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Original article

Selection for acrolein tolerance in *Drosophila melanogaster*

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Summary – Selection, at two temperatures (17 °C and 24 °C), to increase the tolerance of *D. melanogaster* to the toxic action of acrolein, was carried out. At both temperatures, the tolerance increased progressively as a quantitative trait. No major gene implicated in the tolerance was detected. Associated with the increase of tolerance, the lines showed an increase of body size and number of sternopleural bristles, a reduction of fitness, measured as productivity, a lengthening of developmental time and a nearly complete elimination of chromosomal inversions. However, an appreciable number of differences between the lines selected at 17 °C and 24 °C were found.

toxic tolerance – acrolein – selection – *Drosophila melanogaster* – associated responses

Résumé – Sélection pour la tolérance à l'acroléine chez *Drosophila melanogaster*. *On a réalisé, à deux températures (17 °C et 24 °C) une sélection pour accroître la tolérance de* D. melanogaster *à l'action toxique de l'acroléine. Aux deux températures, la tolérance a augmenté progressivement comme un caractère quantitatif. On n'a pas détecté de gène majeur impliqué dans la tolérance. Les lignées ont montré, associées à la tolérance, une augmentation de la taille du corps et du nombre des soies sternopleurales, une réduction de l'aptitude à la reproduction mesurée par la productivité, une augmentation du temps de développement et une presque totale élimination des inversions chromosomiques. On a trouvé, ainsi un grand nombre de différences entre les lignées sélectionnées à 17 °C et 24 °C.*

tolérance à des toxiques – acroléine – sélection* – Drosophila melanogaster – *réponses associées

INTRODUCTION

Acrolein, an unpleasant and troublesome by-product of overheated organic matter, is also a useful substance in important industrial syntheses (Fishbein *et al.*, 1970). Its high reactivity makes acrolein a dangerous substance for the living cell, whose nucleus (Moule and Frayssinet, 1971; Alarcon, 1972) and locomotor apparatus

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(Wynder *et al.*, 1965; Battista and Kensler 1970; Munsch *et al.*, 1973) are both affected. Its environmental presence in industrial fumes, tobacco smoke and car exhaust has stimulated interest in research concerning the toxic and mutagenic effects on a variety of organisms (Izard, 1967, 1973; Brown and Fowler, 1967; Andersen *et al.*, 1972; Izard and Liberman, 1978).

However, despite the facilities offered by *D. melanogaster* as a model in resistance genetics and mutagenesis, as far as we know, only the mutagenicity on larvae (Rapoport, 1948) and the variation of sensitivity to acrolein during development (Comendador, 1984) are known. In this paper, the first of a series, the results of selection to increase the tolerance are shown. In following papers, the genetic architecture and putative mechanisms of resistance will be presented.

MATERIALS AND METHODS

Selection

Selection was carried out on a wild population from Asturias (Spain), with caught within the border which had been of a non-polluted chestnut grove near Oviedo and maintained in a population cage four months before the selection was initiated, this population cage was initiated with all individuals, 150 pairs approximately, that were caught. Two selection lines were derived from this population: one at 24 °C, R24, and another one at 17 °C, R17. The procedure to maintain the selected lines was the following: from R24, as well as from R17, 500 males and 500 virgin females, 2-3 days old, were divided into groups of 50 individuals (10 groups of males and 10 of females, numbered 1 to 10). Each group of flies was placed, without previous etherization, into petri-dishes with agar-maize meal-sugar medium, seeded with an acrolein aqueous solution supplemented with live yeast (4%). These Petri-dishes were placed in a climatic chamber at 24 °C or 17 °C, respectively, for each selection line. After four hours, the surviving individuals were transferred to vials with fresh standard medium (agar-baker's yeast-sugar) and the number of surviving individuals was recorded 16-18 h later. The survival rate for each generation was estimated as the percentage of surviving individuals.

From every group of treated individuals, ten surviving couples were taken and mated, using a circular subpopulation mating system to obtain the next generation. This method, used in each generation, was chosen because it minimizes genetic drift effects (Kimura and Crow, 1963). Two control lines were maintained: C24 as control for R24 and C17 as control for R17. The only difference between selected and control lines was that, in control lines, the individuals used as parentals were randomly taken. To know if the experimental procedure produced mortality *not* due to toxic action, several control experiments were carried out; their only difference with the acrolein treatment was that the toxin was not added. The survival rate was 100% in all these experiments.

In the first selection generation of R24, as well as R17, an acrolein concentration of 195mM was used. When the tolerance level of a generation was considered to be high enough (50% survival or higher), then the acrolein concentration was increased (5, 10 or 20%) with respect to the concentration used in previous generations. In this way, it was possible to maintain a suitable selection pressure (Falconer, 1981),

as well as, similar selection differentials in all generations. When, for any reason, it had been necessary to introduce any modification in the described method, these modifications will be indicated and described in the results section.

In different generations, the lethal concentration killing 50% of flies (LC_{50}) was estimated using the method of Davies (1971) and White and White (1981).

Correlated responses

The mean values of some biometric traits, as well as the frequencies of chromosomal inversions, were estimated in different generations of the selected and control lines.

For this study, 110 virgin females and 110 males were extracted from each line and pairmated avoiding crosses between individuals from the same bottle. Each couple was placed into a vial with standard medium for 48 h; afterwards, they were moved daily for three days, into new vials. The number of sternopleural bristles, as well as, the thorax size as an estimate of body size, were measured on these couples for each line. Likewise, the productivity, measured as the number of emerged adults, and the developmental time in days, were estimated from the emerged offspring in each of the three vials. Furthermore, the chromosomal inversion frequencies were obtained through the observation of polytene chromosomes of one larva descendant of each couple. This analysis was carried out following the method of Levine and Schwartz (1970). We have systematically studied five inversions carried by chromosomes 2 and 3 which were previously detected in this population (Roca *et al.*, 1982). These inversions are: 2Lt, 2RNS, 3LP, 3RP and 3RC. The description of these inversions can be found in Mettler *et al.* (1977), and Inoue and Watanabe (1979).

RESULTS

Tolerance variation during selection

The acrolein concentrations used, and survival obtained in each generation of R24, are shown in Table I. Figure 1 displays the variation of acrolein concentration used in each selection generation. Since the acrolein concentration was increased only if, in the former generation the survival rate was higher than 50%, the variation of concentration used can be seen as an indication of acrolein tolerance variation. The profile of the graph clearly shows a continous response along the selection generations in agreement with what would be expected if the tolerance to acrolein was a quantitative trait. In addition to the increase in concentration used for selection over successive generations, the tolerance increase in R24 is shown when the LC_{50} values of R24 and C24 are compared (Table II). The LC_{50} value is slightly smaller in C24 than in the base population, while R24 has experienced a large increase of its LC_{50} value. On the other hand, between the 14th and 20th and between the 23rd and 27th generations, R24 was maintained without selection, but no fall in the tolerance level was observed. So, it seems that the tolerance to acrolein is not depressed when selection is relaxed. Moreover, the regression coefficients of mortality probit-concentration logarithm are greater in R24 than in C24 and, therefore, a reduction in the genetical variability, which was expected as a consequence of the selection, is shown.

Table I. Acrolein concentration and survival in each generation of R24.

Generation	*Concentration*	*Survival (%)*	
		Females	*Males*
0	195	27.25	25.93
1	195	65.80	60.59
2	214	38.96	47.62
3	214	46.61	56.25
4	214	48.54	43.29
5	214	54.22	59.30
6	257	19.72	22.51
7	257	55.00	50.00
8	283	18.44	25.62
9	283	81.76	70.28
10	297	64.84	46.46
11	312	37.61	38.61
12	312	81.22	84.24
13	327	24.22	37.10
14	327	88.00	89.00
20	368	77.65	84.28
21	405	21.68	31.02
22	405	73.09	67.17
27	446	44.91	51.73
31	490	33.06	20.59
33	490	87.47	78.92
34	540	57.35	35.34

Table II. LC_{50} values, in mM, and regression coefficient of mortality probit-concentration logarithm (in brackets) in the 13th generation of C24 and R24 as well as in the base population.

Generation	*Line*	*Females*	*Males*
0	–	183.78±5.16 (4.09±0.39)	186.62±5.06 (5.18±0.41)
13	C24	112.20±3.90 (3.54±0.20)	144.91±2.97 (4.49±0.19)
13	R24	390.57±4.94 (5.33±0.25)	416.81±5.27 (5.90±0.20)

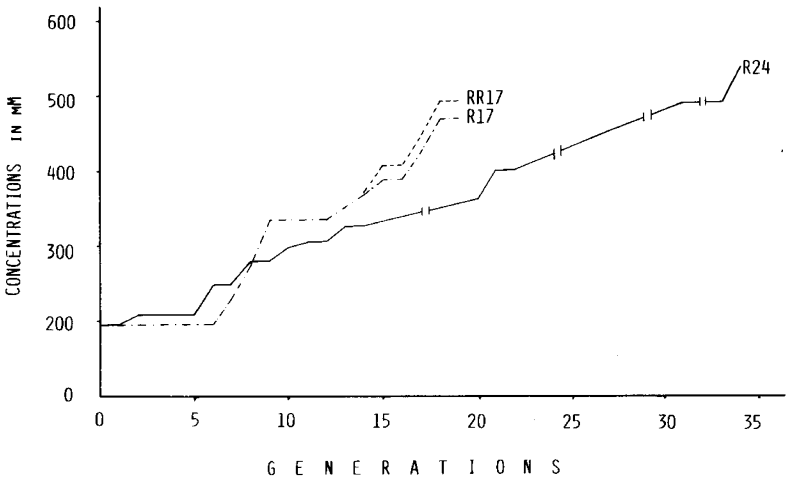

Fig. 1. Variation with generations of acrolein concentrations used for selection in R24, R17 and RR17 lines.

Table III. Acrolein concentration and survival in each selection generation of R17; the results corresponding to RR17 are between brackets (see text).

Generation	*Concentration*		*Survival (%)* *Females*		*Males*	
0	195		21.90		40.78	
1	195		19.37		37.07	
2	195		7.40		16.70	
3	195		73.64		71.83	
4	195		41.09		56.69	
5	195		39.20		40.80	
6	195		63.95		65.22	
7	234		55.49		59.53	
8	280		74.61		4.85	
9	337		32.05		36.99	
10	337		6.29		9.78	
11	337		29.49	(56.32)	27.90	(84.39)
12	337		80.99	(77.80)	81.99	(79.51)
14	370		84.21	(87.68)	86.42	(94.97)
15	389	(407)	44.42	(25.08)	41.80	(18.51)
16	389	(407)	75.61	(59.23)	69.96	(63.20)
17	428	(448)	67.42	(78.25)	82.84	(78.33)
18	470	(492)	23.28	(56.80)	32.52	(52.28)
19	470	(492)	16.97	(50.77)	14.01	(48.31)

Table II shows progress of selection in each generation of R17. Figure 1 also represent the concentration variation with selection generations. The profile of the graph is clearly different from that shown by R24; whereas, in R24, the response was linear from the first selection generation, while, in R17, the survival increase during the first six generations was very little. In fact, in the second generation, the survival rate was so small, that the reduced number of surviving individuals necessitated that we make a reduction in the line size. However, in the 7th and 8th generations, the survival rate underwent a great increase. Then, between the 9th and 11th generations, a new plateau appeared. In the 10th generation, the survival rate was so reduced that the population size was too small again. Because of this, and to prevent as much as possible, the effects of genetic drift, a new line was derived. This line, called RR17, was not selected in the 10th generation, but from this moment onwards, R17 and RR17 were treated in a similar way. A great increase in survival was shown by R17, as well as RR17, between the 14th and 18th generations, but in the last two selection generations, a new plateau seemed to appear.

Although the acrolein concentrations used were lower in R17 than in RR17, both lines showed a close parallelism in their response to selection. In Table IV the LC_{50} values of these lines, as well as of their control, are shown. The LC_{50} is greater in RR17 than in R17, perhaps due to the different selection intensities applied in these lines as a consequence of the greater acrolein concentrations used in RR17, especially in the 15th and 16th generations. From this point onward, all results given from the selected line at 17 °C will be those of RR17. If we compare the responses of R24 to R17 or RR17, it seems that there were different response patterns according to the temperature at which the selection was carried out.

Table IV. LC_{50} values, in mM, and regression coefficients of mortality probit-concentration logarithm (in brackets) in 20th generation of lines selected at 17 °C and their control line.

Generation	*Line*	*Females*	*Males*
20	C17	128.12±3.71 (3.83±0.23)	134.84±3.54 (3.82±0.22)
20	R17	239.15±6.05 (4.73±0.22)	256.18± 5.60 (5.88±0.27)
20	RR17	406.03±9.81 (3.90±0.27)	393.96±6.43 (5.91±0.37)

Variation of chromosomal inversion frequencies

In Table V, the frequencies of all studied inversions in different generations of the selected lines, as well as their control lines, are shown. Chromosomal inversion

frequencies of the control lines show an erratic variation between generations. It may be accepted that this variation is the result of genetic drift. Nevertheless, it cannot be ruled out that some of the observed variation may be due to adaptation to laboratory conditions.

Of interest, is the tendency for inversions to be eliminated in the selected lines. In fact, all inversions were lost in R24, although in RR17 only 2RNS and 3RC remained in the last selection generation. The elimination of the inversions could have possibly resulted from the effects of genetic drift. However, the fluctuations in inversion frequency expected per generation must be minimal since 100 pairs per generation and line were used in the selection experiments, with the exception of the bottleneck in the second generation of RR17. Besides this, in the control lines, which were maintained in a similar way to the selection lines, no chromosomal inversions were lost. Lastly, but no less important, if the changes in inversion frequencies had been the result of random processes, it is unlikely that all these inversions would be lost; or, in other words, the probability that the five inversions are randomly lost in R24 would be very small. Moreover, the bottleneck occurring in the second generation of RR17 cannot explain the frequency changes in this line because most of them appeared in the seventh and following generations. For these reasons, it seems reasonable to suggest that the observed changes during selection cannot be explained only by the effects of genetic drift.

Variation in other traits

In different generations, the mean values of thorax size, number of sternopleural bristles, developmental time, and productivity of the selected and control lines were estimated. The results are shown in Tables VI and VII.

In both selected lines, an increase in thorax size, as well as, in number of sternopleural bristles, can be observed in the first selection generation. But in the following generations, the differences between selected and control lines tend to remain at the level reached in the first generation. Moreover, the differences of R24 with C24 are maintained even if selection for resistance is relaxed (see generations 20 and 35).

On the other hand, a fitness reduction occurred in R24, as well as, in RR17. In both selected lines, there is an increase of development time and a reduction of productivity. Both traits have a great sensitivity to environmental changes (Ohnishi, 1977; Marks, 1982; David *et al.*, 1983), and for this reason, although experimental precautions were taken, some erratic variation can be observed in selected and control lines. However, the comparison between the selected lines and their controls, shows the reduction of fitness mentioned above.

With regard to developmental time, while R24 and C24 diverged from the first generations, RR17 did not differ greatly from control until the end of the selection. By contrast, in relation to productivity, RR17 and C17 are clearly differentiated from the fifth generation onward, whereas, R24 and C24 only diverge after the thirteenth generation. An experiment was performed in which the fecundity and egg-adult survival were estimated. The R24 and RR17 lines showed in both parameters, a similar reduction with respect to their controls. Therefore, the selection to increase acrolein tolerance seems to reduce, in a similar way, the fitness

Table Va. Chromosomal inversion frequencies, in %, for R24 and RR17 lines and their controls (between brackets) and number of chromosomes (n) that were examined. Table Va: R24 and C24; Table Vb: RR17 and C17.

Generation	*2Lt*	*2RNS*	*3LP*	*3RP*	*3RC*	n
0	40.54	14.19	25.68	16.89	4.73	148
3	30.00	14.00	17.00	1.00	7.00	138
	(28.12)	(13.54)	(12.50)	(8.34)	(9.37)	(96)
7	6.97	5.23	8.14	0.00	0.00	172
	(35.05)	(14.36)	(27.01)	(19.54)	(16.66)	(174)
11	0.00	3.33	0.00	0.00	0.00	180
	(13.25)	(15.06)	(24.69)	(14.45)	(24.09)	(166)
15	0.00	0.00	0.00	0.00	0.00	158
	(18.29)	(6.10)	(18.29)	(12.80)	(14.63)	(164)
20	0.00	0.00	0.00	0.00	2.71	184
	(24.19)	(16.13)	(18.22)	(9.68)	(5.38)	(188)
37	0.00	0.00	0.00	0.00	0.00	130
	(17.37)	(38.42)	(6.84)	(23.16)	(23.16)	(190)

Table Vb.

Generation	*2Lt*	*2RNS*	*3LP*	*3RP*	*3RC*	n
0	43.50	12.33	24.02	16.88	7.14	154
3	7.29	19.79	19.79	1.04	27.08	96
	(25.72)	(12.15)	(25.00)	(7.50)	(9.37)	(170)
7	1.38	34.72	25.00	3.47	29.16	144
	(24.00)	(28.00)	(32.00)	(5.33)	(6.66)	(150)
11	0.00	19.09	0.00	0.00	12.72	110
	(28.80)	(22.28)	(42.39)	(3.80)	(14.67)	(184)
15	0.00	10.57	0.00	0.00	15.38	104
	(23.07)	(19.23)	(34.61)	(16.73)	(12.50)	(104)
19	0.00	8.09	0.00	0.00	4.41	136
	(35.53)	(32.89)	(33.55)	(2.63)	(7.89)	(152)

Table VI. Mean values of thorax size (each unit is 1/40 mm), number of sternopleural bristles, developmental time (in days) and productivity for different generations of R24 and C24 lines (between brackets). In each generation, the differences between R24 and C24 are significant at 0.05 level with the exceptions marked as[ns].

	Thorax size		*Number of sternopleural Bristles*		*Developmental time*		*Productivity*
Generation	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	
0	42.87±0.17	37.49±0.16	19.14±0.17	17.79±0.19	11.04±0.07*		139.66±2.77
1	43.38±0.18	38.43±0.15	19.30 ±0.19[ns]	18.09±0.20	12.44±0.01*		186.44±6.22
	(43.09±0.18)	(37.51±0.16)	(18.83±0.15)	(17.52±0.17)	(11.94±0.01)		(212.66±4.66)
3	44.76±0.13	38.91±0.12	19.28±0.18	18.07±0.17	11.87±0.01*		165.09±4.76[ns]
	(42.87±0.17)	(37.69±0.12)	(18.31±0.15)	(17.36±0.19)	(11.60±0.01)		(169.76±3.71)
5	44.77±0.12	39.29±0.12	19.63±0.19	18.28±0.19	11.62±0.01	11.70±0.01	186.81±4.99[ns]
	(42.97±0.17)	(37.94±0.14)	(18.11±0.19)	(17.28±0.19)	(11.14±0.01)	(11.27±0.01)	(186.46±4.38)
7	44.95±0.10	39.40±0.10	19.15±0.15	17.99±0.15	10.62±0.01	10.80±0.01	187.82±5.76
	(43.63±0.15)	(38.10±0.15)	(18.57±0.21)	(17.40±0.16)	(10.18±0.01)	(10.37±0.01)	(163.14±5.01)
9	45.12±0.14	39.44±0.13	20.57±0.20	18.88±0.20	11.56±0.01	11.67±0.01	176.83±4.57[ns]
	(43.70±0.14)	(38.12±0.12)	(18.52±0.16)	(17.86±0.18)	10.98±0.01	11.15±0.01	(180.11±4.00)
11	44.91±0.10	39.07±0.11	19.87±0.22	18.32±0.18	11.12±0.01	11.37±0.01	197.29±6.63
	(43.85±0.11)	(38.71±0.12)	(18.63±0.16)	(17.47±0.17)	(10.72±0.01)	(10.89±0.01)	(168.10±5.93)
13	45.15±0.12	39.80±0.14	20.18±0.18	18.67±0.20	12.36±0.01	12.58±0.01	139.01±4.73
	(43.29±0.15)	(38.02±0.14)	(18.38±0.17)	(17.55±0.20)	(10.97±0.01)	(11.21±0.01)	(185.26±5.20)
15	44.70±0.09	39.52±0.10	19.50±0.19	18.51±0.19[ns]	12.76±0.01	13.03±0.01	166.71±6.04
	(44.40±0.11)	(39.11±0.09)	(18.79±0.20)	(17.99±0.20)	(12.56±0.01)	(12.71±0.01)	(213.90±5.20)
20	44.58±0.15	38.84±0.16	20.27±0.21	18.77±0.21	12.44±0.01	12.64±0.16	164.15±5.25
	(42.54±0.20)	(37.40±0.14)	(18.05±0.18)	(17.23±0.17)	(11.61±0.01)	(11.76±0.01)	(213.52±4.90)
37	45.48±0.12	40.14±0.08	19.80±0.20[ns]	18.55±0.18[ns]	11.30±0.01	11.50±0.01	134.31±3.04
	(43.98±0.10)	(38.33±0.10)	(19.69±0.19)	(18.08±0.19)	(10.73±0.01)	(10.87±0.01)	(150.92±3.68)

*males and females joined.

Table VII. Mean values of thorax size (each unit is 1/40 mm), number of sternopleural bristles, developmental time (in days) and productivity for different generations of RR17 and C17 lines (between brackets). In each generation, the differences between RR 17 and C17 are significant at 0.05 level with the exceptions marked as ^{ns}.

	Thorax size		*Number of sternopleural Bristles*		*Developmental time*		*Productivity*
Generation	*Females*	*Males*	*Females*	*Males*	*Females*	*Males*	
0	45.32±0.16	40.26±0.32	20.10±0.22	19.67±0.17	23.58±0.02	24.41±0.02	34.14±2.44
1	45.02±0.15^{ns}	40.44±0.15^{ns}	20.35±0.18^{ns}	19.35±0.18^{ns}	22.30±0.01	22.99±0.01	75.83±2.34
	(44.85±0.14)	(40.21±0.13)	(20.22±0.18)	(19.46±0.18)	–	–	(96.17±2.57)
3	44.89±0.15	39.34±0.14^{ns}	20.01±0.19^{ns}	19.80±0.20^{ns}	22.23±0.02	22.97±0.02	71.24±2.97^{ns}
	(44.28±0.18)	(39.09±0.18)	(19.96±0.19)	(19.56±0.19)	(22.96±0.01)	(23.78±0.01)	(63.64±3.03)
5	45.43±0.12	40.53±0.13	20.50±0.15	19.73±0.19^{ns}	–	–	77.42±3.81
	(44.31±0.17)	(38.97±0.17)	(19.51±0.19)	(19.21±0.18)	–	–	(88.24±2.99)
7	45.73±0.13	40.74±0.12	20.50±0.17	20.04±0.20	20.20±0.02	20.83±0.02	57.66±2.64
	(44.51±0.17)	(39.62±0.17)	(19.93±0.20)	19.19±0.18	(22.72±0.02)	(23.50±0.02)	(90.65±3.31)
9	46.03±0.12	41.14±0.11	20.99±0.15	20.26±0.18	21.82±0.03	22.54±0.02	33.37±2.20
	(45.15±0.12)	(40.00±0.12)	(19.80±0.17)	(18.73±0.18)	(20.89±0.01)	(21.74±0.01)	(69.97±3.33)
11	46.11±0.11	41.12±0.10	20.62±0.16	19.94±0.19	23.40±0.02	24.09±0.02	55.81±2.76
	(44.29±0.15)	(39.37±0.12)	(18.88±0.18)	(18.26±0.21)	(21.71±0.01)	(22.46±0.01)	(72.19±2.07)
14	46.36±0.09	41.00±0.10	21.15±0.16	20.22±0.19	22.28±0.01	23.63±0.01	51.39±2.41^{ns}
	(44.83±0.12)	(39.79±0.11)	(20.26±0.19)	(19.50±0.20)	(21.96±0.01)	(22.61±0.01)	(53.71±2.20)
17	46.45±0.11	41.07±0.11	20.53±0.16	20.16±0.20	22.17±0.20	22.71±0.02	47.78±2.67
	(45.00±0.10)	(39.97±0.09)	(19.79±0.18)	(19.31±0.18)	(21.41±0.01)	(22.15±0.01)	(59.10±2.77)
19	46.83±0.12	41.37±0.12	20.19±0.19	18.95±0.20	23.04±0.02	23.80±0.02	43.50±2.66
	(44.42±0.13)	(39.32±0.10)	(20.01±0.20)	(19.16±0.16)	(17.12±0.01)	(17.82±0.01)	(71.12±2.42)

*males and females joined

of both selected lines, although the rate of these changes was different according to the temperature at which selection was carried out.

Discussion

In *D. melanogaster*, or at least in the population studied, acrolein tolerance is a trait that has certain similarities with the described responses to a majority of environmental stresses, such as insecticides, ether, or ^{60}Co-γ-radiation (Parsons, 1973). First, almost from the start of the selection, there is an increase of tolerance and this is clearer in R24 than in the lines selected at 17 °C. Furthermore, the profile of the selection responses fits, with some peculiarity, what is expected for a quantitative trait (Finney, 1971). None of the selected lines appear to have reached a limit to their response. There are numerous reported cases in which the resistance to toxic substances can still be increased, although selection has operated during a large number of generations. For example, a *D. melanogaster* line that was selected for DDT resistance for 300 generations, showed a LD_{50} increase of 70 times, and later a new increase of the resistance level was possible by selection (Dapkus and Merrell, 1977). It cannot be deduced from the results obtained that there are major genes involved in the control of resistance to acrolein, as occurs with some other toxic substances (Sawicki and Lord, 1970; Gamo *et al.*, 1980a, 1980b; O'Byrne and Duke, 1980). From the estimates of LC_{50} no evidence emerges to support this point (Wood 1981). However, since the methodological difficulties in revealing the existence of major genes involved in resistance to toxins are well known (Macnair, 1981; Wood, 1981), the presence of such genes cannot be ruled out.

When selection is carried out, it is not easy to discern clearly the effects of selection, and those produced by genetic drift and inbreeding (Falconer, 1981). This difficulty may be overcome if several selection replicates are used. However, in the present work there was a difficult problem to solve. The only way to increase the selection pressure is to increase the toxic action through, for example, an increase of its concentration. But it is not possible to know *a priori* what will be the increase of mortality produced by the toxin. So, there is a great risk of losing the replicates. In fact, and although a great number of individuals were used, in two generations of RR17, the survival was reduced. In the experimental procedure, we have tried to overcome these problems by using only one selection line per temperature, but with a great number of individuals and a crossing system that minimizes the random effects (see Materials and Methods). However, it is not possible to be sure that such effects did not happen.

With regard to developmental time and productivity, R24 and RR17 showed some differences. However, the changes of both selected lines were a reduction of fitness. This type of response is very common in selection experiments. It is known that both traits are very sensitive to inbreeding and, therefore, we cannot be sure that the changes in mean values are not due to an increase of inbreeding in spite of the experimental precautions.

With regard to chromosomal inversion frequencies, thorax size and number of sternopleural bristles, R24 and RR17 behaved in a similar way. This suggests that the changes found in both lines were due to selection effects. Acrolein is a highly volatile liquid, and for the conditions in which the experiments were carried out, it is very likely that its main way to enter into flies was through respiration. As a

consequence, it may be expected that, in a fly, the greater the oxygen consumption, the greater the acrolein consumption. The metabolic rate by unit of weight (T) is related to the weight by the relation $T = aW^{b-1}$ (Gordon, 1972), where a is a constant, W is the weight and b a constant, that for *Drosophila* has a value near to 0.77 (Ellemby 1953, quoted by Locker and von Bertalanffy, 1968). Therefore, it is expected that the biggest flies will show the smallest respiratory demands. In fact, Matheson and Parsons (1973) showed a negative correlation between body weight and mortality produced by CO_2. For all these reasons, it is very probable that an increase of body size favours the greatest acrolein tolerance. There are other facts that support this interpretation. In another population, selected for acrolein tolerance, an increase of body size was also obtained (Comendador, unpublished results). Also, there are significant differences in mean size between acrolein tolerant and sensitive flies (Comendador *et al.*, in preparation). Since there is a relationship between body size and sternopleural bristles (Spicket, 1963), it can be suggested that the higher number of sternopleural bristles is a consequence of the increase of thorax size.

Moreover, it is also necessary to comment upon another aspect; in R24, as well as in RR17, the mean values of these parameters are increased during the first selection generations, but those following the differences between selected and control lines remain almost constant. This is the expected result if some type of stabilizing selection is operating on these traits (Kearsey and Barnes, 1970; Falconer, 1981) as a consequence of the interaction between two opposite forces of selection; one which increases body size as a consequence of the increase in acrolein tolerance, and another imposed by stabilizing selection that acts against the extreme phenotypes.

In the Results section, it was argued that it is not probable that the observed changes in inversion frequencies were due to random effects. However, it is not easy to interpret a relationship between increased acrolein tolerance and the elimination of inversions. Prevosti (1967) found that in *D. subobscura*, selection to increase wing length favoured heterozygous combinations between the standard sequence and several complex inversions, whereas, selection for short wings, generally fixed into homozygous combination – specific complex inversions. Aguade and Serra (1980) did not find any relation between the 2Lt inversion of *D. melanogaster* and body size, and Butlin *et al.* (1982) have shown that in the fly *Coelopa frigida*, the homozygous individuals for the order of chromosome I are significantly bigger.

Watanabe and Yamazaki (1976) have suggested that in populations under the action of mutagenic agents, it is possible to get an elimination of inversions due to the production of mutations within the inversions. As result of these mutations, the complexes of co-adapted genes that could exist, would become destroyed and, consequently, a reduction of fitness could be expected. The mutagenic effects of acrolein have been shown in a variety of organisms (Izard and Liberman, 1978), including larvae of *D. melanogaster* (Rapoport, 1948), but there is no evidence that they occur in adults.

Inoue *et al.* (1984) found a reduction of inversion frequencies in Japanese wild populations of *D. melanogaster*. They have suggested that as a consequence of intensive use of insecticides, the populations have undergone strong selection and that "to become a resistant fly, recombination is an important genetic process since it proceeds to accumulate many resistant genes along the chromosome. Inversion

chromosomes prevent recombination by the action of crossover suppression. Therefore, if the population adapts to the polluted environment, it may become resistant to insecticides at the cost of polymorphic inversions in nature" (*op. cit.* p. 762). Perhaps, the present case may be an experimental support of the hypothesis of Inoue and his associates.

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