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ORIGINAL PAPER

Response to selection and evolvability of invasive populations

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Abstract While natural selection might in some cases facilitate invasions into novel habitats, few direct measurements of selection response exist for invasive populations. This study examined selection response to changes in salinity using the copepod *Eurytemora affinis*. This copepod has invaded fresh water from saline habitats multiple times independently throughout the Northern Hemisphere. Selection response to a constant intermediate salinity (5 PSU) was measured in the laboratory for saline source and freshwater invading populations from the St. Lawrence drainage (North America). These populations were reared under three conditions: (1) native salinities (0 or 15 PSU) for at least two generations, (2) 5 PSU for two generations, and (3) 5 PSU for six generations. Full-sib clutches taken from populations reared under these three conditions were split across four salinities (0, 5, 15, and 25 PSU) to determine reaction norms for survival and development time. Contrasts in survival and development time across the three rearing conditions were treated as the selection response. Selection at 5 PSU resulted in a significant decline in freshwater (0 PSU) tolerance for both the saline and freshwater populations. Yet, evolutionary differences in freshwater tolerance persisted between the saline and freshwater populations. The saline and freshwater populations converged in their high-salinity (25 PSU) tolerance, with an increase in the freshwater population and decline in the saline population. Development time did not shift greatly in response to selection at 5 PSU. For all three rearing conditions, the freshwater population exhibited retarded larval development and accelerated juvenile development relative to the saline population. Results from this study indicate that both the saline and freshwater populations exhibit significant responses to selection for a fitness-related trait critical for invasions into a novel habitat.

Keywords Biological invasion · Selection experiment · Genetic variation · Physiological evolution · Rapid evolution · Evolvability · Exotic · Nonindigenous · Great Lakes

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Introduction

What allows certain populations to invade novel environments, when most populations fail? Only a small proportion of introduced species become successful as invaders (Williamson and Fitter 1996). Much evolutionary research on invasive species has focused on analyzing the interactions between the extrinsic factors that limit range expansions (e.g. enemies, food resource, temperature, salinity) and the intrinsic responses of populations to the extrinsic forces, such as phenotypic plasticity or evolutionary adaptation. Populations vary in their intrinsic responses, with some populations having greater plasticity or evolutionary

potential than others (Huey et al. 2000; Weinig 2000a; Carroll et al. 2001; Reznick and Ghalambor 2001; Lee 2002; Lee et al. 2003; Parker et al. 2003; Blair and Wolfe 2004; Bossdorf et al. 2005; Donohue et al. 2005b). In addition, populations could respond in a complex manner, with both phenotypic plasticity and selection contributing to invasive success (Lee 2002), but aside from a few notable case studies (Weinig 2000a, b; Weinig and Delph 2001), their relative importance and fitness tradeoffs remain unclear.

This study focused on the selection response of the freshwater-invading copepod *Eurytemora affinis* to changes in salinity. Direct measurements of selection response in invasive or colonizing populations are still rare (Gibbs 2002; Lee 2002; Donohue et al. 2005a; Santos et al. 2005). *Eurytemora affinis* is a major component of food webs in estuarine and salt marsh ecosystems in the Northern Hemisphere. Its native range spans salinities from brackish to hypersaline (up to 40 PSU in salt marshes). Within the past century, populations of *E. affinis* have invaded freshwater habitats from saline estuaries and salt marshes multiple times independently in North America, Europe, and Asia (Fig. 1A) (Lee 1999). These freshwater invasions have been mediated through human activity, with the advent of canals, shipping, and fish stocking of reservoirs. For example, *E. affinis* is a dominant grazer in the St. Lawrence estuary of North America and has become abundant in the Great Lakes since the opening of the St. Lawrence Seaway ca. 1959 (Fig. 1B). In addition to large impacts on food web structure, such inland invasions could have implications for disease transmission, as *E. affinis* is a disease vector and a major carrier of Cholera (Colwell 2004; Piasecki et al. 2004).

Previous studies showed that salinity limits geographic distribution of *E. affinis*, and that populations vary in their salinity distribution (Lee 1999; Lee and Petersen 2002; Lee and Petersen 2003; Lee et al. 2003). Neither short-term nor developmental phenotypic plasticity alone could account for the gain in

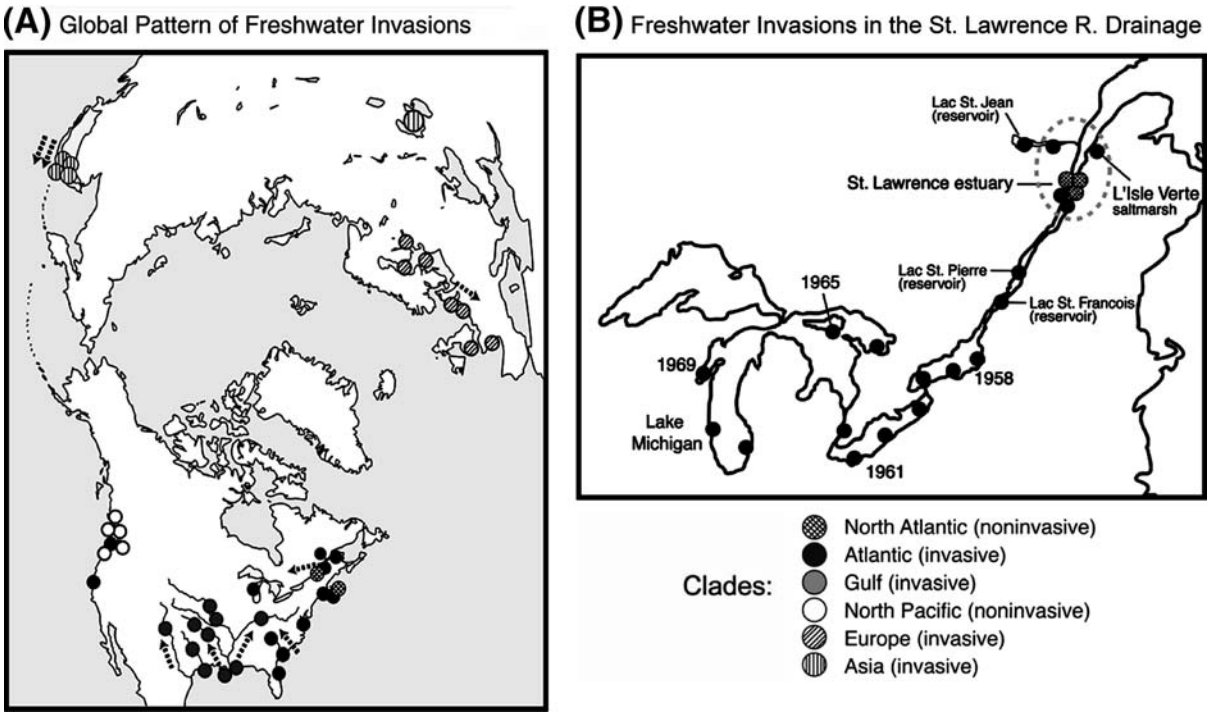

Fig. 1 Geographic pattern of invasions by *Eurytemora affinis* from saline sources into freshwater lakes and reservoirs. Patterned circles represent genetically distinct clades. Details on location and timing of invasions are presented in Lee (1999). (**A**) Global pattern of freshwater invasions by *E. affinis* from genetically distinct saline source populations. Dashed arrows indicate pathways of independent freshwater invasions. (**B**) Populations of *E. affinis* within the St. Lawrence drainage system. Only the Atlantic clade (black dots) has extended its range into fresh water, while the North Atlantic clade has not (hatched dots). The native range for both clades is shown within the dotted circle. Dates are approximate timing of invasions. Populations shown outside the dotted circle are recent invasions into reservoirs and the Great Lakes. Clade identities are known from mitochondrial COI sequence data (562 bp) for ca. 20 individuals per population (Lee 1999; Winkler and Lee, unpubl. data). The clades are reproductively isolated from one another (Lee 2000; Lee and Frost 2002; C. E. Lee, unpubl. data)

freshwater tolerance by the saline populations (Lee and Petersen 2003; Lee et al. 2003). Common-garden experiments using ancestral saline and invading freshwater populations from the St. Lawrence River drainage in North America (Fig. 1B) revealed that the differences in tolerance between the populations were genetically based, and that freshwater invasions were accompanied by evolutionary shifts in freshwater tolerance (Fig. 2A) (Lee et al. 2003). An increase in freshwater tolerance was accompanied by a decline in high-salinity tolerance, suggesting evolutionary tradeoffs between low- and high-salinity tolerance (Fig. 2A) (Lee et al. 2003). Negative genetic correlations between clutch survival at low versus high salinities (0 vs. 5 PSU and higher) provided further support for the tradeoffs (Lee et al. 2003). Both the saline and freshwater populations harbored genetic variation in salinity tolerance, providing genetic substrate upon which selection could act (Fig. 2B) (Lee et al. 2003).

Given the apparent tradeoffs and presence of genetic variation for tolerance, we examined the selection response of both the saline and freshwater populations under a constant salinity of 5 PSU (practical salinity units = parts per thousand). We used a saline ancestral population from the St. Lawrence marsh (L'Isle Verte) and a descendant freshwater population from Lake Michigan (Fig. 1B). A significant selection response would demonstrate the evolutionary potential of these populations in response to salinity change during habitat shifts. Moreover, a significant selection response for the freshwater population placed at 5 PSU would provide more powerful evidence for the tradeoffs between low and high-salinity tolerance than the negative genetic correlations found in the previous study using full-sib clutches (Lee et al. 2003).

We predicted that freshwater tolerance (survival at 0 PSU) would be selected against at 5 PSU in both the saline and freshwater populations, based on the negative genetic correlations between survival at 0 and 5 PSU found in the previous study (Lee et al. 2003). Positive genetic correlations between 5 PSU and higher salinities (Lee et al. 2003) suggest that high-salinity tolerance would increase in response to rearing at 5 PSU. The rate of response would depend in part on the strength of selection and on the heritability of the trait under selection.

We determined selection response by contrasting traits of populations reared under native salinities, measured in the previous study (Fig. 2; Lee et al. 2003), with those of populations reared at 5 PSU. The contrasts were made for saline and freshwater populations reared under three conditions: (1) native salinities for at least two generations, 0 PSU for the freshwater and 15 PSU for the saline populations (Fig. 2), (2) 5 PSU for two generations, and (3) 5 PSU for six generations. Subsequently, full-sib clutches were taken from the populations from each rearing condition and then split across salinities (0, 5, 15, and 25 PSU) to determine reaction norms for survival and development time. We adopted a reaction norm approach, by measuring tolerance for each clutch across a range of salinities, to determine how selection for a trait in one environment would affect the trait in an alternate environment. The contrast in survival and development time across the three rearing conditions was treated as the selection response.

Materials and methods

Population sampling

Common-garden experiments were performed on two populations from the St. Lawrence River drainage (Fig. 1B, Atlantic clade): an ancestral saline population from Baie de L'Isle Verte, 30 km east of Rivière-du-Loup, and a recently-derived freshwater population in Lake Michigan in Racine Harbor, Wisconsin. The L'Isle Verte population occurs in saline pools of a floodplain, where salinity can range from 5 to 40 PSU seasonally, while the Lake Michigan population occurs in relatively constant salinities, at very low conductivities of 200–400 μS/cm (0 PSU). *Eurytemora affinis* was first discovered in the Great Lakes around 1958 (Engel 1962; Faber et al. 1966), and probably originated from one or several saline populations in the St. Lawrence River drainage, based on genetic and geographic proximity (Lee 1999, 2000). Although the L'Isle Verte population might not have been the direct source of the Lake Michigan population, it is probably closely related to the ancestral population. Populations from another clade (North Atlantic) also occurs in the native range, but is not invasive and has not extended its range into fresh water (Fig. 1B) (Lee 1999, 2000).

Selection at 5 PSU for multiple generations

The previous results (Fig. 2; rearing at native salinities) were contrasted with the results in this study (rearing at 5 PSU) to determine effects of selection at 5 PSU. In the previous study (Fig. 2; Lee et al. 2003) the saline ancestral and freshwater invading populations (see above) were reared for at least two generations at

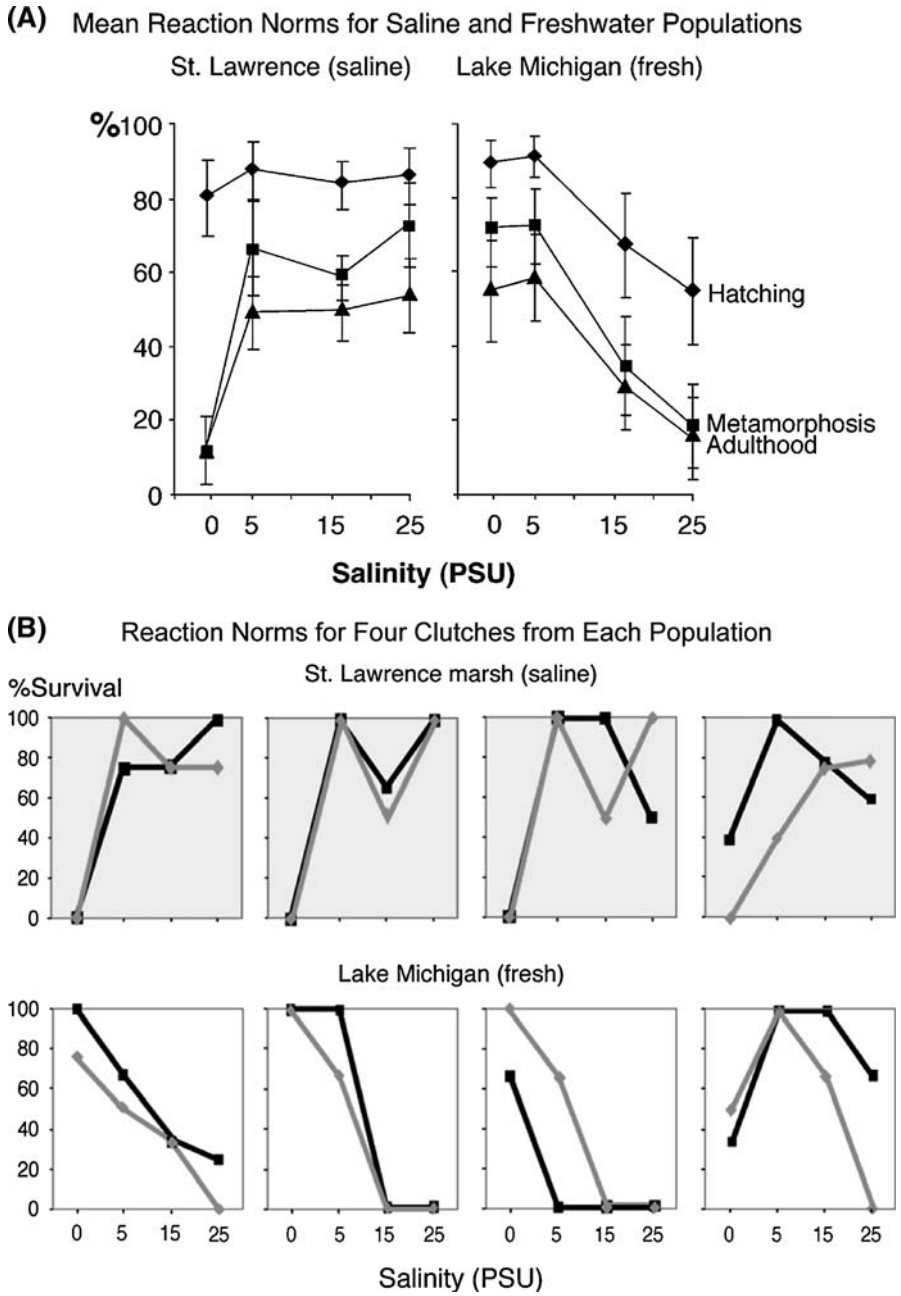

Fig. 2 (**A**) Evolutionary shifts in survival from the ancestral saline St. Lawrence (left) to the freshwater Lake Michigan (right) populations. Graphs show physiological reaction norms for survival across four salinities for both populations. Graphs show mean %survival ± SE for 8 full-sib clutches split across the four salinities. Survival is measured as percentage of eggs that hatched, achieved metamorphosis (nauplius VI to copepodid I), and survived to adulthood. Clutches were taken from parental populations raised at native salinities (15 PSU for the saline and 0 PSU for the freshwater populations) for at least two generations. Data are taken from Lee et al. (2003). (**B**) Genetic variation in reaction norms for the saline and freshwater populations (shown in Fig. 2A). Reaction norms for four representative clutches are shown for each population. Data points are mean survival to metamorphosis for individuals within each clutch. Dark and light curves represent replicated experiments for each clutch (as clutches were split into replicate vials for each salinity). Genotype by environment interaction (G × E) for survival was significant using a mixed-model ANOVA (Lee et al. 2003; St. Lawrence: likelihood ratio $T = 21.8$, $P = 0.00096$; Lake Michigan: $T = 26.5$, $P = 0.0017$)

native salinities, 0 PSU (lake water) for the freshwater population and 15 PSU for the saline population. This previous study, where zero generations were reared at 5 PSU (referred to as ''Generation 0''), was considered the control for this study. In this study, the saline and freshwater populations were reared at a common

salinity of 5 PSU for two and six generations (referred to as ''Generations 2 and 6''). The intermediate salinity of 5 PSU was chosen as the selection regime because it is near the threshold above and below which the fresh and saline populations suffer high larval mortality, respectively (Lee et al. 2003). In general, 5–8 PSU serves as a biogeographic and physiological boundary between fresh and saline copepod species (Khlebovich and Abramova 2000).

Full-sib clutches taken from these populations were split across salinities (0, 5, 15, 25 PSU) to determine reaction norms for survival and development time (see next section for more details). Results from the previous experiment (rearing at native salinities) are reported in Lee et al. (2003) and shown in Fig. 2. The contrasts in survival and development time across the three rearing conditions, representing different numbers of generations (0, 2, 6) at 5 PSU, were treated as the selection response.

The three rearing and reaction norms experiments were conducted sequentially and independently from the laboratory stock populations. Samples collected from the wild saline and freshwater populations were placed in the laboratory at ''native salinities'' of 15 and 0 PSU, respectively. The saline salt marsh population comes from a fluctuating environment, where salinity varies seasonally, such that the native salinities are difficult to replicate in the laboratory (see Discussion). All experiments (including the previous, Lee et al. 2003) were performed on a single collection from the saline habitat, on May 23, 2001, to avoid using populations collected from different salinity regimes. Fifteen PSU was designated as the ''native salinity'' because the population was found at this salinity when collected. For the freshwater populations, the previous study (Lee et al. 2003) used samples collected in October and November of 2001, while the current study used samples collected in July–November 2002. In contrast to the saline habitat, salinity remains relatively constant in the freshwater Lake Michigan (at low levels of 200–400 μS/cm, 0 PSU), such that the selection regime with respect to salinity remains constant.

For the previous experiment (Lee et al. 2003), clutches were taken directly from parents reared at native salinities (0 and 15 PSU) and used for the reaction norm experiments in February of 2002. For the experiment that selected at 5 PSU for two generations, the saline and freshwater populations were placed at 5 PSU in March 2003, and the reaction norm experiments began in May of 2003. For the experiment that selected at 5 PSU for six generations, the saline and freshwater populations were placed at 5 PSU in September 2002, and the reaction norm experiments began in February 2003.

Several hundred individuals were maintained for each population in culture, in at least three 2-l containers per population to avoid effects of genetic drift during selection at 5 PSU. All populations were reared in the laboratory at 13°C. Cultures were fed a combination of freshwater *Rhodomonas minuta* and saline *Rhodomonas salina*, so that all cultures and experimental treatments would have access to cells that were not subjected to osmotic shock. Both species of algae are high in polyunsaturated fatty acids (Vanderploeg et al. 1996; Dunstan et al. 2005) and are more optimal for *E. affinis* growth and development than other algae tested (e.g. *Isochrysis*, *Spirulina*, *Chlamydomonas*, *Thallasiosira*, *Scenedesmus*, *Ankistrodesmus*, *Tetraselmis*) (C. E. Lee, unpublished data). *Rhodomonas minuta* is a dominant algal species in the Great Lakes. Water of different salinity was made from mixtures of water from Lake Michigan and Instant Ocean®.

Effects of selection on survival and development time across salinities

After rearing the populations under the conditions described above, clutches were taken from adult females and then split across salinities to determine reaction norms for survival and development time. More specifically, 8–14 full-sib clutches were excised from adult females with a pin and split into four salinity treatments of 0 (lake water), 5, 15, and 25 PSU, with 6–10 eggs per treatment. Each treatment was split again into two or more replicate vials per clutch to confirm reproducibility of results for each clutch. For the third experiment (Generation 6), clutches were split across only three salinities (0, 5, and 25 PSU), enabling the use of greater number of clutches (14) and more replicate vials per clutch (often 3 or 4).

All solutions were made with Lake Michigan water and Instant Ocean®. Solutions were sterilized either by autoclaving or filtering through a 0.22 μm mesh. For the Lake Michigan population reared at 0 PSU, eggs were transferred gradually to treatment salinities of 15 and 25 PSU to avoid osmotic shock (see Lee et al. 2003). Eggs were placed in 20-ml scintillation vials maintained half-full with caps left ajar to allow for oxygen exchange. Vials were kept at 13°C on a 9D:15L light cycle. Vials were treated with either 10 mg/l Ticarcillin or 20 mg/l Primaxin to avoid mortality due to bacterial infection. To avoid confounding effects of vial position, we placed vials in racks such that

treatment, clutch, and replicates within clutch were spatially interspersed and distributed evenly. Developing copepods were fed in excess every day with a mixture of fresh and saltwater *Rhodomonas* spp. Every 12 days, 75% of the water volume was replaced. Visual inspection of vials was performed daily to determine proportion of hatching, survival to metamorphosis, and survival to adult. Metamorphosis occurred during the transition between the nauplius VI and copepodid I stages. Individuals were classified as adults when males developed geniculate right antennules, and when females developed large wing-like processes on the posterior end of their prosomes (body). Hatching rate and survival to metamorphosis and adult at particular salinities (0, 5, 15, 25 PSU) were used as measures of ''salinity tolerance.'' Development time was measured for individuals by recording days from hatching to metamorphosis (larval development) and from metamorphosis to adult (juvenile development). Development time from metamorphosis to adulthood was obtained by subtraction (hatching to adult-hatching to metamorphosis).

Statistical model for survival data

An ordinal probit model was used to analyze differences in survival (Albert and Chib 2001) across experiments (i.e. 0, 2, 6 generations at 5 PSU) and between saline and freshwater populations. This model was appropriate for this study because it accounts for the discrete nature of survival data and for the cumulative survival at each life history stage. An observation was coded as (a) 1 (if hatched); (b) 2 (if survive to metamorphosis), and (c) 3 (if survived to adult). The probability model can be written as:

$$\begin{aligned}\text{Prob}(y_i = j|\beta, T) = \Phi[T_j - (x_i\beta + z_i u)] \\ - \Phi[T_{j-1} - (x_i\beta + z_i u)],\end{aligned}$$

where $j = 1, 2, 3$, indexing the categories to which the observation belongs; $\Phi(\cdot)$ is the standard cumulative normal density function, and $T = [T_0, T_1, T_2, T_3]'$ is the vector of unknown thresholds. The thresholds must satisfy $-\infty = T_0 \le T_1 \le T_2 \le T_3 = \infty$. The first threshold T_1 is set to zero, because the parameter cannot be identified in a probit analysis, leaving T_2 as the only unknown threshold. The parameter β includes effects of population within each salinity and generation effect and u is a vector of random effects of clutches within population. Variance between clutches within population was estimated. Clutch was nested within salinity, population and experiment when estimating heritability and differences in survival. The survival probability between different populations or experiments (generations at 5 PSU) was computed for 67 clutches using the ''ESTIMATE'' statement in PROC NLMIXED in SAS (version 9.0) (SAS 2003). The ESTIMATE statement computes approximate standard errors for the estimates using the delta method (Billingsley 1986). It uses these standard errors to compute corresponding t statistics, P-values, and confidence limits. For estimating genetic correlations between survival at different salinities, an additional life-history stage 0 (if died at egg stage) with a corresponding threshold was included in the probit model to increase power. For estimates of genetic correlations and also broad sense heritability for survival, we combined the stages into two groups: individuals that did not reach metamorphosis and those that did reach metamorphosis. In this case, the data were binary and no threshold needed to be estimated. The PROC NLMIXED procedure was used to estimate genetic correlations.

Statistical model for development time data

A linear mixed model was fitted to the development time data, which included fixed effects of experiment, population, and salinity, and random effects of clutch, clutch × salinity and residual. The effect of sex was also included in the analysis of development time from hatching to adult. The PROC MIXED procedure in SAS (version 9.0) was used to estimate differences in development time across experiments (generations at 5 PSU) and between populations.

Estimates of broad sense heritability

Intraclass correlations for full-sib clutches (var(clutch)/[var(clutch) + 1]) were used as a measure of broad sense heritability for survival (Falconer and Mackay 1996). For full sib data, intraclass correlations are multiplied by two to gain estimates for broad sense heritability (p. 180, Falconer and Mackay 1996). Estimates based on full-sib data yield upper-bound values for heritability (Falconer and Mackay 1996). The residual variance was set to one in the probit model because of issues regarding identifiability. The PROC NLMIXED statement in SAS produces the approximate covariance matrix of the variance components, which were used to calculate confidence intervals for heritability. Intraclass correlations (var(clutch)/[var(clutch) + var(residual)]) were also computed using PROC NLMIXED in SAS to obtain estimates of broad sense heritability for development time.

Results

Effects of selection at 5 PSU on survival across salinities

Results were generally concordant with our predictions (see Introduction). Both the ancestral saline St. Lawrence (L'Isle Verte) and freshwater Lake Michigan populations experienced a decline in freshwater tolerance (survival at 0 PSU) when reared at 5 PSU (Figs. 3, 4; see below). While freshwater tolerance declined significantly in the freshwater population, it was completely lost in the saline population. These results were congruent with negative genetic correlations for survival between 0 and 5 PSU found for both populations in the previous (Lee et al. 2003) and current (Fig. 5, see next section) studies. Freshwater tolerance declined significantly in the freshwater population after rearing at 5 PSU for two generations (''Generation 2''), relative to the control reared at 0 PSU (''Generation 0'') (Fig. 4; ordinal probit model, see Methods; survival at least to metamorphosis: $P < 0.0001$; to adult: $P < 0.0001$). However, there was no further decline in freshwater tolerance in the freshwater population between Generations 2 and 6, as survival was not significantly different between them ($P > 0.50$). While the decline in freshwater tolerance in the saline population was not statistically significant

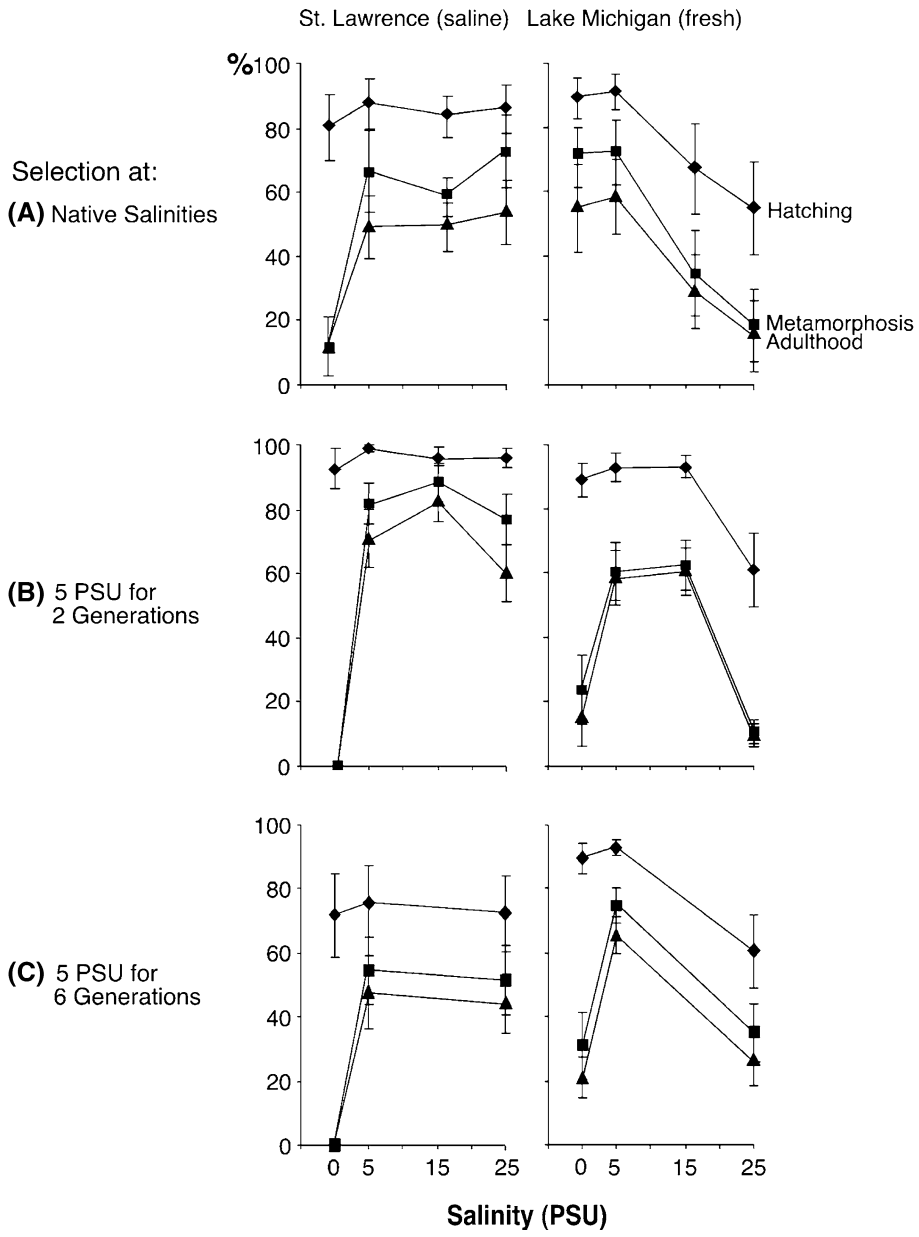

Fig. 3 Physiological reaction norms for survival in response to selection at 5 PSU. Survival across salinities (0, 5, 15, 25 PSU) is shown for the saline St. Lawrence (left) and freshwater Lake Michigan (right) populations. Survival is shown for clutches taken from parental populations raised at (**A**) native salinities (15 PSU for the saline and 0 PSU for the freshwater populations) for at least two generations ($N = 8$ clutches per population), as shown in Fig. 2A, (**B**) 5 PSU for two generations ($N = 12$ clutches), and (**C**) 5 PSU for six generations ($N = 14$ clutches). The contrasts in survival for clutches taken from populations reared under these three conditions constitute the selection response. Graphs show mean %survival ± SE for 8–14 full-sib clutches split among 3–4 salinities. Survival is measured as percentage of eggs that hatched, achieved metamorphosis (nauplius VI to copepodid I), and developed to adulthood

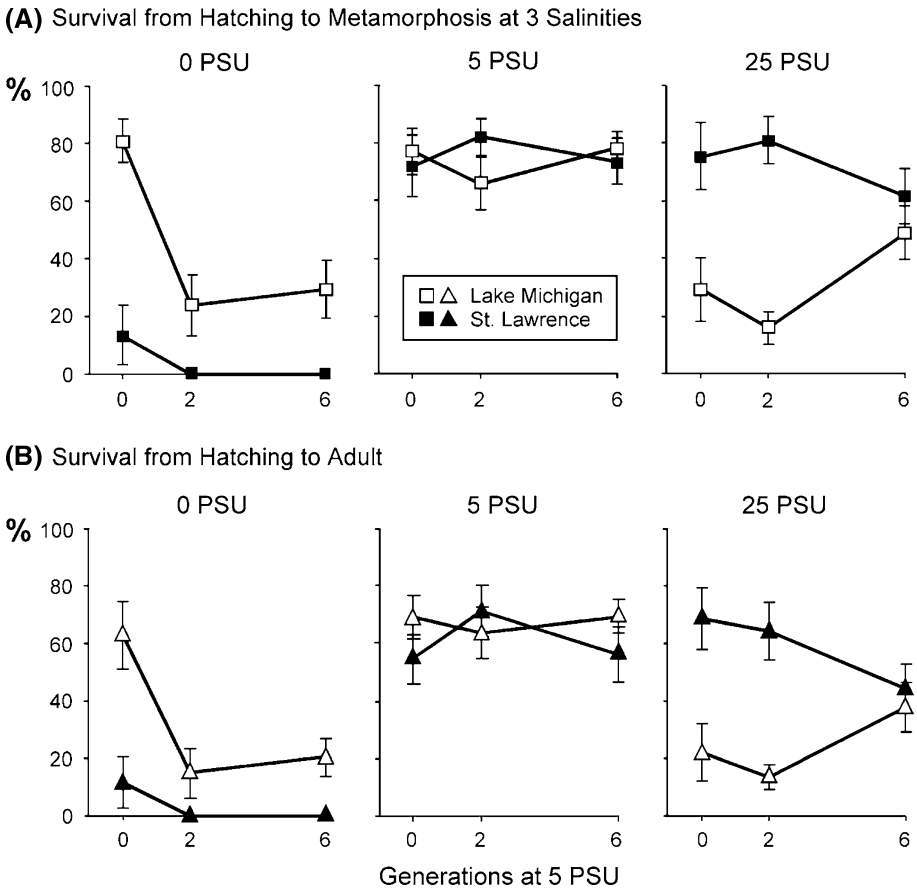

Fig. 4 Shifts in tolerance at three salinities (0, 5, 25 PSU) in response to selection at 5 PSU. Graph shows mean survival (%survival within clutch) for 8 to 14 clutches ± SE for each salinity treatment. Survival was measured for populations from saline St. Lawrence marsh and freshwater Lake Michigan after they were reared at 5 PSU for 0, 2, and 6 generations. (**A**) Survival from hatching to metamorphosis. (**B**) Survival from hatching to adult

(Fig. 4; $P > 0.15$), the presence of any survival at 0 PSU at Generation 0, and the complete lack of survival at Generations 2 and 6 were both notable. At Generation 0, two out of eight clutches contained survivors at 0 PSU, with 83.3% survival in one clutch, and 25% in the other. This genetic variation for freshwater tolerance present at Generation 0 in the saline population (Fig. 2B) was completely lost after rearing at a constant 5 PSU for multiple generations (Figs. 3, 4).

The freshwater population experienced an increase in high-salinity tolerance, consistent with positive genetic correlations between 5 PSU and higher salinities (Lee et al. 2003). High-salinity tolerance increased significantly in the freshwater population between Generations 2 and 6 (to metamorphosis: $P = 0.013$; to adult: $P = 0.019$), though not between Generations 0 and 2 ($P > 0.6$) or between Generations 0 and 6 ($P > 0.1$) (Fig. 4). In contrast, the saline population experienced an unexpected, though nonsignificant ($P > 0.15$), decline in high-salinity tolerance (Fig. 4; see below). This decline was unexpected given the positive genetic correlations between 5 PSU and higher salinities (Fig. 5) (Lee et al. 2003).

Survival at 5 PSU did not change significantly across experiments (generations at 5 PSU) for either population (Fig. 4) ($P > 0.15$). A selection response for increased survival at 5 PSU would be expected in both populations given the heritability for survival at 5 PSU and the presence of genetic variation for tolerance. The lack of a response might have been due to lack of resolution, as survival at 5 PSU was already high (Fig. 4), or experimental error or stochastic effects obscuring patterns of survival. Alternatively, much of the heritability for survival at 5 PSU might be attributable to maternal effects (see below).

Shifts in genetic correlations in response to selection at 5 PSU

There appeared to be a shift in genetic correlations for the freshwater population, from negative between 0 and 5 PSU at Generation 0 toward higher values at Generations 2 and 6 (Fig. 5). The saline population also exhibited negative genetic correlations between 0 and 5 PSU at Generation 0. Shifts in genetic correlations between 0 and 5 PSU were not possible to determine for the saline population, due to lack of survival at 0 PSU at Generations 2 and 6 (Fig. 3). Genetic correlations between the higher salinities (5 vs. 25 PSU)

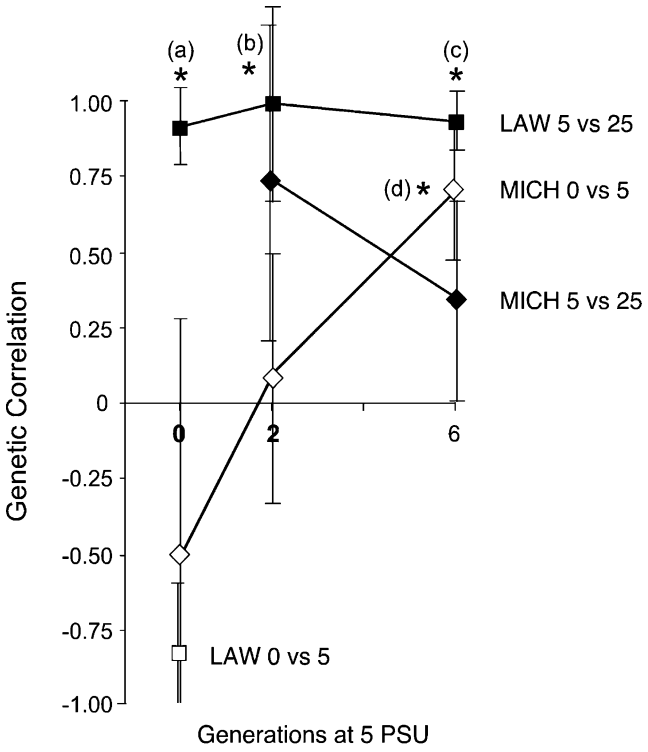

Fig. 5 Genetic correlations between clutch means for survival at different salinities for the saline St. Lawrence (LAW) and freshwater Lake Michigan (MICH) populations after rearing at native salinities (0 generations) and 2, and 6 generations at 5 PSU. Graph shows genetic correlations ± standard error between survival at 0 vs. 5 PSU (white symbols) and 5 vs. 25 PSU (black symbols). Genetic correlations were measured for survival to at least metamorphosis using an ordinal probit model. Stars on graph indicate values that were significantly different from zero using a likelihood-ratio test, where (**a**) $T = 6.92$, $P = 0.0085$ (**b**) $T = 4.65$, $P = 0.031$ (**c**) $T = 11.83$, $P = 0.0005$ (**d**) $T = 4.31$, $P = 0.038$. The genetic correlation between 5 and 25 PSU for the Lake Michigan population failed to converge to a maximum likelihood estimate. No estimates were available for genetic correlations between 0 and 5 PSU for the St. Lawrence population at Generations 2 and 6, as there were no survivors at 0 PSU

were positive for both populations (Fig. 5). In some cases, genetic correlations could not be estimated (e.g. MICH 5 vs. 25 PSU, Generation 0) because algorithmic convergence to a maximum likelihood estimate was not achieved due to small sample size, particularly in cases of high mortality at extreme salinities. Estimates of genetic correlations using a probit model in this study (Fig. 5) were similar to those using a logit model in the previous study (Lee et al. 2003).

Evolutionary differences in survival between saline and freshwater populations

Evolutionary differences in freshwater tolerance between the saline and freshwater populations were maintained following selection at 5 PSU, whereas high-salinity tolerance (at 25 PSU) converged (Fig. 4). While freshwater tolerance declined in response to selection at 5 PSU in both populations, it remained significantly higher in the freshwater population (Fig. 4; Generation 0: $P < 0.0001$; Generation 2: to metamorphosis: $P = 0.029$, to adult: $P = 0.070$; Generation 6: $P < 0.0001$). High-salinity tolerance (at 25 PSU) converged between the populations following selection at 5 PSU, and the significant difference between the populations at Generations 0 and 2 ($P < 0.0001$) was no longer present at Generation 6 ($P > 0.2$). This convergence resulted from an increase in high-salinity tolerance in the freshwater population, along with a decrease in high-salinity tolerance in the saline population (Fig. 4).

Effects of selection at 5 PSU on development time across salinities

There was no clear trend in development time across experiments (generations at 5 PSU), except for a significant increase in juvenile development time (from metamorphosis to adult) for the freshwater population at 0 PSU at Generation 6 (Fig. 6C; Tukey-Kramer: Generation 0 vs. 6, $P = 0.0010$; Generation 2 vs. 6, $P = 0.016$). While juvenile development time was in some cases lower at Generation 2 for both populations, there was no consistent trend across experiments (Fig. 6). For larval development time (from hatching to metamorphosis), there was no significant difference across experiments for both populations (Fig. 6; ANOVA: $P > 0.10$ for all salinities).

Across all experiments and salinities, development time to adulthood was significantly greater for females (27.60 days ± 0.44 SE) than for males (23.33 days ± 0.39 SE) (ANOVA; $F = 49.32$, DenDF = 708, $P < 0.0001$), while development time to metamorphosis did not differ significantly between the sexes (females: 9.98 days ± 0.14 SE, males: 9.93 ± 0.13 SE; ANOVA; $F = 2.60$, DenDF = 708, $P = 0.11$). An uneven sex ratio did not cause spurious significance in the results, as the ratio was fairly constant across treatments and experiments (proportion of females = 0.44% ± 0.03 SE).

Evolutionary differences in development time between saline and freshwater populations

Across all experiments and salinities, the freshwater population showed a general pattern of retarded larval development (from hatching to metamorphosis) (Fig. 6; ANOVA; $F = 45.91$, DenDF = 55.1, $P < 0.0001$) and

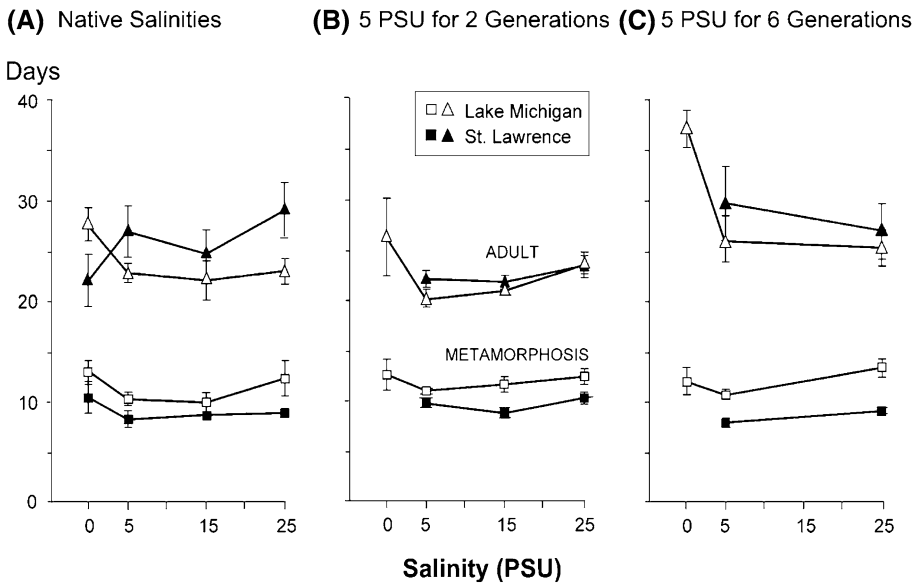

Fig. 6 Development time to metamorphosis and adult stages for the saline St. Lawrence and freshwater Lake Michigan populations. Graphs show mean development time (in days) ± SE for clutches taken from parental populations raised at (**A**) native salinities (15 PSU for the saline and 0 PSU for the freshwater populations) for at least two generations (N = 8 clutches per population), (**B**) 5 PSU for two generations (N = 12 clutches), and (**C**) 5 PSU for six generations (N = 14 clutches). The data point for the St. Lawrence (saline) population at 0 PSU in (**A**) is based on only 2 surviving clutches

accelerated juvenile development (from metamorphosis to adult) (ANOVA; F = 13.39, DenDF = 60.2, P = 0.0005) relative to the saline population. Interestingly, the retarded larval and accelerated juvenile development times resulted in no significant net change in development time from hatching to adult (ANOVA; F = 2.79, DenDF = 59.9, P = 0.100). This pattern was reported previously for the first experiment (Fig. 6A) (Lee et al. 2003). A striking exception to this pattern was the relatively rapid development time of the two surviving clutches in the saline St. Lawrence population at 0 PSU (Fig. 6A), where juvenile development time was not significantly different from that of the freshwater population (Tukey-Kramer, P = 0.57). The saline population had no survival at 0 PSU at Generations 2 and 6, precluding a comparison of development time in these cases.

Estimates of broad-sense heritability for survival and development time

Broad-sense heritability (h^2) was estimated by calculating intraclass correlations among full-sibs clutches and multiplying by two (see Methods). Estimates of broad-sense heritability do not exclude dominance and maternal effects, and yield upper-bound values for heritability (Falconer and Mackay 1996). Estimates of broad-sense heritability tended to be high for some populations, in some cases exceeding 1. These high values suggest that maternal genetic or environmental effects could have contributed to the estimates of clutch effects.

Values for intraclass correlations did not vary significantly across experiments (generations at 5 PSU) for a given salinity (likelihood-ratio test; $P > 0.09$). Values tended to be highest for survival at 0 PSU. For the freshwater population, values for intraclass correlations for survival from hatching to metamorphosis were 0.70 ± 0.10 SE at 0 PSU (N = 33 clutches), 0.27 ± 0.11 SE at 5 PSU (N = 33), and 0.23 ± 0.13 SE at 25 PSU (N = 27). For the saline population, intraclass correlations were 0.79 ± 0.16 SE at 0 PSU (N = 29 clutches), 0.32 ± 0.12 SE at 5 PSU (N = 30), and 0.51 ± 0.12 SE at 25 PSU (N = 30). Survival to metamorphosis was used to estimate heritability for survival because most of the mortality (and selection on salinity tolerance) occurred prior to metamorphosis. Heritability values are not reported for the 15 PSU treatment because polluted water, accidentally introduced in the first experiment (Generation 0), introduced stochastic noise in the survival data. Heritability could not be measured from the selection response, as there was no response to selection at 5 PSU (Fig. 4). As in the case for survival, intraclass correlations for development time often exceeded 0.5, and translated into broad-sense heritabilities exceeding 1.

Discussion

Selection response has seldom been demonstrated for fitness-related traits in the context of range expansions (Donohue et al. 2005a). The presence of such a response would imply that the population could evolve to colonize novel habitats. Most striking in this study was that both saline ancestral (St. Lawrence marsh) and freshwater (Lake Michigan) populations exhibited a significant response to selection following a shift to a constant 5 PSU in the laboratory (Figs. 3, 4). Selection response would depend on genetic variation found in a trait, heritability of a trait, and also on genetic correlations among all selected traits (Lande and Arnold 1983). We would have expected the negative genetic correlations and tradeoffs between saline and freshwater tolerance (Fig. 5; Lee et al. 2003) to have lead to fixation for the optimal phenotypes in the saline and freshwater habitats. Yet, both populations harbored significant levels of genetic variance for tolerance (Fig. 2B; Lee et al. 2003). This presence of genetic variation suggests the presence of mechanisms maintaining variation in both populations in nature (see last section; Fig. 2B; Lee et al. 2003). The mechanisms underlying the maintenance of variation for key quantitative traits might be crucial for understanding factors that allow certain populations to evolve in response to habitat change.

Effects of selection at 5 PSU on salinity tolerance

Genetic correlations between survival at different salinities indicate how selection on survival at a particular salinity would affect survival at other salinities. For example, a negative genetic correlation between 0 and 5 PSU would indicate that selection for increased survival at 0 PSU would also act against survival at 5 PSU. We predicted that such a pattern of selection would operate on both populations, given the negative genetic correlations between 0 and 5 PSU at Generation 0 (Fig. 5; Lee et al. 2003). While these negative genetic correlations were not significantly different from zero using a probit model (Fig. 5; see Methods), genetic correlations between 0 PSU and higher salinities were in several cases significant using a logit model in a previous study (Lee et al. 2003).

For the freshwater population, genetic correlations between 0 and 5 PSU appeared to increase across experiments (Fig. 5). This trend was consistent with selection at 5 PSU acting against polymorphic loci responsible for negative correlations between 0 and 5 PSU, leaving only those with positive correlations. Alternatively, maternal effects resulting from different rearing conditions (native salinities versus 5 PSU) could have contributed to the observed shift in genetic correlations across experiments. However, the increase in genetic correlations between 2 and 6 generations at 5 PSU is consistent with the action of selection rather than the influence of maternal rearing environment. Shifts in genetic correlations during selection would alter predictions for phenotypic evolution across generations (Agrawal et al. 2001). In particular, shifts in genetic correlations following invasions into novel habitats would result in an altered evolutionary trajectory from that of the original source population.

Consistent with the negative genetic correlations between survival at 0 and 5 PSU (Fig. 5) (Lee et al. 2003), freshwater tolerance declined significantly in the freshwater population (Lake Michigan) and was completely lost in the saline population (St. Lawrence marsh) (Fig. 4). Freshwater tolerance declined significantly in the freshwater population following two generations of selection at 5 PSU, but persisted at a low frequency even at Generation 6 (Fig. 4). The slow erosion of freshwater tolerance would be consistent with freshwater tolerance being recessive under saline conditions (Wills 1975; Curtsinger et al. 1994).

Selection response of the saline population might have been affected by rearing the saline population at a constant 15 PSU in the laboratory for 9–22 months prior to the selection experiments at 5 PSU (see Methods). In contrast, the freshwater population was never in the lab for more than a few months prior to each selection experiment. Given the negative genetic correlations between 0 PSU and higher salinities at Generation 0 (Fig. 5; Lee et al. 2003), rearing the saline population at a constant 15 PSU prior to the experiments might have resulted in a reduction in genetic variation for tolerance, particularly for low-salinities. Such an effect might have damped the selection response at 5 PSU and reduced the contrasts between the experiments (generations at 5 PSU).

High-salinity tolerance (at 25 PSU) converged between the saline and freshwater populations after six generations at 5 PSU, with an increase in tolerance in the freshwater population and a decline in the saline population (Fig. 4). While the increase in high-salinity tolerance in the freshwater population was consistent with the positive genetic correlations between 5 PSU and higher salinities (Fig. 5) (Lee et al. 2003), the decline in the saline population was not. It is possible that maternal effects (e.g. infection of clutches) might have contributed to the apparent clutch effects and to the estimates of genetic correlations. A half-sib breeding design could be used to disentangle these effects.

For a selection response to exist, salinity tolerance must be heritable (Falconer and Mackay 1996). Broad sense heritabilities for survival and development time (using intraclass correlations) were generally high across salinities, and were particularly high at 0 PSU in both populations. These high values were consistent with the strong selection response, although the excessively high values (exceeding 1) suggest that maternal effects might have contributed to the estimates of heritability. Aside from maternal nutrition or maternal genetic effects, maternal environmental effects, such as infection of clutches, could be mistaken for clutch effects. A paternal half-sib breeding design would effectively remove maternal effects from estimates of heritable genetic variation. In addition, vial effects could have contributed to the estimates of heritability. Because the experiments used replicate vials per clutch, in some cases up to four vials per clutch, it was possible to determine reproducibility of results for each clutch. Vial effects were not significantly responsible for the clutch effects (Lee et al. 2003).

Evolutionary differences between ancestral saline and derived freshwater populations

While the reaction norms for survival appeared to converge between the saline and freshwater populations following selection at 5 PSU, evolutionary differences between the populations were maintained (Fig. 4). While maternal environment might have had some effects on survival response, particularly at Generation 0 (experiment 1), they were unlikely to account fully for the significant differences in survival and development time between the saline and freshwater populations in all three experiments (Figs. 3, 6).

Reaction norms for development time revealed curious patterns of life-history evolution between the saline and freshwater populations (Fig. 6). Prior to metamorphosis, the freshwater population exhibited retarded development relative to the saline population (Fig. 6). Conversely, the freshwater population exhibited a pattern of accelerated juvenile development (metamorphosis to adulthood; Fig. 6). This pattern was replicated in three independent experiments (Fig. 6). An exception to the pattern was the relatively rapid juvenile development of the saline population at 0 PSU at Generation 0 (Fig. 6A), which resulted from development within the two surviving clutches. Selection for freshwater tolerance might have selected for rapid juvenile development within the saline population.

The opposing patterns of life-history evolution suggest that different selective forces might be operating on early versus later life-history stages. Given that most of the mortality due to osmotic stress occurs at the early life-history stages (Fig. 3; Lee et al. 2003), retarded development at the larval stage might reflect a tradeoff between osmotic tolerance and development rate. Such a result is concordant with studies that show increased development time in response to selection for stress tolerance (Barrera and Medialdea 1996; Chippindale et al. 1998; Harshman et al. 1999). In general, development rate is 1.5–2 times slower in freshwater calanoid copepods relative to their brackish or marine counterparts (Peterson 2001).

In contrast, accelerated development from metamorphosis to the adult stage (Fig. 6) might reflect antagonistic pleiotropy between different life history stages, or the action of other selective forces, such as escape from a high parasite load in fresh water at later stages (Ebert 1995; Stirnadel and Ebert 1997). The parasite load in fresh water is very high. This parasite load appears to be higher on adults than on larvae of *E. affinis* (G. W. Gelembiuk, pers. obs.), such that accelerated development at later stages could shorten the period of susceptibility.

Sources of genetic substrate for selection

The clear selection response at 5 PSU indicates that both the saline and freshwater populations harbor sufficient levels of genetic variation for relevant phenotypic traits upon which selection could act (i.e. additive genetic variance, V_A) (Crow and Kimura 1970). Both the saline and freshwater populations contain significant genetic variation in reaction norms ($G \times E$) for both survival and development time (Fig. 3) (Lee et al. 2003). Given the negative genetic correlations, one would expect selection under constant conditions would act against the maintenance of variation for tolerance in each population. What is interesting and surprising is that suboptimal genotypes are maintained at relatively high frequencies in both saline and freshwater populations (Fig. 2B). The freshwater population should have become fixed for increased freshwater tolerance and reduced high-salinity tolerance over time, given the constant low salinity of freshwater environments. The Lake Michigan population is at least 46-years-old (150–200 generations), which would be expected to provide sufficient time for selection to act against high-salinity tolerance. The presence of genetic variation in tolerance could be due to insufficient time to drive freshwater adaptation to fixation due to overdominance, the

presence of overlapping generations in the form of an egg bank preserving polymorphism, or antagonistic pleiotropy with some other trait maintaining variation.

In the case of the saline population, genetic variation for tolerance could be maintained by some form of balancing selection, either through spatial heterogeneity or fluctuating salinity in the saline source environment (Hairston and Dillon 1990; Schemske and Bierzychudek 2001; Turelli and Barton 2004). Saline environments, such as salt marshes and estuaries tend to have fluctuating salinities over tidal and seasonal time scales. The selection regime in the native source range is likely to affect the evolutionary and invasive potential of populations. Fluctuating selection might constitute a widespread mechanism for the evolution of invasive populations, and might account for the observation that invaders into the Great Lakes originate largely from fluctuating conditions of the Ponto-Caspian Seas (Lee and Bell 1999; Ricciardi and MacIsaac 2000).

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References

Agrawal AF, Brodie ED, Rieseberg LH (2001) Possible consequences of genes of major effect: transient changes in the G-matrix. Genetica 112:33–43

Albert JH, Chib S (2001) Sequential ordinal modeling with applications to survival data. Biometrics 57:829–836

Barrera R, Medialdea V (1996) Development time and resistance to starvation of mosquito larvae. J Nat History 30:447–458

Billingsley P (1986) Probability and measure (2nd edn). John Wiley and Sons, New York

Blair AC, Wolfe LM (2004) The evolution of an invasive plant: an experimental study with *Silene latifolia*. Ecology 85:3035–3042

Bossdorf O, Auge H, Lafuma L, Rogers WE, Siemann E, Prati D (2005) Phenotypic and genetic differentiation between native and introduced plant populations. Oecologia 144:1–11

Carroll SP, Dingle H, Famula TR, Fox CW (2001) Genetic architecture of adaptive differentiation in evolving host races of the soapberry bug, *Jadera haematoloma*. Genetica 112:257–272

Chippindale AK, Gibbs AG, Sheik M, Yee KJ, Djawdan M, Bradley TJ, Rose MR (1998) Resource acquisition and the evolution of stress resistance in *Drosophila melanogaster*. Evolution 52:1342–1352

Colwell RR (2004) Perspectives: infectious disease and environment: cholera as a paradigm for waterborne disease. Int Microbiol 7:285–289

Crow JF, Kimura M (1970) An introduction to population genetics theory. Harper and Row, Publishers, New York

Curtsinger JW, Service PM, Prout T (1994) Antagonistic pleiotropy, reversal of dominance, and genetic-polymorphism. Am Nat 144:210–228

Donohue K, Dorn L, Griffith C, Kim E, Aguiler A, Polisetty CR, Schmitt J (2005a) The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. Evolution 59:740–757

Donohue K, Dorn L, Griffith C, Kim E, Aguilera A, Polisetty CR, Schmitt J (2005b) The evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. Evolution 59:758–770

Dunstan GA, Brown MR, Volkman JK (2005) Cryptophyceae and rhodophyceae; chemotaxonomy, phylogeny, and application. Phytochemistry 66:2557–2570

Ebert D (1995) The ecological interactions between a microsporidian parasite and its host *Daphnia magna*. J Anim Ecol 64:361–369

Engel RA (1962) *Eurytemora affinis*, a calanoid copepod new to Lake Erie. Ohio J Sci 62:252

Faber DJ, Jermolajev EG, Kossiakina EG (1966) A new copepod genus in the plankton of the Great Lakes. Limnol Oceanogr 11:301–303

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics. Prentice Hall, New York, NY

Gibbs AG (2002) Water balance in desert *Drosophila*: lessons from non-charismatic microfauna. Comp Biochem Physiol A-Mol Integ Physiol 133:781–789

Hairston NG, Dillon TA (1990) Fluctuating selection and response in a population of fresh-water copepods. Evolution 44:1796–1805

Harshman LG, Hoffmann AA, Clark AG (1999) Selection for starvation resistance in *Drosophila melanogaster*: physiological correlates, enzyme activities and multiple stress responses. J Evol Biol 12:370–379

Huey RB, Gilchrist GW, Carlson ML, Berrigan D, Serra L (2000) Rapid evolution of a geographic cline in size in an introduced fly. Science 287:308–309

Khlebovich VV, Abramova EN (2000) Some problems of crustacean taxonomy related to the phenomenon of Horohalinicum. Hydrobiologia 417:109–113

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Lee CE (1999) Rapid and repeated invasions of fresh water by the saltwater copepod *Eurytemora affinis*. Evolution 53:1423–1434

Lee CE (2000) Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate ''populations''. Evolution 54:2014–2027

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391

Lee CE, Bell MA (1999) Causes and consequences of recent freshwater invasions by saltwater animals. Trends Ecol Evol 14:284–288

Lee CE, Frost BW (2002) Morphological stasis in the *Eurytemora affinis* species complex (Copepoda: Temoridae). Hydrobiologia 480:111–128

Lee CE, Petersen CH (2003) Effects of developmental acclimation on adult salinity tolerance in the freshwater-invading copepod *Eurytemora affinis*. Physiol Biochem Zool 76:296–301

Lee CE, Petersen CH (2002) Genotype-by-environment interaction for salinity tolerance in the freshwater invading copepod *Eurytemora affinis*. Physiol Biochem Zool 75:335–344

Lee CE, Remfert JL, Gelembiuk GW (2003) Evolution of physiological tolerance and performance during freshwater invasions. Integr Comp Biol 43:439–449

Parker IM, Rodriguez J, Loik ME (2003) An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conserv Biol 17:59–72

Peterson WT (2001) Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance. Hydrobiologia 453–454:91–105

Piasecki W, Goodwin AE, Eiras JC, Nowak BF (2004) Importance of copepoda in freshwater aquaculture. Zool Stud 43:193–205

Reznick DN, Ghalambor CK (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112:183–198

Ricciardi A, MacIsaac HJ (2000) Recent mass invasion of the North American Great Lakes by Ponto-Caspian species. Trends Ecol Evol 15:62–65

Santos M, Cespedes W, Balanya J, Trotta V, Calboli FCF, Fontdevila A, Serra L (2005) Temperature-related genetic changes in laboratory populations of *Drosophila subobscura:* evidence against simple climatic-based explanations for latitudinal clines. Am Nat 165:258–273

SAS (2003) Version 9.1. SAS Institute Inc., Cary, NC

Schemske DW, Bierzychudek P (2001) Perspective: evolution of flower color in the desert annual Linanthus parryae: Wright revisited. Evolution 55:1269–1282

Stirnadel HA, Ebert D (1997) Prevalence, host specificity and impact on host fecundity of microparasites and epibionts in three sympatric *Daphnia* species. J Anim Ecol 66:212–222

Turelli M, Barton NH (2004) Polygenic variation maintained by balancing selection: pleiotropy, sex-dependent allelic effects and GxE interactions. Genetics 166:1053–1079

Vanderploeg HA, Liebig JR, Gluck AA (1996) Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha*): the importance of size and polyunsaturated fatty acid content. J Great Lakes Res 22:36–45

Weinig C (2000a) Plasticity versus canalization: population differences in the timing of shade-avoidance responses. Evolution 54:441–451

Weinig C (2000b) Limits to adaptive plasticity: temperature and photoperiod influence shade-avoidance responses. Am J Bot 87:1660–1668

Weinig C, Delph LF (2001) Phenotypic plasticity early in life constrains developmental responses later. Evolution 55:930–936

Williamson M, Fitter A (1996) The varying success of invaders. Ecology 77:1661–1666

Wills C (1975) Marginal overdominance in *Drosophila*. Genetics 81:177–189