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Rapid evolution of cold tolerance in stickleback

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Climate change is predicted to lead to increased average temperatures and greater intensity and frequency of high and low temperature extremes, but the evolutionary consequences for biological communities are not well understood. Studies of adaptive evolution of temperature tolerance have typically involved correlative analyses of natural populations or artificial selection experiments in the laboratory. Field experiments are required to provide estimates of the timing and strength of natural selection, enhance understanding of the genetics of adaptation and yield insights into the mechanisms driving evolutionary change. Here, we report the experimental evolution of cold tolerance in natural populations of threespine stickleback fish (*Gasterosteus aculeatus*). We show that freshwater sticklebacks are able to tolerate lower minimum temperatures than marine sticklebacks and that this difference is heritable. We transplanted marine sticklebacks to freshwater ponds and measured the rate of evolution after three generations in this environment. Cold tolerance evolved at a rate of 0.63 *haldanes* to a value 2.5°C lower than that of the ancestral population, matching values found in wild freshwater populations. Our results suggest that cold tolerance is under strong selection and that marine sticklebacks carry sufficient genetic variation to adapt to changes in temperature over remarkably short time scales.

Keywords: evolutionary rates; contemporary evolution; climate change; transplant experiment; phenotypic change; adaptation

1. INTRODUCTION

It has been suggested that climate change will have substantial effects on global biodiversity through increased extinction risk for many species [1–6]. Historically, species have been viewed as relatively fixed entities that cannot evolve in response to changing climate [7]. However, accumulating evidence is showing that shifts in climate have led to heritable changes in a wide variety of taxa, rendering the need to incorporate knowledge of evolutionary processes into conservation and management policy [8–10]. Studies investigating evolutionary responses to climate change have generally involved observation of correlated change between an environmental stress (e.g. temperature) and a phenotypic trait associated with tolerance of the stress [11–19]. Experiments are needed to rigorously evaluate the cause and effect relationships underlying adaptation to climate change. Artificial selection experiments have demonstrated that species can be limited in their adaptive potential owing to low levels of genetic variation in traits required for survival [3,20], but these results may not be applicable to natural populations. Field experiments

that directly measure rates of evolution in response to natural selection imposed by changes in temperature will help to determine whether wild populations have the ability to adapt rapidly enough to survive climate change. Here, we combine surveys of temperature tolerance in wild populations of threespine stickleback (*Gasterosteus aculeatus*) with laboratory crosses and transplant experiments to show that heritable differences between populations can permit evolutionary responses of sufficient magnitude to permit adaptation to a changed thermal regime.

2. METHODS

(a) *Environmental temperatures*

Threespine sticklebacks occur in marine and freshwater environments, which differ in temperature regime. We obtained water temperature data from the British Columbia Lighthouse Data Archive of the Canadian Department of Fisheries and Oceans and the Freshwater Lakes Data Archive of the British Columbia Ministry of the Environment (see electronic supplementary material, table S2, for locations). We recorded water temperature from Oyster Lagoon, British Columbia and experimental freshwater ponds at the University of British Columbia, Vancouver, using Hobo Data Loggers (The Weather Shop, Westham, UK). All temperatures were recorded from a depth of 2 m or less.

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(b) Sample populations

We collected adult sticklebacks in April and May 2006, May 2007 and September 2008 from two marine and two freshwater locations in southwestern British Columbia (electronic supplementary material, table S1). Approximately 60 individuals were sampled from each location except Oyster Lagoon, where we sampled approximately 250 individuals. We transplanted all fish to 102 l glass aquaria. We maintained a density of 15–20 fish per aquaria, salinity of 6–10 ppt (gradually decreased to 0 ppt within three weeks), water temperature of $17 \pm 2°C$ and a photoperiod of 14 L : 10 D. To allow individual identification, we injected each fish subepidermally with a fluorescent visible implant elastomer tag (Northwest Marine Technology, Shaw Island, WA, USA) using a 29-gauge syringe.

(c) Crossing design

To test whether any population differences in temperature tolerance are heritable, we generated F_1 crosses from within each population (30 families) and also between a marine and a freshwater population (eight families), and reared offspring in the laboratory under a common constant temperature of $17°C$. To make a cross, we first equally distributed a female's eggs into a Petri dish containing fresh water supplemented with salt (5 ppt, pH 7; Instant Ocean synthetic seasalt, Aquarium Systems, Inc., Mentor, OH, USA). We then sacrificed a male using MS-222 and removed the testes. We placed the testes in a Petri dish and crushed them to release sperm. We left the clutches of eggs and the sperm for 20 min and then placed them into separate plastic egg cups (pint cups with a fine fibreglass mesh lining the bottom) and submerged each into a separate 102 l tank. We added methylene blue to egg tanks to reduce fungal growth and removed any eggs that became inviable owing to fungal growth. After eggs hatched and larvae dropped into the tanks, we removed the cups and any unhatched eggs.

(d) Experimental rearing

We fed larvae live brine shrimp twice per day for six weeks and then frozen *Daphnia* and blood worms once per day until 12 weeks of age, followed by a blood worm diet. After feeding stopped, we removed any remaining food by filtration or manual siphoning, ensuring that each individual was fed to satiation.

(e) Thermal tolerance testing

All fish were acclimated to laboratory conditions for a minimum of three weeks before they were tested for thermal tolerance. Laboratory-raised F_1s were tested once they reached approximately 30 mm in length. We assessed temperature tolerance using critical thermal maximum (CTMax) and critical thermal minimum (CTMin), defined as the upper and lower temperatures, respectively, at which fish lose the ability to escape conditions that will ultimately lead to death [21]. In the laboratory, CTMax and CTMin are usually estimated as the temperatures at which loss of equilibrium occurs following gradual heating or cooling as an empirical endpoint [22]. Our experimental set-up consisted of two rectangular plastic water baths (50 × 35 × 15 cm) each containing 10 individuals in plastic test beakers. The water baths were filled with nitrogen glycol that could be either cooled or heated by adding dry ice or the use of electrical heaters, respectively. Cooling and warming rates were maintained between 0.28 and $0.33°C \, min^{-1}$. We individually aerated each beaker to maintain saturated oxygen

concentration and prevent thermal stratification. We continued testing until each fish reached CTMin or CTMax. CTMin values were highly repeatable over varying lengths of acclimation to the laboratory (electronic supplementary material, figure S2). Repeated CTMax trials were not run on the same individual because reaching CTMax is sometimes lethal.

(f) Selection experiment

To determine the rate at which cold tolerance can evolve in response to a change in temperature regime, we measured cold tolerance in populations of marine stickleback that had been experimentally introduced to three freshwater ponds 2 years previously and that had survived two winters during which water temperatures had dropped below the minimum seen in the marine environment (electronic supplementary material, figure S1). The ponds are located at the University of British Columbia, Vancouver, British Columbia, Canada, and measure 23 × 23 m, with a maximum depth of 3 m in the centre, as described [23]. Like many coastal lakes in British Columbia, the ponds are lined with sand and bordered with limestone. All ponds had been previously drained, cleaned and refilled in 2001, allowing plant and invertebrate communities to re-establish, but remaining free of fish until this experiment. The plants and invertebrates used to seed the ponds were collected from Paxton Lake, Texada Island, British Columbia, an 11 ha lake that contains wild sticklebacks. Apart from their construction, initialization and use in prior experiments, the ponds are unmanipulated environments. In previous experiments, these ponds have sustained large populations of sticklebacks over multiple generations, with life cycles and diets characteristic of their wild source populations [24]. Growth rates of fish in the ponds are similar to those of wild fish in freshwater lakes [25].

On 1 June 2006, we introduced marine sticklebacks from Oyster Lagoon into the ponds (pond 1, $n = 45$; pond 2, $n = 46$; pond 3, $n = 46$). This experimental colonization was part of a study aimed at clarifying mechanisms of selection acting on the lateral plate armour [26], which is greatly reduced in many freshwater populations relative to marine populations [27]. All fish were heterozygous at the *Eda* locus, a gene that controls the lateral plate armour [27]. Within 60 days, we observed larval fish in each colonized pond, indicating that the marine colonizers were breeding. Genotyping of four microsatellite markers confirmed that nearly all alleles present in the parents were at similar frequencies in the progeny, which suggested that founding events did not confer any sampling artefacts (Fisher's combined probability test indicates no significant departure from Hardy–Weinberg equilibrium: parents $\chi^2_{(4)} = 6.303$, $p = 0.178$; progeny $\chi^2_{(4)} = 7.419$, $p = 0.115$). We observed further cohorts of juveniles produced in the ponds in June 2007 and June 2008. In September 2008, we sampled 77 fish (pond 1, $n = 39$; pond 2, $n = 15$; pond 3, $n = 23$) from the third generation (F_3) to test for evolved changes in cold tolerance. We compared the cold tolerance of evolved fish with fish sampled from Oyster Lagoon in September 2008. This Oyster Lagoon sample included both heterozygotes and homozygotes at the *Eda* locus. We found no difference between the mean cold tolerance of this Oyster Lagoon sample and the previous sample from May 2007 (contrast = 0.44 (± 0.58 95% CI)), although there was increased variation in cold tolerance in the 2008 sample (variance ratio test $F_{53,27} = 4.305$, $p < 0.001$). We also found no
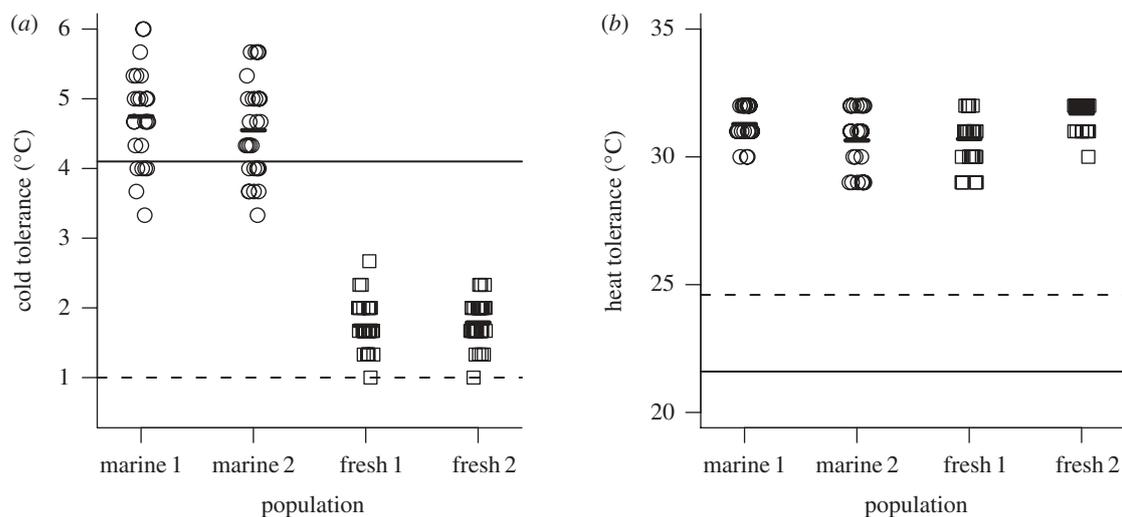
Figure 1. Temperature tolerance of wild stickleback. Circles and squares indicate individual fish from marine and freshwater populations, respectively. (*a*) Cold tolerance of wild stickleback acclimated to 17°C in the laboratory. (*b*) Heat tolerance of laboratory-acclimated wild stickleback. Lines show the (*a*) minimum and (*b*) maximum temperatures from 11 marine sites (solid line) and 14 freshwater lakes (dashed line) in British Columbia (see electronic supplementary material, table S2, for locations). Bars indicate population mean values. All fish were tested within 5 days of each other. Marine 1, Little Campbell; marine 2, Oyster Lagoon; fresh 1, Cranby Lake; fresh 2, Hoggan Lake.

effect of *Eda* genotype on the cold tolerance of Oyster Lagoon or F_3 fish (Oyster Lagoon: ANOVA $F_2 = 0.397$, $p = 0.674$; F_3: ANOVA $F_2 = 0.166$, $p = 0.848$). Before testing, all fish were acclimated in the laboratory for six weeks at 17°C.

(g) *Statistical analysis*

We tested for differences between populations from marine and freshwater by calculating whether the pooled 95% confidence interval for the mean difference exceeded zero. Mean differences were calculated as a vector of constants specifying a linear combination of population means that sum to 1. We used the same method to test for differences between the ancestral population and the F_3 generation in the selection experiment.

3. RESULTS

(a) *Wild populations*

Lakes are warmer in summer and colder in winter than the sea (electronic supplementary material, figure S1). Accordingly, we found significant differences in the cold tolerance between wild marine and freshwater populations (figure 1*a*; mean difference = 2.88 (± 0.20 95% CI); see electronic supplementary material, table S1, for location of populations). Cold tolerance values for laboratory-acclimated marine and freshwater populations overlapped the minimum environmental temperature experienced in their respective habitats (figure 1*a*). In contrast, heat tolerance values for all populations were considerably higher than maximum environmental temperatures, and we detected no significant difference in heat tolerance between marine and freshwater populations (figure 1*b*; mean difference = 0.24 (± 0.33 95% CI)).

(b) *Laboratory-raised populations*

The magnitude of the difference in cold tolerance seen between marine and freshwater populations persisted in the laboratory-raised F_1 generation (figure 2; mean
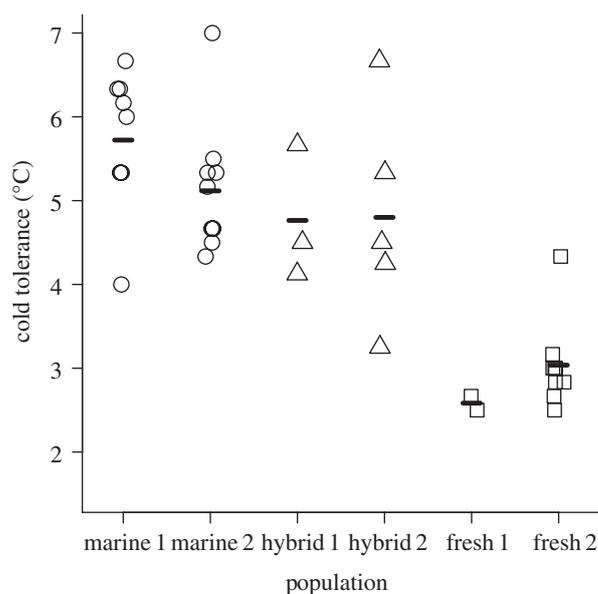

Figure 2. Cold tolerance of laboratory-raised stickleback. Symbols indicate family averages using three or four fish from pure marine (circles), pure freshwater (squares) or marine by freshwater (triangles) crosses. Bars indicate population mean values. All fish were tested within 5 days of each other. Marine 1, Little Campbell; marine 2, Oyster Lagoon; hybrid 1, Oyster Lagoon female crossed to Cranby Lake male; hybrid 2, Cranby Lake female crossed to Oyster Lagoon male; fresh 1, Cranby Lake; fresh 2, Hoggan Lake.

difference = 2.61 (± 0.66 95% CI)), suggesting that population differences measured after an adequate acclimation period in the laboratory are not due to phenotypic plasticity caused by environmental temperatures experienced during development. Cold tolerance values were similar between the reciprocal F_1 crosses between a marine and a freshwater population, suggesting there were no maternal effects on cold tolerance (figure 2; difference = 0.02 (± 1.02 95% CI)).
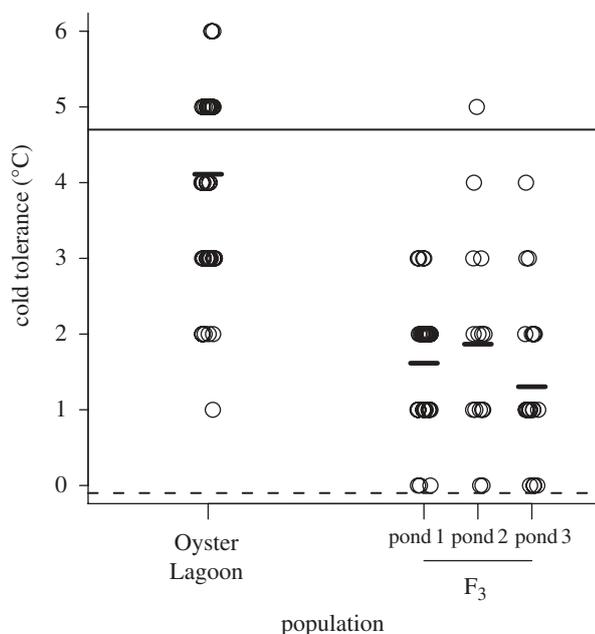
Figure 3. Rapid evolution of cold tolerance in a marine population of stickleback transplanted to freshwater. Circles indicate individuals from the ancestral population (Oyster Lagoon) for the selection experiment and the F_3 generation in the ponds. Lines show the minimum temperature from Oyster Lagoon, British Columbia (solid line), and averaged from three ponds located at the University of British Columbia, Vancouver, British Columbia (dashed line). All fish were sampled in September 2008, acclimated for six weeks in the laboratory at $17^{\circ}C$ and tested within 5 days of each other. Bars indicate population mean values.

(c) Evolved populations

We observed a strong improvement in cold tolerance of evolved fish relative to the ancestral population (figure 3; mean difference = 2.51 (± 0.44 95% CI)), with replicate ponds showing parallel reductions in cold tolerance. Across three generations, cold tolerance evolved at an estimated average rate of 0.63 *haldanes* (a *haldane* is equal to a change of one phenotypic standard deviation per generation) to a value $2.5^{\circ}C$ lower than that of the ancestral population, attaining values found in wild freshwater populations (mean difference = 0.52 (± 0.84 95% CI)). This rate of phenotypic evolution is among the most rapid to be observed in a natural population [28].

4. DISCUSSION

The ability to tolerate increasingly severe temperature extremes will be crucial for species to adapt to the greater variability in temperature expected from climate change [29–35]. Our results suggest that sufficient genetic variation exists in the ancestral marine stickleback population to permit the rapid evolution of a $2.5^{\circ}C$ shift in cold tolerance. However, we caution against interpreting this result as suggesting that natural populations can adapt to climate change without negative consequences. The strong selection required to shift a phenotypic trait so rapidly can result in large changes to population and ecological dynamics, which may in turn negatively affect population persistence [36–38]. This cost of rapid

adaptation may have been manifested during the winter following our F_3 sample, during which all populations went extinct as temperatures reached the lowest minimum recorded in 39 years for the local area [39]. Alternatively, these extinctions could reflect the limits of adaptation to temperature extremes. The populations may not have been able to evolve cold tolerance low enough to survive the large drop in minimum temperatures, or mechanisms to deal with the indirect effects of cold temperature, such as anoxia caused by ice cover.

This work highlights the utility of transplant experiments for testing the feasibility of rapid evolution in response to climate change. The exceptionally high rates of evolution we observed alter our understanding of the tempo at which temperature tolerance in fish can evolve. It remains to be seen whether stickleback populations living in locations with environmental temperatures closer to their maximum heat tolerance will be capable of adapting to shifts towards warmer temperatures. Moreover, it is not clear whether other species of freshwater fish possess sufficient heritable genetic variation to permit rapid evolutionary change in temperature tolerance. Freshwater stickleback have repeatedly evolved substantial phenotypic differences since the last glacial maximum, and contemporary evolution occurring in 40 years or less has been documented for some traits [40–43]. These changes suggest that stickleback may be capable of unusually rapid adaptive evolution; a similar tempo of evolutionary change may not be possible in other taxa. The observed increase in carbon dioxide concentration since 1750 is predicted to cause a minimum warming of $1.4–4.3^{\circ}C$ above pre-industrial surface temperatures [44], suggesting that fish populations that are captive in lakes and unable to migrate northward will require evolutionary responses at least as large as observed in this study to adapt to climate change.

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