Genet. Sel. Evol. (1989) 21, 427-436

Original article

Mechanisms of resistance to acrolein in *Drosophila melanogaster*

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(received 29 November 1988; accepted 26 May 1989)

Summary – The mechanisms of acrolein resistance developed by 2 *D. melanogaster* lines have been studied. The results suggest that there are 2 overlapping mechanisms. One of them is a reduction of breathing requirements, which reduces the amount of acrolein entering the flies, and the other is an increase in aldehyde dehydrogenase activity; probably, the first is the more important.

acrolein – resistance mechanisms – toxic tolerance – *Drosophila melanogaster*

Résumé – Mécanismes de résistance à l'acroléine chez *Drosophila melanogaster*. *Dans ce travail on a étudié les mécanismes de résistance à l'acroléine qu'ont développés 2 souches de* D. melanogaster. *Les résultats suggèrent l'existence de 2 mécanismes superposés. L'un des 2 se présente comme une réduction des exigences respiratoires, ce qui réduit l'entrée d'acroléine dans l'organisme. L'autre montre une élévation de l'activité aldéhyde deshydrogenase. Le premier mécanisme est probablement le plus important.*

***acroléine – mécanismes de résistance – tolérance aux toxiques* – Drosophila melanogaster**

INTRODUCTION

Two main mechanisms of chemical resistance have been described in *Drosophila*:

– an increase in detoxification through the metabolic degradation of the toxin (Togby *et al.*, 1976; McDonald *et al.*, 1977; Kamping and Van Delden, 1978; O'Byrne-Ring and Duke, 1980), for which an increase in the production of the implicated enzyme or enzymes is necessary;

– a modification or alteration in the enzyme action site for which the toxin is the target (Morton and Singh, 1982).

Apart from these 2, other mechanisms have been described in other insects, like *Musca domestica*, which avoid absorption of the toxin by the action of a single gene (Plapp and Wang, 1983; Sawicki, 1974) or by behavioural changes (Wood, 1981).

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We have tried to understand the mechanisms of acrolein resistance in *Drosophila melanogaster*. This compound, an unsaturated aldehyde, is an atmospheric pollutant to which resistance has been developed by 2 lines selected at 2 different temperatures. When selection was carried out (Sierra and Comendador, 1989), several correlated responses suggested that a reduction in the metabolic rate was implicated in this resistance. In this paper, we test this hypothesis as well as the influence on acrolein resistance of 2 enzymes which use aldehydes as substrates, aldehyde oxidase and aldehyde dehydrogenase.

MATERIELS AND METHODS

Strains

The acrolein-resistant lines were R24 and RR17, and their respective controls were C24 and C17; all of them have been described previously (Sierra and Comendador, 1989). Likewise, 4 lines highly sensitive to acrolein (7A, 7A1, 7B and 7C) and 2 natural populations (P15 and P23) from Asturias (Spain) were used to test the aldehyde dehydrogenase (ALDH) activity. The line $Aldox^n$, from Bowling Green, was used to check the influence of the aldehyde oxidase (AO) enzyme in acrolein resistance.

Relationship between body size and acrolein resistance

Thorax size was taken as an estimate of body size, and the measure unit was 1/40 mm. Three different blocks of experiments were carried out. In each block a group of females and another of males were taken from C24. After determination of their size distributions, all these flies were treated with LC_{50} acrolein concentration, following the method previously described (Sierra and Comendador, 1989). The surviving individuals were measured, and the size distribution of dead flies was estimated through the difference between those treated and those surviving.

Moreover, 4 independent lines were started from C24 to carry out bidirectional selection for increased (H1, H2) or decreased (L1, L2) thorax size. In each line 30 pairs were measured every generation, selecting the 5 with an extreme phenotype. After 7 generations, mean thorax sizes of each line, as well as their LC_{50} values, were estimated. These LC_{50} values were calculated following the method described by Barros (1987). This method, easier than that previously used, gives noticeably lower LC_{50} values and thus their comparison is not possible.

Spontaneous locomotor activity measurement

Females and males, 300 in number and all born on the same day, were taken from both C24 and R24 lines and, in groups of 50 individuals (replicates), run for 2.5 min in a countercurrent apparatus like the one described by Benzer (1967). The time elapsed between each of the 11 vials of the apparatus was 15 s. Four different blocks with 6 independent replicates for females and males were carried out for the 2 lines.

These experiments were carried out at 24±1°C and constant humidity, at the same time of day (15.00 h) in order to avoid the effects of daily cycles (Hay, 1972;

Angus, 1974a), without any etherisation during the previous 24 h. The vials were covered with black paper to eliminate phototaxis effects (Grossfield, 1978).

Resistance to CO_2

CO_2 resistance experiments were carried out ot test a possible relationship between acrolein resistance and the ability to reduce breathing requirements. The experimental design used takes into account the fact that an interaction between temperature and acrolein resistance exists (Comendador *et al.*, 1989). So, the lines R24 and C24, developed at 24°C, were tested at 24°C and 17°C, and the lines RR17 and C17, developed at 17°C, were also tested at the 2 temperatures. For every line, individuals of each sex, aged between 2 and 5 days, were placed in vials (104 individuals per vial) which were closed with foam, to allow gas flow. The vials were introduced into a glass dryer, with a wet filter paper inside, in which CO_2 was introduced at atmospheric pressure. After that, the glass dryer was closed with Vaseline and placed in a climatic chamber at the appropriate temperature. When the treatment was finished, the flies were removed to a normal atmosphere, in vials with fresh medium, still at the same temperature. After 24 h, the numbers of surviving and dead were counted. For each line, sex and treatment temperature, 3 different treatment times (4.5, 6.0 and 10.0 h) were used, with 9 replicates per time.

Acrolein sensitivity of Aldox^n *mutants and aldehyde dehydrogenase activity*

The acrolein LC_{50} values of the *Aldox*^{n} line was estimated following the method previously described (Sierra and Comendador, 1989). The aldehyde dehydrogenase activity was determined in the soluble fraction, looking for NADH formation, in order to detect NAD^+ reduction. This method is a modification of that of Libion-Mannaert (personal communication), and uses acetaldehyde as substrate. The aldehyde dehydrogenase activity was estimated in the acrolein-resistant lines R24 and RR17, their controls, and in other lines and populations, mentioned above, for which acrolein sensitivities were previously known.

RESULTS

Relationship between body size and acrolein resistance

Mean values of the size of the C24 individuals which were acrolein resistant or sensitive are shown in Table I, together with the size distribution variances. These mean values are different in different blocks, but this is not strange considering that body size is a trait very susceptible to environmental variations (Marks, 1982; Young, 1970, 1971). Moreover, there are differences for the variances, between surviving and dead individuals, as well as among blocks. For that reason, the comparison of distributions in the same block and sex was carried out by a χ^2 heterogeneity test, within blocks.

With the exception of the comparison between resistant and control males of block I (in which, although the mean size of survivors was higher than that of dead

Table I. Mean thorax length and its variance in survivors or dead flies after treatment with acrolein.

Block		*Sex*	*Mean±S.E.*	*Variance*	*N*	χ^2
I	survivors		42.26±0.16	3.88	149	16.14*
	dead		41.66±0.17	3.62	121	
	survivors		37.33±0.17	2.84	98	6.91 n.s.
	dead		37.06±0.12	2.88	194	
II	survivors		44.01±0.07	1.46	298	17.89**
	dead		43.77±0.15	1.45	97	
	survivors		38.55±0.06	1.06	266	51.73***
	dead		38.08±0.09	0.71	83	
III	survivors		42.97±0.08	1.39	197	39.87***
	dead		41.85±0.17	3.10	107	
	survivors		38.59±0.06	1.06	236	51.77***
	dead		37.50±0.13	1.27	73	

N =number of individuals; S.E. = standard error.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n.s. = not significant.
Three independent blocks were carried out. The heterogeneity of size distributions for surviving and dead individuals of each sex and block have been tested through a χ^2 test because the variances are not homogeneous. When the expected number of any class was less than 5 a grouping was made.

flies, the differences were not significant) the acrolein-resistant individuals were significantly larger than those which died.

The results of the bidirectional selection are shown in Table II. Clearly, the selection to decrease the thorax size has been inefficient. On the other, hand, the mean values of the H1 and H2 lines are both significantly higher than those of the base population and the L1 and L2 lines.

Moreover, the acrolein LC_{50} values of the lines H1 and H2 are also higher than those of lines L1 and L2. (Unfortunately, the base population LC_{50} has not been estimated by a comparable method.) So, not only the larger the individuals the more resistant they are, but, besides, selection to increase body size gives rise to an increase in acrolein resistance. These results agree with previous results, which show that an increase in body size is a response associated with the increase of acrolein resistance (Sierra and Comendador, 1989).

Locomotor activity

The results of the mobility tests are shown in Table III. In 2 of the 4 blocks (I and II) the flies from the acrolein-resistant line (R24) are significantly less mobile than those from the control line (C24), and in the other 2 the differences between lines are not significant. This spontaneous locomotor activity, like many other behavioural

Table II. Mean thorax sizes and LC_{50} values in the lines selected to increase (H1 and H2), or decrease (L1 and L2) the thorax size, as well as the mean size of the base population from which the selected lines were extracted.

Line	*Sex*	*Mean size* ±S.E.	LC_{50}±S.E. (in mM)
H1	females	45.49±0.08	3.48±0.13
	males	39.50±0.08	3.43±0.12
H2	females	44.32±0.12	3.16±0.12
	males	38.79±0.13	3.78±0.13
Base Population	females	41.50±0.22	
	males	36.75±0.19	
L1	females	41.93±0.21	2.34±0.08
	males	36.12±0.15	2.62±0.08
L2	females	42.00±0.17	2.63±0.11
	males	36.86±0.13	1.63±0.16

Table III. Mean mobilities, and their standard errors, in the countercurrent apparatus of C24 and R24 lines.

Block	*Females*			*Males*		
	C24	*R24*	*t*	*C24*	*R24*	*t*
I	1.35±0.11	0.79±0.07	4.30^{**}	1.36±0.15	0.53±0.07	5.37^{***}
II	1.04±0.09	0.82±0.05	2.44^{*}	1.12±0.09	0.79±0.09	2.83^{**}
III	0.74±0.14	0.88±0.12	0.77 n.s.	0.63±0.08	0.82±0.06	1.90 n.s.
IV	0.84±0.10	0.81±0.06	0.25 n.s.	0.68±0.05	0.77±0.06	1.12 n.s.

[a] The values are based on 6 replicates. The significance of the differences between lines within each block is tested by a *t*-test with 10 degrees of freedom.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

traits, is very sensitive to intangible environmental variations (Hay, 1972; Angus, 1974b; Grossfield, 1978). Therefore, it is almost impossible to know the influence of such variations on the experiments; however, the results show some evidence that the acrolein-resistant individuals seem to be less mobile than the control ones.

Resistance to CO_2

Table IV displays the results in the CO_2 resistance experiments. When an ANOVA, with 3 factors and 2 levels per factor, is used to analyse the results after an arcsin transformation, the following facts are clear. First of all, in every case the effects of treatment temperature and doses are significant, although the temperature-dose

interaction is also significant (except in C24 and R24 males). Moreover, there is a significant line effect in all cases, except in C17 and RR17 females, maybe because this is the only case in which the temperature-line interaction is significant. Therefore, taking these results together, it seems clear that there is a relationship between acrolein and CO_2 resistance, although when the temperature is low this relationship has a tendency to disappear, because the CO_2 effects are almost nil. This is simply because there is a negative correlation between temperature and metabolic rate (Hunter, 1964) and, therefore, the CO_2 effects are less drastic at 17°C than at 24°C.

Table IV. Survival, in percent, and standard errors for the CO_2 treatment in the acrolein-resistant lines, and their controls, based on 9 replicas, treated at 24°C or 17 °C for 4.5, 6 or 10 h.

Hours	*Females*		*Males*	
Treatment temperature 24 °C				
	C24	*R24*	*C24*	*R24*
4.5	94.65±0.77	97.64±0.50	67.62±1.56	76.92±1.40
6	87.92±1.08	94.76±0.74	58.97±1.63	73.18±1.47
10	41.45±1.64	47.32±1.66	16.66±1.24	22.22±1.38
	C17	*RR17*	*C17*	*RR17*
4.5	73.50±1.47	78.41±1.37	59.50±1.63	67.73±1.55
6	64.74±1.97	79.27±1.35	42.73±1.64	60.36±1.63
10	45.94±1.66	52.77±1.66	26.60±1.47	34.82±1.58
Treatment temperature 17 °C				
	C24	*R24*	*C24*	*R24*
4.5	99.03±0.33	98.93±0.34	93.58±0.81	97.75±0.49
6	98.18±0.44	98.61±0.39	91.88±0.91	94.65±0.75
10	76.92±1.40	77.77±1.38	37.71±1.61	45.72±2.13
	C17	*RR17*	*C17*	*RR17*
4.5	99.67±0.19	98.50±0.40	99.25±0.28	98.07±0.46
6	98.50±0.40	99.67±0.19	93.80±0.77	98.71±0.37
10	99.46±0.24	99.35±0.26	98.50±0.40	98.71±0.37

Aldox^n *sensitivity and aldehyde dehydrogenase activity*

The acrolein LC_{50} values of the *Aldox* null mutant strain, both for males and females, are not significantly different from those found in natural populations (Gonzalez, 1985) and they can even be considered as relatively high. So, the aldehyde oxidase enzyme can be rejected with respect to acrolein resistance.

The mean values for ALDH activity, detected in the soluble fraction of acrolein-resistant and control lines are displayed in Table Va: each of the resistant lines has an activity significantly higher than that of its controls. Therefore, it seems that one consequence of selection for acrolein resistance has been an increase in ALDH activity.

However, a direct relationship between the acrolein sensitivity of a strain and its ALDH activity cannot be established, as can be deduced from the results shown in Table Vb. The most resistant among the 4 acrolein sensitive lines, 7B, shows an activity that is almost twice that of the others, but the activity of the most sensitive, 7A1, is not different from the activity of the second line in resistance, 7C. Similarly, the differences in activity between the 2 natural populations, P15 and P23, are not significant, while their acrolein LC_{50} values are very different.

Table V. Aldehyde dehydrogenase activity, in mols NADH/ml cm min, in different lines.

	Line	*Survival*[a] *or* LC_{50}^{b}	*Mean±S.E.*
A*			
	C24	110.46[a]	0.54±0.03
	R24	319.50[a]	0.68±0.02
	C17	131.45[a]	0.73±0.03
	RR17	400.00[a]	1.68±0.08
B*			
	7A1	0.00[b]	0.32±0.02
	7A	7.29[b]	0.45±0.05
	7C	45.00[b]	0.36±0.03
	7B	51.22[b]	0.85±0.02
	P15	106.75[a]	0.38±0.04
	P23	276.20[a]	0.35±0.01

A* = acrolein-resistant lines and their controls.
B* = 4 acrolein-sensitive lines and 2 natural populations. The survival of the sensitive line (in percent) against the acrolein dose (100 mM (a)) or the LC_{50} of natural populations (b) are also shown.

DISCUSSION

Previous results have shown that when selection for acrolein resistance is carried out, an increase in thorax size is attained (Sierra and Comendador, 1989). In the present work, we have found that the larger the flies the more resistant they are and, moreover, that selection for body size increase produces an increase in acrolein resistance. Therefore, it seems certain that there is a relationship between body size and acrolein resistance.

The body weight and the metabolic rate are related through the equation $T = k\ W^{6}$ (Gordon, 1972), where T is the metabolic rate, K a constant, W the

body weight and b a constant that is 0.772 for *Drosophila* (Altman and Dittmer, 1968). Because of that, the larger the flies are, the lower metabolic rates per weight unit they have. Since mobility depends on the metabolic rate, the resistant flies (which are larger) would be less mobile than the control ones, and in fact they are.

In agreement with this, it is possible to think that a hypothetical mechanism of resistance, developed during the selection for acrolein resistance, was a metabolic rate depression. So, the breathing requirements of resistant flies would be lower and, therefore, the acrolein flow into the flies would be reduced.

Bearing in mind that the acrolein-resistant flies are also resistant to CO_2, at least, more resistant than control flies, this hypothesis seems to be right.

Parsons (1973) and Matheson and Parsons (1973) have shown that in *D. melanogaster* resistance to CO_2 is a good estimate of resistance to anoxia, and the lower their breathing requirements, the more resistant are the flies. Our results agree with the hypothesis that acrolein resistance depends, at least to an important extent, on a reduction of the breathing capacity of the flies. This reduction is accompanied by a reduction in the metabolic rate, an increase in resistance to anoxia, a reduction in locomotor activity, an increase in body size and, probably, changes in another trait.

In *D. melanogaster*, 2 enzymes that use non-specific aldehydes as substrates catalyzing their oxidation, have been described: aldehyde oxidase (Dickinson, 1970) and aldehyde dehydrogenase (Garcin *et al.*, 1983; Libion-Mannaert *et al.*, 1985). The first does not seem to have any relationship with acrolein resistance, as was shown. On the other hand, ALDH seems to be a good candidate for an enzyme implicated in the acrolein degradation system.

Draminsky *et al.* (1983) have shown that when acrolein is given to rats, they produce and excrete mercapturic-S acid in the urine. This acid is produced by the conjugation between glutathione and methyl acrylate which is produced by acrylic acid methylation. Thus, the fact that ALDH activity is increased in the acrolein-resistant lines suggests that acrolein degradation in flies occurs through its oxidation and integration in a similar metabolic path. Of course, there are too many metabolic differences between rats and flies to assume that the metabolism of this compound is similar in both species but, even so, the known properties of *Drosophila* ALDH enzyme are more similar to those of mammals than to the corresponding one of yeasts.

In short, we propose that in *D. melanogaster* there are at least 2 different mechanisms for acrolein resistance. The first, and more important one, is a kind of barrier against the acrolein flow (the metabolic rate reduction). It is, therefore, a non-specific mechanism that could be valid for other volatile toxins. The second one is the degradation, through the ALDH enzyme, of the acrolein that has passed the barrier.

Finally, although we have no data to suggest the existence of other resistance mechanisms, we cannot discard this possibility.

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