Aust. J. Biol. Sci., 1979, **32**, 127–32

Genetics of Resistance to Tetraethyllead

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Abstract

A *Drosophila melanogaster* population was exposed for 25 generations to 60 μg tetraethyllead per gram of medium. Selection over this period resulted in an increase in fecundity, hatchability and larva-to-adult viability. Chromosome assay showed that response in these traits was generally under additive genetic control in conformity with existing results in the literature on the genetics of resistance to acute environmental stress in *D. melanogaster*.

Introduction

The recent concern of the public to the health and ecological hazards of environmental pollution has generated an interest in research aimed at understanding the biological effects of air pollutants in animals, humans and plants. One of the most harmful air pollutants is tetraethyllead (TEL). It has been reported to be hazardous to plants and animals near highways as well as an environmental poison (e.g. Aronson 1970; Bovary 1971; Hardy *et al.* 1971). In assessing the hazard of a pollutant, consideration should be given to its biological and genetic effects. While the biological effects of air pollutants, including TEL, have been well studied, practically nothing is known about their genetic effects. In assessing the genetic effects of TEL, it is desirable to investigate (1) its mutagenic effects, and (2) the genetics of resistance to the chemical. The first point provides fundamental data for the evaluation of genetic damage. The second point is of interest from the standpoint of knowing the response and the genetic architecture arising from selection as a result of exposure to TEL.

The object of the present study was to investigate the effects of exposure to TEL on fecundity, hatchability and larva-to-adult viability in *Drosophila melanogaster* and to assess the genetic architecture of response in these traits using chromosome assay.

Materials and Methods

The population used in this study was a synthetic population from wildtype laboratory stocks (Oregon, Canton, Urbana, and Swedish). It was maintained for five generations prior to the start of the experiment. From this base population four replications were initiated simultaneously. Two replications were maintained on standard corn meal Karo media with 60 μg TEL added to each gram of medium; the other two were maintained as control on the same media with no TEL added. The choice of 60 μg/g TEL in the media was in a sense arbitrary. The idea was to choose a level of TEL that would generate a strong selection pressure (without causing a very high mortality rate in the population) for genetic changes to occur in a relatively short time. The rate of mortality of adult flies on media with 60 μg/g TEL was about 50%, which was thought to be adequate for this study.

Flies on both TEL and normal media were maintained in $\frac{1}{4}$-pint (*c.* 150 ml) bottles at room temperature. Generations were discrete and parents were allowed to lay eggs for 3 days from time of transfer and were then discarded. The number of adults that was transferred to start the next generation was kept large so that drift effect was minimal. Preliminary investigations on normal food at generation 20 showed that both replications on TEL responded alike with regard to fecundity, hatchability and larva-to-adult viability. Also, the two control replications were alike in their fecundity, hatchability and larva-to-adult viability. There were no differences in fecundity between the TEL and control populations when tested on normal food. However, hatchability and larva-to-adult viability were higher for the TEL than for the control population. Chromosome assay, using the technique of Kearsey and Kojima (1967), was performed on one replicate of tetraethyllead and one control replicate at generation 25 after the initiation of the experiment. The chromosome assay technique consisted of taking the TEL population (T) and the control population (N) and by the use of the inversion stock *C1B*, *Cy*, *UbX* forming the eight lines NNN, NNT, NTN, NTT, TNN, TNT, TTN, TTT [see Kearsey and Kojima (1967) for crossing technique]. Letters T and N denote the source of the first, second and third chromosomes. For example, NNT stands for the genotype with the first and second chromosomes coming from the control population where the flies were not exposed to TEL in the media, and the third chromosome coming from the population that was maintained on media with 60 μg/g TEL. *C1B*, *Cy* and *UbX* are dominant markers associated with the first, second and third chromosome inversions respectively and are lethal when homozygous. The three inversions are good suppressors of crossing over (Lindsley and Grell 1967).

The eight lines (NNN, ..., TTT) were crossed after the scheme of Kearsey and Kojima (1967) to obtain all possible 27 homozygous (NN or TT) and heterozygous (TN) chromosome combinations (Table 1). These 27 genotypes were compared with regard to fecundity, hatchability and larva-to-adult viability on both normal and 60 μg/g TEL media. For each genotype, pair matings were made with one pair to a vial. After 1 day of mating the female was transferred to a new vial that contained a piece of food (1 by 1 by $\frac{1}{2}$ cm) fastened with paraffin wax to the end of a strip of index card. The food was coloured green to facilitate egg counting. The inseminated female was allowed to lay eggs on the green food for 24 h at the end of which the female was discarded and eggs counted. After 2 days the number of eggs that were not hatched were scored and the piece of coloured food transferred to a new vial with food and the number of adults emerging after 14 days was scored. Data were collected on 13 pair matings per genotype (or 13 females per genotype) for each of normal and 60 μg/g TEL food.

Statistical Analysis

A two-way analysis of variance with a fixed model was performed on the transformed data. The best transformations (from the point of view of normality, homogeneity of variances and no correlations between means and variances) were found to be

(1) $\sqrt{X_1}$, where X_1 is the fecundity (= number of eggs laid per female),
(2) $\arcsin[(\sqrt{X_1}-\sqrt{X_2})/\sqrt{X_1}]$, where X_2 is the number of eggs that were not hatched per female, and
(3) $\arcsin\sqrt{[X_3/(X_1-X_2)]}$, where X_3 is the number of adults emerged per female.

The untransformed data for fecundity, hatchability and larva-to-adult viability were expressed as X_1, $(X_1-X_2)/X_1$ and $X_3/(X_1-X_2)$ respectively.

Source of variation in the analysis of variance table included genotypes with 26 degrees of freedom (d.f.), media (1 d.f.), genotypes × media (26 d.f.) and error (648 d.f.). To determine if the number of eggs laid had any effect on hatchability or larva-to-adult viability, a covariance analysis (with X_1 as the covariate) was performed. Results from the covariance analysis were the same as from the variance analysis. The conclusion was that under the present experimental conditions the number of eggs laid in a vial (reflecting the level of crowdedness in a vial) was not a factor in affecting hatchability or larva-to-adult viability.

The interest in the analysis of variance was in testing for additive and dominance effects and for first- and second-order chromosomal interactions on normal and TEL media. Twenty-six orthogonal comparisons (to correspond to 26 degrees of freedom for genotypes within media) of linear (additive) and quadratic (dominance) comparisons and their interactions were computed (using the coefficients in Table 1) on the 27 genotypic means for each of normal (N) and TEL (T) media. The coefficients

for the interaction comparisons were obtained as the cross-products of the coefficients of the appropriate additive and dominance comparisons. Each comparison had one degree of freedom and was tested (using the F statistic) against the pooled error mean square (in the two-way analysis of variance) with 648 degrees of freedom. Since the two error mean squares (one for each medium) were homogeneous, their pooled value from the two-way analysis of variance was the best estimate of the expected error mean square for each comparison.

Table 1. All possible 27 chromosome combinations and the additive (linear) and dominance (quadratic) comparisons for each of chromosomes X, 2 and 3

The interaction comparisons were obtained by the cross-products of the coefficients of the appropriate additive and dominance comparisons. N stands for a chromosome from the control population and T from the tetraethyllead population. $H = \frac{N}{T}$, $N = \frac{N}{N}$, $T = \frac{T}{T}$

Genotype X23	Comparisons					
	Linear (additive)			Quadratic (dominance)		
	X	2	3	X	2	3
NNN	1	1	1	1	1	1
HNN	0	1	1	−2	1	1
HNH	0	1	0	−2	1	−2
HHN	0	0	1	−2	−2	1
NNH	1	1	0	1	1	−2
NNT	1	1	−1	1	1	1
NHT	1	0	−1	1	−2	1
NHN	1	0	1	1	−2	1
NTN	1	−1	1	1	1	1
HTN	0	−1	1	−2	1	1
NHH	1	0	0	1	−2	−2
NTH	1	−1	0	1	1	−2
NTT	1	−1	−1	1	1	1
HHH	0	0	0	−2	−2	−2
TNN	−1	1	1	1	1	1
TNH	−1	1	0	1	1	−2
THH	−1	0	0	1	−2	−2
HNT	0	1	−1	−2	1	1
TNT	−1	1	−1	1	1	1
THT	−1	0	−1	1	−2	1
THN	−1	0	1	1	−2	1
TTN	−1	−1	1	1	1	1
TTH	−1	−1	0	1	1	−2
HHT	0	0	−1	−2	−2	1
HTH	0	−1	0	−2	1	−2
HTT	0	−1	−1	−2	1	1
TTT	−1	−1	−1	1	1	1

Table 2 presents the mean squares for additive, dominance, first- and second-order interaction effects over the three chromosomes. These mean squares were obtained by pooling the sum of squares of the appropriate single degree-of-freedom comparisons. Table 3 presents only the significant comparisons of additive and dominance effects and their interaction for individual chromosomes. Each comparison is presented for transformed data (in parentheses) and untransformed data. A comparison was computed as

$$\sum_{i=1}^{27} \lambda_i \bar{x}_i,$$

where the λs are the coefficients in Table 1 and the $\bar{x}$s are the means of the 27 genotypes within media.

Results

The results in Table 2 show that for fecundity there were significant additive and dominance effects on TEL media. When tested on normal media fecundity showed a significant additive × additive interaction effect. Additive effects were shown for hatchability and larval viability both on normal and TEL media.

The results in Table 3 on specific significant chromosome effects and interactions show that for fecundity on TEL media there was a significant additive effect for the X chromosome and a dominance effect for the second chromosome. In both cases the comparisons were negative, indicating higher fecundity for the TEL population compared with the control population. On normal media there was no significant difference between the TEL and control population with regard to additive and dominance effects of chromosomes. However, positive additive × additive and dominance × dominance interaction effects were shown. With regard to hatchability on TEL media there were negative additive effects for the third chromosome and positive dominance effects for the X chromosome. On normal media negative additive effects were shown for the X and third chromosomes. Also, there was a negative additive X dominance interaction effect involving the second and third chromosomes. Larval viability on TEL media exhibited negative additive effects for chromosomes 2 and 3 and positive additive × additive × additive chromosomal interaction. Larval viability on normal media showed negative additive effects for chromosomes X and 3 and a negative additive × additive chromosomal interaction.

Table 2. Mean squares and degrees of freedom (d.f.) for the main effects and principle interactions for fecundity, hatchability and larval viability on TEL media (T) and on normal media (N)

Included also are the mean square error and degrees of freedom from the variance analysis

Effects	d.f.	Fecundity Mean squares		Hatchability Mean squares		Larval viability Mean squares	
		T	N	T	N	T	N
Additive (a)							
X, 2, 3	3	4·887**	1·546	1·127**	1·503**	2·028**	1·558**
Dominance (d)							
X, 2, 3	3	5·824**	1·601	0·634*	0·046	0·019	0·069
First-order interactions							
a×a	3	2·412	4·806**	0·123	0·035	0·065	0·129
d×d	3	1·593	1·879	0·176	0·061	0·098	0·035
a×d	6	0·807	1·744	0·139	0·138	0·016	0·044
Second-order interactions	8	1·287	1·087	0·244	0·125	0·072	0·042
Mean square error	648	1·218	1·218	0·207	0·207	0·091	0·091

* $0{\cdot}01 < P < 0{\cdot}05$. ** $P < 0{\cdot}01$.

Discussion

Exposure of flies to 60 μg/g TEL in the media caused significant changes in fecundity, hatchability and larva-to-adult viability compared with the control population. Selection resulting from exposure to TEL produced an increase in fecundity compared with the control, but only when egg laying was on TEL media. The increase in fecundity was in the form of additive and dominance effects. On

normal media there were positive additive × additive and dominance × dominance interaction effects and no significant main effects. This indicates that selection was for genotype × environment interaction. Selection on TEL media caused an increase in hatchability and larva-to-adult viability compared with the control. The effects were predominantly additive and were expressed on both normal and TEL media.

These results (of predominantly additive genetic effects for resistance to TEL) are consistent with previous findings on the genetics of resistance to acute stress in *D. melanogaster*. Resistance to DDT (Crow 1954, 1957), phenylthiourea (Deery and Parsons 1972*a*), chloroform and ether (Deery and Parsons 1972*b*) and irradiation with ^{60}Co γ-rays (Parsons *et al.* 1969) were found to be generally under additive genetic control. Mather (1953, 1966) argued that directional selection on a quantitative trait would result in the trait manifesting directional dominance. Stabilizing selection, on the other hand, is expected to result in an additive genetic effect and weak or ambidirectional dominance and interaction effects. If the Mather hypothesis

Table 3. Significant chromosome effects and interactions for fecundity, hatchability and larval viability on normal and TEL media

Comparisons are based on means of 13 observations per genotype. Each comparison is presented for transformed data (in parentheses) and untransformed data. Only the comparisons that were significant are presented

Effect	Chromosome	Fecundity		Hatchability		Larval viability	
		TEL	Normal	TEL	Normal	TEL	Normal
Additive	X	−38·49 (−4·29**)	—	—	−0·64 (−2·67**)	—	−1·10 (−1·74**)
(a)	2	—	—	—	—	−2·59 (−3·12**)	—
	3	—	—	−0·552 (−1·98**)	−0·69 (−2·41**)	−2·19 (−2·74**)	−1·29 (−1·89**)
Dominance	X	—	—	0·674 (2·10*)	—	—	—
(d)	2	−76·21 (−8·19**)	—	—	—	—	—
a × a	2 × 3	—	32·84 (3·21**)	—	—	—	−0·636 (−0·798*)
d × d	X × 3	—	64·29 (6·84*)	—	—	—	—
a × d	3 × 2	—	—	—	−0·533 (−1·78*)	—	—
a × a × a	X × 2 × 3	—	—	—	—	0·459 (0·554*)	—

* $0{\cdot}01 < P < 0{\cdot}05$. ** $0{\cdot}001 < P < 0{\cdot}01$.

is correct, the present results indicate that selection under the acute stress of exposure to TEL was predominantly stabilizing (the rate of mortality of adult flies exposed for the first time to 60 μg/g TEL in the media was about 50%). This is in conformity with previous results on the genetic architecture of resistance to acute stresses (Parsons 1973). Fecundity, hatchability and larval viability are traits directly related to fitness. Studies with *D. melanogaster* showed that such traits were found to exhibit directional dominance indicating directional selection (Kearsey and Kojima 1967). Such studies, however, reflected the results of natural selection under no stress. Based on the Mather hypothesis the present results indicate that under acute stress selection on the present fitness traits was more stabilizing than directional which is in contrast to the case of selection under no stress. It could be argued that selection

under stress due to TEL was not for genes directly controlling fecundity and hatchability, but rather for genes conferring resistance to TEL which in turn had a correlated effect on these fitness traits. This might have been the case for fecundity where the genetics of response was affected to a large extent by the media.

As Kearsey and Kojima (1967) pointed out, the problem in connecting Mather's hypothesis to experimental evidence lies in the scale by which a trait is measured. The theory assumes that the genotype is measured in the same scale as that in which selection is acting. One is normally ignorant of the natural scale in which selection is acting. Genetic effects might be lost or altered if the scale used in measuring a genotype is different from the natural scale in which selection acts.

Acknowledgment

The author wishes to thank the referees for their comments.

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Manuscript received 1 May 1978