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ALLOZYME GENOTYPE AND TIME TO DEATH OF MOSQUITOFISH, *GAMBUSIA AFFINIS* (BAIRD AND GIRARD), DURING ACUTE EXPOSURE TO INORGANIC MERCURY

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Abstract—Genetic plasticity in a mosquitofish (*Gambusia affinis* Baird and Girard) population was examined relative to acute mercury toxicity. Genotypes at eight loci (isocitrate dehydrogenase-1 and -2, mannosephosphate isomerase, glucosephosphate isomerase-2, fumarate hydratase, malate dehydrogenase-1, leucylglycylglycine peptidase and phenylalanylproline peptidase) were scored using starch gel electrophoresis. Two null hypotheses were tested: (a) time to death does not differ between genotypes at individual loci and (b) time to death does not differ with multiple-locus heterozygosity. Genotypes at three of the eight loci displayed significant effects on mosquitofish time to death. Multiple-locus heterozygosity also had a significant effect on time to death. Significant amounts of genetic plasticity were found in a population of mosquitofish with no previous exposure to inorganic mercury.

Keywords—Toxicity Mercury Genetics Mosquitofish Resistance

INTRODUCTION

Populations of plants [1–4] and animals [5,6] exposed chronically to pollutants often show enhanced tolerance to the pollutants relative to populations from uncontaminated areas. Enhanced tolerance can reflect both phenotypic and evolutionary plasticity [7]. Physiological or biochemical acclimation (phenotypic plasticity) involves processes such as metallothionein induction [8–11], phytochelatin production [12] or intracellular granule formation [13–15]. Evolutionary plasticity has an underlying genetic basis and results from selection for individuals with phenotypes (and underlying genotypes) that are resistant to the effects of a particular pollutant.

Recently, Nevo and co-workers [16–18] suggested that contaminants can select for resistant enzyme genotypes in nature. They postulated a variety of single-gene and multiple-gene mechanisms underlying contaminant tolerance. Differential competitive inhibition of magnesium-dependent allozymes by mercury [18] or cadmium [19] has been postulated as one mechanism responsible for shifts in allozyme frequencies in exposed field populations or for differential survival of individual organisms exposed to metals in the laboratory. Favored genotypes were either heterozygotes [17] or homozygotes [19]. Multiple-locus heterozygosity has also been linked to differential survivorship in marine gastropods exposed to trace metals [20].

While instances of enhanced toxicant tolerance are well documented, the genetic or physiological mechanisms are frequently undefined. Nevertheless, Klerks and Weis [21] note that the ability of exposed populations to adapt to toxicant stress has recently led to arguments for more lenient water quality criteria. Water quality criteria are typically derived from exposure of bioassay populations having no previous exposure; thus the response of these populations to toxicant stress probably has little relation to the fate of populations exposed in the field.

The present study was undertaken to determine if mercury-induced mortality was independent of single- and multiple-locus allozyme genotype. The mosquitofish, *Gambusia affinis* (Baird and Girard), was selected as a model organism. This common and widespread species has shown increased tolerance to a spectrum of agricultural pesticides [22,23]. Furthermore, the source population for this study

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was polymorphic for enzymes documented to show selective differences in mercury toxicity in marine species [16,18]. Two null hypotheses were tested in this study: (a) time to death (TTD) for mosquitofish exposed to 1 mg L^{-1} dissolved mercury does not differ among genotypes at eight enzyme loci, and (b) TTD for mosquitofish exposed to 1 mg L^{-1} dissolved mercury does not differ with multiple-locus heterozygosity summed over the eight enzyme loci.

METHODS

Collection site and sampling

Risher Pond, a 1.1-ha pond located at the U.S. Department of Energy Savannah River Plant (Aiken, SC), was chosen as the collection site because it supports a population of mosquitofish with high levels of electrophoretically detectable enzyme polymorphism [24]. The mosquitofish in Risher Pond have never been exposed to elevated levels of mercury or any other toxicants.

Fish were dip-netted from Risher Pond between September and November 1987 and placed into 120-liter plastic coolers filled with pond water. Immature fish and fish larger than approximately 38 mm were culled in the field. Before transport to the laboratory, each cooler was treated with approximately 5 g NaCl and 0.5 ml methylene blue (5.0%, w/v) to minimize stress-related mortality. The fish were held in two 520-liter tanks (Living Streams Model LS700) (18 to 20°C) until the exposure began.

Exposure system

The Living Streams tanks and the exposure system were supplied throughout the experiment with water pumped from Upper Three Runs Creek, a stream with water chemistry similar to that of Risher Pond. Total mercury concentrations are minimal in this stream (5 to 15 ng L^{-1}) (M.C. Newman, unpublished data).

A 38-liter control tank was supplied with a continuous flow of one tank volume per day of creek water. Two 112-liter exposure tanks were supplied with a continuous one-tank-volume-per-day flow of creek water spiked with 1.0 mg L^{-1} Hg as $HgCl_2$. The water used in preparing the toxicant solution was siphoned from one of the Living Streams tanks into a 50-liter carboy to which was added 50 ml of a 1.0 g L^{-1} Hg solution. The resulting 1.0 mg L^{-1} Hg toxicant solution was then emptied into a plastic drum equipped with a Blissfield Model BHL-1116A chiller unit that both mixed and maintained the temperature of the solution. The solution was delivered to the exposure tanks by a variable-speed peristaltic pump, with the flow bisected at a T-fitting. The tanks were well aerated and were covered with nylon netting to avoid loss of fish.

Exposure

Fish were randomly assigned to the control and exposure tanks to obtain a density of approximately 3.5 fish per liter. The mean (±SD) fish sizes (g wet wt.) were 0.20 ± 0.10, 0.18 ± 0.09 and 0.17 ± 0.08 in the control tank, exposure tank 1 and exposure tank 2, respectively. The fish were allowed to acclimate for 48 h and then each of the exposure tanks was dosed by thoroughly mixing 112 ml of 1 g Hg L^{-1} solution into each tank. The toxicant supply tank was filled with a 1 mg Hg L^{-1} solution and the flow was started. Fish were not fed during the exposure period. Dead fish were removed from the tanks every 3 h during the exposure period. Fish that appeared to be dead were gently prodded three to four times and carefully scrutinized for any sign of ventilation or fin movement. Fish judged to be dead were netted from the tank, weighed and the sex determined; the midbody was dissected away and the head and caudal region placed in plastic tubes. Samples were stored on dry ice and transported to a −70°C freezer within 48 h of dissection. All surviving fish were killed and processed as just described at the termination of the exposure period.

Water chemistry

Temperature and dissolved oxygen concentrations were determined daily with a Hydrolab Surveyor II. Total alkalinity (potentiometric titration, APHA 1980) and pH (Orion Research Microprocessor Ionanalyzer 1901, Orion 8130 Ross combination pH electrode) measurements were made daily also. Water samples for subsequent analyses were collected every 24 h and stored at 4°C. Major cation and mercury samples were acidified with 0.5 ml Ultrex nitric acid per 500 ml.

Specific conductance was measured with a Sybron PM-70CB conductivity bridge and a Fisher cell (cell constant = 0.105 cm^{-1}). Magnesium, calcium, sodium and potassium were measured by flame atomic absorption spectrophotometry (Hitachi 180-80 atomic absorption spectrophotometer with Zeeman background correction). Samples and standards used in major anion analysis were sequentially passed through a 0.45-μm membrane filter and then a Sepak reverse-phase column. Concentrations of sulfate and chloride were deter-

mined using a Dionex 4020i ion chromatograph with a conductivity detector and an HPIC-AS4A separator column (0.424 g L^{-1} Na_2CO_3; 0.126 g L^{-1} $NaHCO_3$ eluant). Dissolved organic carbon (DOC) samples were filtered with type A/E glass fiber filters (Gelman Sciences, Inc.) that were ashed at 500°C for 5 h prior to use. Menzel and Vaccaro [25] describe the original persulfate oxidation procedure used in the DOC analysis (OI Corporation Model 5240 ampoule analyzing unit and Model 3300 infrared gas analyzer). Total mercury was determined by a cold vapor technique [26] using a Perkin-Elmer 50A atomic absorption spectrophotometer.

Electrophoresis

Tissue samples were ground in their storage tubes with approximately 0.2 ml of cold grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 M NADP buffer; pH 7.0). Paper wicks were dipped into the homogenate, blotted to remove excess tissue and fluid, and inserted into 12.5% (w/v) horizontal starch gels. The following enzymes and buffers were used: isocitrate dehydrogenase (ICD-1, ICD-2), Tris-citrate, pH 7.1 [27]; malate dehydrogenase (MDH-1), mannosephosphate isomerase (MPI) and glucosephosphate isomerase (GPI-2), Tris-citrate, pH 8.0 [28]; and fumarate hydratase (FH), leucylglycylglycine peptidase (lgg-PEP) and phenylalanylproline peptidase (pp-PEP), Tris-EDTA-borate, pH 8.0 [28]. Gels were stained using methods described by Selander et al. [28] and Harris and Hopkinson [29].

Enzymes are numbered in order of decreasing anodal mobility in multilocus systems. Allozyme mobilities were determined relative to the most common allozyme for each locus, which was arbitrarily designated 100. Thus, the designation $Mdh\text{-}1^{100}/Mdh\text{-}1^{100}$ indicates an individual homozygous for the common MDH allele and $Gpi\text{-}2^{100}/Gpi\text{-}2^{73}$ indicates a heterozygous individual.

Data analysis

The BIOSYS-1 systems of Swofford and Selander [30] were used to determine genotype frequencies, mean heterozygosity and fit of these data to random mating expectations. For the *Icd-1* and *Gpi-2* loci, rare alleles were pooled to test the fit to Hardy–Weinberg expectations. Contingency χ^2 statistics were used to test for homogeneity of genotypic distributions among the control and experimental fish. A measure of fixation, D, was calculated for each locus, where D equals the proportion of heterozygous genotypes observed minus the proportion of heterozygous genotypes expected for a population in Hardy–Weinberg equilibrium.

The time-to-death (TTD) data were analyzed with survival analysis procedures as implemented in the SAS® version 6.03 LIFEREG routine [31]. These methods describe the relationships between predictor variables and the time to failure (death). They include observed TTD information for fish that died and the information that the surviving fish (right-censored individuals) have unobserved TTD longer than the exposure duration.

Since approximately straight parallel lines resulted from plots of ln (−ln (1 − proportion dead)) versus ln (duration of exposure) for various classes, a proportional hazards model was deemed appropriate [32]. This model described the effects of independent variables on time to death as follows:

$$h(\text{class}, t) = h(\text{ref. class}, t)\bar{e}^{x\beta}\bar{e}^{\sigma\epsilon} \qquad (1)$$

where $h(\text{class}, t)$ is the hazard of any class at time t, $h(\text{ref. group}, t)$ is the hazard of any arbitrary reference group, x is the data matrix of continuous and class variables, β is a vector of parameters measuring the influence of each x variable on the hazard, σ is a scale parameter and ϵ is a vector of errors from the assumed distribution.

The hazard function (force of mortality) is the measure of "proneness to fail" for individuals characterized by a continuous variable (weight) and several class variables (individual locus genotypes, sex, number of heterozygous loci). The hazard for a particular class at any time, $h(\text{class}, t)$, is estimated relative to that of an arbitrary reference group for which all x variables have the value 0.

RESULTS

Exposure

Water quality in the exposure and control tanks remained relatively uniform during the experiment (Table 1). Average mercury concentrations in the exposure tanks were approximately 1.0 mg L^{-1} and in the control tank, less than 0.1 μg L^{-1}. A slight increase in chloride concentration was evident in the exposure tanks as a consequence of spiking with $HgCl_2$.

Of the 711 exposed fish, 548 (77%) died during the exposure period. Differences in fish behavior were noted in the control and exposure treatments. Fish exposed to mercury tended to crowd into the bottom corners of the tank while control fish swam vigorously and were regularly distributed

Table 1. Water quality of the exposure and control tank waters

Variable	Tank 1		Tank 2		Control
	Mean	SD	Mean	SD	Mean
Temp. (°C)	17.9	0.8	18.0	0.8	17.7
DO (mg/L)	9.1	0.3	9.1	0.3	9.1
pH[a]	6.74	6.00–6.95	6.86	6.25–7.01	6.78
Specific conductance (μmho/cm)	47.2	8.4	47.6	8.7	45.8
Total alkalinity (mg/L $CaCO_3$)	12.8	3.0	12.8	2.9	12.7
Cl (mg/L)	3.2	0.6	3.3	0.6	2.8
SO_4 (mg SO_4/L)	2.3	0.2	2.3	0.2	2.3
DOC (mg C/L)	3.8	0.9	3.9	1.3	3.4
Mg (mg/L)	1.8	0.3	1.8	0.3	1.7
Ca (mg/L)	3.7	0.5	3.5	0.5	3.3
Na (mg/L)	3.3	1.8	3.4	1.9	3.1
K (mg/L)	0.8	0.3	0.9	0.3	0.8
Hg (mg/L)	0.964	0.124	1.017	0.071	<0.0001

Eleven samples were taken for each variable except specific conductance, for which $N = 10$. DO, diss
gen; DOC, dissolved organic carbon.
[a]Median and range.

throughout the tank. Minor control mortality due to cannibalism was evident after 7 d. No cannibalistic behavior was noted in mercury-exposed fish. Of the 129 control fish, 9 (7%) died during the experiment. Three of the control deaths were clearly attributable to cannibalism.

Genetics

There were no significant differences in genotypic frequencies between the two groups of mosquitofish exposed to mercury or between the exposed and control fish. The overall genotype distributions (Table 2) for the 841 fish from Risher Pond were consistent with the random mating expectation of the Hardy–Weinberg model except for the distribution of MPI genotypes. The *Mpi* locus showed a significant deviation ($\chi^2 = 4.24$, $p = 0.04$) associated with a deficiency of heterozygous genotypes. Six of eight D values were negative and indicated a consistent, although not statistically significant, deficiency of heterozygous genotypes in this sample of the Risher Pond population.

Proportional hazards model

Two statistical models were developed. Sex, size and genotype at the eight enzyme loci were incorporated into a model with the intention of testing the first null hypothesis: TTD for mosquitofish exposed to 1 mg L^{-1} of dissolved mercury does not differ between genotypes at individual loci (Table 3). Sex, size and number of heterozygous loci were then used in a second model to assess the second null hypothesis: TTD for mosquitofish ex-

Table 2. Distribution of genotypes in experimental mosquitofish

Locus	Genotype	Number	p[a]
FH	100/100	801	NA
	100/81	35	
	81/81	2	
GPI-2	100/100	210	0.89
	100/66	282	
	66/66	136	
	100/38	118	
	66/38	66	
	38/38	28	
ICD-1	134/134	7	0.15
	134/116	3	
	134/100	95	
	116/100	34	
	100/100	700	
ICD-2	161/161	53	0.52
	161/100	330	
	100/100	457	
MDH-1	118/118	57	0.23
	118/100	297	
	100/100	481	
MPI	109/109	71	0.04[c]
	109/150	296	
	100/100	440	
lgg-PEP	123/123	15	0.77
	123/100	187	
	100/100	637	
pp-PEP	100/100	794	0.43
	100/91	45	

[a]Probability associated with χ^2 test of fit
mating expectations. NA, not applicable.
[b]$D = H_{obs} - H_{exp}$, where D is a measure of fi
H is the proportion of heterozygous geno
Methods in the text).
[c]Significant at $\alpha = 0.05$.

Table 3. Summary of proportional hazards analysis: Single-locus effects

Variable	Label	*df*	β (SE)	χ^2	$p > \chi^2$	Predicted group[a] median TTD (h) (SE)
Intercept		1	4.820 (0.531)	82.52	0.0001	
Sex		1		65.61	0.0001	
	Female	1	0.361 (0.045)	65.61	0.0001	179 (12)
	Male	0	0 (0)			125 (8)
ICD-1	Overall	4		10.57	0.0318	
	134/134	1	−0.516 (0.228)	5.14	0.0234	75 (18)
	134/116	1	−0.005 (0.291)	<0.01	0.9867	124 (36)
	134/100	1	0.071 (0.070)	1.02	0.3118	134 (11)
	116/100	1	−0.202 (0.102)	3.97	0.0463	102 (11)
	100/100	0	0 (0)			125 (8)
ICD-2	Overall	2		4.25	0.1194	
	161/161	1	0.047 (0.093)	0.25	0.6146	131 (14)
	161/100	1	0.094 (0.046)	4.24	0.0394	137 (9)
	100/100	0	0 (0)			125 (8)
FH	Overall	2		3.25	0.1970	
	100/100	1	−0.668 (0.508)	1.73	0.1882	125 (8)
	100/81	1	−0.525 (0.518)	1.03	0.3110	243 (125)
	81/81	0	0 (0)			144 (19)
MDH-1	Overall	2		6.84	0.0327	
	118/118	1	0.003 (0.083)	<0.01	0.9757	125 (12)
	118/100	1	0.120 (0.047)	6.58	0.0103	141 (10)
	100/100	0	0 (0)			125 (8)
pp-PEP	Overall	1		0.03	0.8666	
	100/100	1	−0.017 (0.100)	0.03	0.8666	125 (8)
	100/91	0	0 (0)			127 (14)
lgg-PEP	Overall	2		2.11	0.3487	
	123/123	1	−0.216 (0.150)	2.09	0.1483	101 (16)
	123/100	1	−0.012 (0.052)	0.06	0.8141	123 (9)
	100/100	0	0 (0)			125 (8)
MPI	Overall	2		1.07	0.5852	
	109/109	1	0.060 (0.081)	0.55	0.4586	127 (11)
	109/100	1	0.041 (0.047)	0.75	0.3875	125 (8)
	100/100	0	0 (0)			120 (7)
GPI-2	Overall	5		13.20	0.0215	
	100/100	1	0.258 (0.117)	4.88	0.0272	111 (8)
	100/66	1	0.375 (0.115)	10.54	0.0012	125 (8)
	100/38	1	0.289 (0.121)	5.72	0.0168	115 (9)
	66/66	1	0.296 (0.124)	5.65	0.0174	116 (9)
	66/38	1	0.227 (0.139)	2.70	0.1006	108 (11)
	38/38	0	0 (0)			86 (11)
Size		1	3.060 (0.353)	75.069	0.0001	
Scale parameter			0.497 (0.018)			

TTD, time to death. Other abbreviations in text (see Methods).
[a]Reference fish: Male, 0.15 g wet wt., *Icd-1*^{100}/*Icd-1*^{100}; *Icd-2*^{100}/*Icd-2*^{100}; *Fh*^{81}/*Fh*^{81}; *Mdh-1*^{100}/*Mdh-1*^{100}; *pp-PEP*^{100}/*pp-PEP*^{100}; *lgg-PEP*^{100}/lgg-PEP^{100}; Mpi^{109}/*Mpi*^{100}; and *Gpi-2*^{100}/*Gpi-2*^{66}.

posed to 1 mg L^{-1} dissolved mercury does not differ with multiple-locus heterozygosity (Table 4).

As there was no significant effect of exposure tank ($\alpha = 0.05$) on the TTD (Fig. 1), this class variable was eliminated during initial model development and data from both experimental tanks were pooled. Fish size (wet wt.) and sex had significant effects on TTD (Table 3), with females and larger fish predicted to have longer TTD than males and small fish. There were predicted differences between median TTD of 42 to 54 h for the two sexes, depending on the reference group used during model generation (Tables 3 and 4).

Genotype at three of the eight loci examined

Table 4. Summary of proportional hazards analysis: Multiple-locus effects

Variable	Label	*df*	β (SE)	χ^2	$p > \chi^2$	Predicted group[a] median TTD (h) (SE)
Intercept		1	5.089 (0.513)	98.53	0.0001	
Sex		1		61.06	0.0001	
	Female	1	0.351 (0.045)	61.06	0.0001	141 (13)
	Male	0	0 (0)			99 (9)
Number of heterozygous loci	Overall	6		14.99	0.0203	
	0	1	−0.753 (0.515)	2.13	0.1441	99 (9)
	1	1	−0.590 (0.509)	1.35	0.2461	117 (6)
	2	1	−0.562 (0.509)	1.22	0.2696	120 (6)
	3	1	−0.454 (0.509)	0.80	0.3722	134 (7)
	4	1	−0.415 (0.512)	0.66	0.4176	139 (11)
	5	1	−0.512 (0.525)	0.95	0.3292	126 (17)
	6	0	0 (0)			211 (107)
Size			2.992 (0.356)	70.48	0.0001	
Scale parameter			0.5065 (0.0189)			

TTD, time to death.
[a]Reference fish: Male, 0.15 g wet wt., 0 heterozygous loci.

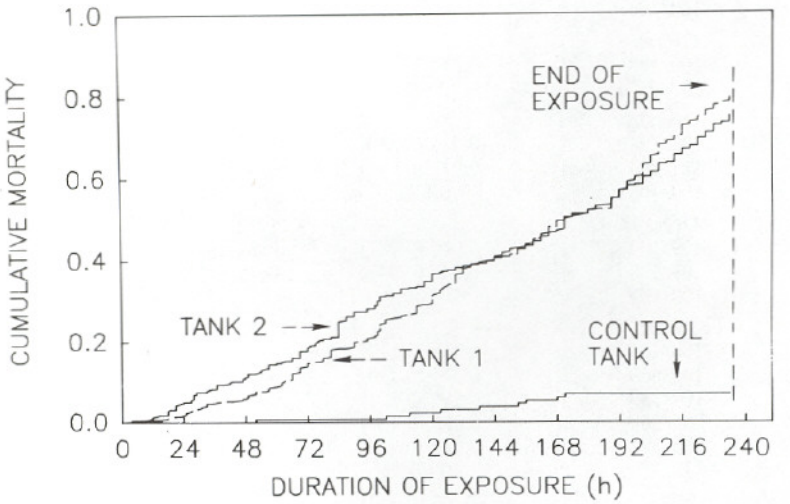

Fig. 1. Cumulative mortality in the control tank and two tanks receiving inorganic mercury.

had significant ($\alpha = 0.05$) effects within the model. Probabilities associated with the overall χ^2 statistics for ICD-1 ($\chi^2 = 10.57$, $p = 0.03$), MDH-1 ($\chi^2 = 6.84$, $p = 0.03$) and GPI-2 ($\chi^2 = 13.20$, $p = 0.02$) suggested that allozyme variation at these loci significantly influenced TTD. The predicted median TTD (Table 3, right-hand column) for the *Icd-1*^{134}/*Icd-1*^{134} genotype was more than 24 h shorter than for the other genotypes at the *Icd-1* locus. The two homozygous genotypes at *Mdh-1* had median TTD estimates approximately 16 h shorter than those for the heterozygous genotype. The *Gpi-2*^{38}/*Gpi-2*^{38} genotype was predicted to have a median TTD lower than that for all other *Gpi-2* genotypes.

Multiple-locus heterozygosity had a significant effect within the regression model (Table 4). The sample sizes for fish heterozygous for zero, one, two, three, four, five or six of the enzyme loci were 39, 181, 221, 172, 69, 17 and 3, respectively.

Despite small sample sizes for classes with high numbers of heterozygous loci, the predicted median TTD increased with increasing number of heterozygous loci.

DISCUSSION

Unpredictable environmental perturbations such as contamination events can severely test the physiological limits of a species. A population sample of mosquitofish exposed to 1.0 mg L^{-1} dissolved mercury showed 77% mortality during the course of a 10-d exposure. Fish sex and size were significant factors in predicted median TTD. All fish dying during the first 24 h were males. The predicted median TTD for males was approximately 2 d shorter than that for females. These differences are apparent in the sex ratio, which was 1.2 females/male among dead fish and 4.3 females/male among fish that survived the exposure period.

Although mosquitofish are sexually dimorphic with respect to size, this dimorphism was normalized in the statistical model and therefore the significant effect of size was not linked to the effect of sex. There was broad overlap in the weight distributions for the male and female fish studied (mean ± SD for control males, 0.19 ± 0.09 g; for control females, 0.20 ± 0.10 g; for experimental males, 0.17 ± 0.07 g; for experimental females, 0.17 ± 0.10 g).

Genotypic variation at single electrophoretic loci has increasingly been associated with differences in performance under osmotic [33,34], thermal [35–37] and trace metal [18,20] stress. In addition to the strong effects of fish sex and size on predicted median TTD during acute mercury exposure, three of the eight enzyme loci showed significant effects. For the *Mdh-1* locus, the two homozygous genotypes, $Mdh\text{-}1^{118}/Mdh\text{-}1^{118}$ and $Mdh\text{-}1^{100}/Mdh\text{-}1^{100}$, had predicted median TTDs approximately 16 h earlier than the heterozygous genotype, $Mdh\text{-}1^{118}/Mdh\text{-}1^{100}$. The greater mortality among the *Mdh-1* homozygotes was apparent from the outset and became more marked as exposure time increased.

Five genotypic classes were observed for the *Icd-1* locus. Only five individuals of the $Icd\text{-}1^{150}/Icd\text{-}1^{150}$ homozygous genotype were present in the exposure tanks; however, it is apparent that these fish had significantly earlier mortality than other genotypes. The predicted median TTD was only 75 h and all were dead at 138 h. Additionally, individuals of the $Icd\text{-}1^{116}/Icd\text{-}1^{100}$ genotype had a predicted median TTD at least 27 h earlier than fish of other *Icd-1* genotypes.

The *Gpi-2* locus had six genotypes and, again, individuals of the least common homozygous genotype, $Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$, had the poorest survival. Of 23 fish with this genotype in the exposure tanks, only 1 survived the exposure period (>95% mortality).

These data lead to a rejection of the hypothesis that median TTD following acute exposure to mercury is independent of single-locus genotype. Rare homozygous genotypes, $Icd\text{-}1^{134}/Icd\text{-}1^{134}$ and $Gpi\text{-}2^{38}/Gpi\text{-}2^{38}$, and both $Mdh\text{-}1^{118}/Mdh\text{-}1^{118}$ and $Mdh\text{-}1^{100}/Mdh\text{-}1^{100}$ homozygotes had significantly earlier predicted median TTD than other genotypes for these enzyme loci.

Evidence is accumulating that fitness or a surrogate measure of individual success (growth rate, metabolic efficiency, survivorship) can be positively correlated with heterozygosity summed over many electrophoretic loci [38–41]. Fish heterozygous for zero to six of the eight enzyme loci were available from the exposure tanks. There was a significant effect of the number of heterozygous loci on the predicted median TTD for mosquitofish exposed to mercury. Predicted median TTD showed an essentially monotonic increase with increasing number of heterozygous loci. Individuals homozygous for all of the eight enzyme loci had predicted median TTD 40 h earlier than that of individuals heterozygous for four of the loci. The predicted median TTD was 211 h for the highly heterozygous fish. The second hypothesis, that TTD is independent of multiple-locus heterozygosity, is rejected.

Organism size and sex are well recognized as factors influencing population responses to toxicant stress. In the present study, fish sex and size were important factors in predicted median TTD during acute mercury exposure; additionally, genetic variability present in naturally occurring populations may also be an important factor. Differences in predicted median TTD among genotypic classes were as much as two to three d, comparable to effects attributable to sex and size.

Local populations often have gene frequencies different from geographically distant populations. These differences have been shown to correlate with environmental factors such as temperature [35,42–44], salinity [33,34] and, recently, heavy metal pollution [20]. Exposure of populations to acute levels of mercury in the environment is restricted to episodic spills or releases; however, there is direct evidence that long-term exposure to

low concentrations of mercury can be associated with genotypic responses at enzyme loci [18,45].

Environmental contaminants are often associated with high mortality in affected populations. If populations are composed of individuals with a range of contaminant tolerance, the most tolerant genotypes should increase in frequency as pollution persists. Populations from contaminated areas have frequently been reported to tolerate higher levels of contaminants than populations from unaffected areas [46]. The mechanism of selection for tolerant allozymes may involve direct effects on enzyme activity. Mercury and other heavy metals are able to successfully compete with enzyme cofactors, such as magnesium or zinc, for enzyme cofactor sites [47,48]. If the enzyme structure allows access to sulfhydryl groups, mercurials will crosslink sulfhydryl groups with a rigid, linear R′—S—Hg—S—R bond [47]. In the present study, one of the three enzymes showing significant effects (*Icd-1*) is Mg-dependent. Different forms of an enzyme (allozymes), because of slight variation in structure, are likely to exhibit different sensitivities to the effects of trace metals. Indeed, whenever allozymes have been studied in sufficient detail, they have been shown to differ in kinetic properties, substrate specificity and sensitivity to inhibitors [49–51]. The observation that there were three loci showing strong genotypic effects in mosquitofish exposed to high levels of mercury is consistent with the hypothesis that allozymes at these loci may differ in their sensitivities to mercury.

In this study, the number of heterozygous loci was positively related to the predicted median TTD. For six of the eight enzyme loci, the longest predicted median TTD was associated with a heterozygous genotype. Superior performance of heterozygotes (heterosis) may reflect subtle differences in physiological or biochemical performance. If mercury toxicity is associated with competition for enzyme cofactor binding sites or crosslinking of sulfhydryl groups, then heterozygotes may show greater tolerance because they possess multiple allozyme forms. Recent work of Trehan and Gill [52] suggests that this might be especially true for multimeric enzymes, where heterozygotes possess both parental homopolymers and one or more heteropolymers.

The present study indicates that many factors [illegible] differences among individuals within populations are only rarely considered. For mosquitofish, genetic polymorphism at specific enzyme loci or closely linked loci have significant effects on TTD during mercury exposure.

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