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EVOLVED ENVIRONMENT-DEPENDENT EXPRESSION OF DETOXICATION ENZYME ACTIVITY IN *DROSOPHILA MELANOGASTER*

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Enzyme induction by environmental cues is potentially a key adaptive process. This environment-dependent response may underlie phenomena such as physiological acclimation (Magnum and Towle, 1977), morphological plasticity (Schlichting, 1986), or detoxication of xenobiotic compounds (Nebert, 1979). However, there are virtually no studies on the evolutionary dynamics of this regulatory phenomenon.

Detoxication enzymes probably play an important role in adaptation to chemical challenges in the environment and consequently are expected to be a useful model for the study of inducible enzyme expression. Two major detoxication enzymes that have been studied in various organisms, including *Drosophila melanogaster,* are epoxide hydrolases (EH, E.C.3.3.2.3) and glutathione *S*-transferases (GST, E.C.2.5.1.18) (Oesch, 1972; Baars et al., 1979; Chasseaud, 1979; Jansen et al., 1984; Wixtrom and Hammock, 1985; Cochrane and LeBlanc, 1986; Jansen, 1986; Cochrane et al., 1987; Ottea et al., 1987*a*; Meijer and DePierre, 1988).

The objective of this study was to characterize the activity of epoxide hydrolases and glutathione *S*-transferases in *D. melanogaster* reared on standard laboratory medium as well as on lemon, which can be used by flies as a resource in the field. It was observed that all lines reared on lemon, but none of the control lines, evolved a substantial increase in expression of one glutathione *S*-transferase activity in response to the presence of lemon.

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MATERIALS AND METHODS

Thirty isofemale lines of *D. melanogaster* were initiated from flies collected on fallen lemons in a lemon grove near Orange Cove, California (Fresno County). The lines were pooled by mixing approximately the same number of flies from each isofemale line to produce a population of at least 3,000 individuals that was maintained on a standard laboratory medium for 15 generations. This population was then used to form six lines of at least 500 individuals each; three control lines were cultured on standard medium in bottles and three lines on lemon. For selection, flies were placed in bottles with 10 grams of freshly cut pesticide-free lemon on filter paper and usually held 7 to 10 days at room temperature. Approximately 50% mortality occurred at the end of this time, which may have been a result of natural insecticidal activity in lemons (Su and Horvat, 1987), toxins, or both produced by microbes growing on the fruit. The survivors were transferred to bottles with 30 grams of freshly cut lemon on a bed of vermiculite to produce the next generation. The control flies were held in bottles with medium and then transferred to fresh medium for propagation. Generally, 35 to 70 flies were used to initiate selected and control bottles after intraline randomization of adults in each generation.

EH specifically hydrolyses epoxides while GST forms a covalent glutathione reaction product with epoxides as well as other toxins. In *D. melanogaster* it is known that both enzyme activities can be induced by exposure of flies to exogenously administered compounds (Hällstrom and Grafstrom, 1981). Based on column chromatography, substrate selectivity, and subcellular

TABLE 1. Mean specific activities (with standard deviations) of glutathione *S*-transferase (GST) and epoxide hydrolase (EH) in microsomal and cytosolic preparations using *trans*-stilbene oxide (TSO) or *cis*-stilbene oxide (CSO) as substrates. Enzyme activity is in pmoles/minute/mg protein. Mean activity is the average of the three lines reared on medium or the three lines reared on lemon with standard deviations in parentheses. Enzyme activities of flies reared on medium was measured after 24 hours of exposure to medium (medium on medium) or 24 hours exposure to lemon (medium on lemon). Enzyme activities of flies reared on lemon was measured after 24 hours of exposure to medium (lemon on medium) or 24 hours of exposure to lemon (lemon on lemon). There is no detectable (ND) activity of microsomal GST toward TSO where the detection of 10 units of activity was considered to be above background.

	EH			
	TSO		CSO	
	Microsomes	Cytosol	Microsomes	Cytosol
Medium-reared on medium	311.7 (60.6)	70.7 (23.3)	1,213.9 (226.2)	742.6 (150.7)
Medium-reared on lemon	259.5 (154.2)	78.0 (17.9)	1,190.6 (188.7)	742.4 (80.8)
Lemon-reared on medium	167.7 (66.8)	51.0 (4.4)	918.0 (623.7)	1,059.5 (122.2)
Lemon-reared on lemon	165.7 (53.8)	62.0 (25.0)	886.4 (153.8)	1,417.9 (79.8)
	GST			
	TSO		CSO	
	Microsomes	Cytosol	Microsomes	Cytosol
Medium-reared on medium	ND	282.1 (75.6)	862.9 (160.6)	1,063.4 (60.3)
Medium-reared on lemon	ND	326.6 (104.5)	719.7 (281.4)	979.6 (229.2)
Lemon-reared on medium	ND	237.3 (14.5)	1,382.9 (651.7)	889.6 (399.6)
Lemon-reared on lemon	ND	612.6 (131.0)	986.2 (177.5)	639.2 (246.5)

location there is evidence for multiple forms of EH and GST in *D. melanogaster* (Cochrane and LeBlanc, 1986; Jansen et al., 1986; Ottea et al., 1987*a*). For this reason enzyme activities in this study were measured from two subcellular locations and with two substrates. Activity was determined radiometrically using *trans*- or *cis*-stilbene oxide (TSO or CSO) as substrates (Gill et al., 1983). It is a routine procedure to use substrate isomers, such as TSO and CSO, to identify various enzyme activities. The relative rate of hydrolysis of these substrates will depend on enzyme mechanism and the isozymes present. Approximately 100 females were homogenized, processed, and assayed in the manner described by Ottea et al. (1987*a*). For determination of specific activity protein concentration was measured by the method of Bradford (1976), using BSA as a standard. In addition, enzyme activity was standardized by the number of flies used ("fly equivalents," Ottea et al., 1987*a*) and by fly weight.

Enzyme activities were determined after 20 generations of culture on lemon or medium. For each line, three different samples of flies were assayed. All flies were reared one generation on medium at a controlled low density prior to enzyme assays to standardize the rearing environment of the flies prior to enzyme assays. Density was controlled by transferring 40 eggs or first instar larvae to vials with 10 ml of medium. Virgin females were collected after eclosion and held 100 per bottle at 25°C. Three days post-eclosion the females were aspirated onto fresh medium. Five days post-eclosion half the females were aspirated onto 30 grams of freshly cut pesticide-free lemon in bottles and the remainder aspirated into bottles with medium. No mortality occurred when the flies were held on medium or freshly-cut lemon for 24 hours. Six days post-eclosion the females were assayed for EH and GST activities.

RESULTS

Table 1 presents the mean specific activities of females from lines reared for 20 generations on either lemon or medium. By comparing activities of lemon-reared and control flies exposed to medium just prior to the assay it is possible to determine if baseline activity in lemon lines is different from control lines. The only statistically significant lemon-reared versus medium-reared difference ("group," Table 2) is for cytosolic EH on CSO where lemon-reared flies have higher baseline activity. All lines reared on lemon had higher cytosolic EH activity on CSO than any of the lines reared on medium, regardless of exposure prior to the assays.

The measure of environment-dependent enzyme expression is the activity of flies exposed to lemon just before the assay compared to activity after exposure only to medium. For cytosolic GST on TSO, lines reared on medium show only a 16% relative increase in activity when exposed to lemon, whereas the lines reared on lemon show 258% relative increase after exposure to lemon (Table 1). This difference between lines reared on lemon and control lines is shown by the significant "group by exposure" interaction for cytosolic GST on TSO (Table 2). The magnitude of the environment-dependent response is responsible for most of the variation that is shown as a significant "exposure" effect (Table 2). There is a possibility of a Type I error because multiple tests were conducted and

TABLE 2. Statistical analysis of specific activities of epoxide hydrolase and glutathione *S*-transferase. The tests are based on a factorial analysis of variance of ln transformed variates. The group factor is the food used to rear lines and the exposure factor is the food the flies were exposed to just before the assay. The factors tested are group averaged across exposures, exposure averaged across groups, and group-by-exposure interaction that measures whether the lemon and medium exposure response is the same in both groups of lines. Results that are insignificant at the 0.05 probability level are indicated by NS.

Enzyme activity	Group (rearing condition)	Exposure (prior to assay)	Group × Exposure
EH TSO microsomes	NS	NS	NS
EH TSO cytosol	NS	NS	NS
EH CSO microsomes	NS	NS	NS
EH CSO cytosol	0.0010	NS	NS
GST TSO cytosol	NS	0.0023	0.0068
GST CSO microsomes	NS	NS	NS
GST CSO cytosol	NS	NS	NS

by chance at least one would be expected to be positive at $\alpha = 0.05$. However, the significant P values were relatively small and continue to be significant after a Bonferroni correction for the number of enzyme activities tested. Moreover, each line reared on medium is insensitive to lemon as an inducer; in contrast, each line reared on lemon responds to lemon as an inducer of cytosolic GST activity on TSO (Table 3). The environment-dependent activity in flies reared on lemon is probably an evolved response because this pattern of expression was not present in the base population just prior to subdivision into lines (Table 3). Calculating enzyme activities on a per fly basis instead of per milligram protein does not alter the magnitude of the increase in expression of cytosolic GST on TSO (Table 3).

DISCUSSION

The adaptive role of "detoxication enzymes" is usually considered in terms of herbivore detoxication of plant toxins or insecticide resistance. However, these enzymes may be involved in a variety of essential physiological processes based on the metabolism of exogenous and endogenous substrates. The broad substrate range of detoxication gene families and differential induction in response to particular toxins (Jones et al., 1985) suggests the presence of a flexible defense system. The potentially extensive role of detoxication enzymes in evolution has largely been overlooked.

This study supports the hypothesis that there are multiple forms of glutathione transferase in *D. melanogaster* because environment-dependent activity of cytosolic GST activity toward TSO changed independently of cytosolic or microsomal GST activity on CSO. Cochrane et al. (1987) have noted that one purified form of GST from larvae accounts for most of the cytosolic activity against 1-chloro-2,4-dinitrobenzene (CDNB) and that other forms of GST must constitute minor fractions of the enzyme present. Testing GST activity in microsomes and cytosol from the lines employed in the present study using CDNB as a substrate indicated that over 90% of the activity was in the cytosolic fraction and there was no stimulation of CDNB cytosolic GST activity by lemon in lines reared on medium or lemon (data not shown). This observation agrees with a previous study indicating that as a GST substrate TSO may have unique properties (Ottea et al., 1987*b*). Multiple genes for GST are found in vertebrates where it is observed that each locus has its own promoter and some inducers affect only specific loci in a GST gene family (Pickett et al., 1984). One gene in rats has two *cis*-acting regulatory units; one for baseline activity and one for inducible expression (Telakowski-Hopkins et al., 1988).

After 20 generations on lemon there was a pro-

TABLE 3. Activity of cytosolic glutathione S-transferase on *trans*-stilbene oxide (TSO). Activity was measured in three independent replicate fly samples from the base population before subdivision (G_0). The lines are identified by the first letter which indicates whether they were cultured on medium (M) or lemon (L), the number identifies each separate line and the second letter indicates whether the females were exposed to medium (M) or lemon (L) prior to the assay. Activity per fly equivalent is based on the number of flies used in the assay.

						$\bar{x}$	SD
G_0 Activity (pmoles/minute/mg protein)							
On medium						335.1	73.6
On lemon						315.8	49.6
G_{20} Activity (pmoles/minute/mg protein)							
M1M	253.5	M2M	225.0	M3M	367.9	282.1	75.6
M1L	300.8	M2L	237.4	M3L	441.5	326.6	104.5
L1M	249.7	L4M	240.5	L5M	221.7	237.3	14.5
L1L	600.0	L4L	488.4	L5L	749.4	612.6	131.0
G_{20} Activity (pmoles/minute/fly equivalent)							
M1M	8.1	M2M	10.4	M3M	13.0	10.5	2.4
M1L	10.5	M2L	11.3	M3L	11.6	11.1	0.6
L1M	14.0	L4M	10.4	L5M	8.3	10.9	2.9
L1L	31.6	L4L	18.9	L5L	32.8	27.8	7.7

nounced change in environment-dependent expression of cytosolic glutathione *S*-transferase activity on TSO. The response appeared independently in all three lines on lemon (L1, L4, and L5 in Table 3). The fact that all three lines responded similarly is significant and would become increasingly important if the same pattern of selection is eventually observed in flies with different genetic backgrounds.

In this study, enzyme activity was measured in vitro, which does not necessarily indicate transcriptional change in vivo. One possibility is that a catalytically more efficient enzyme has evolved in the lemon lines and if synthesized at the same rate as in the control lines, there would be an apparent induction effect. However, when one compares the activity of lemon flies placed on medium just prior to the assay with control flies on medium there is no difference. Also arguing against this hypothesis is the observation of higher activity in lemon-selected flies held on lemon versus those held on medium just prior to the assay. An antibody derived from purified enzyme for this GST activity is not available to determine if the observed response is correlated with an increase in the abundance of a specific protein. In addition, kinetic analyses without purified enzyme are problematic when there are multiple forms of an enzyme or competing activities (such as EH) are present in homogenates. Another possibility is that the lemon lines have evolved a form of the enzyme whose activity is enhanced by a lemon derivative, perhaps by allosteric modification. A third possibility is that the environment-dependent change in activity in the lemon lines is due to an evolved increase in induction response to the presence of lemon.

In most cases nothing is known about the genetics of an inducible response. This includes the induction of a trout acetyl cholinesterase isozyme in response to a temperature shift (Baldwin and Hochachka, 1970) and the induction of insect detoxication enzymes after exposure to host plants (Yu et al., 1979; Farnsworth et al., 1981). An exception is the work by Yamazaki and Matsuo (1984) who have demonstrated the presence of genetic variation for amylase induction in *D. melanogaster* and also have related the induction response to fitness differences in a defined environment.

If selection varies either spatially or temporally the modulation of enzyme activity on the basis of environmental cues has several possible advantages. The first could be the ability to produce a "physiologically appropriate" amount of enzyme. The second may be termed the "energy conservation hypothesis" (Zamenhof and Eickhorn, 1967; Dykhuizen, 1978), which postulates an advantage to limiting expenditure for biosynthesis in those environments or at those times when the enzyme activity is not needed. In the present study the culturing regime used was ostensibly continuous, unless the process of lemon rotting every generation constitutes temporal variation. Normally, one would anticipate selection for change in environment-dependent enzyme expression to occur in variable environments but the results of the present study suggest it can evolve in a relatively consistent regime. In general, the evolution of environment-dependent expression of enzyme activity may occur under a variety of selection regimes and this response may underly an otherwise diverse set of adaptations.

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