

Mating success at high temperature in highland- and lowland-derived populations as well as in heat knock-down selected *Drosophila buzzatii*

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Abstract

Thermal-stress selection can affect multiple fitness components including mating success. Reproductive success is one of the most inclusive measures of overall fitness, and mating success is a major component of reproduction. However, almost no attention has been spent to test how mating success can be affected by thermal-stress selection. In this study, we examine the mating success in the cactophilic *Drosophila buzzatii* Patterson & Wheeler (Diptera: Drosophilidae) derived from two natural populations that nearly represent the ends of an altitudinal cline for heat knock-down resistance. Furthermore, we extended the analysis using laboratory lines artificially selected for high and low heat knock-down resistance. Mating success at high temperature was found to be higher in the lowland than the highland population after a heat pre-treatment. Moreover, individuals selected for heat knock-down resistance showed higher mating success at high temperature than did individuals selected for low knock-down resistance. These results indicate that adaptation to thermal stress can confer an advantage on fitness-related traits including mating success and highlight the benefits of earlier heat exposure as an adaptive plastic response affecting mating success under stress of higher temperature.

Introduction

Environmental temperature is one of the most important variables that influence the distribution and abundance of species (Hoffmann & Sgrò, 2011; Franks & Hoffmann, 2012). Consequences for fitness after thermal stress can include failure to mate, the cessation of locomotion, and changes in the developmental and survival rates (Hoffmann et al., 2003; Fasolo & Krebs, 2004; Jørgensen et al., 2006). Given that reproductive success is the most inclusive measure of overall fitness (Brooks & Endler, 2001), reproductive fitness components such as mating success are of interest to test for adaptive responses to thermal-stress selection. In contrast to an increasing number of important findings on genetic associations between heat-stress resistance and longevity (reviewed in Vermeulen & Loeschcke, 2007; Bowler & Terblanche, 2008), fewer studies have tested whether mating success is also affected by

thermal-stress selection. Dolgin et al. (2006) found that male mating success consistently responds to artificial selection for culture temperature in the laboratory. However, whether mating success at elevated temperature responds to selection on heat-stress resistance is still unknown.

Knock-down resistance to high temperature (hereafter referred to as KRHT) is one ecologically relevant trait for measuring the response to selection on heat stress in small insects like *Drosophila* species (Huey et al., 1992; Hoffmann et al., 2003; Sørensen et al., 2005). KRHT can regulate the activity of organisms in nature with heat-tolerant individuals being heat-knocked less often than more heat-susceptible ones. Thus, at high temperature, an increased heat-tolerance could result in a demographical advantage for reproduction, as heat-tolerant individuals may be more active to locate resources and better able to pursue females during courtship than heat-sensitive individuals (Kristensen et al., 2007; Loeschcke et al., 2011). Adaptive geographical patterns of KRHT for *Drosophila* species have evolved on different continents,

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where KRHT and other thermal stress-related traits exhibit clinical variation with altitude or latitude of population (Hoffmann et al., 2002; Sørensen et al., 2005; Sarup et al., 2006). For example, an altitudinal cline for KRHT was found in the cactophilic *Drosophila buzzatii* Patterson & Wheeler (Diptera: Drosophilidae) from north-western Argentina (Sørensen et al., 2005), where other stress-related traits as well as longevity and size-related traits also vary clinically with altitude (Norry et al., 2006; Sambucetti et al., 2006). Mating success is an important trait too, that has not yet been explored in altitudinal populations.

Artificial selection experiments have shown moderate to relatively high levels of heritability for resistance to high temperature in *Drosophila* species (Hoffmann et al., 2003; Reusch & Wood, 2007; Bowler & Terblanche, 2008). Experiments of artificial selection in *Drosophila* species offer the possibility to evaluate not only direct, but also correlated responses to selection (Harshman & Hoffmann, 2000), making it an experimental evolutionary tool to identify the relevant traits that are most likely involved in ectothermal adaptation to environmental temperature (Hoffmann et al., 2003). In artificial selection experiments, KRHT has the advantage of allowing selection in both directions (low vs. high resistance) in a simple way. Recently, we found a direct response to bidirectional selection on KRHT in lines of *D. buzzatii* recently derived from a clinal population (Sambucetti et al., 2010). Correlated responses were also tested for other ecologically relevant stress traits such as chill-coma recovery and starvation resistance, but not for mating success.

Here, we examine mating success in *D. buzzatii* derived from two natural populations that represent the ends of an altitudinal cline for heat knock-down resistance in north-western Argentina (Sørensen et al., 2005). We used a competitive design by releasing marked flies from each population within a mating cage at both high and low temperatures. This design is useful to test for sexual selection resulting from either male–male competition or mate choice (Andersson, 1994). This mating design is also suitable for adaptive traits even if natural and sexual selection may oppose each other (Andersson, 1994). We tested the hypothesis that the lowland population of high KRHT has higher mating success at high temperature than the highland population of low KRHT. This hypothesis was also tested after flies were exposed to a heat pre-treatment. Furthermore, we tested this hypothesis by also using laboratory lines artificially selected for high and low KRHT, as the line selected for high KRHT could be expected to have higher mating success at high temperature than the low KRHT line.

Materials and methods

Natural populations

The flies used in this study were collected using banana baits at two localities of north-western Argentina in September 2009. These two populations of *D. buzzatii* are Chumbicha (28.52°S, 66.15°W, 401 m a.s.l.; hereafter referred to as the lowland population) and Quilmes (26.28°S, 65.52°W, 1850 m a.s.l.; highland population) and represent the ends of an altitudinal cline for heat knock-down resistance in north-western Argentina (Sørensen et al., 2005). Mass cultures were set up using 40–50 flies for each locality. Inseminated flies from the G2 laboratory generation were transferred from mass cultures to individual vials to set up isofemale lines. About 20 isofemale lines were derived from the wild flies and inter se crossed to set up the laboratory stock used in this study. This laboratory stock was maintained at 25 °C and L12: D12 photoperiod, with five standard bottles and a mean number of 70 flies per bottle. Standard bottles were 125 ml, containing 30 ml of instant *Drosophila* medium based on instant mashed potato plus water and methylparaben (Parafarm; Droguería Saporiti, Buenos Aires, Argentina).

Artificially selected lines

The lines used in this study were selected from a laboratory stock that was set up in April 2003 from a single wild population sampled at Chumbicha, as described in Norry et al. (2006). The laboratory stock and the selection regime for these lines are described in detail in Sambucetti et al. (2010). Briefly, three sets of replicated lines were set up from the laboratory stock. One set were the control (non-selected) lines which were maintained as three independent replicates (denoted C lines). The other two sets were selected for knock-down resistance to high temperature (KRHT) in three replicated lines each. One of these sets was selected for decreased KRHT (denoted K– lines) and the other set was selected for increased KRHT (K+ lines). After 12 generations of selection, the lines diverged in their KRHT values (Sambucetti et al., 2010). In this study, replicated lines were crossed within each selection regimen (hereafter referred to as F3-hybrid) to reduce any possible inbreeding effects in the inferred correlated responses to selection on mating success (Falconer & Mackay, 1996). To do this, 30 males and 30 females from each replicate line were placed in a standard bottle with fresh medium in the same culture conditions as described above. The F2 was allowed to lay eggs in standard bottles for 2 days to avoid larval crowding and flies emerged from these cultures (F3-hybrids) were used as experimental flies.

Knock-down resistance measurement

Experimental flies were reared avoiding larval crowding as described above. Flies of 1–2 days of age were collected from the cultures and placed in standard bottles with fresh medium. At the age of 3–4 days, 60–100 flies (1:1 sex ratio) were released within a knock-down tube (65 cm long, 3.8 cm diameter) at 37.5 ± 0.1 °C (Huey et al., 1992). KRHT was scored at intervals of 30 s until the last fly was knocked down as described in Norry et al. (2008).

Mating experiment

To obtain experimental individuals, flies were allowed to lay eggs in standard bottles for 2 days to avoid larval crowding as described above. All virgin flies emerging from these cultures were collected, sexed under slight CO₂ anaesthesia, and placed in vials with fresh medium at 25 °C and L12:D12 photoperiod. At the age of 4–5 days, 20 flies per sex and line were released into a transparent rectangular cage (20 × 12 × 10 cm) containing rotting tissues of *Opuntia vulgaris* Mill. (Cactaceae) (a natural host for *D. buzzatii*) and covered with a porous gauze. Three cages were used simultaneously in each assay (see below). Just before release, flies were transferred into vials with 0.0015 (\pm 0.0005) g of fluorescent micronized dust and lightly shaken (Loeschke & Hoffmann, 2007). Dust colours were randomly assigned to the different populations/lines and changed between cages. During the next 4 h after the release within the mating cages, all copulating pairs were collected from these cages without perturbing the surrounding flies by using an aspirating tube. Each fly of every copulating pair was observed at 10× magnification to determine both population/line of origin and sex. The assays were performed at two test temperatures, 25 ± 1 °C as non-stressing temperature and 33 ± 1 °C as stress temperature. The 33 °C assay for the lowland vs. highland population was performed with and without a heat pre-treatment. The heat pre-treatment consisted of exposing flies at 30 ± 1 °C (water bath) for 1 h (13:00–14:00 hours) every day during three consecutive days before the mating assay. The assays were performed twice at each temperature resulting in six replicated mating cages for each treatment.

Statistical analysis

A two-way ANOVA was performed to test for differences in KRHT using sex and population of origin (highland vs. lowland population) or line (K+ vs. K–) as fixed factors. For analysis of the F3-hybrid lines, KRHT was ln-transformed, as this transformation improved normality and removed dependence of variances on means. All analyses were performed with STATISTICA (StatSoft, 1999).

For mating assays, χ^2 tests were performed to test for differences in mating success between either highland vs. lowland populations or K+ vs. K– flies, separately for each sex and temperature by pooling data from replicated mating cages, as there was no differential trend among replicated assays.

Results

Lowland and highland populations differ in KRHT, the lowland population being more heat resistant than the highland population (ANOVA; population: $F_{1,370} = 6.62$, $P < 0.05$; sex: $F_{1,370} = 1.76$, $P = 0.18$; population*sex: $F_{1,370} = 3.33$, $P = 0.07$; Figure 1A). Likewise, K+ flies were more heat-resistant than both C and K– flies (line: $F_{2,290} = 17.88$, $P < 0.001$; sex: $F_{1,290} = 0.41$, $P = 0.52$; line*sex: $F_{2,290} = 0.58$, $P = 0.56$; Figure 1B).

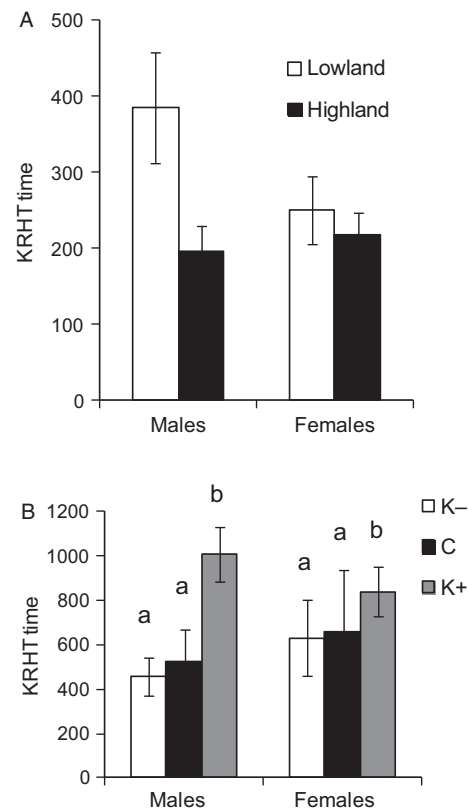


Figure 1 Mean (\pm SE) *Drosophila buzzatii* knock-down resistance to high temperature (KRHT; s) for (A) lowland and highland populations, and (B) flies selected for increased KRHT (K+), decreased KRHT (K–), and their respective controls (C). Means within sex capped with different letters are significantly different (two-way ANOVA, followed by Tukey's pairwise comparisons: $P < 0.001$).

The number of copulating pairs differed between lowland and highland populations at each experimental temperature (Table 1). Importantly, lowland males at 33 °C exhibited a substantially higher mating success than highland males when flies received a heat pre-treatment ($\chi^2 = 20.32$, d.f. = 1, $P < 0.001$; Figure 2). Heat pre-treated females showed no differences in mating success ($\chi^2 = 0.27$, d.f. = 1, $P = 0.6$; Figure 2). In sharp contrast, there was no mating advantage of lowland males at 33 °C without heat pre-treatment, with no significant differences in the proportion of observed matings between lowland and highland flies ($\chi^2 = 2.05$, d.f. = 1, $P = 0.15$; Figure 3). Non-heat pre-treated females at 33 °C also showed no differences in mating success ($\chi^2 = 0.02$, d.f. = 1, $P = 0.89$; Figure 2). At 25 °C (i.e., control temperature), there was no difference in mating success between lowland and highland flies (males: $\chi^2 = 2.32$, d.f. = 1, $P = 0.13$; females: $\chi^2 = 0.83$, d.f. = 1, $P = 0.36$; Figure 2).

Mating assays for artificially selected lines were performed between K+ and K- lines. The number of copulating pairs between K+ and K- males and females at each experimental temperature is shown in Table 2. Importantly, K+ males at 33 °C exhibited a substantially higher mating success than K- males ($\chi^2 = 8.02$, d.f. = 1, $P < 0.01$; Figure 3). In sharp contrast, there was no mating advantage of K+ males at 25 °C, with no significant differences in the proportion of matings between K+ and K- males ($\chi^2 = 1.18$, d.f. = 1, $P = 0.29$; Figure 3). In contrast to males, there was no differential mating success between K+ and K- females (at 25 °C: $\chi^2 = 0.67$, d.f. = 1, $P = 0.41$; at 33 °C: $\chi^2 = 3.07$, d.f. = 1, $P = 0.08$; Figure 3) although K+ females tended to mate more often than K- females at 33 °C (Figure 3).

Discussion

Knock-down resistance to heat was found to be higher in the lowland than in the highland population. This result is consistent with previous findings about negative associations between KRHT and population altitude (e.g., Sørensen et al., 2005). In our mating tests, males from the high

KRHT lowland population displayed a higher mating success than males from the low KRHT highland population at high temperature. The mating success difference was significant only for flies exposed to a heat pre-treatment. This result shows that adaptation to previous and

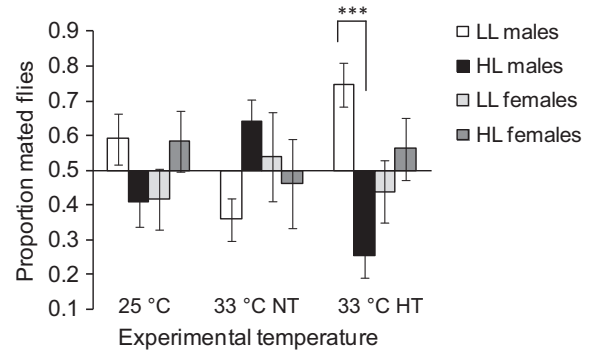


Figure 2 Mean (\pm SE; n = 6) proportion of mated *Drosophila buzzatii* males and females relative to the total number of matings from lowland (LL) and highland (HL) populations at control temperature (25 °C) and heat stress (33 °C), without (NT) and with (HT) a heat pre-treatment. Proportions with asterisks are significantly different (χ^2 -test: $P < 0.001$).

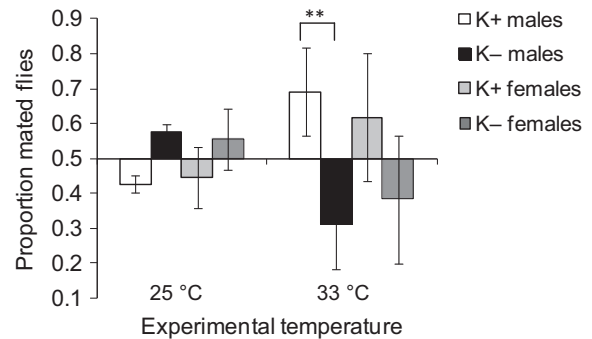


Figure 3 Mean (\pm SE; n = 6) proportion of mated *Drosophila buzzatii* males and females relative to the total number of matings from lines selected for increased (K+) and decreased (K-) knock-down resistance to heat at control temperature (25 °C) and heat stress (33 °C). Proportions with asterisks are significantly different (χ^2 -test: $P < 0.01$).

Table 1 Number of matings between *Drosophila buzzatii* males and females from lowland (LL) and highland (HL) populations at control temperature (25 °C) and heat stress (33 °C), without and with a heat pre-treatment (NT and HT, respectively)

		25 °C			33 °C NT			33 °C HT		
		LL	HL	Total	LL	HL	Total	LL	HL	Total
Females	LL	19	25	44	13	17	30	34	9	43
	HL	37	16	53	11	18	29	33	15	48
	Total	56	41	97	24	35	59	67	24	91

Table 2 Number of matings between *Drosophila buzzatii* males and females of lines selected for increased (K+) and decreased (K-) heat knock-down resistance at control temperature (25 °C) and heat stress (33 °C)

		25 °C			33 °C		
Males		K+	K-	Total	K+	K-	Total
Females	K+	11	13	24	24	10	34
	K-	12	18	30	14	7	21
	Total	23	31	54	38	17	55

repeated exposure to moderate heat can confer not only an advantage in survival but also on other fitness-related traits including mating success in heat-resistant males. Temperature can regulate the activity level of small insects, including the cessation of mobility by heat knock-down. In this study, males from the lowland population persisted active for about twice as long as males from the highland population at high temperature. Thus, heat-tolerant males may be more active and better able to vigorously pursue females during courtship than heat-sensitive males at high temperature, resulting in a demographical advantage for reproduction under relatively high temperature.

Improved thermotolerance after short-term exposure to heat is commonly referred to as heat hardening. Hardening is a plastic change that can be triggered by environmental factors, such as high temperature. Heat-hardening temperature typically induces the heat-shock response, including high expression of molecular chaperones (heat-shock proteins, HSPs). Heat hardening increases heat-stress resistance (Sørensen et al., 2003). Our heat pre-treatment improved mating success at high temperature differentially in lowland as compared to highland populations. Heat hardening might be ecologically relevant particularly in lowland environments, where heat stress occurs more often than in highland populations (Sørensen et al., 2003). Therefore, heat-hardening effects appear to include adaptive plastic responses that influence mating success. The beneficial effects of heat hardening have been tested previously in other fitness-related traits. In *Drosophila melanogaster* Meigen, Loeschcke & Hoffmann (2007) reported beneficial effects of heat hardening on the likelihood of locating resources under warm conditions in the field. Beneficial effects of hardening treatments on male reproductive traits have also been reported in *D. buzzatii* (Jørgensen et al., 2006). However, beneficial effects of hardening are dependent on both the trait and the hardening treatment applied (Jørgensen et al., 2006; Loeschcke & Hoffmann, 2007), as negative effects have also been found (Hoffmann et al., 2003). Our results highlight the benefits

of heat hardening as an adaptive plastic response affecting mating success under heat stress.

Artificial selection for increased KRHT dramatically increased male mating success at high temperature. At our stressful experimental temperature (33 °C), males selected for increased KRHT had a much higher mating success than males selected for low KRHT. This is consistent with the extended knock-down time that was shown by K+ vs. K- males at high temperature. Furthermore, the difference in mating success between K+ and K- males disappeared at benign (25 °C) temperature, indicating that K+ selection chiefly improved mating success at high (33 °C) but not at benign (25 °C) temperature. This clear-cut result was sex-specific, although K+ selected females also showed a similar pattern as K+ selected males (but not significantly so). It is well-known that sexual selection is typically higher in males than in females (Andersson, 1994; Brooks & Endler, 2001). In contrast to highland vs. lowland populations, mating success differences between selected and control lines were detected without any heat pre-treatments. Our results with highland and lowland populations suggest that plastic changes in thermotolerance should be induced by previous exposure to heat stress, with substantial effects expected in natural populations that are more often exposed to high temperature, as discussed above. Our results indicate that selection for increased KRHT can confer an advantage on mating success at elevated temperature. These results are consistent with a previous study in *D. melanogaster* under a totally different thermal selection regime. Specifically, Dolgin et al. (2006) found that laboratory populations adapted to different rearing temperatures exhibit increased mating success in the same thermal environment in which they had evolved.

Field release experiments showed that selection for heat resistance increases the likelihood of locating feeding and breeding resources in *D. melanogaster* under warm conditions in the field (Kristensen et al., 2007; Loeschcke & Hoffmann, 2007). Loeschcke et al. (2011) found consistent effects of a major quantitative trait locus (QTL) for thermal resistance in field-released *D. melanogaster*. Flies carrying the QTL genotype for heat resistance were significantly better able at locating resources in field releases, and males of this genotype mated more often than flies carrying the alternative genotype of low resistance under high temperatures (Loeschcke et al., 2011). Our present results show that male mating success at high temperature also significantly increases by KRHT selection in *D. buzzatii*.

It is well-known that body size is positively correlated with mating success in *Drosophila* species (Partridge et al., 1987; Norry et al., 1995). Larger males tend to court females more often and have a higher mating success than

smaller males (Partridge et al., 1987). Temperature has been implicated as a major factor for the evolution of body size (Brakefield et al., 2003). However, there is no variation in body size between the K+ and K- flies (Sambucetti et al., 2010), which is consistent with many previous studies on heat selection in *Drosophila* species (e.g., Bublly & Loeschcke, 2005). Therefore, even though mating success is typically correlated with size, we can conclude that K+ vs. K- differences in mating success were not related to body size itself. This conclusion can be extended to the lowland and highland population studied here, where thermal adaptation is evident and temperature is the main climatic variation along an altitudinal gradient in northwestern Argentina where these populations came from (Sørensen et al., 2005; Sambucetti et al., 2006). Along this elevation gradient, KRHT and developmental time were negatively and positively correlated with altitude, respectively (Sørensen et al., 2005; Sambucetti et al., 2006). However, there was no altitudinal cline for body size in the above mentioned altitudinal gradient (Sambucetti et al., 2006). Thus, differences in mating success between lowland and highland populations cannot be attributed to differences in body size itself.

KRHT has been shown to consistently form adaptive thermal clines in different continents and species, indicating its ecological relevance (Hoffmann et al., 2002; Sørensen et al., 2005). However, direct fitness components such as mating success at elevated temperature have not been previously tested as correlated responses to heat knock-down selection in insects. This study suggests that mating success can be strongly influenced by selection for KRHT, with important implications of thermal adaptation for reproductive success.

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