Competition moderates the benefits of thermal acclimation to reproductive performance in male eastern mosquitofish

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The reproductive behaviour of the sexually coercive male eastern mosquitofish (Gambusia holbrooki) offers an excellent model system for testing the benefits of reversible thermal acclimation responses to mating success. We acclimated male mosquitofish to either 18 or 30°C (14 h light : 10 h dark) for six weeks and tested their ability to obtain coercive copulations in the presence and the absence of male–male competition. Based on the beneficial acclimation hypothesis, we predicted for both sets of experiments that 18°C acclimated males would outperform 30°C acclimated males when tested at 18°C, and vice versa when tested at 30°C. We found that copulation success was greater for acclimated than non-acclimated males at both temperatures when individual males were tested without competing males. In contrast, when males from the different acclimation treatments were competed against each other for copulations with a single female, the 30°C acclimated males were more aggressive and obtained a greater number of copulations at both test temperatures. Thus, we found a clear benefit for acclimation when fish were tested in a non-competitive environment, but acclimation to cool temperatures was associated with a decrease in aggressive behaviour that reduced mating performance at both test temperatures in a competitive environment. In contrast with the long-held assumption that reversible plasticity is beneficial, the adaptive significance of reversible physiological plasticity is affected by a variety of other ecological factors and is more complex than previously suggested.

Keywords: temperature; beneficial acclimation hypothesis; Gambusia holbrooki

1. INTRODUCTION

Whole-animal traits such as metabolic rate and locomotor performance govern much of the behavioural activities of ectotherms and are highly dependent on body temperature (Angilletta et al. 2006). Many ectotherms display phenotypic plasticity to seasonal changes in environmental temperature (Johnston & Temple 2002). Two broad categories of phenotypic plasticity can be distinguished based on the reversibility of the response. During early development, temperature often produces irreversible changes in the phenotype due to the rapid pace of ontogenetic change, resulting in altered developmental outcome (developmental plasticity). In adult stages, phenotypic plasticity to temperature is generally considered reversible and does not lead to the fixation of specific physiological traits (reversible acclimation or phenotypic flexibility; Johnston & Wilson 2005). The distinction between developmental plasticity and reversible acclimation is important when discussing the evolutionary significance of these responses (Wilson & Franklin 2002; Woods & Harrison 2002; Fischer et al. 2003; Angilletta et al. 2006).

The physiological mechanisms underlying thermal acclimation of locomotion have been studied across a range of ectotherms (Johnston & Temple 2002; Deere & Chown 2006). In contrast, the adaptive significance of thermal acclimation responses has only relatively recently received experimental attention. Most such studies have used model organisms to test the long-held assumption that all acclimation responses (developmental and reversible) enhance the fitness of an individual organism, referred to as the beneficial acclimation hypothesis (BAH; Leroi et al. 1994). In contrast with predictions, most experimental tests of the BAH have rejected the generality of the hypothesis (Leroi et al. 1994; Zamudio et al. 1995; Huey & Berrigan 1996; Bennett & Lenski 1997; Huey et al. 1999). Previous tests of the BAH have often used organisms with short generation times, such as the bacterium Escherichia coli and the fruit fly Drosophila. In the majority of these studies, organisms were reared at different temperatures over one or more generations at constant or fluctuating temperatures and as a consequence, developmental plasticity and reversible acclimation responses were confounded (Wilson & Franklin 2002). Thus, the adaptive benefits of reversible acclimation responses still remain to be investigated (Wilson & Franklin 2002).

Clearly, analyses of the benefits of reversible acclimation responses must also use traits that are good proxies of fitness. The effect of temperature on the reproductive behaviour of the male eastern mosquitofish (Gambusia holbrooki) is an excellent system for examining questions related to temperature acclimation and adaptation (Wilson 2005). The mosquitofish is a small live-bearing poeciliid fish native to the southeastern waterways of the United States and has been introduced as a combatant against mosquito
populations across most of the world’s continents. Throughout their native range, *G. holbrooki* experience a wide seasonal range of temperatures and they possess the capacity to acclimatize their locomotor system to different thermal environments (Hammill et al. 2004). Although there is some evidence that cryptic female choice may occur in this species (Langerhans et al. 2005; Condon & Wilson 2006; Wilson et al. in press), males almost solely rely on a coercive strategy to obtain copulations and often use extended bouts of male–male combat to obtain territories around uncooperative females (McPeck 1992; Pilastro et al. 1997). As males attempt these sneaky copulations across a wide seasonal range of temperatures (at least 14–38°C; Wilson 2005), thermal acclimation of mating ability could potentially enhance fitness.

In this study, we tested the benefits of thermal acclimation to the maximum aerobic swimming performance of male *G. holbrooki* (traditional measure of physical capacity) and their ability to obtain coercive copulations in the presence and the absence of male–male competition. Based on the BAH, we predicted for both sets of experiments that 18°C acclimated males would outperform 30°C acclimated males when tested at 18°C, and vice versa when tested at 30°C.

2. MATERIAL AND METHODS

We collected eastern mosquitofish (*G. holbrooki*) from introduced populations along the water channels surrounding Montpellier, France, in July 2003 (water temperatures typically ranged from less than 10°C in winter to greater than 30°C in summer). Fish were maintained in mixed-sexed groups in 40–200 l aquaria at the Gatty Marine Laboratory, St Andrews. Gravid females were kept separate during the latter stages of pregnancy and fry were collected from at least 30 broods. Randomly selected fry were reared individually to maturity in 51 containers at 25 ± 0.5°C. Fish were fed daily on newly hatched brine shrimp. At maturity (approx. four months), 70 males and 70 females were separated into two acclimation temperature treatment groups and maintained in individual containers at 30°C (*N* = 35 males and females) or 18°C (*N* = 35 males and females; 14 h light : 10 h dark). A large number of mature females were also separated into the two acclimation treatment temperatures and maintained in groups of 15 fish in 50 l aquaria.

We tested the sustained swimming performance of each individual fish at both 18 and 30°C after a six-week acclimation period. Following swimming trials, we measured the mating behaviour of each individual male under both non-competitive and competitive conditions at 18 and 30°C. For all experimental tests, the first temperature tested for each individual (or pair) was selected at random. For acute temperature transfers, we changed the temperature at a rate that did not exceed 5°C d⁻¹, which natural populations of *G. holbrooki* routinely experience (Wilson 2005).

(a) Swimming performance

We tested the sustained swimming performance of each individual mosquito fish at 18 and 30°C, as previously described (Hammill et al. 2004). The total time to exhaustion and the water velocity at exhaustion were recorded for each fish and used to calculate *U*ₘₑₓ with the equation (Brett 1964)

\[ U_{\text{max}} = U_t + \left( \frac{T_t}{T_i} U_i \right) \]

where *U*ₜ is the highest speed maintained for an entire 5 min interval; *T*ₜ is the time taken to exhaustion in the final speed interval; *T*ᵢ is the time-interval length (5 min); and *U*ᵢ is the speed increment (2 cm s⁻¹).

(b) Individual mating performance

One week after the completion of swimming trials, we tested the effect of thermal acclimation on individual male mating behaviour in a non-competitive environment. These experiments involved allowing an individual male the opportunity to copulate with two mature females at both 18 and 30°C. The observation tank consisted of a 25 × 20 × 10 cm deep bare glass aquarium with aged tap water (pH 7.0), a corner aeration stone and a 50 W Jager heater in a small section of the tank separated by plastic gauze. We recorded mating behaviour using a high-speed video camera recording at 200 Hz (NAC). We selected separate groups of two female *G. holbrooki* (body lengths 3.7–3.9 cm) for each male observation period (females only used at their acclimation temperature). We used two females for each male to allow multiple mate choices. We used only females that had not given birth during the previous 5 days to avoid the effects of post-partum females on male behaviour (Farr 1989). We introduced females into the observation tank first and following a 20 min settling period, we introduced males and recorded their behaviour for 10 min.

Mating behaviour of each male was recorded for a total of 6 min and the observation time was started when the males first began to follow one of the females. We analysed tapes using a playback function and behaviours were entered into the Behavioural software program ETHOM v. 1.0. Total time each male spent following females (total following time, TFT), the total number of mating attempts (unsuccessful mating attempts and successful copulations) and copulations, and the mating efficiency were recorded for each individual. Mating efficiency was the proportion of total mating attempts (unsuccessful + successful attempts) that resulted in successful copulations. Mating attempts were defined as when a male swings the gonopodium forward and attempts to insert it into the female’s genital opening. We deemed copulations to occur when the gonopodium was inserted into the female’s genital opening and was followed by the characteristic twisting motion to remove the barbed tip (Wilson 2005).

(c) Male–male competition

We tested the effect of thermal acclimation on male mating performance in a competitive environment 5 days after they were used in the non-competitive experiments. Competitive experiments involved competing one male from each acclimation group against each other for the opportunity to copulate with a single female at both 18 and 30°C. As body length is an important determinant of mating success in this species (McPeck 1992; Bisazza & Pilastro 1997), we size-matched the males from each acclimation group. Total body lengths of each fish were determined by recording a lateral image using a Panasonic digital camera attached to a Leica dissection microscope and analysing the images using the Video Pro 32 morphometric program. Each pair of size-matched males differed in body length by less than 0.1 mm.

We assessed the mating behaviour of each competing male during a 20 min observation period in a 30 × 30 × 20 cm deep aquarium containing Java moss and a base of gravel to increase structural complexity. For each pair of fish, we used a
different size-matched virgin female for each test temperature. After introducing males into the aquarium, the observation period was started as soon as one male first began to follow the female. Following each initial test for a pair, we returned males to their host acclimation temperature at a rate of 5 °C d⁻¹ and retested them at the next test temperature after 3 rest days. During the 20 min observation period, we recorded the total time each male spent following the female (TFT), the number of mating attempts and the total number of copulations for each individual. We also counted the total numbers of dominant behaviours displayed by each male towards the competing male (e.g. chasing male away, biting male).

3. STATISTICAL ANALYSIS
We analysed all data using the statistical programming package R or STATISTICA. For all data, the effects of acute changes in temperature and acclimation group were analysed using repeated measures ANOVA (assuming a Poisson distribution for the behavioural ‘count’ data only). For analyses of male–male competitive behaviour, we used data only from one ‘focal’ male from each pair (selected at random) at both test temperatures. We used post hoc Holm–Sidak multiple comparisons to test for significant effects between treatment groups at each test temperature. All results are presented as means ± s.e. Significance was taken at the level of p < 0.05.

4. RESULTS
(a) Swimming performance
We found that $U_{\text{crit}}$ of 30°C acclimated $G. \ holbrooki$ was greater at 30°C than 18°C acclimated fish, and vice versa when both acclimation groups were tested at 18°C ($F_{1,62} = 70.4; p < 0.001$; figure 1). When tested at 30°C, the $U_{\text{crit}}$ of the 30°C acclimated group was 24.5 ± 0.5 cm s⁻¹, which was more than 15% greater than the 18°C acclimated fish (19.1 ± 0.2 cm s⁻¹; Holm–Sidak; T = 4.0; p < 0.001). In contrast, when tested at 18°C, we found that the $U_{\text{crit}}$ of 18°C acclimated $G. \ holbrooki$ was 14.5 ± 0.2 cm s⁻¹, which was more than 20% greater than the 30°C acclimated fish (Holm–Sidak; T = 6.5; p < 0.001).

(b) Individual mating performance
We found that thermal acclimation benefited the mating performance of individual male $G. \ holbrooki$ when tested at their host acclimation temperature in a non-competitive environment (figure 2). The total time male $G. \ holbrooki$ spent following females (TFT) was significantly influenced by test temperature ($F_{1,64} = 9.1; p = 0.004$). In addition, we detected a significant interaction between temperature and acclimation group for TFT ($F_{1,64} = 16.0; p < 0.001$). No difference was detected in TFT between the acclimation groups at the test temperature of 30°C; however, the 18°C males spent significantly more time in pursuit of copulations (302.7 ± 14.3 s) than the warm males ($p < 0.01$; 212.5 ± 20.6 s) at 18°C (figure 2a). The relationship between temperature and the total number of mating attempts was also significantly influenced by acclimation group ($F_{1,64} = 14.6; p < 0.001$). The 30°C males exhibited more copulation attempts when tested at 30°C than the 18°C males ($p < 0.001$), while the 18°C males attempted more copulations at their host temperature ($p < 0.001$; figure 2b).

Figure 1. Effect of temperature on the maximum sustained swimming speeds ($U_{\text{crit}}$) of male eastern mosquitofish ($G. \ holbrooki$) acclimated to either 18 or 30°C. Data represent means ± s.e. (N = 31 for both 18 and 30°C treatments). Significant differences were detected between acclimation groups.

The effect of test temperature on the number of coercive copulations obtained by male $G. \ holbrooki$ was influenced by acclimation group ($F_{1,64} = 11.7; p < 0.001$; figure 2c), with males obtaining a greater number of copulations at their host acclimation temperature. For example, when tested at 30°C, the 30°C males obtained 3.22 ± 0.53 copulations during the observation period, which was significantly greater than the cool males that obtained 1.89 ± 0.29 ($p < 0.001$; figure 2c). We found a significant influence of acclimation group on the relationship between test temperature and mating efficiency ($F_{1,64} = 4.16; p = 0.05$; figure 2d).

(c) Male–male competition
We found that male $G. \ holbrooki$ exposed to 30°C were more aggressive, more sexually active and obtained a greater number of copulations than 18°C males across both test temperatures (figure 3). The total time male $G. \ holbrooki$ spent following females was significantly influenced by test temperature ($F_{1,30} = 3.8; p = 0.05$) and the 30°C males spent a greater amount of time in pursuit of copulations at both test temperatures ($F_{1,30} = 11.8; p = 0.002$; figure 3a). Similarly, temperature markedly influenced the total number of mating attempts for males from both treatment groups ($F_{1,30} = 25.2; p < 0.001$). The 30°C males exhibited almost twice as many copulation attempts as the 18°C males when tested at both temperatures ($F_{1,30} = 14.6; p < 0.001$; figure 3b). At 18°C, 30°C males made 6.5 ± 1.6 attempts during the 20 min observation period while the 18°C males only made 2.1 ± 0.8 attempts (figure 3b).

The 30°C males displayed a greater number of dominant behaviours than the 18°C males at both test temperatures ($F_{1,30} = 8.7; p = 0.006$). Dominant behaviours usually consisted of chasing another male from within the vicinity of the female, fin nipping and gonopodial chopping. When tested at 30°C, the 30°C males displayed 18.3 ± 6.1 dominant behaviours during the observation period, while the cool males only displayed 4.7 ± 3.4 (figure 3c). The total number of coercive copulations obtained by male $G. \ holbrooki$ was dependent on test temperature ($F_{1,30} = 6.95; p < 0.001$) and treatment group ($F_{1,30} = 5.6; p = 0.01$). Thus, the
30°C males obtained a greater number of copulations than the 18°C males at both test temperatures (figure 3d).

5. DISCUSSION
The present study represents the first experimental investigation of the benefits of reversible thermal acclimation responses for reproductive performance. In the absence of male–male competition, we found that cool-acclimated males exhibited greater sexual activity and obtained more copulations at the lower test temperature than warm-acclimated males, and vice versa at the higher temperature. Improvements in the coercive mating ability of male *G. holbrooki* with thermal acclimation were most probably associated with modifications in underlying physiological traits such as aerobic capacity (Johnston & Maitland 1980) and muscle contractile properties (Johnston et al. 1985, 1995). Maximal aerobic performance (Johnston 1993; Hammill et al. 2004) and maximum anaerobic performance (Beddow et al. 1995;
Johnston et al. (1995) are both modified following reversible temperature acclimation in many species, resulting in improved locomotor capacity in the new environment. As with previous studies, we found that male G. holbrooki could thermally acclimate their sustained swimming performance across the temperature range studied. For male G. holbrooki, improved aerobic capacity would most probably support increased sexual activity and higher rates of mating attempts over an extended period.

Despite the advantages of greater aerobic capacity for cool-acclimated males in cool environments, these benefits to mating performance disappeared when tested in a competitive environment. We found that when males from both treatment groups were competed against each other, 30°C acclimated males were more aggressive, more sexually active and subsequently obtained a greater number of copulations than the cool-acclimated males across both temperatures. Thus, it appears that physical capacity is not the only important determinant of mating success for male G. holbrooki when tested under male–male competition. Simply assuming that improvements in locomotor function with acclimation transfers into benefits in fitness-associated measures is not justified as it also depends on the behavioural context. There is clearly a complex interplay between modifications in physical performance and behaviour that determines the fitness consequences of thermal acclimation.

Increases in aggressive behaviour with temperature have been reported for a variety of other fish taxa (Ratnasabapathiet al. 1992). Both acute and long-term exposure to high temperatures was associated with increased aggression in male G. holbrooki. Remarkably, warm-acclimated mosquitofish displayed approximately double the number of aggressively dominant behaviours towards competing males at both test temperatures. Temperature acclimation may generate sustained changes in the output of the neuroendocrine system resulting in altered concentrations of testosterone (Black et al. 2005), serotonin (Lepage et al. 2005) and other hormones controlling aggressive behaviour. Whatever the mechanism, the consequence of this increased aggression was an improved ability for warm-acclimated males to maintain a territory around a female and dominate mating opportunities. Thus, the advantages of improved aerobic capacity and coercive mating ability for the cool-acclimated males at low temperatures may have been directly negated by the aggressive behaviour of the warm-acclimated males. The inability of the cool-acclimated males to spend substantial time within the vicinity of a female resulted in a reduced number of copulations at both temperatures.

Interpreting the ecological significance of reversible acclimation responses is difficult (Wilson & Franklin 2002; Woods & Harrison 2002), especially given the benefits for the mating behaviour of male mosquitofish appear to be context dependent. Clearly, in the absence of male competition, we found a benefit for thermal acclimation to male mating success. However, acclimation to cool temperatures was associated with the suppression of aggressive behaviours that was costly in a competitive environment across both test temperatures. Modifications in physiology and behaviour with acclimation may not only be related to seasonal changes in environment temperature but also variations in biotic factors such as sex ratio. For example, the sex ratio of natural populations of G. holbrooki is more biased towards males in the peak of summer (Vargas & de Sostoa 1996), but often heavily biased towards females in the cooler months (commonly four females to one male; Vargas & de Sostoa 1996). Thus, there may be intense male–male competition for mating attempts in the warm temperatures of summer, which may be greatly diminished during the cooler periods. It is possible that the benefits of cool-temperature acclimation should only be interpreted within the context of limited (or absent) male–male competition, while the benefits of warm acclimation should be assessed in a competitive environment. We suggest that future tests and interpretations of the adaptive benefits of seasonal acclimation responses in mating behaviour would profit from closely matching the ecological circumstances of male–male competition with the corresponding season in nature.

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REFERENCES


