Genetic differences in thermal tolerance of eastern mosquitofish (Gambusia holbrooki; Poeciliidae) from ambient and thermal ponds

Gary K. Meffe, Stephen C. Weeks, Margaret Mulvey, and Karen L. Kandl

Abstract: Two populations of eastern mosquitofish (Gambusia holbrooki; Poeciliidae) in South Carolina, one in an ambient temperature pond and the other in a pond heated to near-lethal temperatures by nuclear reactor effluents for 60–90 mosquitofish generations, offered an excellent opportunity to observe selection for increased thermal tolerance. We performed three experiments. First, we determined the critical thermal maximum of each population and, as predicted, found the thermal population to have a higher one. We then exposed fish from both populations to an acute thermal LD$_{50}$ stress and compared genetic diversity of fish that died and fish that survived. Survivors had higher heterozygosities, indicating that genetic diversity may contribute to thermal tolerance. Finally, we used a half-sib – full-sib experimental design to estimate heritabilities for temperature tolerance in fish from the heated pond. We calculated a narrow-sense heritability for temperature at death of over 32%, indicating that selection has not depleted the population of genetic variation associated with thermal tolerance. Our results have implications for climate change because adaptations to higher thermal regimes must, in part, come from selection on genetic variation for temperature tolerance within populations.

Introduction

Williams (1966) warned that “evolutionary adaptation is a special and onerous concept, that should not be used unnecessarily, and an effect should not be called a function unless it is clearly produced by design and not by chance.” That is, we need to be cautious in calling something an adaptation unless we can clearly demonstrate that it was produced by selection, rather than a result of chance events. One of the best opportunities to examine presumptive adaptations, and avoid this pitfall, is in extreme environments, where survival or reproduction is obviously dependent upon appropriate responses to exceptional environmental challenges, and an adaptive response is more evident (e.g., Antonovics 1971). A good opportunity to pursue such an approach is in thermally altered environments.
where natural populations have been exposed for many generations to unusual temperatures, and yet have survived or even prospered under conditions that might be lethal to unexposed populations. We exploited such an opportunity here to test hypotheses regarding adaptation through thermal tolerance in a common fish species.

We examined thermal tolerance and genetic variation in populations of the eastern mosquitofish (Gambusia holbrooki; Poeciliidae) exposed to different thermal environments. Specifically, we used critical thermal maxima (CTM) of two populations that differed in their thermal histories to examine possible adaptation to thermal extremes. One population was exposed to high, semilethal temperatures for 30 years (60–90 generations), while the other existed in an unheated pond. First, we compared CTMs of fish from these two populations to determine whether there was increased tolerance to high temperature in the thermally stressed population. We predicted that selection for higher temperature tolerance would result in higher CTMs of the thermally stressed population if genetic variation for such a response exists. Second, because multiple-locus genetic heterozygosity has been shown to be important in surviving environmental challenges such as thermal stress (Mitton and Koehn 1975; Feder et al. 1984), we predicted that individuals with higher heterozygosity should better survive high temperatures. We tested this by exposing fish from each population to an acute heat shock designed to kill about half of the individuals, and examining whether survivors had a significantly higher level of heterozygosity. Finally, we used a quantitative genetics design to determine whether heritable variation still exists for thermal tolerance in the heat-stressed population. If so, this approach could be used to gauge the effects of long-term selection on thermal tolerance and to estimate potential genetic responses to increased temperature.

The three experiments thus addressed the following specific questions relative to responses of mosquitofish to thermal stress: (i) is there a difference in thermal tolerance between isolated populations with different thermal histories? (ii) does individual genetic diversity, in the form of heterozygosity, correspond to greater thermal tolerance? and (iii) is variation in thermal tolerance heritable, and does the thermally selected population retain genetic variation upon which further natural selection may act? Results of these experiments have implications for responses of biota to global warming because such responses require a genetic basis upon which selection may act. Thus, we will relate our findings to possible global warming effects.

Materials and methods

Study organism, field sites, and thermal histories

The eastern mosquitofish, a member of the live-bearing family Poeciliidae, has a natural distribution along the Atlantic slope from southern New Jersey through Florida, west to the Mobile basin, and north through the Mississippi Valley to Illinois. Females grow to a maximum of about 60 mm, while males reach about 40 mm. Fertilization is internal, and embryos develop to parturition within a female’s single, fused ovary in 3–5 weeks, depending on temperature (Constandz 1989). Clutch sizes depend on female size and may range from just a few to well over 75. Mosquitofish are hardy and abundant fish, and seem to adapt well to rigorous environments (Courtenay and Meffe 1989).

Pond C is a 67-ha, man-made reservoir on the U.S. Department of Energy’s Savannah River Site near Aiken, South Carolina. The system was used as a precooling reservoir for the larger Par Pond, and intermittently received thermal effluent from a nuclear production reactor from 1958 to 1988. During periods of effluent release, ranging from several hours to several months, temperatures throughout most of the pond exceeded 40°C, which is lethal to all species of fish present, and in many places exceeded 50°C. However, several refugia existed near springs or feeder streams where temperatures remained low enough to support mosquitofish, but still approached lethal levels. For example, mosquitofish could typically be seen along the edges of the pond near an inlet stream in a narrow band of 35–39°C water. When disturbed, they fled into deeper and hotter water and would succumb to the higher temperatures of 40–42°C (G.K. Meffe, personal observation). Mortality associated with thermal events was often significant (Parker et al. 1973), although fish populations persisted in refuge areas. Fish surviving in refuges might be a random subset of those in Pond C, or might be individuals with higher thermal tolerance. If individual differences in thermal tolerance have a heritable genetic basis, then we would expect selection for thermal tolerance in mosquitofish to have occurred during the 30 years of reactor operation.

Risher Pond, a 1.1-ha ambient farm pond with no history of supplemental heating, is 15 km from Pond C. Fish in Risher Pond are expected to be naive with respect to high temperatures. Mosquitofish life histories have been studied in these two systems and many differences exist in reproductive, demographic, and energetic characteristics (Meffe 1990, 1991, 1992; Meffe and Snelson 1993a, 1993b).

Experiment 1: population differences in CTM

This experiment was designed to test the null hypothesis of no difference in CTM between a population exposed for many generations to periodic bouts of semilethal temperatures, and a population in ambient waters.

Mosquitofish from Pond C and Risher Pond were seeded, returned to the laboratory in insulated coolers, and placed into separate plastic jugs (3.7 L) in 100-L aquaria (24 jugs/aquarium) with under-gravel filters. Jugs had 4 × 8 cm mesh-covered holes in each of the four walls to allow free water exchange within the aquarium. Two 50-W heaters were placed in each aquarium to maintain the temperature at 25 ± 1°C. Fish were allowed to acclimate for 7–10 days before testing, during which time they were fed ad libitum quantities of frozen brine shrimp and flake food daily.

To determine CTMs, fish were individually placed into 500-mL beakers in a heating water bath (model 270, Precision Scientific Inc., Chicago, Ill.), starting at 25°C and raised at a rate of 1°C every 2.5 min. Temperature was measured with a multichannel thermometer (YSI Thermometer, model 44TF, Yellow Springs, Ohio), and each bath was supplied with an air stone that kept water...
oxygenated. CTM was measured at two end points: temperature at first loss of orientation, determined as the point at which the fish turned on its side (90° or greater) and immediately righted itself, and at loss of righting response, determined as the point at which the fish did not right itself after being nudged twice with a blunt probe. Fish were then fixed in small vials of 5% formalin, and size (standard length (SL) in millimetres) was later measured. Thirty-nine fish from Pond C and 41 fish from Risher Pond were tested.

Orientation loss was analyzed using a two-way ANOVA or ANCOVA, with pond site and sex as the two independent variables, and SL as the covariate where appropriate. All analyses were conducted using PROC GLM (SAS Institute Inc. 1985).

**Experiment 2: alzyme genotype and acute heat stress**

This experiment tested the null hypothesis of no difference in mean heterozygosity between fish that survived an acute thermal stress and those that died. Mosquitofish of both sexes and all size-classes (n > 700) were collected from Pond C and Risher Pond, returned to the laboratory in insulated coolers, and established in large, fiberglass tanks of over 1000 L inside a greenhouse. Fish were held at ambient greenhouse temperatures (23–25°C) for at least 10 days to allow acclimation to captivity and to cull initial mortalities. All fish were fed ad libitum quantities of frozen brine shrimp and flake food. Preliminary tests with subsamples of fish established the temperatures at which approximately 50% of the fish would die in 30 min (i.e., a thermal LD50); this temperature was 37–38°C for Risher Pond fish and 39–40°C for Pond C fish.

To test heterozygosities of fish that survived and died during an acute thermal stress, approximately 50 individuals at a time per population were immersed in a water bath for 30 min at 37.5 ± 1°C for Risher Pond fish and 39.5 ± 1°C for Pond C fish. Baths were provided with a supplemental oxygen supply so that oxygen stress was not a factor. After 30 min, containers were returned to ambient temperature over a period of about 1 min; after 5 min to allow recovery, fish were separated into dead (no detectable movement) and surviving (some movement) groups. Fish were immediately frozen at −70°C for later electrophoretic analysis.

Because individual size or reproductive state could affect survival, all fish were measured (SL), and clutch sizes of females were determined by removing the ovary and counting all fertilized ova and developing embryos. Fish were then dissected for electrophoresis. Somatic tissues were ground in approximately 0.2 mL of cold grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP buffer, pH 7.0). Homogenate fluid was absorbed onto paper wicks, which were blotted to remove excess fluid and inserted into 12.5% horizontal starch gels. Buffer and stain combinations were (i) isocitrate dehydrogenase (IDHP 1,2) with Tris–citrate–EDTA (Ayala et al. 1972); (ii) malate dehydrogenase (MDH 1), mannose-phosphate isomerase (MPI), and glucose phosphate isomerase (GPI 2) with Tris–citrate, pH 8.0 (Selander et al. 1971); and (iii) fumarate hydratase (FH), leucylglycylglycine peptidase (PEPB), and phenylalanine proline peptidase (PEPD) with Tris–EDTA–borate, pH 8.0 (Selander et al. 1971). Staining procedures followed those in Selander et al. (1971) and Richardson et al. (1986). Alzyme mobilities were determined relative to the most common alzyme for each locus, which was designated 100. Enzymes were numbered in order of decreasing anodal mobility in multilocus systems.

Mean percent heterozygosities were compared among outcome groups (living and dead individuals) from both populations using two-way ANOVA, after determination by probability plots that heterozygosities were normally distributed. Fisher’s least significant difference multiple comparison test was used to examine differences among groups.

A linear regression analysis was used to determine if there was a relationship between level of heterozygosity and survivorship. Where sample sizes for a given heterozygosity class were small, adjacent classes were combined and averaged. The relationships of fish size and clutch size of pregnant females to survivorship were analyzed with ANOVA.

**Experiment 3: heritable variation in thermal tolerance**

This experiment tested the null hypothesis of no measurable heritable genetic variation for thermal sensitivity, as measured by CTM, in Pond C mosquitofish.

The following half-sib – full-sib experimental design with Pond C females was used in this experiment. Thirty pregnant G. holbrooki (P generation) were collected from Pond C and transported to the laboratory, where they were placed in individual 3.7-L plastic jugs, contained within 220-L aquaria with under-gravel filters. Several offspring (F1) from each P female (4–10 offspring per P female) were reared to maturity in 18.5-L aquaria, one aquarium per F1 family. A single female was chosen from each of the 30 F1 families, and each was isolated in the 3.7-L plastic jugs. When mature, these 30 F1 females (dams) were mated with 10 wild-caught males (sires) in the design shown in Fig. 1; each male was mated with 3 females. A single brood of offspring (F2) from each dam was reared separately in family groups (30 F2 groups) in 18.5-L aquaria until mature. Nearing the age of maturity (at approximately 4 weeks), the 30 family groups were monitored daily to remove any developing males, thereby eliminating the possibility of brother–sister matings within family groups. When the F2 females matured, they were individually placed in 3-L plastic jugs. From 2 to 12 females per family (depending on availability) were placed into separate blocks (the 220-L aquaria described above), 1 F2 female per jug per block. The females were mated with wild males and allowed to have offspring for several months prior to the thermal stress experiment. Mating of females produced fish that better represented the condition of wild females, and the offspring collected were used in a separate experiment (S.C. Weeks and G.K. Meffe, unpublished data).

Mosquitofish CTMs were randomly measured within each family, and CTM of at least one member of each half-sib family was measured on each day of testing. Only
Table 1. ANOVA results for temperatures at which orientation loss and loss of righting response were observed for *G. holbrooki* from Pond C and Risher Pond.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orientation loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pond</td>
<td>1</td>
<td>2.101</td>
<td>3.805</td>
<td>0.055</td>
</tr>
<tr>
<td>Error</td>
<td>78</td>
<td>43.056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of righting response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard length</td>
<td>1</td>
<td>3.322</td>
<td>5.248</td>
<td>0.015</td>
</tr>
<tr>
<td>Pond</td>
<td>1</td>
<td>4.275</td>
<td>8.017</td>
<td>0.006</td>
</tr>
<tr>
<td>Error</td>
<td>73</td>
<td>38.909</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

29 of the 30 full-sib families were measured, because 1 full-sib family was all males. Fish were removed from their jugs and placed in individual 500-mL glass beakers containing 225 mL of water at 25°C. Five to seven beakers were then placed in the water bath at 25°C, each with an air stone to maintain oxygen level throughout the measurement period. The temperature of the water bath was then raised to 30°C and the beakers were allowed to equilibrate for 20 min, after which the temperature was raised 1°C every 2 min. Two measurements were taken for each fish during this time (with the multichannel thermometer described above): temperature at orientation loss and temperature at death. Definition of orientation loss was when the fish first lost its bearing, either tipping to one side or “falling forward,” and then righting itself. Temperature at death was defined by the total lack of fin, gill, or mouth movement when prodded with a probe. After death, SL was recorded and fish were preserved in 5% formalin. Three or four separate runs were performed each day for 10 days.

Possible differences in CTM among half- and full-sib families were determined using a nested ANCOVA with two levels: sires, and dams nested within sires. Both sires and dams were considered random effects, and thus the mean squares for sires were compared against the mean squares for dams within sires in tests of significance. Partitioning of the variance components was done with the restricted maximum likelihood procedure in PROC VARCOMP (SAS Institute Inc. 1985), which constrained all components to nonnegative values. Because VARCOMP does not allow covariates, residuals were constructed by regressing the two dependent variables on their respective covariates (brood size or SL), and these residuals were then used in the VARCOMP procedure.

Results

Experiment 1: population differences in CTM
There were no significant differences between sexes in CTM, so sexes were combined in analyses. SL was not significantly correlated with orientation loss, and was not used as a covariate, but it was a significant covariate for loss of righting response, and therefore a two-way ANCOVA was used for this dependent variable. The relationship between loss of righting response and SL was homogeneous across sites and sexes, as assumed by the ANCOVA procedure. A residual analysis showed the data to be distributed normally and showed no correlation of variance with mean.

The temperature at which mosquitofish lost orientation was higher for Pond C than for Risher Pond fish (Table 1; Fig. 2; *p* = 0.055). Pond C fish retained their righting response to a higher temperature than did Risher Pond fish (Table 1; Fig. 2; *p* = 0.006); this relationship was also dependent upon size of fish (significant covariate SL), with smaller fish succumbing first, at lower temperatures.

Experiment 2: allozyme genotype and acute heat stress
We used 546 fish from Pond C and Risher Pond in the acute thermal stress experiment, of which 59% died as a result of our treatment and 41% survived. Not all seven allozyme loci were resolved in every individual; thus, we calculated percent heterozygosities only for those individuals
in which five or more allozymes were resolved, 229 from Pond C and 280 from Risher Pond.

Mean heterozygosity level was significantly different between populations (higher in Pond C), and there was a marginally significant interaction between population and outcome (Table 2; \( p = 0.084 \)). The higher heterozygosity level in Pond C is not biologically significant to this experiment. Populations of mosquitofish on the Savannah River Site show great diversity in heterozygosity levels (Smith et al. 1989), and there is no particular biological meaning attached to the differences seen here. However, mean heterozygosity level was marginally higher in surviving than in dead fish \( (p = 0.082; \text{Table 2}) \), a biologically meaningful result that was due entirely to Pond C, the only group to have a significant difference in heterozygosity level between outcome groups (mean percent heterozygosity was 32.2 in dead and 37.5 in surviving fish). Fish size did not affect outcomes \( (p = 0.944 \text{ in ANOVA with } 1 \text{ and } 475 \text{ df}) \), nor did clutch size \( (p = 0.657 \text{ in ANOVA with } 1 \text{ and } 261 \text{ df}) \), indicating that size (roughly equivalent to age) and reproductive state had no direct bearing on survival.

Regressions of percent survivorship as a function of percent heterozygosity indicated strong tendencies toward a positive relationship (Fig. 3). For both populations combined, over 37% of the variance in survivorship was attributable to variance in heterozygosities, and the probability level for rejecting the null hypothesis of no relationship was 0.081. For individual populations, \( r^2 \) values were 0.498 \( (p = 0.072) \) for Pond C and 0.187 \( (p = 0.284) \) for Risher Pond. In both cases, the trend was for higher survivorship in higher mean heterozygosity classes.

### Experiment 3: heritable variation in thermal tolerance

Using the half-sib – full-sib design, we distinguished two types of genetic variation: additive variation and nonadditive plus maternal variation. The additive portion of genetic variation was most relevant for determining the capability of natural selection to modify a trait such as CTM over time and was used for the calculation of narrow-sense heritabilities (see Falconer (1981) for further details).

Differences among half-sib families were primarily caused by differences in the expression of additive genes among families, whereas differences among full-sib families were caused by differences in the expression of both additive and nonadditive genes, as well as localized environmental effects (Falconer 1981).

Differences among blocks (large holding tanks) were not significant for either orientation loss or temperature at death, and therefore the term blocks was removed from the model. Female size and the number of broods of offspring within a female's life-span were both positively correlated with orientation loss, and thus used as covariates.

Differences among half-sib families (sire effect) were not significant for orientation loss (Table 3); however, differences among full-sib families (dam within sire effect) were significant (Table 3). These results were reflected in the breakdown of the variance components. Nearly 10% of the total variation in orientation loss was explained by differences among full-sib families \( (V_{dm}) \), whereas no variance was explained by differences among half-sib families \( (V_{hm}) \) (Table 3). Calculations of the causal components of variation for orientation loss showed no additive genetic variance \( (V_A) \) but a substantial portion of variance as a result of nonadditive and maternal sources of variation \( (V_{NA} + M) \).

In contrast with the data for orientation loss, temperature at death differed significantly among half-sib families, but not among full-sib families (Table 3). Almost 11% of the total variation in temperature at death was explained by differences among half-sibs, whereas no variation was explained by differences among full-sibs (Table 3). These results translated into a narrow-sense heritability of over 32% for temperature at death, indicating that natural selection had not depleted the selectable genetic variation for this trait. There was no evidence of nonadditive or maternal sources of variation for temperature at death.

### Discussion

Populations exposed to high temperature for many generations should be selected for increased thermal tolerance as a local adaptation. Comparisons of CTM between Pond C and Risher Pond mosquitofish support this prediction. Both

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**Table 2.** (A) Mean heterozygosities \((H)\) and sample sizes \((n)\) for groups defined by population of origin, and experimental outcome \((D, \text{ died}; S, \text{ survived}); \) (B) corresponding ANOVA results; and (C) pairwise comparisons using Fisher's least significant difference.

(A) Mean heterozygosities and sample sizes

<table>
<thead>
<tr>
<th>Population</th>
<th>Outcome</th>
<th>( H(%) )</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond C</td>
<td>D</td>
<td>0.322</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.375</td>
<td>90</td>
</tr>
<tr>
<td>Risher Pond</td>
<td>D</td>
<td>0.253</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>S</td>
<td>0.254</td>
<td>130</td>
</tr>
</tbody>
</table>

(B) ANOVA results

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Sum of squares</th>
<th>( F )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population</td>
<td>1</td>
<td>1.111</td>
<td>38.767</td>
<td>0.000</td>
</tr>
<tr>
<td>Outcome</td>
<td>1</td>
<td>0.087</td>
<td>3.034</td>
<td>0.082</td>
</tr>
<tr>
<td>Population (\times) Outcome</td>
<td>1</td>
<td>0.086</td>
<td>2.989</td>
<td>0.084</td>
</tr>
<tr>
<td>Error</td>
<td>505</td>
<td>14.477</td>
<td></td>
<td></td>
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</tbody>
</table>

(C) Pairwise comparisons using Fisher's least significant difference

<table>
<thead>
<tr>
<th></th>
<th>Pond C (D)</th>
<th>Pond C (S)</th>
<th>Risher (D)</th>
<th>Risher (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pond C (D)</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pond C (S)</td>
<td>0.021</td>
<td>1.000</td>
<td></td>
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</tr>
<tr>
<td>Risher (D)</td>
<td>0.001</td>
<td>0.000</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Risher (S)</td>
<td>0.001</td>
<td>0.000</td>
<td>0.992</td>
<td>1.000</td>
</tr>
</tbody>
</table>

**Note:** The only comparisons of direct biological relevance are between outcomes within a population (shown in boldface).
measures of CTM were higher in the thermally impacted population (Pond C), although this difference was only strongly significant for loss of righting response. Although we are only able to compare these two populations and do not have replicated systems, these results suggest that selection has favored mosquitofish with higher temperature tolerance in the thermal environment. Because thermal tolerance was significantly higher in Pond C mosquitofish, the next step was to test predictions concerning its genetic basis. Other studies have examined the relationship between allozyme genotype and response to stress in mosquitofish (Diamond et al. 1989). A number of studies indicate that higher levels of multilocus heterozygosity may confer increased fitness on individuals (Mitton and Grant 1984; Allendorf and Leary 1986; Mitton 1993). This observation led to our prediction that greater heterozygosity could impart greater resistance to thermal stress for individual mosquitofish. There was some evidence from both populations that multilocus heterozygosity conferred increased thermal tolerance. The relationship of percent survival to percent heterozygosity was positive in both populations, and the overall percentage of surviving mosquitofish was marginally significant across the heterozygosity groups. The relationship was much stronger in the Pond C population, however.

Because increased thermal tolerance appears to have been selected for in the Pond C population, one can ask whether this selection pressure has depleted the population of heritable genetic variation for thermal tolerance. Continued strong selection for a trait will eventually lead to a depletion of genetic variation for that trait (Falconer 1981). Our estimates of genetic variation in the thermally impacted population reveal that heritable variation was zero for orientation loss, but that there was a significant amount of heritable variation in temperature at death. Our estimate of this variation (32%) reveals a sufficient amount for future responses to selection (Falconer 1981). Therefore, it appears that the 60–90 generations of presumed selection for thermal tolerance have not completely depleted the Pond C population’s genetic variation for that trait, and that future selection for increased thermal tolerance would be possible in this population because additive genetic variation for the trait remains.

Two caveats qualify our estimates of heritable variation. First, the number of half-sib families (10) used in this study was small. Larger designs would have greater power to detect additive variation than the design used here. Therefore, our estimates of additive variation for the two traits measured are conservative. Second, because additive variation was measured in the laboratory, we cannot be certain that our estimates equal those that would be expressed in the field (e.g., see Trexler and Travis 1990; Trexler et al. 1990). However, laboratory estimates of genetic variation are our best estimates of heritable genetic variation and predict well the responses of populations to natural and artificial selection (Clayton et al. 1957).
reflected in significant changes in the postulated gradual rise in global temperatures would pre-
by heritable genetic variation for that trait.

Our results indicate that mosquitofish have the genetic variation necessary to adapt to local temperature increases over a long time. The Pond C population has adapted to an acute stress within three decades (60–90 generations), reflected in significant changes in the CTM accompanied by heritable genetic variation for that trait. A currently postulated gradual rise in global temperatures would presumably pose even less of a challenge to this species.

These results should be interpreted with caution relative to the potential effects of global warming because not all organisms will quickly adapt to a changing environment, whether in real or generational time. There are a number of factors that limit inference of our conclusions to other species. First, *G. holbrooki* is a highly adaptive species, able to adjust to a wide range of environments. Along with its close relative, western mosquitofish (*Gambusia affinis*), it is one of the most widespread freshwater fish in the world, caused by rampant stocking for its purported (but erroneous) ability to control mosquitoes (Courtenay and Meffe 1989). Mosquitofish now occur in a wide variety of habitats, ranging from rivers and streams to ponds, lakes, springs, canals, temporary ditches, and thermal effluents. Therefore, although our results suggest that such a tolerant species can adapt to a thermal environment, extrapolation to less tolerant species is not warranted. Second, the recent thermal history of Pond C is different than what is predicted by global warming models. Thermal impacts on Pond C were acute temperature fluctuations rather than the gradual temperature increase expected via greenhouse warming. Finally, we have concentrated our efforts on only one of many species in the thermal environment. Community characteristics of Pond C, as well as other thermal habitats on the Savannah River Site, have been drastically altered by high temperatures, and many species have been eliminated from these systems (Parker et al. 1973). Thus, the work reported here furthers our understanding of the effects of increased temperatures on one species, including its genetically based responses, but much more comprehensive analyses are needed to better predict potential biological effects of global warming.

**Acknowledgments**

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**References**


