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Opposing Modes of Selection on the Alcohol Dehydrogenase Locus in *Drosophila melanogaster*

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Abstract

Three experiments have been carried out which show that exogenous environments of ethanol impose selection on the alcohol dehydrogenase (*Adh*) locus of *D. melanogaster*. This locus is widely polymorphic for two alleles, Adh^F and Adh^S, and Adh^F generally produces about twice as much alcohol dehydrogenase activity as Adh^S. In the first experiment, Adh^F/Adh^F and Adh^F/Adh^S flies survived equally often and Adh^S/Adh^S flies less frequently after exposure for 7 days to medium impregnated with ethanol. The same pattern of survival differences was found in the second experiment in which flies were exposed for 1 day to an aqueous solution of ethanol and sucrose. In contrast, in the third experiment survival was scored after exposure for 45-min to ethanol fumes, and Adh^S/Adh^S flies survived more often than Adh^F/Adh^S, both these genotypes surviving more frequently than Adh^F/Adh^F. We doubt whether any one of the three experiments by itself adequately represents the ecology of natural populations of *D. melanogaster* exposed to ethanol. It is likely that mixtures of the three experimental conditions approximate more closely the natural environments and therefore we suggest that, overall, selection might favour intermediate levels of alcohol dehydrogenase activity, producing a net advantage for heterozygotes at the *Adh* locus.

Introduction

One of the first observations of systematic changes in gene frequency at a locus segregating allozymes was made by Gibson (1970) who found that ethanol selected between the Adh^F and Adh^S alleles (hereinafter denoted *F* and *S*) at the alcohol dehydrogenase (*Adh*) locus in *Drosophila melanogaster*. This locus produces the enzyme alcohol dehydrogenase (ADH, EC 1.1.1.1) and in two laboratory populations that Gibson (1970) maintained on medium supplemented with 6% (v/v) ethanol, the frequency of *F* increased above the frequency in populations maintained on medium without added ethanol. It is known that *F* produces more ADH activity than *S* and Gibson (1970) suggested that the additional ADH activity was advantageous during exposure to ethanol.

Briscoe *et al.* (1975) tried to relate Gibson's (1970) findings to the environments of the winery habitats of wild *D. melanogaster*. They found that *F* frequency was higher in a Spanish winery cellar than it was in a rubbish tip about 1 km away. However, McKenzie and McKechnie (1978) and Gibson *et al.* (unpublished data) were unable to detect differences in the frequencies of *F* between the inside and outside populations of four Australian winery cellars. Two other reasons also make it doubtful whether winery cellars should generally maintain higher *F* frequencies. Firstly, biochemical work (Oakeshott *et al.*, unpublished data) indicates that appreciable levels of ethanol occur in many *D. melanogaster* food sources and the levels in wine exudates often differ

very little from those in other food sources. Secondly, further fitness tests in the laboratory have indicated that the optimum ADH activity for fitness on media supplemented with ethanol is not always the maximum level. In general, such tests have indicated that *F*/*F* are fitter than *S*/*S* flies but at least in some tests the relative fitness of *F*/*S* flies has exceeded those of both homozygotes.

Thus, there are now several reports of *F* rising in frequency over several generations on medium supplemented with ethanol, but in only one are there data showing that the *F* allele went to fixation. Gibson (1970) found that *F* frequency rose from 0·5 to about 0·8 after 18 generations on medium impregnated with 6% (v/v) ethanol, although it was later reported (Gibson *et al.* 1979) that one of the two experimental lines eventually became fixed for *F*. Cavenar and Clegg (1978) found that *F* rose from 0·30 to about 0·60 after 18 generations on 10% (v/v) ethanol medium, although admittedly they did not establish whether the latter was an equilibrium frequency. However, Van Delden *et al.* (1978) found that on 15% (v/v) ethanol medium, *F* frequency rose from 0·5 to about 0·68 after 10 generations, and 15 generations later was still at about the latter frequency. Furthermore, Oakeshott (1979) found that when the initial *F* frequency was higher than 0·5 it increased very little on ethanol medium: from 0·59 to 0·61 after 20 generations on 7·5% (v/v) ethanol.

In a different kind of experiment, Bijlsma-Meeles and Van Delden (1974) found that stocks polymorphic for *F* and *S* survived for eight generations on 20% (v/v) ethanol medium slightly more often than did stocks fixed for *F*. Similarly, two populations selected by Gibson *et al.* (1979) for increased ethanol tolerance over 12 generations maintained mean *F* frequencies of about 0·7, which were not significantly higher than those in the unselected control populations.

Despite these relatively consistent results from the various many-generation experiments, studies on individual fitness components within a generation have produced conflicting results on the relative fitness of *Adh* heterozygotes. Libion-Mannaert *et al.* (1974) found that the time which *F*/*S* adults survived exposure to a solution of ethanol and sucrose was intermediate between the times for *F*/*F* and *S*/*S*, and Oakeshott (1976) found that, on a range of media impregnated with ethanol, the development rate of *F*/*S* pre-adults was also intermediate between those for the two homozygotes. However, Briscoe *et al.* (1975) reported that *F*/*F* and *F*/*S* adults were equally likely to survive exposure for 1 day to media containing 12·5% (v/v) ethanol while Oakeshott (1976) found that *F*/*S* adults survived more often than *F*/*F* adults on a variety of media impregnated with ethanol.

The present experiments were carried out to try to reconcile these discrepancies by investigating adult survival under three different conditions of exposure to ethanol: 7 days exposure to medium impregnated with ethanol, 1 day exposure tc a mixture of ethanol liquid and vapour, and a 45-min exposure to ethanol vapour. The results test the hypothesis that the optimum ADH activity for fitness diminishes as the conditions of exposure become more acute. This expectation derives from work on mammals which indicates that the acetaldehyde produced by ADH acting on ethanol is potentially more toxic than the ethanol. Usually the acetaldehyde is removed by aldehyde dehydrogenase almost as soon as it is produced, but when high ethanol concentrations occur acetaldehyde can also accumulate; high levels of ADH are then disadvantageous since they allow acetaldehyde to accumulate more rapidly (Stamatoyannopoulos *et al.* 1975; see Li 1977 for a review). Little work has been done in this area in insects but the conditions for the three exposures used in this study should

stimulate different biochemical responses and test the possibility that conditions also occur for *D. melanogaster* under which maximum ADH activity is not optimum for fitness.

Materials and Methods

Experiment 1

The first of the three experiments in this study compared the survival of *F/F*, *F/S* and *S/S* flies after exposure for 7 days to medium impregnated with ethanol. The method was based on the one used by Oakeshott (1976) except that *Adh* genotypes were determined by 'Cellogel' electrophoresis as in Lewis and Gibson (1978) and the recipe for the food medium was: 10 g agar, 26 g sucrose, 50 g glucose, 22 g wheat germ, 50 g maize meal, 6 g dead yeast, 5 ml propionic acid, 1 litre of water and a particular percentage (v/v) of ethanol. The experiment involved three stocks, *M1*, *M2* and *M3*, which were isofemale lines captured from Mudgee, N.S.W. in 1978. The frequency of *F* was $0{\cdot}40 \pm 0{\cdot}05$, $0{\cdot}48 \pm 0{\cdot}05$ and $0{\cdot}51 \pm 0{\cdot}05$ in *M1*, *M2* and *M3* respectively. From each of the three stocks, several cohorts of about 300 flies were collected as they emerged as adults. The cohorts, each of which contained a mixture of males and females, were aged a further 6–8 days on medium lacking ethanol. About 200 'tester' flies were taken at random from each cohort and then transferred to two 200-ml vials (100 per vial) each containing 50 ml of medium supplemented with 9–12% ethanol. The other 100 'control' flies from each cohort were left in a 200-ml vial containing 50 ml of normal food medium. Seven days later the *Adh* genotypes of all control flies and all surviving testers were scored. (Mortality among controls was negligible.) The proportion of each genotype surviving among the testers from each cohort was then calculated by assuming that the initial frequencies of each genotype among the testers and controls were the same. As the frequencies of *F* and *S* were both about 0·50 in each cohort, the testers in each cohort initially comprised a total of about 50 *F/F*, 100 *F/S* and 50 *S/S* flies and the survival proportions recorded were based on denominators of about these magnitudes.

Experiment 2

The second experiment compared the survival of *F/F*, *F/S* and *S/S* flies after exposure for 1 day to a solution of sucrose and 8–10% ethanol absorbed on filter paper. It involved the *M1*, *M2* and *M3* stocks used in the first experiment as well as *M4*, which was also an isofemale line taken from Mudgee in 1978 and had an *F* frequency of $0{\cdot}54 \pm 0{\cdot}05$. (*M4* was not used in the first experiment because it produced too few 6–8-day-old adults in synchrony with those from cultures of *M1–M3*.) As in the first experiment, several cohorts of about 300 flies were collected from each stock and aged 6–8 days on normal food medium after emergence. About 200 tester flies from each cohort were then placed in 100-ml vials (50 per vial) each containing 150 cm^2 of filter paper soaked in 4 ml of an aqueous solution of 30% (w/v) sucrose and a particular percentage (v/v) of ethanol. The other 100 flies from each cohort were used as controls and were treated similarly except that the solution lacked ethanol. One day later the survivors in all vials were typed for *Adh*. Mortality among controls was negligible and the proportions of each genotype surviving among the testers were calculated as in the first experiment.

Experiment 3

The third experiment tested the survival of *F/F*, *F/S* and *S/S* genotypes after exposure for 45 min to ethanol vapour in air. *M1*, *M3* and *M4* flies were used, which as before had been aged 6–8 days on normal food medium. (The *M2* stock produced too few flies for this experiment.) Flies from each of *M1*, *M3* and *M4* were placed unetherized in cohorts of about 15 in cylindrical, clear plastic chambers 13 mm in diameter and 20 mm high with gauze bases and lids. About 40 such chambers were placed around the perimeter of a clear glass dessicator which was then sealed. The volume of the dessicator was 10·5 litres and 1·05 ml of absolute ethanol was added to a filter paper in a Petri dish on the floor of the dessicator. The ethanol evaporated quickly and an even distribution of its vapour in the dessicator was ensured by using a magnetic stirrer on the filter paper. Most flies were immobile within 45 min, after which time each chamber was removed from the dessicator and placed with its lid open in a 200-ml vial containing 50 ml of normal food medium. One day later, both the survivors

and casualties in all cohorts were typed for *Adh* so the proportions of flies of each genotype which survived in each cohort could be calculated directly. (Since the casualties had been typed for *Adh*, this experiment obviously did not require the control flies that were necessary in previous experiments.)

Table 1. Survival percentages of *F/F*, *F/S* and *S/S* flies after exposure for 7 days to medium impregnated with ethanol

Survival percentages for each cohort are shown as well as the mean and standard error of the percentages for all cohorts at each ethanol concentration

Ethanol concn (% v/v)	Stock and cohort	Percentage survival *F/F*	*F/S*	*S/S*
9	*M1.1*	80	100	52
	M2.1	100	81	39
	M3.1	90	82	81
Mean		90±6	88±6	57±12
11	*M1.2*	78	69	78
	M1.3	61	82	72
	M2.2	51	76	26
	M2.3	77	63	33
	M2.4	76	74	20
	M2.5	88	73	72
	M3.2	45	63	39
	M3.3	72	59	53
Mean		69±5	70±3	49±10
12	*M1.4*	62	69	32
	M2.6	50	63	22
	M2.7	76	80	18
Mean		63±8	71±5	24±4

Analysis of variance on angular transforms of the data

Source of variation	d.f.	m.s.	*F* ratio	*P*
Ethanol concn (E)	2	898·8	11·66	<0·001
Stock (S)	2	127·2	1·65	n.s.[A]
Adh genotype (A)	2	1457·2	18·90	<0·001
E × S	3	51·2	0·67	n.s.
E × A	4	106·9	1·39	n.s.
S × A	4	214·6	2·78	n.s.
E × S × A	6	134·9	1·75	n.s.
Residual	18	77·1		

[A] n.s., not significant.

Results

Experiment 1. Exposure for 7 Days to Ethanol in the Medium

In this experiment, survival percentages were recorded for *F/F*, *F/S* and *S/S* flies in each of five or six cohorts from each of *M1*, *M2* and *M3*. The medium on which each cohort was tested contained either 9, 11 or 12% ethanol and the results are summarized together with the analysis of variance in Table 1.

Percentage survival did not differ significantly between the three stocks but it did decrease with increasing ethanol concentration and also clearly differed between

Table 2. Survival percentages of *F/F*, *F/S* and *S/S* flies after exposure for 1 day to a solution of ethanol and sucrose

Survival percentages for each cohort are shown as well as the mean and standard error of the percentages for all cohorts at each ethanol concentration

Ethanol concn (% v/v)	Stock and cohort	Percentage Survival *F/F*	*F/S*	*S/S*
8	*M1.1*	83	77	42
	M2.1	59	75	20
	M3.1	82	67	49
	M4.1	72	65	55
Mean		74±6	71±3	42±8
9	*M1.2*	11	6	3
	M1.3	4	5	4
	M2.2	19	39	9
	M2.3	7	5	6
	M3.2	55	55	33
	M3.3	34	32	20
	M4.2	31	32	36
Mean		23±7	25±7	16±5
10	*M1.4*	1	1	2
	M1.5	0	2	2
	M2.4	9	4	0
	M2.5	0	2	6
	M3.4	21	28	20
	M3.5	15	22	8
	M3.6	2	1	1
	M4.3	0	0	0
	M4.4	23	24	26
	M4.5	24	25	22
Mean		10±3	11±4	9±3

Analysis of variance on angular transforms of the data

Source of variation	d.f.	m.s.	*F* ratio	*P*
Ethanol concn (E)	3	6055·6	47·96	<0·001
Stock (S)	2	1074·9	8·51	<0·001
Adh genotype (A)	2	423·0	3·35	<0·05
E × S	6	174·5	1·38	n.s.[A]
E × A	6	270·5	2·14	n.s.
S × A	4	60·6	0·48	n.s.
E × S × A	12	62·4	0·49	n.s.
Residual	27	126·3		

[A] n.s., not significant.

Adh genotypes. There were no significant interaction effects and the significant main effect of *Adh* genotype was due to the generally poorer survival of *S/S* flies. The survival percentages of *F/F* and *F/S* flies were very similar and if the *S/S* data were

omitted from the analysis, the effect of *Adh* genotype was no longer significant ($F^1_{12} = 0{\cdot}10$, $P > 0{\cdot}05$).

Experiment 2. Exposure for 1 Day to Ethanol Solution

This experiment tested five or six cohorts from each of the four stocks *M1–M4* and the solution tested for each cohort contained either 8, 9 or 10% ethanol. The

Table 3. Survival percentages of *F/F*, *F/S* and *S/S* flies after exposure for 45 min to ethanol vapour

Survival percentages for each group of cohorts are shown as well as the means and standard errors of the percentages for various combinations of groups

Occasion	Stock	Percentage survival		
		F/F	*F/S*	*S/S*
1	*M1*	18	39	50
	M3	58	72	77
	M4	62	56	69
2	*M1*	5	17	50
	M3	36	27	53
	M4	54	47	55
3	*M1*	1	3	19
	M3	31	33	50
	M4	40	50	57
4	*M1*	1	9	17
	M3	22	21	18
	M4	19	39	37
Means of occasions	*M1*	6±4	17±8	34±9
	M3	37±8	38±12	50±12
	M4	44±9	48±4	55±7
Means of occasions and stocks		29±6	34±6	46±6

Analysis of variance on angular transforms of the data

Source of variation	d.f.	m.s.	*F* ratio	*P*
Occasion (O)	3	829·1	45·23	<0·001
Stock (S)	2	1450·0	79·11	<0·001
Adh genotype (A)	2	458·6	25·02	<0·001
O × S	6	60·0	3·28	<0·05
O × A	6	21·9	1·20	n.s.[A]
S × A	4	84·4	4·61	<0·05
O × S × A	12	18·3		

[A] n.s., not significant.

results are summarized together with their analysis of variance in Table 2. All three main effects—concentration, stock and *Adh* genotype—were significant, but once again none of the interaction terms were. The largest of the significant main effects was the differences between stocks.

As expected, the significance of the main effect of concentration was due to percentage survival decreasing with increasing ethanol concentration. The significance of the main effect of stock was due to *M3* and *M4* performing better than *M1* and *M2*. This result was in contrast to that in experiment 1, which did not examine *M4* but found *M1*, *M2* and *M3* equally likely to survive. This suggests that traits inherited independently of the *Adh* locus were important in determining survival after exposure for 1 day to ethanol solution, but not after exposure for 7 days to medium impregnated with ethanol.

The significant main effect of *Adh* genotype was largely due to the poorer performance of *S*/*S* flies: if the *S*/*S* data were omitted from the analysis, the effect of *Adh* genotype was no longer significant ($F^1_{27} = 0{\cdot}05$, $P > 0{\cdot}05$).

Experiment 3. Exposure to Ethanol Vapour for 45 Minutes

In this experiment between 10 and 15 cohorts were tested for each of *M1*, *M3* and *M4* on each of four different occasions. As each cohort contained about 15 flies, a group of about 200 flies was tested for each stock on each occasion. This was similar to the number of tester flies in each cohort in experiments 1 and 2 so that, in terms of accuracy and amount of information, a group of cohorts as defined in experiment 3 corresponded to a single cohort in experiments 1 or 2. Accordingly, the basic measure for analysis in experiment 3 was the overall proportion of survivors of each *Adh* genotype in each group of cohorts and these proportions are shown together with the analysis of variance in Table 3.

The main effects of occasion, stock and *Adh* genotype were all highly significant while two interaction terms just reached the threshold of significance ($P \simeq 0{\cdot}04$ for both). As no simple trends which would explain the two interactions were obvious in the data, and as they accounted for so much less of the total variance than the main effects, they will not be discussed further.

Regarding the main effects, the differences between occasions might be due to experimental differences affecting the effective dose of ethanol administered to the flies, for example differences in the efficiency of the stirrer. The significance of the effect of stock reflected the poorer performance of *M1* relative to *M3* and *M4*. In fact, *M1* flies had also survived less often than *M3* and *M4* flies in experiment 2, suggesting that the genetic differences apart from *Adh* which affected survival in experiment *2* (but not experiment 1) might also have affected performance in experiment 3. The significance of the main effect of *Adh* genotype in experiment 3 was mainly due to the higher percentage survival of *S*/*S* flies relative to the other two genotypes, although the survival of *F*/*S* flies was also significantly higher than that of *F*/*F*. If the *S*/*S* data were omitted from the analysis, the main effect of *Adh* genotype remained statistically significant ($F^1_{12} = 6{\cdot}07$, $P < 0{\cdot}05$).

In summary, the results of experiments 2 and 3 were similar and both differed from those of experiment 1 in terms of differences in survival between stocks, but experiment 3 differed from experiment 2 as well as experiment 1 in terms of differences between *Adh* genotypes within stocks. Whereas experiment 1 ordered the stocks according to percentage survival as

$$M1 = M2 = M3$$

and the genotypes as

$$F/F = F/S > S/S,$$

experiment 2 ordered the stocks as

$$M3 = M4 > M1 = M2$$

and the genotypes as

$$F/F = F/S > S/S$$

while experiment 3 ordered them as

$$M3 = M4 > M1$$

and

$$S/S > F/S > F/F.$$

Discussion

In the first experiment, in which the conditions closely resembled those used by Briscoe *et al.* (1975) and Oakeshott (1976), ethanol tolerance was examined in terms of adult survival after several days on media impregnated with ethanol. Under these conditions, ethanol is likely to have been ingested with the food and there was probably relatively little ethanol vapour in the atmosphere which could be absorbed through the cuticle. This experiment showed that, overall, *F/F* and *F/S* flies were equally likely to survive but *S/S* flies were less successful. The same pattern had been reported by Briscoe *et al.* (1975) but Oakeshott (1976), although agreeing that *F/F* was superior to *S/S*, found that *F/S* in his stocks did better than either homozygote. This latter observation is not inconsistent with the present results because Oakeshott (1976) only observed heterozygote advantage at ethanol concentrations causing relatively high mortality. At the highest ethanol concentration used in the present study, a higher proportion of heterozygotes survived than either homozygote, although the excess of heterozygotes was not statistically significant.

In the second experiment, which was similar to a test used by Libion-Mannaert *et al.* (1974), ethanol tolerance was investigated as the survival of adults after exposure for 1 day to filter paper soaked in ethanol and sucrose solution. As in the conditions of the first experiment, ethanol was allowed to be ingested with the food, but compared to the first experiment it seems likely that there were higher levels of ethanol vapour which could be absorbed through the flies' cuticles. It might be that response to this additional factor in the test environment was the reason why genetic differences apart from *Adh* (which were manifest as the differences between stocks) were important for survival in this experiment but not in the first.

These genetic differences at loci other than *Adh* were only important in the second experiment but the pattern of survival differences between the three *Adh* genotypes applied in both experiments. *F/F* and *F/S* flies had similar survivals but *S/S* flies had lower survivals.

Ethanol tolerance in the third experiment was examined exclusively by investigating adult survival in ethanol fumes. Under such conditions, in which ethanol is likely to be absorbed through the cuticle rather than ingested, factors other than ADH such as surface area, respiratory rate or general metabolic activity may also be important for survival. In this respect it is interesting that the differences between stocks which were found in the second experiment (but not the first where alcohol vapour would have been minimal) also occurred in the third experiment.

With respect to genotypes at the *Adh* locus, the results of the third experiment opposed those of both the other two experiments. In the third experiment, *S/S* flies performed better than *F/S* flies and both did better than *F/F*. This held in all three

stocks studied (*M1*, *M3* and *M4*) and three additional lines of evidence suggest it would hold for other stocks exposed to these conditions. Firstly, we have tested 17 other isofemale lines from Mudgee under the same conditions as the third experiment. *F* frequency varied from 0·00 to 1·00 among the 17 stocks and percentage survival also differed between them, regressing negatively and significantly on *F* frequency ($F^1_{119} = 34{\cdot}03$, $P < 0{\cdot}001$). (We did not quantify the relative survival scores of the *Adh* genotypes within each of these stocks.)

The second additional line of evidence comes from the report of Briscoe *et al.* (1975) who exposed *F*/*F*, *F*/*S* and *S*/*S* adults to medium supplemented with ethanol and monitored their survival at intervals of about 2 h for 24 h from the beginning of exposure. Briscoe *et al.* (1975) concluded that *F*/*F* and *F*/*S* performed comparably and *S*/*S* worse on the basis of the results after 24 h, but inspection of their graphs shows that at 2 h *S*/*S* flies had higher survival than *F*/*S* flies and both these genotypes had higher survival than *F*/*F*. These results, obtained from short-term exposure to medium supplemented with ethanol, are similar to those in our third experiment in which short-term exposure to ethanol vapour was tested. This suggests the possibility that the advantage to *S*/*S* in the third experiment may derive from the relatively short duration of exposure rather than from the fact that the ethanol vapour was absorbed through the cuticle.

The third additional line of evidence derives from work on the lines *13m 8d 2a*, *14k 12h* and *14k 12j 1a*, the ADH's of which were characterized biochemically by Lewis and Gibson (1978). *13m 8d 2a* is fixed for *F* and like most *F* lines has relatively high levels of ADH activity. *14k 12h* is fixed for *S* and like most *S* lines has much less ADH activity. *14k 12j 1a* is fixed for *F* but is peculiar in showing the low ADH levels usually characteristic of *S* lines. We have found that after exposure for 45 min to ethanol vapour as in the third experiment, percentage survival for *13m 8d 2a*, *14k 12h* and *14k 12j 1a* flies was 35 ± 4, 58 ± 5 and 55 ± 6 respectively, and the percentages for the three stocks regressed negatively and highly significantly on their respective ADH activities ($F^1_{60} = 42{\cdot}34$, $P < 0{\cdot}001$). This suggests that the advantage to *S*/*S* flies under the test conditions is in fact a reflection of, and conditional upon, their relatively low ADH activities.

Overall, the results of this paper indicate that the mode of selection on *Adh* and the optimum ADH activity for fitness appear to depend on some of the conditions of exposure to ethanol. The experiments show that the low activity in *S*/*S* flies is optimal during short-term exposure to ethanol vapour and the high activity in *F*/*F* flies is optimal during longer exposure to ethanol in the food. We suspect that the conditions of exposure in the wild are more complex than those presented by any one of the three experiments and probably more closely approximate a mixture of the three. Therefore we suggest that selection in natural environments does not act directionally to favour either *F* or *S* or high or low ADH activity, but overall intermediate ADH activity is optimal and this will often be produced by *F*/*S* heterozygotes. This suggestion provides a mechanism which would explain the selective maintenance of the polymorphism at the *Adh* locus commonly found in natural populations of *D. melanogaster*.

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