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Allozyme genotype and time to death of mosquitofish, *Gambusia affinis* (Baird and Girard) during acute toxicant exposure: a comparison of arsenate and inorganic mercury

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Genotypic frequencies at 8 enzyme loci were examined in a population of mosquitofish, *Gambusia affinis* (Baird and Girard) during acute arsenate exposure. Genotypes at 2 loci (fumarate hydratase and glucose-phosphate isomerase-2) and multiple locus heterozygosity (male fish) were significantly correlated with time to death (TTD). The results from arsenate exposures were contrasted with those reported earlier for acute inorganic mercury exposure. Earlier TTD were associated with a rare homozygous genotype for the *Gpi*-2 locus in both arsenate and inorganic mercury exposures; however, no other single locus effect on TTD was common to both toxicants. The results of acute exposures of mosquitofish to mercury and arsenate suggest that most of the effects of multiple locus heterozygosity can be attributed to the summation of single locus effects.

Key words: Mosquitofish (*Gambusia affinis*); Mercury; Arsenate Toxicity; Genetics; Resistance

INTRODUCTION

Populations chronically exposed to pollutants often exhibit enhanced contaminant tolerance. Numerous mechanisms involving phenotypic or evolutionary plasticity could account for this acquired tolerance; however, most studies fail to clearly define the basis for such population responses to contaminants. This ambiguity is unfortunate as the probability of tolerance acquisition within a particular population, the rate of acquisition, inter- and intrapopulation variability and permanence of the enhanced tolerance are linked to the underlying mechanism(s). This lack of understanding has led to difficulty in evaluating proposals to relax discharge regulations through

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incorporation of population adaptation into national water quality criteria (see Klerks and Weis, 1987). Clearly, an understanding of the nature of enhanced tolerance is as important as confirmation of its presence in a population.

The rich literature for plant tolerance to toxicants in soils from seleniferous (Brown and Shift, 1982), metalliferous (Baker, 1978), mining (Lefebvre, 1975; Rocovich and West, 1975; Shaw et al., 1987), smelting (Pitelka, 1988), agricultural (Erickson et al., 1985) or heavily urbanized (Ehinger and Parker, 1979) regions describes a diversity of tolerance mechanisms including physiological and genecological mechanisms (Antonovics et al., 1971; Antonovics, 1975; Baker, 1978; Pitelka, 1988). In contrast, the genetic, physiological or biochemical mechanisms imparting enhanced tolerance in populations of aquatic fauna remain unclear in most studies (Klerks and Weis, 1987). Nevo and coworkers (Lavie and Nevo, 1982; Nevo et al., 1981; Nevo et al., 1984; Lavie and Nevo, 1986) have examined pollutant tolerance in various marine animals and its relation to electrophoretically determined enzyme variation. These authors report significant differences in allozyme frequencies in populations from polluted and nonpolluted areas, and differential survival among individuals of various enzyme genotypes during laboratory exposure to pollutants. Several single gene and multiple gene mechanisms were suggested to explain the relation between enhanced tolerance of pollutants and allozyme genotype. They reasoned that single gene effects might be associated with differential, competitive inhibition of magnesium-dependent allozymes by heavy metals. We (Diamond et al., 1989) clearly demonstrated sufficient latent genetic variability in a population of mosquitofish (*Gambusia affinis*) to allow significant differences in individual tolerance to inorganic mercury. Allozyme genotypes in 3 of 8 loci were significantly associated with differences in time to death (TTD). Genotypes associated with lowest median TTD involved one (isocitrate dehydrogenase or ICD-1 and glucosephosphate isomerase-2 or GPI-2) or both (malate dehydrogenase or MDH-1) of the homozygotes. Least tolerant genotypes tended to be the rare homozygotes. Multiple locus heterozygosity also had a significant influence on TTD. Median TTD increased with increasing number of heterozygous loci.

Although we (Diamond et al., 1989) did demonstrate sufficient latent genetic variability within a naive population to foster enhanced mercury tolerance, the direct linkage of response (TTD) to the scored loci and specificity of differential allozyme responses remained untested. The genotype effects could be specific responses to inorganic mercury or a nonspecific response to a stressor. To address this question, the acute exposure experiment was repeated with an alternate toxicant, sodium arsenate. Mercury and arsenate are characterized by a different mode of toxicity (Beliles, 1975). If the response reported in the preceding paper (Diamond et al., 1989) was a specific one to inorganic mercury then we predict dissimilar patterns of single and multiple locus effects on TTD in both mercury and arsenate exposures. Alternately, if this response was toxicant nonspecific then there should not be differences in responses associated with the mercury and arsenate exposures. The results of this sec-

ond exposure are described herein and contrasted with those reported for mercury exposures.

MATERIALS AND METHODS

Mosquitofish

Mosquitofish used in these studies were designated *Gambusia affinis* (subspecies *holbrooki*) (Baird and Girard). Recently, Wooten et al. (1988) suggested a systematic modification that would designate the mosquitofish in South Carolina as *Gambusia holbrooki*. Although there is convincing evidence to support such action, no formal reassignment of species has been provided to date. Therefore, we have retained the species name *affinis* in this report to facilitate comparison with past work.

The mosquitofish were collected from a small (1.1 ha), abandoned farm pond situated on the Department of Energy's Savannah River Plant (Aiken, SC) during the first 2 wk of June 1988. This genetically well-defined mosquitofish population (Smith et al., 1983; Diamond et al., 1989) has never been exposed to elevated concentrations of mercury or arsenate. Fish taken from waters of 27°C were acclimated to the experimental temperature (20°C) for at least 3 d in a 520-l holding tank (Living Streams Model LS700) prior to exposure. Details of collection and maintenance techniques are outlined in Diamond et al. (1989).

Arsenate exposure

The holding tank and tanks used in the flow-through exposure system received water from Upper Three Runs Creek as detailed in Diamond et al. (1989). The exposure system was comprised of 3 tanks: 1 38-l control tank and 2 112-l exposure tanks. Each tank received a continuous flow of Upper Three Runs Creek water at a rate of one tank vol per day. Both exposure tanks received Upper Three Runs Creek water spiked to a nominal concentration of 100 mg As l^{-1} from a common dosing reservoir.

Fish were randomly assigned to the 3 tanks to a density of 2.5–3.5 fish per l. Dosing of the 2 experimental tanks began after 24 h of fish acclimation to the exposure system. Fish were not fed during the period of acclimation to the exposure system nor the period of arsenate exposure.

Tanks were checked for dead fish every 3 h during the 102 h exposure period. A fish was scored as dead when it showed no signs of ventilation or fin movement, and it failed to respond to repeated, gentle prodding. Dead fish were removed from the tanks, weighed, sexed and dissected. The midbody was removed to avoid incorporation of food stuffs and embryos in the tissue samples. Plastic tubes containing the head and caudal sections of each fish were placed on dry ice until they could be stored at −70°C (less than 24 h). At 102 h, all surviving fish were killed and processed as described above.

(1989). Rare alleles were pooled during tests of fit to Hardy-Weinberg expectations for the *Icd-1* and *Gpi-2* loci. Contingency χ^2 statistics were used to test for homogeneity of genotypic distributions. A measure of fixation (D) was estimated for each locus. The measure was defined as the difference between the proportion of heterozygous genotypes observed and the proportion of heterozygous genotypes expected under the assumption of random mating.

Survival analysis procedures were used to describe the relations between TTD with fish size, sex and genotypes. The SAS Version 6.03 LIFEREG routine (SAS Institute, Inc., 1987) was used assuming a Weibull distribution for the hazard function. The assumption of a Weibull distribution was supported by the approximately straight, parallel lines resulting from plots of Ln(−Ln(proportion dead)) versus Ln(duration of exposure) for the various classes (Miller, 1981). The hazard (proneness to fail or die) for a particular class at any time, h(class, t) was estimated relative to that of an arbitrarily selected reference group (h(reference group, t)). Details of these methods are provided elsewhere (Diamond et al., 1989).

PROC LIFEREG does not accept models including interaction terms (SAS Institute, Inc., 1987). However, the importance of interactions between size and sex, size and genotype, sex and genotype, and number of heterozygous loci and sex was tested by constructing dummy variables corresponding to these interactions. A log-likelihood was computed for models including different interactions. The hypothesis that an interaction was zero was tested by comparing twice the change in log-likelihood when an interaction is included against the appropriate χ^2 distribution.

RESULTS

Water quality

Most water quality variables were similar for all tanks (Table I). Apparent alkalinity was higher in the exposure tanks than that of the control tank due to significant proton acceptance by the $HAsO_4^{-2}$ and $H_2AsO_4^{-1}$. The pH and sodium concentration were also elevated by the addition of Na_2HAsO_4 to Upper Three Runs Creek water. However, the change in pH was within the range of those seen daily in Risher Pond. Further, the increase in sodium concentration was insignificant relative to the salinity tolerance of this species. Measured arsenate concentrations were approximately 93–94 mg l^{-1} as As in the exposure tanks and <2 mg l^{-1} in the control tank. Past work in this stream (Newman, unpubl. data) has defined a median dissolved arsenic concentration <0.4 μg l^{-1} (n: 57, range: <0.4–4.1 μg As l^{-1}) in Upper Three Runs Creek water. Routine quality control measures and the minimal deviation from estimated perfect ionic balance indicated acceptable analytical control for the water quality variables.

TABLE I
Water quality in exposure system tanks ($n = 5$).

Variable	Tank 1		Tank 2		Control tank	
	X	SD	X	SD	X	SD
Temperature (C)	19.4	0.5	19.4	0.5	20.4	0.5
D. Oxygen (mg O_2/l)	9.3	0.5	9.3	0.4	9.0	0.4
pH*	7.40	7.34–7.83	7.38	7.33–7.86	6.22	5.61–6.33
S. conductance (μmho/cm)	266.0	24.7	264.6	25.8	32.6	2.4
T. alkalinity (mg/l $CaCO_3$)	73.7	6.3	73.6	6.0	7.8	1.0
Cl (mg/l)	2.6	0.5	2.6	0.6	2.4	0.2
SO_4 (mg/l)	1.9	0.3	1.9	0.3	1.6	0.2
Mg (mg/l)	0.7	0.2	0.7	0.2	0.7	0.1
Ca (mg/l)	1.8	0.2	1.7	0.1	1.6	0.1
Na (mg/l)	44.6	4.5	40.5	7.3	1.1	0.1
K (mg/l)	0.4	0.1	0.4	0.1	0.3	0.1
As (mg/l)	94	8	93	8	<2	
Deviation from perfect ionic balance (%)	6	2	7	3	2	2

*Median and range.

Genetics

The genotype distributions for the 8 loci of the 851 fish were consistent with random mating expectations and were essentially identical to those in the mercury exposure experiment (Diamond et al., 1989) (Table II). Six of the 8 D-values were negative and indicated a statistically insignificant, yet consistent deficiency of heterozygous genotypes in this sample of Risher Pond fish. There was no significant ($\alpha = 0.05$) deviation from random assignment of genotypes, sexes or fish sizes between control and exposure tanks. Contingency χ^2 tests confirmed that genotypes were homogeneously distributed among the control and exposure treatments.

Proportional hazards models

Eighty-seven percent (658 of 754) of the fish exposed to arsenate died. One of the 97 control fish died during the 103 h experiment. This control mortality was assumed to be insignificant and control fish were excluded from any further statistical consideration.

Two null hypotheses were tested with proportional hazards models. The genotypes at the 8 loci, fish size (wet weight) and sex were used to test the null hypothesis that genotype at individual loci had no significant effect on mosquitofish TTD during acute exposure to arsenate. The sex, size and total number of heterozygous loci for

TABLE II
Distribution of genotypes in experimental mosquitofish.

Locus	Genotype	Number	χ^{2a}	D[b]
FH			NA	0.069
	100/100	794		
	100/81	53		
	81/81	3		
GPI-2			0.62	−0.029
	100/100	215		
	100/66	286		
	66/66	127		
	100/38	116		
	66/38	86		
	38/38	19		
ICD-1			0.01	−0.014
	134/134	4		
	134/100	85		
	116/100	21		
	100/100	740		
ICD-2			0.01	−0.004
	161/161	53		
	161/100	316		
	100/100	481		
MDH-1			0.08	−0.010
	118/118	58		
	118/100	322		
	100/100	470		
MPI			0.18	−0.015
	109/109	60		
	109/150	322		
	100/100	466		
lgg-PEP			0.12	0.012
	123/123	17		
	123/100	215		
	100/100	618		
pp-PEP			0.88	
				0.012
	100/100	793		
	100/91	53		

[a] χ^2 test of fit to random mating expectations.

[b] $D = 1 - \frac{H_{obs}}{H_{exp}}$.

each fish were used to test the null hypothesis that the level of heterozygosity had no significant effect on TTD during acute arsenate exposure.

TABLE III
Summary of proportional hazards analysis – single locus effects.

Variable	Label	*d.f.*	β(SE)	χ^2	$P > \chi^2$	Predicted group median TTD (h) (SE)
Intercept		1	2.316 (0.454)	26.04	0.0001	
Sex		1		18.36	0.0001	
	Female	1	0.575 (0.134)	18.36	0.0001	58 (4)
	Male	0	0 (0)			43 (4)
Sex-specific Size effect	Male-size	1	0.048 (0.115)	0.17	0.6777	
	Female-size	1	1.812 (0.677)	7.17	0.0074	
ICD-1	Overall	3		1.59	0.6624	
	134/134	1	−0.213 (0.376)	0.32	0.5721	35 (13)
	134/100	1	0.079 (0.090)	0.77	0.3809	46 (5)
	116/100	1	−0.119 (0.179)	0.44	0.5076	38 (7)
	100/100	0	0 (0)			
ICD-2	Overall	2		1.44	0.4878	
	161/161	1	0.089 (0.113)	0.62	0.4312	47 (6)
	161/100	1	−0.040 (0.056)	0.52	0.4704	41 (3)
	100/100	0	0 (0)			43 (4)
FH	Overall	2		14.89	0.0006	
	100/100	1	1.084 (0.376)	8.32	0.0039	43 (4)
	100/81	1	0.801 (0.389)	4.24	0.0395	32 (4)
	81/81	0	0 (0)			14 (6)
MDH-1	Overall	2		2.70	0.2592	
	118/118	1	−0.034 (0.112)	0.09	0.7597	41 (5)
	118/100	1	−0.091 (0.055)	2.70	0.1005	39 (3)
	100/100	0	0 (0)			43 (4)
pp-PEP	Overall	1		0.33	0.5645	
	100/100	1	0.062 (0.108)	0.33	0.5645	43 (4)
	100/91	0	0 (0)			40 (6)
lgg-PEP	Overall	2		1.94	0.3800	
	123/123	1	−0.175 (0.180)	0.94	0.3312	36 (7)
	123/100	1	−0.065 (0.060)	1.16	0.2818	40 (4)
	100/100	0	0 (0)			43 (4)
MPI	Overall	2		2.81	0.2456	
	109/109	1	0.061 (0.109)	0.32	0.5727	49 (6)
	109/100	1	−0.079 (0.055)	2.05	0.1517	43 (4)
	100/100	0	0 (0)			46 (4)

TABLE III
Summary of proportional hazards analysis – single locus effects.

Variable	Label	*d.f.*	β(SE)	χ^2	$P > \chi^2$	Predicted group median TTD (h) (SE)
GPI-2	Overall	5		24.93	0.0001	
	100/100	1	0.392 (0.177)	4.92	0.0266	45 (4)
	100/66	1	0.338 (0.174)	3.75	0.0528	43 (4)
	100/38	1	0.050 (0.181)	0.08	0.7811	32 (3)
	66/66	1	0.227 (0.184)	1.53	0.2168	38 (4)
	66/38	1	0.114 (0.187)	0.37	0.5437	34 (4)
	38/38	0	0 (0)			31 (6)

Reference fish: Male, 0.15 g wet wt, ICD-1 100/100, ICD-2 100/100, FH 100/100, MDH-1 100/100, pp-PEP 100/100, lgg-PEP 100/100, MPI 100/100, GPI-2 100/66.
$N = 749$.

Since there was no significant effect of exposure tank assignment ($\alpha = 0.05$) on TTD, data for fish from the two exposure tanks were pooled prior to model development. Initial data exploration also indicated that the influence of size on TTD was different for the two sexes; but the sex*genotype interaction was not significant. Therefore, we fit one model for genotypes which included two size coefficients, one for each sex. Separate models were developed for each sex to test the second hypothesis as preliminary exploration of these data suggested that sex*size and sex*heterozygosity interactions were significant.

Genotype at 2 of the 8 loci had significant effects on TTD (Table III). Probabilities associated with the overall χ^2 statistics for Fh ($\chi^2 = 14.89$, $P = 0.0006$) and *Gpi-2* ($\chi^2 = 24.93$, $P = 0.0001$) suggested that genotype at these 2 loci significantly affected TTD for mosquitofish acutely exposed to arsenate. The predicted median TTD for the rare genotype Fh^{81}/Fh^{81} was 14 h. The heterozygote (Fh^{100}/Fh^{81}) had an intermediate median TTD (32 h) and the homozygous genotype for the most common allele, Fh^{100}/Fh^{100} had the longest median TTD (43 h). The individuals homozygous for the least common allele ($Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$) had the shortest predicted median TTD and the individuals homozygous for the most common allele ($Gpi\text{-}2^{100}/Gpi\text{-}2^{100}$) had the longest predicted median TTD. The most sensitive of the GPI-2 genotypes to arsenate intoxication ($Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$) was the same as predicted for inorganic mercury intoxication (Diamond et al., 1989). Thus, the first null hypothesis (TTD for mosquitofish exposed to arsenate does not differ among genotypes) was rejected.

Multiple locus heterozygosity was the sum of all heterozygous loci over the 8 scored loci and ranged from 0–6 per individual. Multiple locus heterozygosity had a significant effect for male fish only (Tables IV and V). The predicted median TTD for male fish decreased as the number of heterozygous loci increased. For example,

TABLE IV
Summary of proportional hazards analysis – multiple locus effects for females.

Variable	Label	*d.f.*	β (SE)	χ^2	$P > \chi^2$	Predicted group median TTD (h) (SE)
Intercept		1	4.107 (0.440)	87.07	0.0001	
Size		1	0.029 (0.109)	0.07	0.7895	
Number of	Overall	6		4.55	0.6028	
Hete. Loci	0	1	0.322 (0.456)	0.50	0.4804	67 (8)
	1	1	0.082 (0.445)	0.03	0.8536	53 (4)
	2	1	0.077 (0.443)	0.03	0.8616	53 (3)
	3	1	0.103 (0.444)	0.05	0.8159	54 (3)
	4	1	0.008 (0.451)	<0.01	0.9862	49 (5)
	5	1	0.146 (0.487)	0.09	0.7638	56 (12)
	6	0	0 (0)			49 (21)

Reference fish: 0.15 g wet wt, 0 heterozygous loci.
$N = 539$.

TABLE V
Summary of proportional hazards analysis – multiple locus effects for males.

Variable	Label	*d.f.*	β (SE)	χ^2	$P > \chi^2$	Predicted group median TTD (h) (SE)
Intercept		1	3.411 (0.728)	21.95	0.0001	
Size		1	2.211 (0.726)	9.26	0.0023	
Number of	Overall	6		16.85	0.0098	
Hete. Loci	0	1	0.418 (0.766)	0.30	0.5856	49 (13)
	1	1	0.324 (0.728)	0.20	0.6559	45 (6)
	2	1	0.206 (0.722)	0.08	0.7750	40 (4)
	3	1	0.016 (0.723)	<0.01	0.9820	33 (3)
	4	1	−0.264 (0.735)	0.13	0.7197	25 (4)
	5	1	−0.585 (0.774)	0.57	0.4496	18 (5)
	6	1	0 (0)			32 (23)

Reference fish: 0.15 g wet wt, 0 heterozygous loci.
$N = 210$.

TABLE IV
Summary of proportional hazards analysis – multiple locus effects for females.

Variable	Label	*d.f.*	β (SE)	χ^2	$P > \chi^2$	Predicted group median TTD (h) (SE)
Intercept		1	4.107 (0.440)	87.07	0.0001	
Size		1	0.029 (0.109)	0.07	0.7895	
Number of	Overall	6		4.55	0.6028	
Hete. Loci	0	1	0.322 (0.456)	0.50	0.4804	67 (8)
	1	1	0.082 (0.445)	0.03	0.8536	53 (4)
	2	1	0.077 (0.443)	0.03	0.8616	53 (3)
	3	1	0.103 (0.444)	0.05	0.8159	54 (3)
	4	1	0.008 (0.451)	<0.01	0.9862	49 (5)
	5	1	0.146 (0.487)	0.09	0.7638	56 (12)
	6	0	0 (0)			49 (21)

Reference fish: 0.15 g wet wt, 0 heterozygous loci.
$N = 539$.

TABLE V
Summary of proportional hazards analysis – multiple locus effects for males.

Variable	Label	*d.f.*	β (SE)	χ^2	$P > \chi^2$	Predicted group median TTD (h) (SE)
Intercept		1	3.411 (0.728)	21.95	0.0001	
Size		1	2.211 (0.726)	9.26	0.0023	
Number of	Overall	6		16.85	0.0098	
Hete. Loci	0	1	0.418 (0.766)	0.30	0.5856	49 (13)
	1	1	0.324 (0.728)	0.20	0.6559	45 (6)
	2	1	0.206 (0.722)	0.08	0.7750	40 (4)
	3	1	0.016 (0.723)	<0.01	0.9820	33 (3)
	4	1	−0.264 (0.735)	0.13	0.7197	25 (4)
	5	1	−0.585 (0.774)	0.57	0.4496	18 (5)
	6	1	0 (0)			32 (23)

Reference fish: 0.15 g wet wt, 0 heterozygous loci.
$N = 210$.

a 0.15 g male with 0 heterozygous loci had predicted median TTD 37% longer than the same size male with 6 heterozygous loci. Therefore, the second null hypothesis that TTD for mosquitofish exposed to arsenate does not differ with multiple locus heterozygosity was rejected for male mosquitofish.

DISCUSSION

Sex and size effects

Fish sex and size are well established as factors that can influence TTD during toxicant exposures (Rand and Petrocelli, 1985). The influences of fish sex and the sex-specific effects of fish size were apparent in mosquitofish exposed to arsenate (Tables III–V). Fish used in the experiment had the following wet weights (g): female controls – 0.376 ± 0.286 ($n = 73$); male controls – 0.175 ± 0.096 ($n = 24$); female experimentals – 0.389 ± 0.283 ($n = 540$), and male experimentals – 0.167 ± 0.077 ($n = 213$). The female fish tended to be larger and more variable in wet weight. Plots of size versus TTD suggested that these differences contributed to the perceived sex*size effects.

Single locus effects

It is often argued that most of the electrophoretically detectable enzyme polymorphism found within populations is selectively neutral (Pemberton et al., 1988). However, allozyme variation has been correlated with differences in response to osmotic (Burton and Feldman, 1983; Hilbish and Koehn, 1985), thermal (Hines et al., 1983; Watt, 1977, 1983) and pollution conditions (Nevo et al., 1984, 1986). Further, electrophoretic variance has been linked to juvenile survival in natural populations (Pemberton et al., 1988). We demonstrated a significant correlation between allozyme genotype at 3 (*Gpi-2*, *Idh-1*, *Mdh-1*) of 8 enzyme loci studied and TTD during acute exposure of mosquitofish to inorganic mercury (Diamond et al., 1989). The present study involved mosquitofish from the same source population and 2 (*Gpi-2*, *Fh*) of these 8 enzyme loci were correlated significantly with TTD during acute arsenate exposure. Thus, enzyme polymorphisms in a naive population were correlated with differences in survivorship during acute exposure to either toxicant.

Arsenate was used as an alternate toxicant with which to assess if the single locus effects on TTD of mosquitofish exposed to inorganic mercury were genetic differences in tolerance specifically to mercury or a nonspecific, differential response between genotypes to stress. If some genotypes were more prone to succumb to stress than others, then these genotypes would display the same patterns of sensitivity whether the toxicant were arsenate or mercury. This was the case for the *Gpi-2* genotypes. Among the 6 genotypes scored at this locus, the shortest median TTD was associated with the rare, homozygous genotype ($Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$) regardless of the toxicant. The *Icd-1* and *Mdh-1* loci which showed significant effects during the mercury exposures were not significantly related to TTD during arsenate exposure. Also, sig-

nificant differences in TTD between genotypes for FH were noted during the arsenate exposure only.

Where allozymes have been studied in detail, they have been found to differ in temperature optima, substrate specificity and sensitivity to inhibitors. Therefore, the high percentage of loci significantly correlated with differences in TTD is not an unreasonable finding. The present data suggest that allozyme genotype in mosquitofish can have significant effects on TTD during acute exposure; these effects can be general (*Gpi-2*) or specific (*Fh*, *Icd-2* and *Mdh-1*) relative to the toxicant. The specific response associated with *Icd-2* and *Mdh-1* during mercury exposure could be related to allozyme inactivation through Hg crosslinking of thiol groups. An alternative explanation would not involve the scored allozymes; rather, scored loci may be in linkage disequilibrium with unscored loci that impart differential response. To date, insufficient information is available to determine which of these potential explanations is correct for the influence of genotype on TTD. Further, the relevance of the results of these acute, short-term exposures to effects during chronic, low-level exposures to these toxicants remains ambiguous.

Multiple locus effects

Multiple locus heterozygosity has been correlated with several measurements of success that appear to be related to Darwinian fitness; individuals associated with higher scores for "fitness" are generally characterized by high levels of heterozygosity. Hypothesized mechanisms, principally inbreeding depression and overdominance, are discussed by Turelli and Ginzburg (1983), Mitton et al. (1986) and Smouse (1986). Fitness measurements including growth rate (Bush et al., 1987; Koehn et al., 1988), developmental rate (Danzmann et al., 1986), developmental stability (fluctuating asymmetry) (Ferguson, 1986; Leary et al., 1987), oxygen consumption rates (Mitton et al., 1986; Danzman et al., 1988), juvenile survival (Samallow and Soule, 1983; Pemberton et al., 1988) and survival during exposure to pollutants (Nevo et al., 1986; Diamond et al., 1989) were significantly enhanced by multiple locus heterozygosity. However, active metabolism of tiger salamanders was positively correlated with multiple locus heterozygosity (Mitton et al., 1986) suggesting higher metabolic cost for more heterozygous individuals. Mitton et al. (1986) suggested that the increased levels of multiple locus heterozygosity enhanced fitness by increasing an individual's aerobic scope (the increase in metabolic rate between rest and maximal activity).

A significant, positive relationship was derived between TTD during mercury exposure and mosquitofish multiple locus heterozygosity; however, a similar relationship was not noted for TTD during exposure to arsenate. No significant relationship was apparent for female mosquitofish and an inverse relationship was noted between male mosquitofish TTD and multiple locus heterozygosity. This suggests that among male mosquitofish more heterozygous individuals have shorter median TTD than less heterozygous fish. This conclusion is contrary to most studies in which multiple locus heterozygosity imparts an advantage to the individual.

The apparent inconsistency regarding the multiple locus heterozygosity effects on TTD during exposure to inorganic mercury versus arsenate can be resolved by assuming that the multiple locus effect was the sum of single locus effects. This initial assumption is similar to that espoused by Koehn et al. (1988) in their treatment of multiple heterozygosity effects on growth rates of the coot clam. The "sum of single locus effects" (SOSLE) and "multiple locus effects" (MLE) models derived for the two toxicants can then be used to test the validity of this assumption.

In a proportional hazards model, the effect of a set of predictor variables (x) is additive on a log hazard scale (Miller, 1981) i.e.

$$\log(\text{TTD}) = X\beta + \log(\text{baseline hazard}).$$

Our models include three sets of parameters: covariates such as sex and size, genotype effects and multiple locus heterozygosity effects. The $X\beta$ value for each individual is a linearly scaled measure of the effect of covariates and genotypes in the genotype model (Table III) and the effect of covariates and multiple locus heterozygosity in the heterozygosity model (Tables IV and V). The effects of only genotypes or heterozygosity can be obtained by subtracting the effects due to covariates such as size or sex from the total $X\beta$.

For each heterozygosity level, the sum of single locus effects was estimated by averaging $X\beta$ for genotypes across all individuals with that heterozygosity level. Pearson correlation coefficients were calculated between these averages and the corresponding $X\beta$ value for the number of heterozygous loci in the MLE model. Multiple locus effects are highly correlated with the sum of single locus effects in both the inorganic mercury data set (pooled sexes: $r=0.89$) and the sex-specific models for the arsenate data set (male: $r=0.81$; female: $r=0.85$) (Fig. 1). Sixty-six to 79% of the variance in these analyses could be attributed to the models of SOSLE versus MLE.

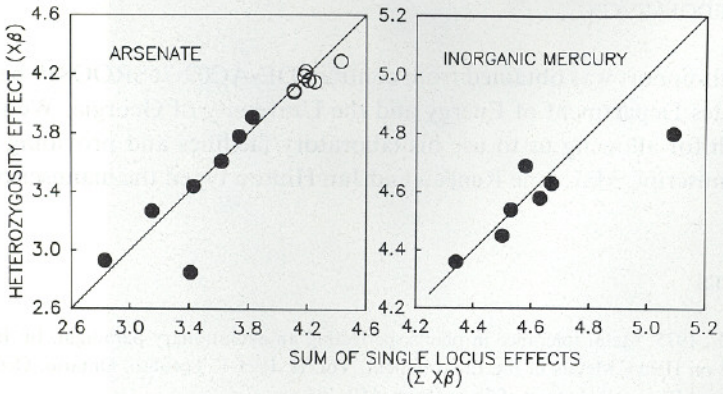

Fig. 1. Plots of heterozygosity effect against the sum of single locus effects during inorganic mercury and arsenate exposures. The estimates from the models for female (○) and male (●) mosquitofish were presented on the same plot for arsenate. Sexes were pooled (●) for the inorganic mercury models.

The assignment of most of the variance to the models and the general lack of patterns within the regression residuals suggests that the effect of multiple locus heterozygosity noted for inorganic mercury and arsenate induced mortality in mosquitofish simply reflects the sum of the single locus effects. Thus, the apparently contradictory effects of multiple locus heterozygosity reflect the differences in TTD for several loci. The genotypes with shortest TTD during mercury exposure were rare homozygotes and heterozygous genotypes tended to have the longest TTD of all measured genotypes. However, the high number of individuals with the favorable *Fh* genotype (Fh^{100}/Fh^{100}; $n=794$) and the relatively long TTD for the $Gpi\text{-}2^{100}/Gpi\text{-}2^{100}$ genotype produced an apparent multiple locus heterozygosity disadvantage during arsenate exposures.

SUMMARY

Significant differences between genotypes in TTD during arsenate or inorganic mercury acute exposure were present for a high percentage of allozymes (arsenate: 2 of 8 loci; inorganic mercury: 3 of 8 loci). Only differences in TTD at one locus seemed to be shared by both toxicants (*Gpi-2*). Therefore, differences in TTD associated with genotypic variation can be a specific for the toxicant (*Fh*, *Icd-1* and *Mdh-1*) or a nonspecific response to chemical stressors (*Gpi-2*). As suggested by the current study and the study of Diamond et al. (1989), the most sensitive genotypes can be homozygotes or heterozygotes; but, rare homozygotes were associated with relatively short TTD estimates. The influence of multiple locus heterozygosity on TTD during arsenate or inorganic mercury exposures reflected the summation of single gene effects and suggested no effect attributable to multiple locus heterozygosity per se.

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