (*ru, h, th, st, cu, sr, e^s, Pr, ca*) chromosomes (for a more detailed description see

G0   ♂ Problem Line X ♀♀ TM3/Pr

G1   ♂ Pr/+   X   ♀♀ TM3/III$_i$ Complementation test

if complements

G2   ♂♂ TM3/+ X ♀♀ Canton S   ♂♂ TM3/+ X ♀♀ TM3/+

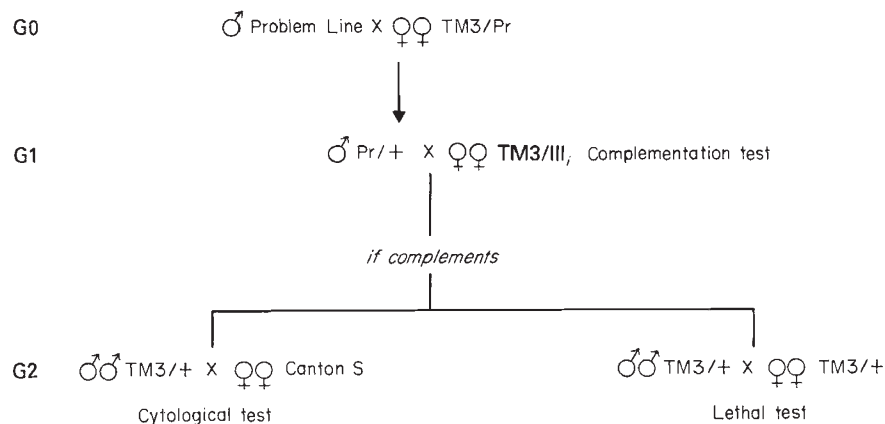   Cytological test   Lethal test

Fig. 1 General mating scheme to test chromosomes III for lethality and inversions.

Lindsley & Zimm, 1992). Males from the $III_i/TM3$ representative stocks were crossed to *rucuca* females. $III_i/rucuca$ females from their progeny were crossed to *ruPrica*/$TM3$ males. Recombinants between the lethal and *rucuca* chromosomes were recognized when heterozygous with *ruPrica* and tested for lethality by crossing individually with females from the balanced stock from the corresponding lethal ($III_i/TM3$).

Results

The evolution of frequencies of lethals III_1 and III_2 in lines S-27S, S-27P and Ac-27S is shown in Table 1. They were maintained at frequencies near $1/3$ for a large number of generations. Lethal III_3 was found at a frequency of 0.12 in line Ac-27P at generation 107, but it was not detected in successive tests.

Lethal III_1 was associated with the inversion $In(3L)P$ (63B8-11; 72E1-2). It is located in the left arm of chromosome III. No recombinants were found between *rucuca* and the left arm of the chromosome bearing the representative copy of lethal III_1. All III_1-carrying chromosomes extracted from line Ac-27S at generations 135, 170 and 185 bore the inversion, and all homozygotes for standard chromosomes were viable and fertile.

Table 2 shows the results of mapping the representative copy of lethal III_2. The results suggest a complex of at least two lethals, one located between the markers *curled* (*cu*, 50 cM) and *stripe* (*sr*, 62 cM) and the other between *ebony* (*e*, 70.7 cM) and *claret* (*ca*, 100.7 cM). It is possible that there may be some other lethals between these two, but this method only allows detection of the two extremes. The recombinant class $+ +$ $+ sr \, e^s \, ca$ was heterogeneous because some chromosomes were recombinant between the marker *cu* and the left lethal, and others between the lethal and the *sr* marker. The same can be said for the $h \, st \, cu \, sr \, e^s \, +$ class, but if there were another lethal between these two, for instance in the sr-e^s region, it would not be detected because the $h \, st \, cu \, sr \, + \, +$ recombinants

would bear the right-most lethal and the $+ \, + \, + \, + \, e^s$ ca the left-most.

Two lethal recombinant chromosomes ($h \, st \, cu \, sr \, e^s$ $+$ and $+ \, + \, + \, sr \, e^s \, ca$) bearing the two extreme lethals of the complex were balanced with $TM3$ and crossed with all the III_2-bearing chromosomes extracted from lines S-27S and S-27P at generation 170. There was no complementation in any case, showing that all the III_2-bearing chromosomes shared at least the two extreme elements of the III_2 complex. Lines S-27S and S-27P were both polymorphic for inversion $(3R)C$ (92D1-E1; 100F2-3). Lethal III_2 was always found associated with the standard ordination, whereas all $In(3R)C$ chromosomes were lethal-free except one sampled from line S-27S at generation 185. Homozygotes for non-lethal chromosomes were all fertile.

The two other lines were not polymorphic for chromosome III inversions. N-21 was homozygous for $In(3R)C$, and Ac-27P for the standard ordination. No third chromosome lethals were detected in either line at generations 135 and 170.

Lethals III_1 in Ac-27S and III_2 in lines S-27 were not balanced by deleterious recessive genes with sizeable influences on viability. In lethal tests (Fig. 1) of generation 185, 15 vials were set up, each with a pair of a virgin $TM3$ heterozygous female and a male. Offspring from eggs laid in the first 5 days were scored and compared with the 2:1 theoretical segregation by χ^2 tests. Results departed significantly from expected ($P < 0.001$) in the three lines. The viability of homozygotes, calculated as the number of observed wild-type flies divided by the expected number, was larger than 1 in every case (Table 3), denoting that standard homozygotes from line Ac-27S and $In(3R)C$ homozygotes from lines S-27 were more viable than the corresponding $TM3$ heterozygotes.

Table 4 shows mean k scores for lethals III_1 and III_2 in heterozygous males and females. Errors are based on observed variances. Preferential transmission of lethals in males was tested for by comparing the k

Table 1 Frequencies of chromosome III lethals (number of tested individuals in parentheses)

| Lethal | Line | Generation | | | | | | |
		107*	135	138	150	170	185	200*
III_1	Ac-27S	0.28	0.07 (28)	—	—	0.42 (19)	0.32 (34)	0.25 (57)†
III_2	S-27S	0.17	0.31 (29)	0.38 (58)†	0.28 (60)†	0.43 (58)	0.23 (30)	—
III_2	S-27P	0.14	0.30 (30)	0.39 (60)†	0.36 (57)†	0.32 (55)	0.33 (30)	—

*Data from Domínguez *et al.*, 1987.
†Scored from genotypic frequencies in meiotic drive tests.

values yielded in males and females by means of one-tailed Student's t-tests. In line S-27S the lethal III_2 segregation ratio was significantly higher in males than in females. Though not significant, the same trend could be observed for line S-27P. If results from the two lines are considered jointly, preferential transmission of the lethal III_2 in heterozygous males is significant at the $P = 0.01$ level ($\chi_8^2 = 22.90$). This result points to a distortion of segregation similar to that of the SD complex.

In most segregation distorter systems, preferential transmission takes place by the interaction between a

Table 2 Localization of lethal III_2

Recombinant chromosomes						Non-lethal	Lethal
h	st	cu	sr	e^s	ca	13	0
$+$	st	cu	sr	e^s	ca	13	0
$+$	$+$	cu	sr	e^s	ca	10	0
$+$	$+$	$+$	sr	e^s	ca	4	2
$+$	$+$	$+$	$+$	e^s	ca	0	7
$+$	$+$	$+$	$+$	$+$	ca	0	13
$+$	$+$	$+$	$+$	$+$	$+$	0	13
h	$+$	$+$	$+$	$+$	$+$	0	13
h	st	$+$	$+$	$+$	$+$	0	13
h	st	cu	$+$	$+$	$+$	0	10
h	st	cu	sr	$+$	$+$	0	7
h	st	cu	sr	e^s	$+$	6	6

Table 3 Viability of homozygotes for non-lethal chromosomes relative to that of TM3 heterozygotes (see text for more details)

Line	$+/+$	$+/TM3$	χ^2	Viability of $+/+$
S-27S	932	1328	63.56*	1.24
S-27P	793	1255	26.75*	1.16
Ac-27S	841	1454	11.32*	1.10

*$P \leqslant 0.001$.

distorter locus in the drive chromosome and a target locus in its homologue (Lyttle, 1991). Target alleles have different degrees of sensitivity to the distorter, and sensitive alleles are abundant in populations. To test further the possibility of preferential transmission of lethal III_2, we have tested the transmission ratio of III_2-bearing chromosomes against several recessive-marked chromosomes III from laboratory strains (*rucuca, ebony, sepia* and *scarlet*) and the *cinnabar brown* strain sensitive to Segregation distorter (*Sd*), but we failed to find any distortion. Tests for sensitivity of chromosomes III from lines Ac-27S, Ac-27P and N-21 to recessive-marked III_2-bearing chromosomes also provided negative results. It thus seems probable that the observed differences in transmission between sexes were actually due to differences in viability between the male progeny of the reciprocal crosses that could be attributed to the different genetic constitution of the X chromosome.

Discussion

The stability of the polymorphism after relaxation of selection proves that it is not maintained by artificial selection. This corroborates the assumptions of Domínguez *et al.* (1987) based on the lack of effect of lethals on the selected trait. Lethals on chromosome III have been submitted to forces different from artificial selection. However, in contrast to lethals on chromosome II (Domínguez *et al.*, 1993b), preferential transmission cannot account for their maintenance. Although it was statistically significant in lines S, failure in the search for sensitive chromosomes points to the possibility that it was an artefact.

The selection coefficient necessary to maintain a balanced polymorphism can be obtained from the equation $\hat{q} = s_1/(s_1 + s_2)$. Lethals in this study fluctuated around frequencies near 1/3 and, therefore, the selection coefficient against non-lethal chromosomes (s_1) must be around 0.5. Nevertheless, they do not bear

Table 4 Segregation ratios of lethal versus non-lethal bearing chromosomes (k) from heterozygotes (number of tested heterozygotes in parentheses)

Lethal	Line	Generation	k Males	k Females	d.f.	t
III_1	Ac-27S	200	0.46 ± 0.04 (14)	0.49 ± 0.04 (15)	27	0.49
III_2	S-27S	138	0.52 ± 0.04 (21)	0.43 ± 0.03 (23)	42	1.87*
		150	0.50 ± 0.04 (18)	0.40 ± 0.04 (16)	32	1.76*
III_2	S-27P	138	0.46 ± 0.03 (24)	0.41 ± 0.03 (23)	45	1.22
		150	0.50 ± 0.03 (20)	0.43 ± 0.03 (21)	39	1.65

*$P \leqslant 0.05$.

genes with clear-cut negative effects on viability nor fertility. The genetic load of III_1 and III_2 lethals must be counteracted by accumulation on the opposite chromosomes of genes with negative effects on other components of fitness, probably prezygotic ones such as fecundity or sexual activity.

Our results can be interpreted on the basis of Müller's (1964) 'ratchet mechanism'. Polymorphism for inversions and small population size must be responsible for the accumulation of detrimentals. Chance fixation of detrimental alleles in haplotypes closed to recombination can increase the heterokaryotype advantage (Ohta, 1971; Felsenstein, 1974; James, 1992) and finally lead to a balanced system. The process will be favoured if heterokaryotypes initially show a selective advantage large enough to maintain the polymorphism in the face of drift, which is likely because overdominance is very frequently observed for paracentric inversions in natural populations (Sperlich & Pfreim, 1986).

Overdominance of inversions can, in turn, be interpreted as a consequence of lack of recombination (Ohta, 1971). Coincident occurrence of the III_2 complex in two lines suggests that the polymorphism for $In(3R)C$ in the base population was maintained by associative overdominance. Inoue & Watanabe (1992) showed that natural populations must be considered as sets of divided or semidivided small populations. In such subpopulations inversion polymorphism is maintained by heterokaryotype advantage, and is destroyed when inversions from different subpopulations recombine. Although they interpret their results on the basis of coadaptation, the same would be expected if chromosomal heterosis were due to deleterious recessive genes. The small size of such subpopulations would favour the ratchet mechanism.

We have no similar data to decide whether the elements involved in the balance of $In(3L)P$ came from the base population, but the persistence of the polymorphism in the face of artificial selection and drive should require strong balancing in the early stages of artificial selection. On the other hand, some data such as the loss of lethal III_3 in line Ac-27P and the variety of associations between $In(2L)t$, elements of the SD complex and detrimental genes found by Domínguez *et al.* (1993b) in chromosome II of the lines Ac suggest that some associations had been established throughout the selection and relaxation processes.

Some cases of lethal polymorphism associated with inversions in natural populations (Coyne *et al.*, 1991) and selected lines (García-Dorado & López-Fanjul, 1987) can also be explained on the basis of the ratchet mechanism, but most other cases of accumulation of lethals in artificially selected lines have not been investigated for chromosomal abnormalities.

Table 5 Mean number of dorsocentral bristles in females and chromosome sequences found in the lines under study (ST = standard ordination)

| Line | No. of dorsocentral bristles* | Types of chromosome sequence | |
		II†	III
Ac-27S	16.9 ± 0.5	$In(2L)t + ST$	$In(3L)P + ST$
Ac-27P	16.5 ± 0.6	$In(2L)t + ST$	ST
S-27S	38.4 ± 1.0	ST	$In(3R)C + ST$
S-27P	36.8 ± 1.1	ST	$In(3R)C + ST$
N-21	35.6 ± 1.3	$In(2L)t + ST$	$In(3R)C$

*Data from Domínguez *et al.* (1993a).
†Data from Domínguez *et al.* (1993b).

Finally, it is interesting to note the high degree of polymorphism found in these lines despite the long and successful selection process to which they were subjected (Table 5). The three lines with the higher selected phenotype (S-27S, S-27P and N-21) seem to share the same selected alleles distributed among the three major chromosomes (Domínguez *et al.*, 1993a), but they have different inversion contents. It is thus clear that inversions were independent of selection response. The same can also be deduced from the fact that lethals in these lines had no influence on the selected trait (Domínguez *et al.*, 1987). As most lethals were linked to inversions, neither inversion has any influence on the number of bristles.

In consequence the extreme phenotypes attained (even more than eight times the normal number of bristles, in lines S-27 and N-21) have been achieved without exploiting the potential from large genomic regions that had yielded to forces other than artificial selection. These results agree with theories relating selection response to a small number of genes with large effect (Thompson, 1975) and with recent experimentation on the distribution of effects of spontaneous mutations on quantitative traits denoting the presence of major genes (Santiago *et al.*, 1992; López & López-Fanjul, 1993).

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