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Original article

Genetic architecture of tolerance to acrolein in *Drosophila melanogaster*

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Summary – Two different methods were used to study the genetic architecture of acrolein tolerance under 2 different temperature conditions. At 17 °C, a temperature considered less stressful than 24 °C, only additive effects were detected, while at 24 °C dominant effects were also found. No reciprocal effect was detected and at both temperatures chromosomes 2 and 3 appeared to play more important roles that the X chromosome.

acrolein – *D. melanogaster* – toxic tolerance – genotype-environment interaction – genetic architecture

Résumé – Architecture génétique de la tolérance à l'acroléine chez *Drosophila melanogaster*. *On a utilisé 2 méthodes pour étudier l'architecture génétique de la tolérance à l'acroléine sous deux températures. A 17 °C on identifie seulement des effets additifs tandis qu'à 24 °C on trouve aussi des effets de dominance. On ne détecte pas d'effets réciproques, à aucune température. Les chromosomes 2 et 3 montrent des effets plus importants que le chromosome X.*

acroléine – Drosophila melanogaster – *tolérance aux toxiques* – *interaction génotype-environnement* – *architecture génétique*

INTRODUCTION

During the last 20 years important information has emerged suggesting that the genetic architecture of a trait may be different depending on the environmental conditions. In line with this idea, Orozco and Bell (1974) showed that in *Tribolium castaneum* an increase in dominant effects occurs under stress conditions and in *Drosophila melanogaster* a similar effect for longevity was found (Parsons, 1966). In a review, Barlow (1981) concluded that "the evidence indicates that the heterosis is environment dependent, but the nature of interactions does depend on the species and on the trait under consideration." Recently, Domínguez and Albornoz (1987)

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have found that heterosis for fecundity in *D. melanogaster* is greater in optimal environments than in stressful ones.

Parsons (1973, 1987) concluded that in *D. melanogaster* under acute stresses produced by different chemical and physical agents (anoxia ^{60}Co γ-rays, anaesthetics and DDT), the additive genetic control was predominant, whereas for less stressful doses the dominant effects were more important. An important consequence of the above facts is that selection may act in different ways according to the specific environmental conditions under which selection is carried out. When a population of *D. melanogaster* was selected for increased tolerance to the polluant acrolein, an unsaturated aldehyde, at 2 different temperatures, 17 °C and 24 °C, results suggested a different temperature action under each condition (Sierra and Comendador, 1989). This paper presents a study of the genetic architecture of acrolein tolerance under the 2 temperature conditions.

MATERIALS AND METHODS

Strains

Tolerant and control lines. The acrolein tolerant lines R24 and RR17, as well as their controls, were obtained by Sierra and Comendador (1989). Briefly, R24 and RR17 were obtained by selection at 24 °C and 17 °C respectively; C24 and C17 are the lines used as controls of R24 and RR17. The LC_{50} (semilethal concentration) values (in mM) for these lines, at the time when the experiments were carried out, were:

Line	*Females*	*Males*
R24	330.24	308.79
C24	138.69	82.17
RR17	406.03	393.96
C17	128.13	134.84

Inbred lines. Six independent inbred lines were obtained from females caught in Teverga (Asturias, Spain), through sister-brother matings for more than 100 generations. Their inbreeding coefficient is close to 1. The lines were maintained through mass cultures until the beginning of the experiments.

Chromosome substitution analysis

A chromosome substitution analysis, similar to that described by Dapkus and Merrell (1977), was carried out as follows. Through a series of crosses between each tolerant line, its control and a balanced strain for chromosomes 2 and 3 *(SM1/Pm;TM3/D)* the following chromosomal combinations were obtained: RRR, HRR, RHR, RRH, HHR, HRH, RHH, HHH, CHH, HCH, HHC, CCH, CHC, HCC, CCC (R = homozygous for chromosomes from selected line; C = homozygous for chromosomes from control line; H = heterozygous). The first letter is for the X chromosome, the second for the 2, and third for the 3; (see figures 1 and 2).

The 3-fold heterozygous combination, HHH, can be obtained through 3 different crosses: ♀ RRRx ♂ CCC (HHH1), ♀ CCCx ♂ RRR (HHH2) and ♀ CCCx

♂ *R/Y;SM1/R;TM3/R* (HHH3). The comparison between HHH1 and HHH2 indicates if there have been reciprocal effects, and that between those 2 and HHH3 it shows if a double crossover within the inversions has happened during the chromosome substitution process, to produce recombinant chromosomes between R and C (for more details, see Dapkus and Merrell, 1977).

Every chromosomal combination from R24 and C24 was obtained and treated at 24 °C, whereas those from RR17 and C17 were analysed at 17°C. There were 7 independent replicates for each chromosomal combination. Each replicate was obtained from 20 pairs in every cross necessary to get the different chromosomal combination, except in the last in which this number fluctuated between 5 and 20. For each replicate and chromosomal combination, 4 groups of 50 females were 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, depending on the line. After 4h, the individuals were transferred to vials with fresh standard medium and the number of survivors was recorded 16-18 h later. The survival rate for each generation was estimated as the percentage of surviving individuals. The acrolein concentration used was 250 mM, which is intermediate between the LC_{50} values of tolerant and control lines, because it was the best one to discriminate among the different combinations.

The 15 chromosomal combinations can be considered as 2 different factorial substitution series. In series I, C chromosomes are replaced by R chromosomes in HHH individuals (from HHH to RRR), and in series II, R chromosomes are replaced by C chromosomes (from HHH to CCC). Each series is analysed by an ANOVA with 3 factors (chromosomes), 2 levels per factors (H or R in series I, and H or C in series II) and 7 repetitions by level. The comparison between these 2 series indicates if the net effects of each chromosome are dominant or additive.

Diallel analysis

Two 6 × 6 diallel crosses, with 2 blocks per diallel, were carried out with the 6 inbred lines; 1 of them at 17 °C and the other at 24 °C. For every cross and block, 400 females were treated with acrolein in the same way as in the chromosome-substitution analysis. The concentration used was 80 mM because in previous tests it was observed that this produced enough differences among lines. The results were analysed according to Hayman's model (1954).

Crosses between selected lines

The offspring of the R24 × RR17 cross, and its reciprocal, were developed at 24 °C or 17 °C, and were treated also at 2 temperatures. For every growth and treatment temperature, 6 replicates, with 100 females per replicate, were carried out. Two different acrolein concentrations were used: 300 and 400 mM, similar to the LC_{50} values of R24 and RR17, respectively, at that time. This experimental design leads, for each concentration, to an ANOVA with 3 factors (cross direction, development temperature and treatment temperature), 2 levels per factor and 6 replicates per level.

Survival estimation

In each case, the survival was estimated as percentage of surviving individuals with respect to the number of treated individuals. For ANOVAs, this percentage was normalized through an arcsin square-root transformation.

Survival in control tests

For these tests some control experiments have been carried out to study treatment effects not due to the toxin. Systematically, the survival in each test was 100%; therefore, deaths due to other effects can be excluded, and thus, it was not necessary to correct the results in any experiment (Finney, 1971).

RESULTS

Chromosome substitution analysis

R24 and C24 lines. The comparison between HHH1 and HHH2 combinations showed that there were no reciprocal effects, because their survival rates were not significantly different ($t = 0.36$, d.f. $= 12$, $P > 0.60$). Moreover, if these two combinations are compared with HHH3, it is evident that there was no recombination during the chromosome manipulation process, because the differences between their survival rates are not significant ($t = 1;39$, d.f. $= 19$, $P > 0.20$). Therefore, from now on we take the data HHH3 as representative of the HHH combination.

The average survival values for the 15 genotypic combinations, as well as transformed values of mean and variance, are shown in Figure 1. The variances are homogeneous in a Barlett's test ($\chi^2 = 14.01$, d.f. $= 14$, $P > 0.30$). The factorial ANOVAs for series I and II are shown in Table I, and also the values of the effects due to each chromosome or to their interactions.

The effect of each chromosome has been estimated as the difference between the mean values of the genotypic combinations that differ for that chromosome. So, for instance, the X-chromosome effect is estimated as the difference between the R-- and H-- mean values (R-- = R homozygous combinations for X chromosome; H-- = heterozygous combinations for this chromosome). The interaction effects between 2 or 3 chromosomes have been estimated as the differences between the observed and expected values assuming that there is no interaction, according to a factorial ANOVA model (Sokal and Rohlf, 1981). All these values can be calculated from the transformed values in Fig. 1.

Comparison between the results of series I and II infers that there are additive effects in chromosomes 2 and 3, since the variation due to each of them, in both series, is significant. However, since the X chromosome does not show significant effects in series II but does in series I, it may be concluded that the X chromosome from R24 shows recessivity; that is, the tolerance genes which are on the X chromosome from R24 are recessive in the relation to their alleles from C24. Moreover, in series II the only significant first-degree interaction is the one in which the X chromosome is involved. Therefore, a clear interaction exists between the X chromosome dominant effects and those from chromosomes 2 and 3. These

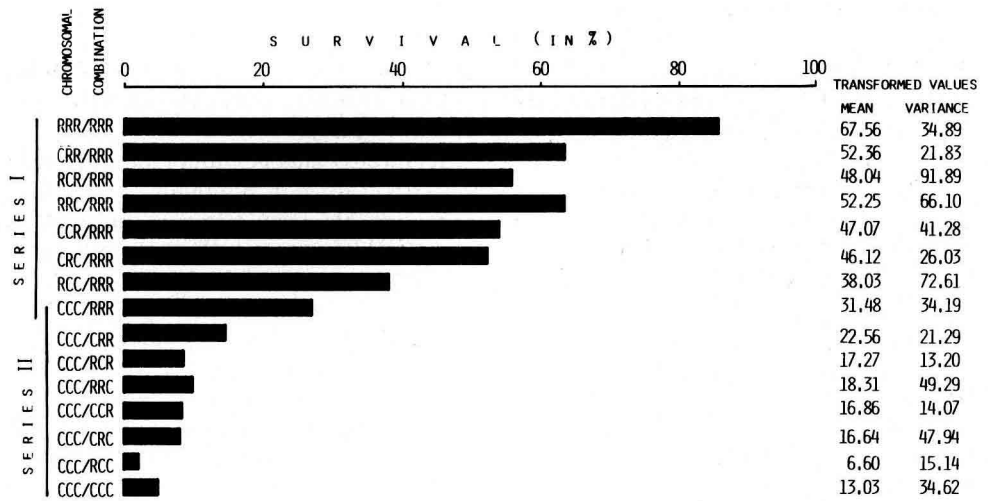

Fig 1. Survival, in percent, of 2 chromosomal combinations of series I and II from C24 and R24 lines. At the right are shown mean and variance of the arcsin square-root transformed values.

Table I. Results of ANOVAs for the series I and II data corresponding to the analysis of C24 and R24 lines. The values of each effect or interaction (see text) as well as the percents of total variation attributable to each effect or interaction are indicated. [a]

Source of variation	*d.f.*	*Series I*			*Series II*		
		M.S.	*Effect of interaction*	*percent*[b]	*M.S.*	*Effect of interaction*	*Percent*[b]
X	1	728.12***	7.22	12.93	16.89	1.14	0.66
2	1	2520.28***	13.32	44.75	1092.44***	8.80	42.73
3	1	1954.03***	11.78	34.56	976.82***	8.39	38.20
X × 2	1	166.95	3.45	2.96	246.28**	4.16	9.63
X × 3	1	10.74	0.87	0.19	170.82*	3.53	6.68
2 × 3	1	14.35	−1.01	0.25	19.84	1.15	0.78
X × 2 × 3	1	188.38	3.66	3.35	0.25	0.95	0.01
error	48	56.79		1.01	33.51		1.31

[a] X, 2 and 3 mean chromosome X, 2 and 3, respectively; d.f. = degree of freedom; M.S. = mean square

[b] Percent of total variation attributable to cach effect or interaction.

* Significant at 0.05 level; ** significant 0.01 level; *** significant 0.001 level.

interactions are positive, so that the effects of chromosomes 2 and 3 are greater for the level H than for the level C of the X chromosome.

The main effects are those due to chromosomes 2 and 3, since they explain jointly around 80% of the observed variance, in both series, although the effects of chromosome 2 are slightly greater than those of chromosome 3.

RR17 and C17 lines. As shown previously, reciprocal effects cannot be detected because the differences between HHH1 and HHH2 are not signifiant ($t = 0.35$, d.f. $= 12$, $P > 0.70$), and the crossover suppression has been effective during the experiment as can be seen from the fact that HHH3 is not significantly different from HHH1 and HHH2 ($t = 0.52$, d.f. $= 19$, $P > 0.60$).

The mean survival values of every genotypic combination, as well as the transformed values of mean and variance, are given in Fig. 2. These variances may be considered as homogeneous in a Barlett's test ($\chi^2 = 18.40$, d.f. $= 14$, $P > 0.10$). In table II the ANOVAs of series I and II, respectively, are given. In both series, the effects of each chromosome are significant, therefore, no dominance is observed in them, although as in the R24 line the X chromosome shows the lowest effects, while those most important are from chromosome 2, specially in series II.

Diallel analysis

The ANOVA for crosses at 24 °C is shown in Table III. From this Table we can deduce that acrolein tolerance, at this temperature, has an important additive component; but dominant effects can also be detected, mainly due to directional dominance and also, but less importantly, to the residual one. In contrast to this, the ANOVA for the 17 °C results shows (Table IV) that at this temperature only the additive effects are significant.

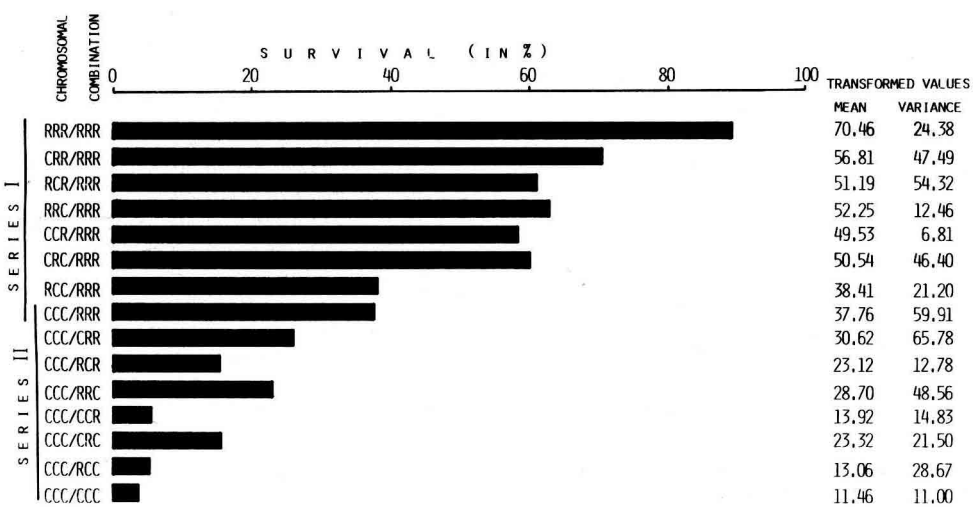

Fig 2. As for Fig. 1 but referring to the C17 and RR17 lines.

Table II. Results of ANOVAs for the series I and II data corresponding to the analysis of C17 and RR17 lines. [a]

Source of variation	*d.f.*	*Series I*			*Series II*		
		M.S.	*Effect of interaction*	*percent*[b]	*M.S.*	*Effect of interaction*	*Percent*[b]
X	1	280.50*	4.41	5.31	477.03	5.84	10.84
2	1	2466.13***	13.29	46.27	3032.05***	14.72	68.91
3	1	2123.49***	12.25	40.17	730.17***	7.23	16.59
X × 2	1	155.68	3.27	2.94	2.69	0.43	0.06
X × 3	1	141.62	3.25	2.68	77.26	2.34	1.76
2 × 3	1	0.04	0.01	0.00	13.11	0.96	0.30
X × 2 × 3	1	99.26	2.72	1.88	29.54	1.45	0.67
error	48	39.62		0.75	38.36		0.87

[a] Symbols are as in Table I.

[b] Percent of each variation attribuable to each effect or interaction.

Table III. Results of ANOVA corresponding to diallel crosses carried out at 24 °C.

Source of variation	*d.f.*	*M.S.*	*F*
Additive (a)	5	1207.11	13.67**
Dominance (b)	15	83.82	2.62*
b1	1	96.31	200.64***
b2	5	7.65	< 1
b3	9	124.75	4.18*
Maternal reciprocal (c)	5	67.80	1.86
Non-maternal reciprocal (d)	10	55.56	1.20
Blocks (B)	1	53.05	
B × a	5	88.28	
B × b	15	31.47	
B × b1	1	0.48	
B × b2	5	40.65	
B × b3	9	29.82	
B × c	5	36.41	
B × d	10	47.05	

* Significant at 0.05 level; ** significant at 0.01 level; *** significant at 0.001 level.
The dominance component b is divided into 3 sources of variation: b1 tests directional dominance, b2 tests if some parental lines contain considerably more dominants alleles than others, and b3 tests residual dominant effects. Following Hayman (1955), the interactions with blocks have been used as error for each source of variation; for example, the error of the additive component (a) is the interaction component B × a.

Since at both temperatures the same genotypes were analysed, it is possible to say that the genetic systems that are active at each temperature are, at least partially, different.

Table IV. Results of ANOVA corresponding to diallel crosses carried out at 17 °C. [a]

Source of variation	*d.f.*	*M.S.*	*F*
Additive (a)	5	2830.23	26.84**
Dominance (b)	15	55.05	1.91
Maternal reciprocal (c)	5	61.34	< 1
Non-maternal reciprocal (d)	10	17.71	< 1
Blocks (B)	1	1.55	
B × a	5	105.43	
B × b	15	28.80	
B × c	5	64.48	
B × d	10	20.90	

[a] Interpretation and symbols are as in Table III. b has not been decomposed because it is not significant.

On the other hand, the heterosis detected in the crosses carried out at 24 °C was in the direction of a lesser tolerance in hybrids than in parental lines ($\overline{F}_1 - \overline{P} = -7.23\%$; $\overline{F}_1$ = mean of hybrids, $\overline{P}$ = mean of parental lines). So, the dominant alleles are those which produce acrolein sensitivity. This agrees with the results from chromosome substitution analysis using R24 and C24 lines.

Crosses between selected lines at different temperatures

Table V shows the average survival of female offspring of the reciprocal crosses between resistant lines, when these females were treated with acrolein at 300 or 400 mM under 4 different conditions: developed and/or treated at 24 °C or 17 °C. The corresponding ANOVAs are in Table VI.

When the acrolein concentration was 300 mM, a clear tendency was observed (Table V): the individuals developed at 1 specific temperature were more tolerant when they were treated at the same temperature, and this is particulary true at 17°C. Because of this, the effects of developmental temperature, as well as the interaction between both temperatures (development and treatment) are significant (see table VI).

When the hybrid flies were treated with 400 mM, a somewhat different picture was shown. A clear effect of development and treatment temperatures can be detected, meaning that in any case, the survival was smaller in the treatments at 24 °C and, furthermore, the individuals developed at 17 °C were more tolerant than those developed at 24 °C.

Table V. Survival of the offspring of crosses between R24 and RR17 lines developed and/or treated at 24 or 17 °C when the 300 or 400 mM concentrations were used.

Cross ×	*Development temperature* (°C)	*Treatment temperature* (°C)	*Concentration*	
			300 *mM*	400 *mM*
R24 × RR17	24	24	0.89 ± 0.01	0.18 ± 0.02
		17	0.83 ± 0.02	0.59 ± 0.02
	17	24	0.78 ± 0.02	0.33 ± 0.02
		17	0.87 ± 0.01	0.75 ± 0.02
RR17 × R24	24	24	0.91 ± 0.01	0.25 ± 0.02
		17	0.89 ± 0.02	0.74 ± 0.02
	17	24	0.74 ± 0.02	0.47 ± 0.02
		17	0.85 ± 0.01	0.82 ± 0.02

Table VI. Factorial ANOVAs corresponding to data of Table V.

Source of variation	*d.f.*	*Concentration*	
		300 *mM* *MS*	400 *mM* *MS*
Cross direction (a)	1	0.21	195.16
Development temperature (b)	1	325.58*	2505.76**
Treatment temperature (c)	1	34.26	6098.66**
a × b	1	90.73	211.14
a × c	1	22.25	74.42
b × c	1	359.27*	46.89
a × b × c	1	5.37	24.19
error	40	70.06	114.94

* Significant at 0.05 level; ** significant at 0.01 level.

DISCUSSION

It is necessary to take into account that the method used in chromosome analysis substitution considers complete chromosomes as units, whereas the diallel analysis estimates the net effects of complete haploid chromosome sets. However, these independent methods lead to the same conclusion: while at 17 °C the acrolein tolerance shows only the additive effects, at 24 °C dominance effects, in favour of acrolein sensitivity genes, are detected.

While in each chromosome substitution analysis, average effects of chromosomal samples from each selection line were estimated, in the diallel analysis identical genotypes were analysed at 2 temperatures. In spite of this, the same conclusions

were reached with both methods; so the differences in genetic architecture observed between R24 and RR17 were not an added effect of the tolerance selection, but a consequence of the genotype-environment interaction, since the genetic architecture of acrolein tolerance was different depending on the temperature at which the flies were treated.

When R24 and RR17 lines were obtained, a number of differences could be observed between both lines (Sierra and Comendador, 1989); these differences could be a consequence of the different genetic control of the tolerance according to the temperature at which each line was selected.

A number of authors have presented evidence showing that, in general, heterosis is more pronounced in stressful environments than in optimum ones (for a review, see Barlow, 1981). It has been suggested that the optimum temperature for *D. melanogaster* is between 20 °C and 25 °C (David *et al.*, 1983). Nevertheless, there are several reasons to think that for the populations used and under the experimental regime imposed, 17 °C must be a temperature less stressful than 24 °C. The mean temperature during the months of highest population density in the localities in which the present populations were caught is 17.5 °C (Felicísimo, 1980). Besides this, since acrolein is a volatile liquid, an important proportion must be taken through respiration, and since respiration is increased with temperature (Hunter, 1964) it may be assumed that for a given concentration, the flies will consume more acrolein at 24 °C. Therefore, in the present case, it seems that heterosis only arises when the environmental conditions are more stressful. This conclusion is contrary to the generalisation of Parsons (1973; 1987) mentioned in the introduction.

An additional commentary is necessary. The presence of directional dominance is often considered as a consequence of directional selection, but it seems improbable that here this was the origin; first of all because the detected dominance was in favour of genes that produce a tolerance decrease; and secondly, because in natural conditions the atmospheric acrolein concentrations must be very low. We have evidence that acrolein tolerance is negatively correlated with mobility and respiratory rate and positively with body size (Sierra et al., 1989). So, a probable hypothesis is that observed heterosis at 24°C is due to heterosis in a trait correlated with acrolein tolerance.

In this work we have obtained evidence showing that acrolein sensitivity depends not only on the supplied acrolein dose, but also on the treatment temperature, as well as on the temperature during egg-adult development. There are many data which show the important role of development on morphology and physiology of *D. melanogaster* adults (for a review, see David *et al.*, 1983). So, it seems reasonable to assume that the complexity of the genetic architecture of acrolein tolerance is due to a correlation between the tolerance and other physiological traits sensitive to environmental changes.

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