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Analysis of lethals in selected lines of *Drosophila melanogaster*

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Summary. Five lines of *Drosophila melanogaster* that reached an extreme phenotype after long-term selection for increased dorsocentral bristle number, were analysed for the presence of lethals. Seven chromosome II and three chromosome III lethal types were detected in four of the lines, at frequencies ranging from between 6% and 36%. No lethal had any demonstrable effect over the selected trait. In one line, where almost every chromosome II was a lethal carrier, it was shown that the main lethal (at a frequency of 36%) was associated with the transmission ratio distortion in males. The processes which could lead to the accumulation of this lethal and others linked in disequilibrium to it is discussed. Some results suggest similar mechanisms for the accumulation of lethals in the other lines. These findings show that causes other than the direct effect of artificial selection must be taken into account when trying to explain the accumulation of lethals in selected lines.

Key words: Artificial selection – Lethals – Segregation distortion – Dorsocentral bristle number – Drosophila melanogaster

Introduction

The presence of lethals at high frequencies in selected lines of *Drosophila* has been documented on several occasions (Reeve and Robertson 1953; Clayton and Robertson 1957; Frankham et al. 1968; Hollingdale 1971; Madalena and Robertson 1975; Yoo 1980; García-Dorado and López-Fanjul 1983; Skibinski 1986).

When a lethal has an effect on the selected trait or is closely linked to another gene with such an effect, selection will change the frequency of the lethal allele until an equilibrium is reached where all selected parents are lethal heterozygotes and the lethal frequency on the offspring is, therefore, one-third (Hollingdale 1971).

An additional consequence (Madalena and Robertson 1975) of selecting lethal heterozygotes at a given locus is a lower probability of losing lethal alleles at other loci that are linked in disequilibrium to the first and thus protected from natural selection. This will occur whether or not those alleles affect the selected trait. A progressive accumulation of lethals at high frequencies in the selected line will then ensue.

Most work on lethal accumulation in selected lines showes that one or more of the detected lethals have an effect on the selected trait (Clayton and Robertson 1957; Frankham et al. 1968; Hollingdale 1971; Madalena and Robertson 1975; Yoo 1980; García-Dorado and López-Fanjul 1983). In some of these cases, however, the frequency at which lethals were found could not be completely explained by their effect on the trait (Frankham et al. 1968; Hollingdale 1971; Yoo 1980).

The fact that the same lethal was sampled repeatedly on a number of males of selected lines (Reeve and Robertson 1953; Skibinski 1986) suggests other mechanisms of lethal maintenance. This observation was interpreted by Reeve and Robertson (1953) as lethality being dependent on the genetic background of the stock used for detection, while Skibinski (1986) suggested segregation distortion associated with lethal alleles. Whether the identified lethals were non-lethals in the selected lines, or were associated with segregation distortion, they were able to reach high frequencies in selected lines without having an effect on the selected trait.

We present here an analysis on lethals, carried out in five *Drosophila melanogaster* lines selected for in-

creased dorsocentral bristle number. The effect of lethals on the selected trait, the lethality dependence on background and the association of lethals with segregation distortion have also been considered in order to explain the high frequencies attained.

Materials and methods

The selected lines were kindly provided by Dr. Nuez (Universidad Politécnica de Valencia). From a population caught in a wine cellar, three lines selected for increased dorsocentral bristle number were set up: N-21, Ac-27 and S-27. N-21 was raised in a normal culture medium at 21 °C, Ac-27 in a medium rich in acetic acid (12.8 ml/l water) at 27 °C and S-27 in a medium rich in sugar (416 g/l water) at 27 °C. At generation 16, Ac-27 was split into two sublines: Ac-27S and Ac-27P. At generation 17, S-27 was also subdivided (S-27S and S-27P). All lines were selected during 90 generations followed by 10 generations of relaxation. After that, we reassumed selection in all lines with a selected proportion of 30%. Normal culture medium at 24 °C was used throughout the experiments. Table 1 shows the realised heritability of each line over the 11 former generations in this second period of selection, with the dorsocentral bristle average at the end.

Lethals on the second and third chromosomes were scored using a SM5/Sp; TM3/Pr stock (Lindsley and Grell 1968). A second and third chromosome from each of the 30 selected males in every line were scored for complete lethals at generation 7 of the second period of selection. In this first lethal test, all lethal chromosomes were kept as balanced stocks with SM5 or TM3 chromosomes to test their allelic relationships by half diallel crosses, within and between lines. Finally, one representative lethal from each allelic group was kept for further allelism tests. When the general situation had become clear, a second test was carried out to calculate the frequency and effect of every lethal in each line. This was carried out after three (S-27S and Ac-27S) or six (S-27P and Ac-27P) further generations of selection. A random sample of 60 males per line was scored for dorsocentral bristle number. Then, four second and four third chromosomes from each male were extracted by means of the SM5/Sp; TM3/Pr stock and tested for lethality by crossing with a representative of each lethal allelic group determined in the first test. When a line showed more than one type of lethal chromosome for a given pair, the four extracted chromosomes from each male were replicated by further crossing to the balancer stock and then tested separately for lethality against each lethal representative. Moreover, the II chromosomes extracted from the Ac-27P line were tested in homozygosis to see if they were self-lethals. Genotypes were assigned according to the lethal content of the four chromosomes extracted from each male.

Results

In the first test, which was carried out on selected males, a total of nine lethals represented in the sample by more than a single copy were detected. Six were located on the second chromosome and the other three on the third (Table 2). Some inconsistencies were found in this first test. All Ac-27P second chromosomes were self-lethal with one exception; however, this non-lethal chromosome was lethal with the entire II_3 allelic group. The chromosome which was kept as a representative of lethal group II_5 was later shown to be incomplete since some homozygote individuals were observed in subsequent generations.

Table 1. Realised heritability in the former 11 generations of the second period of selection and means of dorsocentral bristles in the last generation

Line	h^2	$\bar{X}$ males	$\bar{X}$ females
Ac-27S	0.05±0.02	11.57±0.15	14.66±0.18
Ac-27P	−0.01±0.02	11.19±0.17	14.49±0.23
S-27S	0.28±0.04	28.63±0.41	36.26±0.49
S-27P	0.14±0.02	26.06±0.35	34.73±0.47
N-21	0.02±0.02	23.95±0.35	29.36±0.40

Table 2. Lethal frequencies in the first test. Frequencies were calculated from 25–30 chromosomes per line

	Lines				
	Ac-27S	Ac-27P	S-27S	S-27P	N-21
Chromosome II					
II_1	0	0	0	0.28	0
II_2	0	0	0.30	0.12	0
II_3	0	0.63	0	0	0
II_4	0	0.21	0	0	0
II_5	0	0.13	0	0	0
II_6	0	0.13	0	0	0
Chromosome III					
III_1	0.28	0	0	0	0
III_2	0	0	0.17	0.14	0
III_3	0	0.12	0	0	0

Table 3 shows the results of the second test, where four (or three, if not wholly successful) chromosomes per male were extracted. No significant effects on the selected trait were detected. Frequencies of lethals II_2 (S27S), II_1 and II_4 were somewhat different from those in the first test. Lethals II_2 and III_2 from S-27P and III_3 from Ac-27P were not found and therefore their effect could not be calculated.

Lethal frequencies in line Ac-27P ought to be analysed further. For 23 out of 39 males carrying the lethal II_3, this was the only cromosome sampled. The probability of such repeated sampling under the hypothesis of equal transmission frequencies is less than 10^{-10}, which shows that either lethal II_3 is non-lethal in the selected line (and most of these males were homozygous), or that the chromosome carrying this lethal is transmitted preferentially. A survey on the segregation frequencies for males known to be heterozygous showed preferential transmission. Lethal II_3 and a distinct chromosome had been sampled from 16 males; the remainder extracted were 19 II_3 chromosomes and 6 which were non-carriers of this lethal ($X^2_{1\,d.f.}=2.96$;

Table 3. Lethals detected in each line and their frequencies and effects on the selected trait

Line	Lethal	No. of males tested	No. of males l/?[a]	Lethal frequency	Effect on selected trait
S-27S	II_2	53	4 (0.03)	0.17	0.07 ± 1.05
	III_2	53	1 (0.64)	0.14	-0.20 ± 1.12
S-27P	II_1	56	0	0.07	-0.67 ± 1.63
	II_2	55	–	0	–
	III_2	56	–	0	–
Ac-27S	III_1	43	3 (0.20)	0.27	0.04 ± 0.38
Ac-27P	II_3	54	23 ($< 10^{-10}$)	0.36	0.32 ± 0.60
	II_4	54	2 (0.05)	0.05	1.04 ± 1.00
	II_5	54	0	0.05	0.05 ± 1.01
	II_6	54	1 (0.67)	0.16	1.07 ± 0.73
Cromosome II lethals not found		54	0	0.08	–
in the first test	II^+	54	0	0.06	–
	II?[b]	54	0	0.24	–
	III_3	54	0	0	–

[a] The probability of obtaining at least that number when the gene is lethal is given in brackets
[b] From males in which chromosome II_3 was the only one sampled

$P < 0.01$). The number of II_3 chromosomes sampled from males with at least one of these lethal-bearing chromosomes was 114 out of a total of 136 chromosomes extracted. Thereafter, the sampling frequency of chromosome II_3 (instead of its homologue) is 0.84 ± 0.05. Given such a preferential sampling, the gametic frequency of II_3 in the first test agrees with the genic frequency in the second test.

Significant repeated sampling was observed for two other lethals (II_2 from S-27S and II_4 from Ac-27P), but the segregation data in the known heterozygous could not be analysed because of the lower frequencies of these lethals and as sampling from a male was stopped when two chromosome types were detected.

A further analysis to confirm the preferential transmission hypothesis was carried out in line Ac-27P after 15 generations of relaxed selection. Eight second chromosomes were extracted from each specimen in a ramdom sample of 22 males and 20 females, using the SM5/Sp stock, and tested for lethality with the representative copy of lethal II_3. The pooled frequency (from males and females) of lethal II_3 (0.42) did not differ from that calculated in the second lethal test. The distributions of sampling frequencies of chromosomes II_3 in males and females are shown in Fig. 1. Heterozygous males transmited chromosome II_3 with a higher frequency than females ($P < 0.01$). Comparison of observed variances with those expected in a binomial distribution (data were arc sin transformed), showed significant differences only in males ($P < 0.001$) and therefore errors in k estimates were based on variance between males.

Frequencies and effects of Ac-27P lethals other than II_3 (Table 3) are underestimated due to the impossibility of assigning genotypes to those males in which II_3 was the only second chromosome sampled.

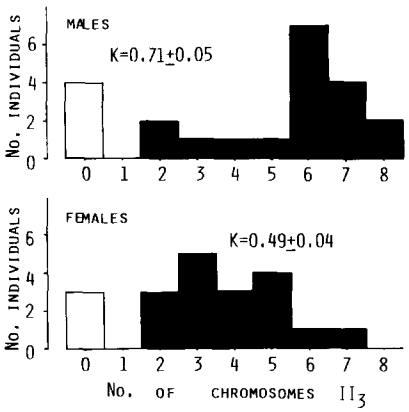

Fig. 1. Distribution of sampling frequencies of chromosome II_3; 8 chromosomes were extracted for each of 22 males and 20 females

Some inconsistencies in the second test on line Ac-27P suggest that lethality is somewhat dependent on genetic background. Three apparently different types of second chromosomes were obtained from the same male in three cases; thus II_6 (or perhaps II_6 and II_3) may be an incomplete lethal in some instances. In two other cases, chromosomes behaving as lethals with representative copies of II_5 and II_6 were not self-lethals.

It is interesting to note that only 6% of the chromosomes from Ac-27P in the second lethal test were lethal free. Lethals showed an important linkage disequilibrium. Only 3 II chromosomes out of 25 carried more than one lethal (II_5 and II_3) in the first test, while no II chromosomes carried more than one lethal in the

second test. The other line with more than one second chromosome lethal type (S-27P) did not show an important linkage disequilibrium since chromosomes with the three possible lethal combinations were present.

Discussion

The frequencies of lethals observed here are in line with the values reported in other studies which relate their high frequencies to an effect on the selected trait. However our results are in sharp contrast with most previously reported work. No lethal had any demonstrable effect over the selected trait. Of course, their non-significant effects do not exclude their existence, but if they had an effect large enough to be maintained by artificial selection, it would be rather unlikely that this would not have been shown in the analysis.

Lethals II_2 and III_2 were maintained in lines S-27S and S-27P for nearly 100 generations; besides, frequencies of lethals III_1 and II_3 were maintained from the first test to the second and even through 15 further generations for the latter. These facts rule out random drift as the main operating factor.

Some inconsistencies found when assigning genotypes to males from line Ac-27P can be explained if lethality is somewhat dependent on genetic background, as in Yoo (1980), rather than resulting from the clustering of small overlapping lethal deletions, as in Hollingdale (1971). Nevertheless, it was supposed to be the cause of repeated sampling of lethals by Reeve and Robertson (1953). It is not supported by our study, which clearly shows that lethal II_3 from line Ac-27P is transmitted preferentially by males.

Preferential transmission associated with a lethal would self-select it and then increase its frequency until equilibrium is reached. The self-selection for lethal heterozygotes at that locus would protect lethal alleles at other loci from natural selection when they are linked in disequilibrium to it (Madalena and Robertson 1975). The accumulation of these lethals would increase the frequency of the main lethal. The equilibrium frequency of the main lethal and the accumulation of other lethals would depend on the segregation ratio.

The segregation ratio found in the distortion test was 0.71. This would lead lethal II_3 to an equilibrium frequency of 0.18 if it were the only lethal in the line. This frequency can hardly explain the accumulation of other lethals linked in disequilibrium to the first. Nevertheless, we have found differences between males for k values, which suggests that it may be also subject to selection. Indeed, the segregation ratio of lethal II_3, as estimated from the second lethal test, was higher than that estimated later ($P < 0.05$). Therefore, we must suppose that the k value associated with lethal II_3 when it first occurred in the line was higher than later estimates. After the accumulation of this lethal and others linked in disequilibrium to it, selection of modifiers with smaller sensitivity to distortion would lead to a reduction in the segregation ratio. We do not know what mechanisms are implied in the maintenance of lethals in the other lines, nor why lethals II_2 and III_2, which were maintained in line S-27P for so many generations, have been suddenly eliminated from it in only six generations. However, the repeated sampling of some of the lethals in these lines suggests that similar mechanisms could be implicated.

Preferential transmission was suggested Skibinsky (1986) as the possible cause of repeated sampling for some of the lethals in his lines, and here it was shown to be so for at least one of our lines. Therefore, this is another mechanism to take into account when explaining lethal accumulation in selected lines.

Transmission ratio distortion has been shown in *Drosophila* for the so called "segregation distorter" system (Hartl and Hiraizumi 1976), which is frequently found in natural populations, and is also associated with hybrid dysgenesis (Kidwell et al. 1977). It is not clear whether the effect we have found is in any way related to one or the other. However, there was an unusual, high response in artificial selection (the phenotype of females has been increased 9 times for some of the lines) and most lethals were detected in only one of the lines. Together, these point to a great deal of new variation originating during selection. It is known that transposable elements can create additional variation for quantitative traits and contribute to the realized response to selection (Mackay 1985). On the other hand, they may be implicated in the origin of lethal mutations and transmission ratio distortion (Kidwell et al. 1977), just as transpositions were related to the drastic increase of fitness in inbred lines of *D. melanogaster* (Pasyukova et al. 1986). Therefore, we suggest that transposable elements could satisfactorily explain the intuitively puzzling observations of lethals at high frequencies and the very large response to selection in the studied lines, although this topic should be the object of experimentation in the future.

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