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DIRECT AND CORRELATED RESPONSES TO ARTIFICIAL SELECTION ON ACUTE THERMAL STRESS TOLERANCE IN A LIVEBEARING FISH

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Abstract.—Tradeoffs in performance or fitness across environments have important implications regarding the nature of evolutionary constraints. It remains controversial whether tradeoffs such as these reflect genetic correlations that are genuine evolutionary constraints. However, if such long-term genetic constraints do exist, they must be due to underlying pleiotropy such that alleles that confer high performance in one environment invariably confer low performance in another. The distribution of genetic correlations within and among populations can provide insight about the existence of such pleiotropic tradeoffs.

The long-term association of certain teleost fish taxa with particular abiotic environments suggests that tradeoffs in performance across environments have constrained the geographic distribution of those taxa. Here we report the results of an experiment in which we artificially selected on acute heat- and cold-stress tolerance in two stocks of the poeciliid fish *Heterandria formosa* from source populations with different thermal histories. Unexpectedly, we observed no direct responses to selection. Under certain conditions, fish from the different source populations differed significantly in cold tolerance, but not in heat tolerance. The results suggest there are no strong pleiotropic tradeoffs between heat- and cold-stress tolerance in these populations.

Key words.—Correlated evolution, evolutionary constraint, *Heterandria formosa*, selection experiment.

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Although tradeoffs in performance or fitness across environments have important implications throughout evolutionary biology, they are central to hypotheses in biogeography and the determination of species ranges. For example, tradeoffs between heat and cold tolerance among closely related species from different latitudes or altitudes are well documented (Prosser 1986, ch. 7; Cossins and Bowler 1987, ch. 6) and similar tradeoffs have been observed among conspecific populations (Hoffman and Parsons 1991; Hoffman and Watson 1993). Such tradeoffs imply negative genetic correlations between performance and/or fitness in different abiotic environments and are the heart of the argument that species distributions are constrained by tradeoffs in the response to abiotic selective forces (Dunson and Travis 1991).

If and when evolutionary constraints will be honestly reflected by observed genetic correlations has been the subject of substantial research and debate (e.g., Rose and Charlesworth 1981; Rose 1982; Via and Lande 1985; Cheverud 1988; Gillespie and Turelli 1989; Charlesworth 1990; Houle 1991; Perrin and Travis 1992; Curtsinger et al. 1994; Schluter 1996). Indeed, tradeoffs such as those observed between heat and cold tolerance can have at least three genetic foundations, only one of which will generate long-term evolutionary constraints.

Consider two conspecific populations, one inhabiting a warm environment and the other a cool environment, that exhibit the type of tradeoff described above (individuals from the warm environment are more heat tolerant and less cold tolerant than individuals from the other environment). First, there can be general pleiotropic effects such that physiological properties (e.g., enzyme kinetics, membrane fluidity) cannot be simultaneously optimized in the two different environments (e.g., Hochachka and Somero 1984; Quinn 1989; Gillespie 1991; ch. 1) and the underlying loci have different alleles fixed in the two populations. The alleles that increase heat (cold) tolerance have pleiotropic effects that decrease cold (heat) tolerance. If this were true, selection for increased heat tolerance should lead to decreased cold tolerance and vice versa, and the results should be consistent in both populations. A correlated response might arise through random linkage disequilibrium in any particular case, but such a random response should not be consistent among populations.

Second, some subset of the loci that affect heat (cold) tolerance may not affect cold (heat) tolerance. The genetic composition of those loci will be constrained in the warm (cool) environment, but not in the cool (warm) one. The remaining loci that affect heat (cold) tolerance are unconstrained and will accumulate neutral alleles with a variety of effects on heat (cold) tolerance, most of which will have suboptimal effects in the constrained environment. The thermal tolerance properties of the two populations will diverge through mutation and drift. If this subset of loci is large, then selection for increased heat (cold) tolerance should have, on average, no correlated effect on cold (heat) tolerance, even though the phenotypic properties of the two populations would suggest otherwise.

Third, a subset of loci affect tolerance of only one factor, heat or cold, but alleles that confer high heat (cold) tolerance have deleterious pleiotropic effects that are unrelated to the tolerance of cold (heat). These effects could be exhibited in life-history traits, morphology, or responses to other abiotic factors. As in the second explanation, selection on tolerance to heat (cold) should produce no correlated effect on tolerance to cold (heat). Moreover, in both explanations, any additional correlated responses to selection may not be the same in the two populations.

Two similar mechanisms could also generate a *positive* relationship between heat and cold tolerance without pleiotropy (positive relationships are described in Travis et al. 1999). First, most loci may be nearly fixed for alleles that

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are optimal in any realistic environment, and most variation is due to rare alleles that are uniformly deleterious (i.e., mutation-selection balance; Houle et al. 1996). In this case, selection for increased heat (cold) tolerance could simply constitute a finer selective sieve, and tolerance to the opposite stress would also increase. Second, there may be certain genotypes that perform well in thermal extremes, but have deleterious pleiotropic effects in intermediate thermal environments (e.g., due to reduced metabolic rates; Service 1987; Hoffman and Parsons 1989). Again, selection for increased heat (cold) tolerance will result in a correlated increase in cold (heat) tolerance. Note, however, that even when a specific correlated response is expected there are theoretical reasons to expect the correlated response to be less consistent than the direct response (Bohren et al. 1966; Gromko 1995).

Teleost fishes are a taxon in which abiotic factors appear to be important in determining species distributions and for which the elucidation of these types of tradeoffs and their genetic foundation would be particularly important. For example, there are widespread families whose member species are almost all tropical (e.g., cichlids, lutjanids, serranids, gerreids) or temperate (e.g., salmonids, scombrids, clupeids). There are also sets of closely related species whose distributions appear causally associated with the distribution of specific abiotic conditions (Dunson and Travis 1991) and sets of conspecific populations that appear to demonstrate a variety of tradeoffs in performance across abiotic conditions (White et al. 1970; Bulger and Schultz 1979; Ceccarelli 1989; Vrijenhoek et al. 1992).

Here we report the results of an experiment in which we investigated the comparative genetic architecture of thermal performance by artificially selecting for acute heat- or cold-stress tolerance in stocks derived from either a subtropical or temperate population of the poeciliid fish *Heterandria formosa*. Our purpose was to describe the correlated evolution in the opposite stress tolerance (heat or cold) and in a suite of life-history traits. Our goal was to use the distribution of correlated responses within and among populations to indicate whether genuine genetic constraints on thermal tolerance might exist. Because of the nature of the results, we will not discuss life-history traits further; we mention them to establish the motivation for the project.

Materials and Methods

Natural History

Heterandria formosa (Pisces: Poeciliidae) is a small (12–30 mm), prolific livebearing fish, common throughout the coastal plain of the southeastern United States, ranging from central Louisiana to southern North Carolina (Martin 1980). It inhabits almost exclusively shallow, slow-moving, heavily vegetated habitats. We have collected specimens from water hotter than 39°C and colder than 8°C (Forster-Blouin 1989; Leips and Travis 1999); thermal stress is likely to be a very relevant ecological factor in the life history of this species.

The family Poeciliidae is neotropical, with approximately 200 species in 22 genera (Parenti and Rauchenberger 1989). Individuals are small (the largest species reaches ≅ 12 cm) and are found in a variety of freshwater and brackish habitats, but typically in shallow backwaters. Only a few representative species of three genera occur north of the Río Grandé; one species (*Gambusia holbrooki*) is found as far north as New Jersey. *Heterandria formosa* is the only North American representative of its genus; all congeners occur from the Yucatan Peninsula southward into Central America.

Experimental Design

Our goal was to establish four replicate lines of three selection treatments (heat tolerance, cold tolerance, controls) from each of two regions within the range of *H. formosa*. In late 1994 we collected approximately 1000 adults from two source populations, the Peace River, DeSoto County, Florida (henceforth FL) and from the Savannah River, Jasper County, South Carolina (henceforth SC). These populations were chosen to represent the heart of the species range on the Florida Peninsula and the northernmost extension of the species' range. Populations north of the St. Johns River drainage in northeastern Florida are genetically distinguishable from populations to the south and west and are likely to be more recent in origin (Baer 1998). In April 1995, 12 lines from each source population were established in 1.3-m diameter wading pools in a heated greenhouse. Each line was initially assigned to one of three treatment groups: heat stress (H), cold stress (C), or control (CN). Lines were started with 15 males and 15 gravid females.

By midsummer 1995, several lines from the FL source population had died out. We reestablished these lines, but to decrease the chances of having all lines in a treatment group die out, we increased the number of replicate lines in the FLH and FLC treatments from four to six and eliminated the control treatment from the FL source population.

Selection Protocol

We subjected the selected lines (H or C) to thermal stress selection at approximately eight week intervals from April 1996 to October 1996 and again from April 1997 to October 1997, for a total of eight rounds of selection. At the beginning of a round of selection, we collected all fish from a pool by dipnet and separated them by class (male, female, juvenile) into 10-by-14-in plastic tubs. We then introduced a manageable number of individuals of a particular class (≅ 10 adults, 20 juveniles) into a 10-by-14-in plastic tub containing 1.5 l of water at ambient temperature, which we then placed into either a hot water (52°C, ± 3°C) or ice bath. Our criterion for selection was the critical thermal limit (CTmax, CTmin). CTmax was defined as the point at which ventilation (flaring of the gills) ceased. CTmin was defined as the point at which the righting response was lost; this is a traditional measure of thermal stress tolerance in physiological zoology (Prosser 1973; Bulger and Schultz 1979; Fields et al. 1987; Forster-Blouin 1989; Elliot et al. 1994; Elliot and Elliot 1995; Meffe et al. 1995). Thermal stress was continued until half the individuals in a trial had reached the critical thermal limit (analogous to an LD50). Survivors were saved as parents for the next generation. We repeated this protocol until all adult individuals and approximately 120 juveniles from a line had been subjected to thermal stress. In April 1995, we only selected on juveniles because of the small number of mature

TABLE 1. Analysis of variance model. Here σ^2 is the variance for a random effect and ϕ is the magnitude of a fixed effect. Because the effect of replicate was not significant ($\alpha = 0.05$) in any case (see text), the term is dropped from the model.

Factor	Expected mean square	Effect tested over[1]
Block	$\sigma^2(E) + 2n\sigma^2(L) + 16n\phi(B)$	Error[2]
Source	$\sigma^2(E) + 10n\sigma^2(L) + 40n\phi(S)$	Line (source)
Line (source)	$\sigma^2(E) + 10n\sigma^2(L)$	Error
Treatment	$\sigma^2(E) + 5n\sigma^2(L) + 40n\phi(T)$	Treatment × line (source)
Source × treatment	$\sigma^2(E) + 5n\sigma^2(LT) + 20n\phi(ST)$	Treatment × line (source)
Line (source) × treatment	$\sigma^2(E) + 5n\sigma^2(LT)$	Error
Error	$\sigma^2(E)$	

[1] If the probability of an effect at a lower hierarchical level was > 0.15, that effect was pooled with the error variance.
[2] Strictly speaking, there is no exact test for block.

females; in subsequent rounds, we selected on individuals of all classes.

After a round of selection, all lines were reestablished with the same number of individuals of each class, the number of which was determined by the line with the fewest individuals of a class, subject to the constraint that there be at least 10 males, 15 females, and 20 juveniles. If there were fewer than those numbers of survivors in a line at 50% selection intensity, selection intensity was relaxed to the degree that allowed those numbers in the next generation. The reason for relaxing selection intensity rather than census size is that if population sizes differ, levels of expected inbreeding load will differ, thereby complicating the interpretation of apparent responses to selection. If selection intensities differ, however, response to selection can straightforwardly be interpreted as a function of selection intensity.

Control lines were handled and maintained in the same way as selected lines except with respect to the thermal stress treatment.

Protocol to Remove Effects of Unique Environment

To unambiguously interpret differences in phenotype as reflective of underlying genotypic differences, we raised all stocks in a common environment for two generations to remove the potential effects of unique environmental experience and/or environmentally induced maternal effects (Falconer 1989). Upon completion of the final round of selection in October 1996, we transferred fish into tanks in a climate-controlled room (14:10 L:D cycle, 30°C). Each line was divided into four replicate 5-gal aquaria, into which were placed five adult females and three adult males (generation P). Offspring (generation G1) were collected and pooled within lines, and four replicate aquaria within each line were established with 20 G1 juveniles on 2 January 1997. We repeated this procedure for a second generation (G2), beginning on 8 March 1997. On 5 August 1997, we collected mature G2 individuals from each replicate aquarium and established male/female pairs in 1.5-L plastic tubs in the same room as the natal tanks. Offspring (G3) were collected beginning on 18 August 1997 and daily thereafter until 28 October 1997. Upon removal from the natal tub, G3 newborns were transported to the greenhouse and placed in 1.5-L tubs, to a maximum of four individuals per tub.

Thermal stress tolerance was measured on G2 females and G3 males and females. In addition, we estimated the heritability of heat- and cold-stress tolerance from full-sib families of G3 fish; heritability estimated from full-sibs will overestimate the narrow-sense heritability (h^2) if effects of dominance are substantial (Falconer 1989).

Protocol for Measurement of Thermal Stress Tolerance

Beginning on 5 November 1997, G2 females were subjected to cold-stress treatment. Individual fish were placed in a 10-by-14-in plastic tub containing 1.5 L ambient temperature water, which was then placed in an ice bath and allowed to cool. Temperature was recorded by placing the probe of a digital thermometer (Model #15-077, Fisher Scientific, Santa Clara, CA) at a standardized location on a 0.7-cm-thick rubber disk glued to the bottom of the tub; CTmin was determined as before. Upon completion of a cold-stress test, fish were returned to their holding tubs.

Beginning on 9 December 1997, the same G2 females were subjected to heat-stress treatment. The protocol was the same as the cold-stress treatment, using a hot water bath (52°C, ± 3°C) instead of an ice bath, except that two individuals were tested per trial instead of one. Criteria for CTmax were as before. All stress tests of G2 females were conducted in the climate-controlled room in the laboratory.

Beginning on 23 January 1998, G3 fish (males and females) were subjected to cold-stress treatment. The protocol was exactly the same as for the G2 fish. Beginning on 8 March 1998, G3 fish were heat stressed, following the same protocol as for G2 fish except fish were tested individually instead of in pairs. All tests on G3 fish were conducted at the uncontrolled-climate greenhouse facility.

Data Analysis

The experiment was designed as a doubly nested, split-plot, mixed-model ANOVA, with treatment (heat/cold/control) and source population (FL/SC) as fixed effects and line nested within source population (*plot*) and replicate nested within line as random effects. The statistical model is presented in Table 1 (Hicks 1973; Underwood 1997). For G3 fish, greenhouse bench (five total) was considered a block effect. We did not initially include dam as an effect in the analysis of G3 fish. Interactions with block were pooled with the error variance. If the effect of replicate was not significant ($\alpha = 0.05$), it was dropped from the model and the data were reanalyzed. Average maternal length ([initial length + final length]/2) was initially included as a covariate and retained if significant ($\alpha = 0.05$). Nuisance variables (holding tub

temperature, maternal length, block, sex) were identified by Type I sums of squares and retained if significant; subsequent analyses used Type III sums of squares. Analyses were done using either Systat 7.0 or SAS 8.1 software. Full ANOVA tables are available online at http://neuro.fsu.edu/faculty/travis/website/.

In all cases there was a highly significant effect of holding tub (i.e., the tubs in which the fish were kept) temperature on CTmax/min. CTmax/min was first regressed against holding tub temperature and the residuals retained for subsequent analyses. In the heat-stress treatment of G2 mothers, there was a highly significant positive correlation between the CTs of the two individuals in a given trial (pairwise $r = 0.561$, $P < 0.001$). To account for these correlations, residual CTs of the first individual in a trial to succumb were converted to standard normal deviates (SND), CTs of the second individual in the trial to succumb were regressed against the SND value of the first individual, and the normalized residuals retained. Thus, for every ''first'' fish *i* with (normalized) CT_i, there is a ''second'' fish *j* with $CT_{j|i}$. Normalized critical thermal values (CTmax[std]) were used in subsequent analyses.

As an independent assessment of the genetic architecture of acute thermal stress tolerance, we calculated the heritability for CTmax and CTmin from the relationship between full-sibs in the G3 generation. If a trait is heritable, there will be a significant effect of dam in an ANOVA. In these analyses, the sexes were pooled; block and sex were initially included in the analyses and retained if significant. Dams with only a single surviving offspring were excluded from the analysis. We performed power analyses where advisable following procedures for random effects models described in Underwood (1997).

Several lines died out subsequent to the summer of 1995, and several others went through population bottlenecks (discussed below). We used a two-way contingency table to test if extinction was nonrandom with respect to source population and/or treatment and ANOVA with ''bottleneck'' as the independent variable.

Results and Discussion

Cold-Stress Tolerance

After eight rounds of selection on thermal stress tolerance, there was apparently no lasting effect of selection for cold-stress tolerance in G2 females, that is, there was no main effect of treatment on CTmin ($F_{2,6} = 0.25$, $P = 0.777$; Table 2). Raw CTmins were on the order of 10°C, which are similar to those found by Forster-Blouin (1989) for this species. There was a significant main effect of source population on CTmin ($F_{2,229} = 9.96$, $P = 0.002$); SC fish had a mean CTmin that was approximately 1.5° lower than FL fish. There was also a significant interaction of holding-container temperature with source population, with FL fish being more sensitive to initial conditions (slope of log[CTmin] vs. log[holding temp]) = 0.229 for SC fish, 0.582 for FL fish). However, the unequal numbers of lines in the different treatments for the two source populations complicates the interpretation of the results. Of the eight FL lines surviving, six were from the H treatment, and five of those six had higher CTmins than the two C treatment lines. No FL line had a mean CTmin lower than the mean value of the line means; eight of the 11 SC lines were below the mean (Table 2). If there was even a weak response to selection in the FL lines, (Tr*Line[Source] $F_{6,229} = 1.71$, $P = 0.119$] some of the apparent difference between source populations could actually be due to a treatment effect in the FL lines.

The pattern of cold-stress tolerance among G3 fish was even less straightforward (Table 2). For males and females considered together, there was a weak but significant Tr*Source interaction ($F_{1,327} = 4.44$, $P = 0.036$). There were no main effects of treatment, source, or sex, but there was a significant block effect ($F_{4,327} = 4.63$, $P = 0.002$). SC males were more cold tolerant on average; CTmin was again approximately 1.5° lower for SC fish than for FL fish. For G3 females there was no difference in CTmin between the two source populations ($\cong 0.1°$ lower for SC fish). For the FL source population, five of the six H lines (all but line 21) had higher CTmin than the two C lines; the result was the same for both males and females, again suggestive of a weak response to selection on cold-stress tolerance. The sample size from line 21 was particularly small (Table 2), perhaps explaining the atypical result. For SC males, three of the four C lines had lower CTmins than the three CN lines and two of the three H lines. For SC G3 females, however, the result was different; three of the four C lines were substantially *less* cold tolerant than two of the three high lines and all three of the CN lines. There is no obvious explanation for this result, other than either an idiosyncratic sex-by-autosomal genotype-by-environment interaction or sampling error.

There was no effect of dam on CTmin among full-sib families of G3 fish ($F_{104,140} = 0.98$, $P = 0.546$), that is, we cannot reject the null hypothesis that $h^2 = 0$. Power analyses indicated that our probability of detecting a genuine $h^2 \geq 0.2$ was 0.84; probability for $h^2 \geq 0.3$ was 0.99. Power fell sharply for $h^2 \leq 0.15$, however; our power to detect $h^2 = 0.15$ was 0.63. If CTmin is heritable under the conditions in which G3 fish were raised, that heritability is not likely to exceed 0.15. Dams were pooled across source populations and treatments in this analysis, so any differences among those groups would artificially increase h^2. Because the full-sib families were derived from only about 40% of G2 mothers, the power of our selection experiment to detect a heritable response would have been considerably greater than the full-sib design. The lack of response to selection is consistent with a very low heritability.

Heat-Stress Tolerance

There were no significant effects on CTmax in G2 females. Raw CTmaxs were on the order of 41–42°C, again similar to those found by Forster-Blouin (1989) for this species. Examination of line means (Table 2) reveals that FL lines are somewhat more heat tolerant than SC lines and that the three SC H lines had higher CTmaxs than three of the four C lines and three of the four CN lines. There is no strong pattern among the FL lines, but the fact that only two FL C lines survived limits our statistical power.

Among G3 fish, there were no statistically significant ef-

TABLE 2. Unweighted mean values for standardized critical thermal (CT) values at different hierarchical levels (see Materials and Methods for description of the standardization procedure). For example, under CT max (G2) the value 0.102 (0.131) is the mean of the six H treatment (Tr) lines from the FL source population. SEM is given in parentheses; sample size follows line means. Lines marked with an asterisk experienced a population bottleneck (defined in text). Values in bold type are significantly different ($\alpha = 0.05$) at that hierarchical level.

Source	Tr	Line	CTmax (G2)	CTmin (G2)	CTmax (G3 male)	CTmax (G3 female)	CTmin (G3 male)	CTmin (G3 female)
FL			0.138 (0.118)	**0.804** (0.131)	−0.007 (0.197)	0.082 (0.195)	0.345 (0.407)	0.303 (0.342)
	H		0.102 (0.131)	0.900 (0.154)	−0.021 (0.260)	0.187 (0.241)	0.801 (0.407)	0.489 (0.430)
		2	0.384, 14	1.105, 14	0.446, 12	0.079, 12	1.862, 9	1.734, 10
		5	0.337, 14	0.899, 14	0.115, 10	0.272, 10	0.444, 10	0.996, 7
		9*	−0.495, 14	1.353, 15	0.000, 14	0.376, 15	0.421, 13	0.669, 12
		12*	0.221, 9	1.081, 9	0.353, 7	0.761, 15	0.421, 13	0.669, 12
		14	0.147, 13	0.281, 14	0.120, 6	0.444, 12	0.494, 6	0.519, 8
		21*	0.016, 12	0.683, 13	−1.159, 7	−0.813, 4	−0.361, 6	−1.148, 4
	C		0.245 (0.345)	0.514 (0.119)	0.034 (—)	−0.231 (—)	−1.021 (—)	−0.255 (—)
		15	−0.100, 13	0.394, 13	0.352, 17	0.037, 16	−0.087, 16	−0.634, 10
		24	0.591, 13	0.633, 15	−0.284, 11	−0.498, 8	−1.955, 10	0.125, 9
SC			−0.112 (0.107)	**−0.422** (0.345)	−0.038 (0.128)	−0.067 (0.123)	−1.318 (0.276)	0.728 (0.708)
	H		0.081 (0.092)	−0.513 (0.720)	−0.072 (0.396)	0.087 (0.299)	−2.028 (0.433)	0.574 (1.113)
		3	0.059, 11	−1.039, 12	−0.388, 11	−0.061, 9	−1.829, 11	1.488, 9
		18*	−0.066, 14	0.912, 14	−0.402, 8	−0.243, 15	−2.716, 8	−1.245, 9
		22	0.250, 16	−1.411, 16	0.574, 3	0.564, 5	−1.540, 3	1.478, 2
	C		−0.261 (0.236)	−0.314 (0.718)	−0.082 (0.209)	−0.116 (0.273)	−1.603 (0.501)	2.176 (0.971)
		4	−0.367, 10	−1.326, 10	−0.306, 4	−0.223, 9	−2.340, 4	2.209, 7
		10	−0.181, 13	−0.142, 15	−0.343, 8	0.442, 8	−2.333, 7	2.888, 6
		17	−0.820, 14	−1.748, 15	0.440, 7	0.009, 4	−2.343, 7	3.766, 4
		19*	0.323, 9	0.676, 9	−0.120, 5	−0.694, 7	0.603, 6	−0.159, 9
	CN		−0.109 (0.165)	−0.463 (0.556)	0.056 (0.264)	−0.156 (0.154)	−0.228 (1.041)	−1.047 (1.518)
		1*	0.301, 13	−0.367, 13	−0.312, 4	−0.351, 11	1.202, 4	0.914, 8
		11*	−0.509, 9	0.763, 9	—	—	—	—
		16	−0.105, 14	−0.310, 15	0.044, 13	−0.196, 16	−0.145, 13	−0.715, 16
		20*	−0.122, 13	−1.936, 13	0.435, 8	0.079, 6	−1.740, 9	−3.340, 3

fects on CTmax except of sex ($F_{1,299} = 23.25$, $P = 0.0001$) and of block ($F_{4,299} = 5.42$, $P = 0.0003$). CTmax of G3 females was approximately 1.2° greater than that of males (Table 2), and the difference was not due to differences in standard length. Our data and those of Forster-Blouin (1989) consistently show that the largest fraction of the variance in CTmax is explained by temperature of the container in which an individual fish has been kept prior to testing. The thermal environment of the greenhouse in which G3 fish were housed is subject to large daily and even hourly fluctuations, and position within a particular bench affects the thermal properties of a holding container. It is quite likely that small-scale environmental effects had a substantial effect on CTmax of G3 fish; the highly significant block effect reinforces that interpretation.

There was no effect of dam on CTmax among full-sib families of G3 fish ($F_{102,181} = 1.15$, $P = 0.211$). Power analyses indicated that although we could have detected $h^2 \geq 0.18$ with substantial power (exceeding 0.88), we lacked power to detect a genuine $h^2 \leq 0.15$. Our power to detect $h^2 = 0.15$ was 0.78; power to detect $h^2 = 0.1$ was only 0.50. As with CTmin, if CTmax is heritable under the conditions in which G3 fish were raised, the heritability is likely to be low. The lack of response to selection is consistent with a low heritability.

Line Extinctions and Bottlenecks

The dying out of several lines provided an unwelcome source of data. Of the 24 original lines, five had died out by July 1995, after two rounds of selection. Five new lines were established in August 1995, all from the FL source population; of those lines, three died out by September 1995, before the next round of selection. Two of the remaining original lines died out over the winter of 1995–1996 and were not reestablished. The pattern of dying out was not random with respect to source population or treatment. Of the 10 lines that died out, nine were from the FL source population and none of those were from the heat treatment group; the one SC line that died out was from the heat treatment. The effects of source and treatment were both significant (source, $\chi^2 = 6.196$, df = 1, $P = 0.013$; treatment, $\chi^2 = 8.076$, df = 2, $P = 0.018$). Clearly, the laboratory environment, even in the absence of applied thermal stress, was more stressful for the FL fish than for the SC fish, although we cannot say why. The heat-stress treatment may have had a ''hardening'' effect on the H lines, rendering them better able to withstand the laboratory environment; there is some evidence that exposure to heat shock renders individuals more resistant to other environmental stresses (Kapoor and Lewis 1987; Michel and Starka 1987).

A complicating factor is that eight of the 19 surviving lines went through population bottlenecks (defined as fewer than 12 adults and/or 25 total individuals at a census) at some time during the first year of the experiment, although no line went through more than one bottleneck. For G2 females, one-way ANOVA with presence/absence of bottleneck as the grouping variable revealed no significant differences in CTmax ($P > 0.5$). Two-way ANOVA with source population and bottleneck as the grouping variables showed no significant effects of bottleneck on CTmin (main effect, $P = 0.16$, interaction, $P = 0.30$; source population was included in the model because of the previously determined significant difference in CTmin). Although bottlenecks will not substantially reduce heterozygosity unless maintained for several generations (Nei et al. 1975), a single-generation bottleneck may either decrease or increase additive genetic variance for a trait if the genotypic variance is largely due to the effects of rare alleles (Goodnight 1988).

Conclusions

The lack of response to selection was unexpected, given the heritabilities typically observed for a wide variety of phenotypic traits (Houle et al. 1996). The difference in CTmin between source populations in G2 females indicates that there is a genetic basis to variation in acute cold tolerance under some conditions, but that there is little, if any, heritable variation in cold tolerance within either source population. The highly significant difference in CTmax between the sexes in G3 fish also indicates a genetic basis for heat tolerance, but that there are no differences either within or among source populations.

The absence of detectable direct responses to selection obviously limits our ability to test the hypotheses regarding correlated responses outlined in the introduction. However, the differentiation between the two source populations in cold tolerance but not in heat tolerance is not consistent with the presence of strong pleiotropic relationships involving heat and cold tolerance. We have discussed elsewhere (Travis et al. 1999) how purely environmental effects could create apparent tradeoffs among populations.

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