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Allozymes reflect the population-level effect of mercury: simulations of the mosquitofish (*Gambusia holbrooki* Girard) GPI-2 response

MICHAEL C. NEWMAN*[1] and ROSEMARY H. JAGOE[2]

[1]*College of William and Mary, Virginia Institute of Marine Science, Gloucester Point, VA 23062, USA*

[2]*University of Georgia, Savannah River Ecology Laboratory, PO Drawer E, Aiken, SC 29802, USA*

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Measurements of the differential tolerance between enzyme genotypes and shifts in allozyme frequencies in populations from contaminated habitats have prompted the use of allozymes as markers of population-level toxicant effects. However, such studies often do not consider other factors that influence allele frequencies, including natural clines, migration, the intensity and specificity of selection and toxicant-induced genetic bottlenecks. In addition, selection components other than survival are not included. Consequently, the associated conclusions remain speculative. To assess this approach rigorously, a simulation study was conducted with the mosquitofish (*Gambusia holbrooki*) GPI-2 locus. Laboratory studies have shown the GPI-$2^{38/38}$ homozygote at this locus to be less tolerant than other genotypes during acute exposure to mercury. The GPI-$2^{100/100}$ genotype has also been shown to have a reproductive disadvantage at lower mercury concentrations. Simple and then more complex models were used to quantify the relative effects of viability selection, random genetic drift and migration on the GPI-2^{38} allele frequency. Simulations were also performed to assess the contribution of sexual and fecundity selection. A simple population model suggested that viability selection plays a greater role than does mortality-driven, genetic drift in the decrease of the sensitive allele under the conditions of this study. A more complex, stochastic model indicated that no significant mortality-driven drift was taking place in this system. In both models, migration mitigated the effect of selection. Sexual and fecundity selection had little effect on the allele frequencies in these simulations. We conclude that, provided the system under study is clearly understood, shifts in allele frequency can indicate the population-level effects of pollutants.

Keywords: fish; mercury; natural selection; allozyme; population.

Introduction

Allozyme analysis has been advocated for assessing the effects of pollution on populations (e.g. Nevo *et al.*, 1977, 1983; Battaglia *et al.*, 1980; Chagnon and Guttman, 1989). Levels of environmental contamination have been correlated with the allele or genotype frequencies of field populations in many studies supporting such use of allozymes (e.g. Nevo *et al.*, 1978, 1984). In some studies, the correlations were then compared to the differential survival of the genotypes under acute laboratory exposure (e.g. Nevo *et al.*, 1981; Gillespie and Guttman, 1989; Kopp *et al.*, 1992). Still other studies documented only the differential survival during acute laboratory exposures and speculated that the differences in genotype mortality could shift allele frequencies in field populations (e.g. Lavie *et al.*, 1984; Nevo *et al.*, 1986; Diamond *et al.*, 1989; Newman *et al.*, 1989; Hughes *et al.*, 1992).

Questions regarding such use of allozymes arise from several sources. Advocates of neutralism in the neutralist–selectionist debate generally question studies putatively measuring selection with allozymes. However, studies of several species (e.g. Watt, 1977, 1983; Watt *et al.*, 1983, 1985; Carter and Watt, 1988) have clearly demonstrated that some allozyme genotypes can display differential fitness and these differences influence the genetic composition of field populations. More serious criticism arises from the inferential weaknesses of the work described above. First, a reliance on correlation analyses of field data precludes clear assignment of causation. Second, even conclusions from studies including laboratory components remain equivocal because the exposure concentrations are much higher than those experienced by field populations. Third, other covariates influencing the phenotype (survival), such as animal size and sex, often remain unquantified. Fourth, the mechanism of genotype effect is frequently undefined and considered only speculatively. Finally, although there are several components of an organism's life cycle upon which natural

*To whom correspondence should be addressed.

selection can act (selection components), only survival (viability selection) is measured and other potentially important selection components such as fecundity or sexual selection are not studied. Consideration of all the selection components is critical because selection can take place at several stages of the organism's life cycle, frequently in opposing directions (Hartl and Clark, 1989). Endler (1986) cited these last two shortcomings as the most common flaws in field studies of natural selection.

Because of these shortcomings, the reliability of biomonitoring with allozymes remains difficult to judge. Consequently, Newman, Mulvey and co-workers designed more detailed studies using mosquitofish (*Gambusia holbrooki* Girard) exposure to mercury as a model for allozyme genotype effects under toxicant stress. The adaptation to toxicants by both *G. holbrooki* (formerly *Gambusia affinis holbrooki*) and the phylogenetically similar *Gambusia affinis* has been studied extensively (Vinson *et al.*, 1963; Boyd and Ferguson, 1964; Kynard, 1974; Yarbrough, 1974; Chambers and Yarbrough, 1979; Angus, 1983; Keklak *et al.*, 1994): much information is available for this common and widespread species which has demonstrated evolutionary plasticity relative to toxicants. Newman, Mulvey and co-workers found differential survival for genotypes at three loci including glucosephosphate isomerase (GPI-2) exposed to acute concentrations of mercury (Diamond *et al.*, 1989). Multiple locus heterozygosity was also correlated with the time to death but this putative relationship was later shown to result from the summation of single allele effects, not heterozygosity *per se* (Newman *et al.*, 1989). Although the GPI-2 effect on the time to death was observed consistently (Heagler *et al.*, 1993), the other locus effects were not observed in further experiments and were assumed to have been artefacts arising from an undefined population structure (Lee *et al.*, 1992). However, the GPI-2 effect was obscured in exposures above approximately $1\ \mu g\,l^{-1}$ of mercury (Heagler *et al.*, 1993). The effect of the GPI-2 genotype on the time to death was not toxicant specific because it was also noted during acute exposure to arsenate (Newman *et al.*, 1989). The non-specific nature of the GPI-2 effect is supported by the work of Mulvey *et al.* (1995a) which demonstrated that the mosquitofish GPI-2 genotype also influences life history traits under different temperature regimes. In all of the toxicity studies, the phenotype (time to death) is also influenced by the fish sex and size.

Shifts in glycolysis and Krebs cycle metabolites (Kramer *et al.*, 1992a,b) indicated that, during mercury exposure, mosquitofish with the sensitive GPI-2 genotype (GPI-$2^{38/38}$) have enhanced glycolytic activity in contrast to that of the other GPI-2 genotypes. Subsequent *in vitro* studies failed to support the hypothesis that direct mercury inhibition of the allozyme was responsible for the higher sensitivity of this genotype (Kramer and Newman, 1994). These results were consistent with Watt's (1977, 1983; Watt *et al.*, 1983, 1985; Carter and Watt, 1988) work in which GPI-2 was a coupling enzyme critical in regulating metabolic reactions. The various allozymes exhibited distinct kinetic qualities that imparted differential fitnesses to specific genotypes. Speculating from this work, the GPI-2 effect on mosquitofish survival was likely a product of the lowered metabolic fitness of the GPI-$2^{38/38}$ genotype during acute stress. However, the possibility of a closely linked gene causing the GPI-2 effect cannot be rejected.

Low-level exposures ($18\ \mu g\,l^{-1}$ of mercury) in mesocosms revealed other significant selection components at the GPI-2 locus (Mulvey *et al.*, 1995b). The genotype affected the probability of a female mosquitofish being gravid (female sexual selection) and the number of late stage embryos carried by a female (fecundity selection). In contrast to the GPI-$2^{38/38}$ genotype survival disadvantage during acute exposure, the GPI-$2^{100/100}$ genotype had a lower reproductive fitness at sublethal concentrations. This is consistent with the assumption that metabolic allocation underlies the GPI-2 effect and reinforces the cautionary statement made above that speculation based on only one selection component (differential survival = viability selection) can be misleading.

It remains untested whether the GPI-2 effects quantified in the above studies could result in an observable shift in GPI-2 allele frequencies over many generations of toxicant exposure. Therefore, the quantified GPI-2 genotype effects (GPI-$2^{38/38}$ survival disadvantage and GPI-$2^{100/100}$ reproductive disadvantage) were used to explore this possibility with numerical simulations. Two different models were used. A parsimonious model described the effects of acute exposure to the toxicant on the population size and allele frequencies with simple equations. A second, more complex and individual-based model included competing selection components, population demographics combined with genetic drift and selection and a phenotype (time to death) that was a product of the genotype, sex and size.

With these parsimonious and complex models, the question of whether or not the reported GPI-2 effects would result in observable allozyme frequency shifts in the field was addressed. It was addressed with and without immigration that could potentially obscure trends by adding alleles back to the pool of alleles who's frequencies are changing in the exposed population. Although impossible to do with the simple model, the complex model addressed the questions of whether observable allozyme frequency shifts would occur in the presence of complicating phenotypic and demographic factors and in the presence of potentially balancing selection components (viability versus fecundity and female sexual selection).

Materials and methods

The parsimonious model

The software package Time-Zero[&] (Kirchner, 1987) simulated the temporal changes in allele frequencies during

acute exposure with simple difference equations. Selection due to the toxicant was modelled with the following equation (Crow and Kimura, 1970),

$$q_{t+1} = \frac{q_t^2 w_{AA} + q_t(1 - q_t)w_{Aa}}{q_t^2 w_{AA} + 2q_t(1 - q_t)w_{Aa} + (1 - q_t)^2 w_{aa}} \quad (1)$$

where q_{t+1} is the frequency of allele a at time $t + 1$, q_t is the frequency of allele a at time t and w_{AA}, w_{aa} and w_{Aa} are the relative fitnesses of the genotypes AA, aa and Aa with respect to toxicant exposure. Migration was included in this equation by adding the term $m(q_0 - q_t)$ (Li, 1955), where m is the fraction of the population replaced by immigrants each generation and q_0 is the initial allele frequency.

Even in the absence of selection, the toxicant can affect the allele frequencies by reducing the population size. To accommodate this possibility, the logistic equation for population dynamics (May, 1974) was modified to include a toxicant-induced mortality term to calculate the total population size.

$$N_{t+1} = N_t\left[1 + r\left(1 - \frac{N_t}{K}\right) - I\right] \quad (2)$$

where N_{t+1} is the population size at time $t + 1$, N_t is the population size at time t, r is the intrinsic rate of population growth, K is the carrying capacity of the unexposed population and I is the exposure duration-dependent fraction of the population killed by toxicant each generation.

Random fluctuations in allele frequencies are always present, but in an infinite (indefinitely large) population, the resultant net frequency change is zero. However, these changes can be significant (accelerated genetic drift) in a population whose size has been reduced by toxicant exposure. The change in the frequency of a neutral allele due to drift was estimated by Equation 3 (Li, 1955; Mettler and Gregg, 1969).

$$q_t = \frac{1 \pm \sqrt{1 - 4q_0(1 - q_0)\left(1 - \frac{1}{2N_e}\right)^t}}{2} \quad (3)$$

where q_t is the allele frequency at time t, q_0 is the initial allele frequency and N_e is the effective population size (approximated here by one half the total population size at any given time).

Here we assumed a decline in the allele frequency so a minus sign was used in the numerator. The decline in allele frequency was studied because the probability of loss of a neutral allele is determined by its initial frequency and the allele of most interest here (GPI-2^{38}) had a low frequency in the population simulated. It had a much higher probability of dropping to 0 (loss) than increasing to 1 (fixation). The decrease in allele frequency depends on the effective population size (N_e), the number of individuals contributing genes to the next generation. A drastic reduction in the effective population size can cause a genetic bottleneck or accelerated genetic drift resulting in the loss of a rare allele.

The complex, individual-based model

Although the above model is useful because of its simplicity and ease of computation, it is a highly idealized representation. The population is not treated as a collection of individual fish, but of genes stripped of external characteristics. The effects of selection and drift were treated separately, the effective population sizes were only approximately estimated and the populations were assumed to be non-overlapping. Although there were three alleles at the GPI-2 locus of the mosquitofish, two were pooled to implement the parsimonious two-allele treatment used.

The second model included more detail and the computations were accordingly more laborious. For the individual-based model, simulations were performed with STRESS, a FORTRAN program designed to model the effects of toxicant exposure on the demographic and genetic characteristics of a population. This application incorporated life history traits specific to mosquitofish (*G. holbrooki* Girard) but could be adapted for other species. The toxicant effect on the time to death depends on the fish size, sex and genotype, as well as the toxicant concentration and frequency and duration of exposure. The program includes three selection components: viability selection (differential survival), sexual selection (differential mating success) and fecundity selection (differential fecundity). In field populations, toxic exposure is often both acute and periodic, i.e. seasonal. Therefore, a periodic acute exposure was chosen to investigate how allele frequencies would change with and without selection over many generations. Copies of the program and the associated user's manual can be obtained from the senior author.

Natural mortality. Each fish was assigned a time to death due to natural causes under the assumption that a Weibull distribution described the variation in times to death among fish (Pinder *et al.*, 1978). The equation below (Kennedy and Gentle, 1980) generated random times to death with a Weibull distribution:

$$\mathrm{TTD}_{nat} = a\cdot[-\ln(1 - z)]^{1/b} \quad (4)$$

where Z is the random number drawn from a normal distribution and a and b are the user-specified scale and shape parameters, respectively. Separate scale and shape parameters were used for males and females because males tend to die earlier than females (Table 1). The natural time to death generated for each fish with this equation was then multipled by a factor to adjust for population density-

dependent effects on the individual's probability of dying (Table 1).

Exposure mortality. The cumulative mortality and median time to death due to acute toxicant exposure were estimated with a proportional hazard model (Dixon and Newman, 1991; Newman and Aplin, 1992; Newman, 1995), where the hazard (the probability that an individual will die at time interval (t)) is described by Equation 5.

$$h(X, t) = e^{(\mu + X \cdot \beta)} h_0(t) \quad (5)$$

where μ is the hazard model intercept, X is the matrix of covariates (e.g. sex, log weight and genotype), β is the vector of parameters that estimate the effect of X on the hazard (e.g. β_s, β_w, β_g) and $h_0(t)$ is the baseline hazard function. The equation describing the cumulative mortality (CM) depends on the error distribution assumed. For a Weibull distribution (Pinder *et al.*, 1978),

$$\mathrm{CM} = e^{-\alpha \cdot t^{Y}} \quad (6)$$

where Y is $1/\sigma$ with σ described in Equation 7, α is $e^{-(\mu + \mathrm{sex} \cdot \beta_s + \mathrm{weight} \cdot \beta_w + \mathrm{genotype} \cdot \beta_g)/\sigma}$ and t is the duration of exposure. The median time to death due to toxicant exposure is described by Equation 7.

$$\text{Median } \mathrm{TTD_{tox}} = e^{\mu + X \cdot \beta} e^{\sigma \cdot \varepsilon_{0.50}} \quad (7)$$

where σ is a scale factor and $\varepsilon_{0.5}$ is the 50th percentile of the assumed error function (e.g. −0.3665 for the Weibull distribution).

Breeding. The alleles at each locus were randomly chosen from the female and from a male assigned to that female. The assignment of males to females for mating could include sexual selection during the chronic, low-concentration exposures between pulses of acute exposures. The probability of mating was genotype specific in the case of female sexual selection. (See Mulvey *et al.* (1995b) for details.) Otherwise, mating was random.

A linear relationship was defined between the weight of a female fish and the maximum possible number of offspring in her brood (B_{max}) (Vondracek *et al.*, 1988; Mulvey *et al.*, 1995b).

$$B_{max} = B_{slope} \cdot W + B_0 \quad (8)$$

where W is the female's wet weight and B_{slope} and B_0 are the user-supplied slope and intercept.

If fecundity selection was included, a separate slope and intercept for Equation 8 were supplied to each female genotype. Brood sizes (B) were also adjusted for population density.

$$B = B_{max}\left(1 - \frac{P_{bnm}}{K}\right) \quad (9)$$

Table 1. Selected parameters used in computer simulations

Variable	Equation	Description	Value(s) used	Reference
N_0	2	Initial population	1000	–
B_{slope} and B_0	8	Slope and intercept of weight–brood size Relationship		Mulvey *et al.* (1995b)
β_g	5	Genotype β	See Table 3	Reanalysed data from Diamond *et al.* (1989)
β_s	5	Sex β	0.38060	Reanalysed data from Diamond *et al.* (1989)
β_w	5	Size β	1.29707	Reanalysed data from Diamond *et al.* (1989)
μ	5	Hazard model intercept	5.71908	Reanalysed data from Diamond *et al.* (1989)
σ	5	Hazard model scale parameter	0.50822	Reanalysed data from Diamond *et al.* (1989)
W	–	Mean wet weight (mg)	Males 30.0 ± 100.0; Females 160.0 ± 1000.0	Mulvey *et al.* (1995b)
G	10	Growth rate (mg day^{-1})	Males 0.060 ± 0.0250; Females 0.060 ± 0.0250	Mulvey *et al.* (1995b)
r	2	Intrinsic rate of growth of population	0.2	R. Chesser (verbal communication) estimated from May, 1974, similar to result from Scribner (1993)
q_0	1 and 3	Initial 38 allele frequency	0.1425	Smith *et al.* (1983), Diamond *et al.* (1989), Newman *et al.* (1989)
$w_{38/38}$, $w_{38/X}$ and $w_{X/X}$	1	Fitness parameters for genotypes 38/38, 38/X and X/X	0.42, 0.90 and 1.00	–
K	2	Carrying capacity of population	3000	–

where P_{bnm} is the population size before natural mortality and K is the population carrying capacity.

The total number of neonates born each gestation period was the sum of the brood sizes (B) over all adult females.

Fish growth. The growth of individual fish was described with the logistic equation (May, 1974)

$$W_{t+1} = W_t\left[1 + G\left(1 - \frac{W_t}{W_{max}}\right)\right] \quad (10)$$

where W_t is the individual's weight at the current time step, W_{t+1} is the weight at the next time step, W_{max} is the maximum weight (different for males and females) and G is the randomly assigned growth rate. The growth rates were normally distributed among individuals with separate means and standard deviations for males and females. The time steps were gestation periods of approximately 28 days (Krumholz, 1948) rather than generations because generations overlap in mosquitofish populations.

Density- and size-dependent mortality. A linear relationship was assumed between a fish's size (W) and the probability that it would die due to size-dependent mortality, e.g. predation including cannibalism. For $W > W_{min}$

$$C = C_{max} - C_{slope} \cdot W \quad (11)$$

where C is the adjusted probability of death, C_{max} is the maximum probability of death, C_{slope} is the slope and W_{min} is the minimum fish weight to be adjusted. For $W \leq W_{min}$, C is equal to C_{max}. The C is then multiplied by the factor P/K, the population size divided by the carrying capacity, to account for the effect of population density on the size-dependent mortality. This is analogous to assuming a type 1 functional response and approximates a type II functional response of a predator as described by Holling (1959).

Migration. The computer model allows for the addition of a specified number of migrating fish to the population each gestation period. The immigrants are assigned genetic and demographic qualities reflecting those of the mosquitofish in the initial population.

Model implementation

General. The initial population used was 1000 fish and the carrying capacity was 3000 individuals for both models. Each simulation was run for 150 time steps representing generations (simple model) or gestation periods of 28 days each (complex model). The values of the specific parameters used in the simulations are listed in Table 1. Fish mortality data from a previous study (Diamond *et al.*, 1989) were fitted to a proportional hazards model assuming a log transformation for the effect of fish weight on the time to death (Newman, 1995). These data were generated during exposure to concentrations of 1.0 mg l^{-1} of mercury (as Hg^{2+}). The SAS LIFEREG procedure (Version 6.03) (SAS, 1987) was used to estimate the β, σ and μ values (see Table 2).

The parsimonious model. The genotype β estimates were used to derive the relative fitness parameters with the following method (Newman, 1995). The relative hazard or risk of death to a fish of a particular genotype due to acute toxicant exposure was $e^{-(\beta_g/\sigma)}$ (Dixon and Newman, 1991). The relative fitness of each genotype is the ratio of lowest relative risk to the risk of the genotype in question (Newman, 1995). Consequently, the fitness of the lowest risk genotype equals 1 and the fitness of each of the other genotypes was some value less than 1. There were three alleles at the GPI-2 locus of mosquitofish, denoted as GPI-2^{38}, GPI-2^{66} and GPI-2^{100}, giving six possible genotypes. For the purpose of simplification, the GPI-2^{66} and GPI-2^{100} alleles were pooled and referred to as the X allele (see Table 3). The resulting fitnesses were 0.46, 0.90 and 1.00 for the GPI-$2^{38/38}$, GPI-$2^{38/X}$ and GPI-$2^{X/X}$ genotypes, respectively.

From Equation 2, toxicant exposure alters the effective carrying capacity (N_{eq}) of the population to the following:

$$N_{eq} = K\left(1 - \frac{I}{r}\right) \quad (12)$$

Table 2. Results of proportional hazards analysis

Locus	Genotype	Genotype frequency[a]	Genotype β	Hazard model intercept	Hazard model scale parameter
GPI-2	100/100	0.2545	0.35713		
	100/60	0.3357	0.45437		
	100/38	0.1405	0.35505		
	66/66	0.1619	0.37370		
	66/38	0.0786	0.32353		
	38/38	0.0333	0.00000		
				5.71908	0.50822

[a]Based on the population of mosquitofish in Risher Pond at the US Department of Energy Savannah River Site in Aiken, SC. (Smith *et al.*, 1983; Diamond *et al.*, 1989; Newman *et al.*, 1989).

Table 3. Calculation of relative risks to different genotypes using a two-allele rendering (used in the simple model)

Genotype	New genotype β	Relative risk $= e^{-(\beta_g/\sigma)}$	Relative fitness
38/38	0.0	1.00	0.46/1 = 0.46
38/X	(0.35505 + 0.32353)/2 = 0.3393	0.51	0.46/0.51 = 0.90
X/X	(0.35713 + 0.45437 + 0.37370)/3 = 0.3951	0.46	0.46/0.46 = 1

where I is the fraction killed by the toxicant each generation. The proportion dead due to the toxicant is

$$M = 1 - \frac{N_{eq}}{N_0} \tag{13}$$

Equation 7 was modified to estimate the exposure time leading to this extent of mortality.

$$TTD_M = e^{(\mu + X \cdot \beta)} e^{\sigma \cdot W_M} \tag{14}$$

where W_M is the Mth percentile of the assumed error distribution.

The simulations included either viability selection (differential survival) (Equation 1) or mortality-driven, genetic drift (Equations 2 and 3) only. The simulations including drift were run for $I = 0.0$, 0.1350, 0.1400, 0.1500, 0.1600, 0.1667, 0.1750 and 0.1825. Assuming a Weibull distribution in Equation 14, this corresponded to acute exposure durations of 0, 25, 50, 75, 100, 125, 150 and 175 h, respectively, every 28 days to 1 mg l^{-1} of Hg^{2+}. Simulations with viability selection were also run with the number of immigrants being added each gestation period equivalent to 0, 1, 3, 10 and 30% of the population size.

The complex, individual-based model. Six or more simulations were run for acute exposure durations of 0, 25, 50, 75, 100, 125, 150 and 175 h per gestation period with or without selection. The mean frequency of the 38 allele and the median total number of alleles at eight loci were calculated after 150 gestation periods. Simulations were also run with both viability selection and migration with the exposure duration held at 50 h. Six simulations each were run with 0, 30, 90 and 300 immigrants joining the population each gestation period. In addition, six simulations incorporating viability (acute, pulsed exposure) and sexual and fecundity selection (low-concentration, chronic exposures between the acute, pulsed exposures) were also run at each of the above exposure durations.

Results

The parsimonious model

Selection had a substantial effect on the GPI-2^{38} allele frequency but genetic drift acceleration by mercury-related mortality was more subtle (Fig. 1). The results of the genetic drift simulations are shown with exposure durations ranging from 0 h (curve at the top of the cluster) to 175 h (curve at the bottom of the cluster). Selection eliminated

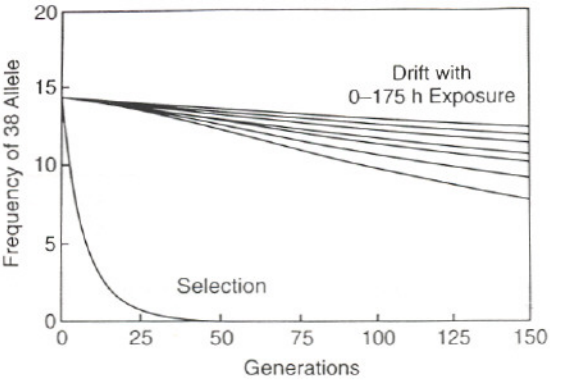

Fig. 1. The simple model. The change in frequency of the GPI-2^{38} allele due to selection (bottom curve) and mortality-driven drift (top cluster of curves) over 150 time steps.

the GPI-2^{38} allele from the population in approximately 50 generations, while mortality-driven drift reduced the frequency of the GPI-2^{38} allele by less than half over the course of 150 generations. Instead, the GPI-2^{38} allele frequency decreased slowly and the slope changed slightly with increasing exposure duration. A comparison of the GPI-2^{38} allele frequencies under the influence of selection with and without migration is shown in Fig. 2. With a small number of immigrants (1% of the original population) entering the population each generation, the GPI-2^{38} allele frequency decreased rapidly until it reached an equilibrium level. Increasing the rate of immigration resulted in a higher equilibrium frequency for the GPI-2^{38} allele, although an initial drop in frequency was noticeable even with high migration intensities. The GPI-2^{38} allele was never completely eliminated whenever migration was present.

The complex, individual-based model

Note that, while the viability selection curve in Fig. 3 resembles the one in Fig. 1, different variables are plotted on the horizontal axes in the two graphs. Figure 1 shows the allele frequency changes over time. In the simple model, the exposure time cannot be changed so there is only one curve for selection. The complex model produced a curve analogous to Fig. 1 for each exposure duration. Figure 3 plots the final allele frequency (after 150 gestation periods) from each of these curves against the exposure duration. When this more inclusive and realistic model was used to compare the change in frequency of the GPI-2^{38} allele in

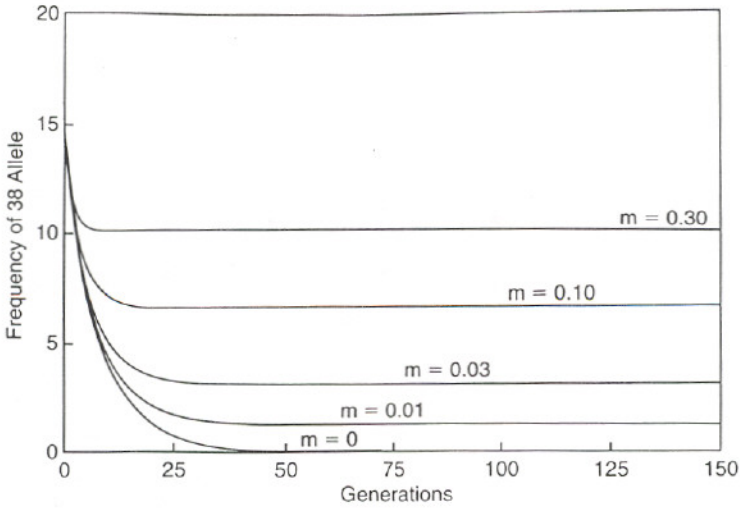

Fig. 2. The simple model. The effect of migration when selection is in effect, where m is the fraction of the population size augmented by immigrants each gestation period.

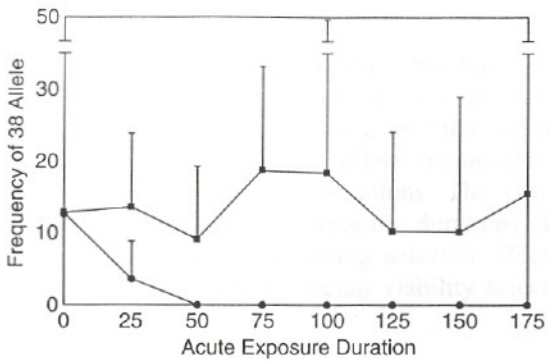

Fig. 3. The individual-based model. The frequency of the GPI-2^{38} allele after 150 gestation periods as a function of exposure duration (note that the x-axis is different to Figs 1 and 2). The lower curve (●) shows the effect of selection. The top curve (■) shows the dynamics without selection. Error bars indicate standard errors.

the presence and absence of selection, the results generally agreed with those of the simple model. The simulations with selection were each repeated six times and the results averaged. In these simulations, selection had a clear effect on the 38 allele frequency (Fig. 3, lower curve) despite the confounding effects of population demographics on the phenotype (time to death). The GPI-2^{38} allele decreased rapidly, more so with increasing exposure duration. The final allele frequency was zero for exposure durations of 50 h and longer. The final allele frequency was independent of the exposure duration in the simulations without selection (upper curve). The allele frequencies were extremely variable, in particular at long exposure durations. For this reason, 12 simulations were used to estimate the mean final GPI-2^{38} allele frequency in the absence of selection. On average, the GPI-2^{38} allele frequency remained constant through time for all exposure durations.

A set of curves for 50 h of exposure with selection and varying numbers of immigrants per gestation period are shown in Fig. 4. The population size fluctuated over time, ranging from approximately 2000 to approximately 10 000 fish. Compared to the simple model, the proportion of the population immigrating was lower here. As before, the allele frequency decreased even in the presence of high migration intensities, but never reached zero as long as there was some immigration. The combined effects of viability selection with sexual and fecundity selection on the final frequencies of the GPI-2^{38}, GPI-2^{66} and GPI-2^{100} alleles are shown in Fig. 5. The addition of sexual and fecundity selection did not alter the response observed with viability selection alone. At exposure durations of 50 h or longer, the GPI-2^{38} allele was essentially eliminated by the

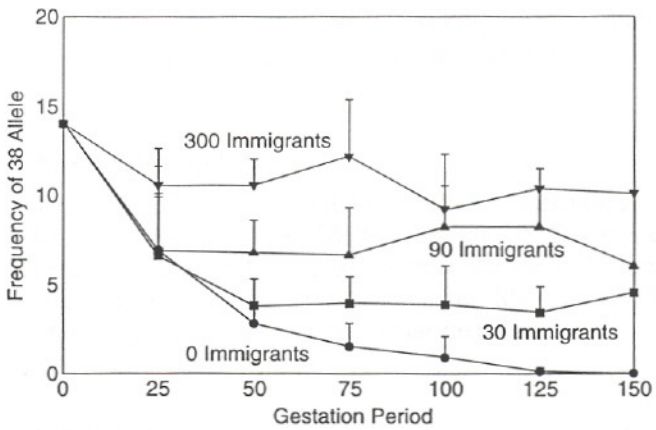

Fig. 4. The individual-based model. The effect of migration on selection over the course of 150 gestation periods. The exposure duration is 50 h per gestation period. The four curves reflect 300 (top curve), 90, 30 and 0 (bottom curve) immigrants per gestation period.

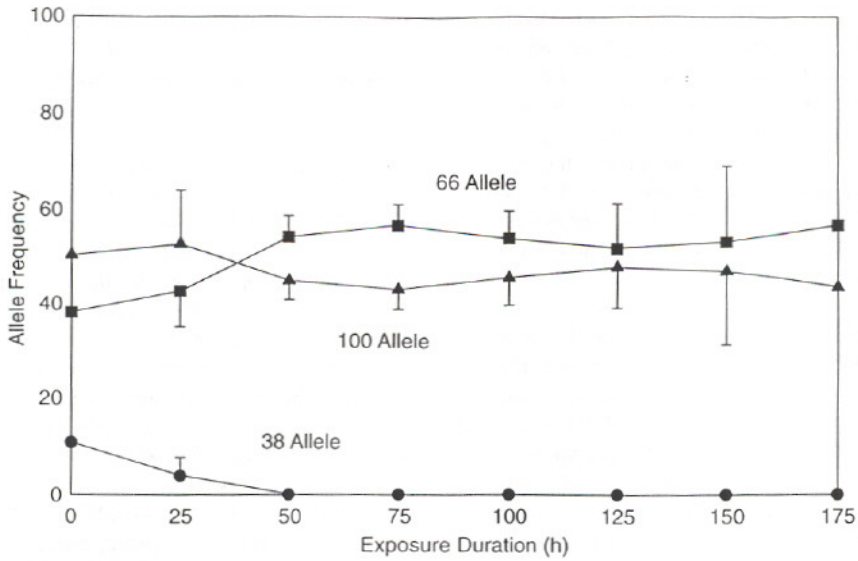

Fig. 5. The individual-based model. The effects of sexual and fecundity selection on the final frequencies of the GPI-2^{38} and GPI-2^{100} alleles (after 100 gestation periods).

fiftieth gestation period. In contrast, the final frequency of the GPI-2^{100} allele decreased slightly as the exposure duration increased from 1 to 50 h and that of the GPI-2^{66} allele increased slightly. The allele frequencies remained constant at higher exposure durations. The GPI-2^{38} allele frequency dropped at all exposure durations despite the presence of potentially balancing selection (fecundity and female sexual selection balancing viability selection).

Discussion

Simulations using our simple model produced results in which the effect of viability selection was much larger than that of mortality-driven, genetic drift. The effect was still clearly evident after the addition of the complex genotypic, phenotypic and demographic detail contained in the second, more realistic model. In the presence of viability selection, even periodic short pulses of toxic exposure had a great influence. With longer exposure durations, the sensitive allele decreased more rapidly. The frequencies of all three alleles at the GPI-2 locus were affected. The addition of other potentially balancing selection components (female sexual and fecundity selection) did not alter this relationship. In fact, the results indicate that female sexual and fecundity selection did not exert a great enough influence on the outcome of viability selection (differential survival).

Both models produced results in which migration mitigated the effect of viability selection. Even a small number of immigrants was enough to prevent the loss of the rare allele. The more immigrants there were, the less the effect of viability selection. However, even in the case of high migration intensities, a clear effect was present.

The results suggest that the overall reduction of the GPI-2^{38} allele and loss of the GPI-$2^{38/38}$ genotype which has been observed in mercury-exposed laboratory and field populations of mosquitofish may be explained by viability selection rather than random fluctuations. Provided the system under study is clearly understood, shifts in allele frequency at the GPI-2 locus can be used to indicate the population-level effects of mercury. There may be a temptation to extrapolate results like these without thoughtful modification to specific situations in the field or laboratory. We reiterate that the results reported here apply to a limited context: a 1 $\mu g\,l^{-1}$ of mercury exposure for 0–175 h every gestation period (28 days). While this model has only been applied to the effects of mercury on mosquitofish populations, it is applicable to other populations, species, toxicants and exposure scenarios. Future research may reveal other sensitive alleles that may indicate a population response to the presence of toxicants.

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