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Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline

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Abstract. In fewer than two decades after invading the Americas, the European fly *Drosophila subobscura* evolved latitudinal clines in several traits. Moreover, its chromosomal inversion frequencies at given localities have shifted with climate warming. Temperature may have driven the evolution of both geographic clines and within-site shifts. Nevertheless, whether body temperature (T_b) of active flies actually varies geographically and temporally is unknown: if these flies are effective behavioral thermoregulators, they might maintain relatively constant T_b when active, independent of season and latitude. To evaluate these possibilities, we monitored activity and estimated T_b of active flies in all seasons and at five sites (37–49° N) in western North America. Latitudinal and seasonal shifts in activity are conspicuous. Flies have longer activity seasons (and are much more active) at higher latitudes. Flies are generally active only at midday in cool seasons, and only early and late in the day (if active at all) in warm seasons. Despite these behavioral shifts active flies have much lower T_b in cooler seasons and at higher latitudes. The observed pattern is consistent with the hypothesis that geographic shifts in T_b may be an evolutionary driver of latitudinal clines in this invading species.

Key words: *body temperature; Drosophila subobscura; European fruit fly; invasive species; latitudinal clines; North America; thermoregulation.*

INTRODUCTION

The invasive fly *Drosophila subobscura* serves as a classic example of rapid clinal evolution (Prevosti et al. 1985). Within a decade after its invasion of South and North America from Europe, flies evolved clines in genetic, morphological, and physiological traits that were generally parallel with clines in the Old World (Prevosti et al. 1985, Balanyà et al. 2003, Gilchrist et al. 2004, 2008). For latitudinal clines to evolve so quickly and in parallel with the Old World clines, directional selection must be strong and must vary with latitude. Temperature is a likely selective agent (but see Bradshaw and Holzapfel 2006) for two reasons. First, inversion frequencies correlate with ambient temperature (Balanyà et al. 2006) on all three continents. Second, frequencies of “warm-adapted” inversions have recently increased at numerous sites on three continents,

paralleling observed climate warming at those sites (Balanyà et al. 2006).

Although these correlative patterns strongly suggest a selective role for temperature, its role can be questioned on three grounds. First, *D. subobscura* evolving by laboratory natural selection at different constant temperatures have not evolved the expected shifts in inversion frequencies or in wing size (Santos et al. 2005). Second, the role of temperature as a selective agent in nature is rarely implicated (Feder et al. 1997a, Hoffmann et al. 2003, Loeschcke and Hoffmann 2007). Third and most importantly, latitudinal variation in body temperatures (T_b) of *Drosophila* has always been assumed, but never quantified. Although this assumption might seem reasonable, behavioral adjustments enable some ectotherms to achieve relatively constant T_b at different seasons or latitudes (Stevenson 1985b, Huey et al. 2003). *Drosophila* thermoregulate carefully in laboratory gradients (Dillon et al. 2009) and adjust activity patterns in nature (Pascual et al. 1993, Noor 1998, Argemí et al. 2000). Nevertheless, whether such behavioral shifts compensate fully for seasonal and latitudinal shifts in their thermal environment needs to

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be evaluated. If these shifts are fully compensatory, such that T_b during activity is independent of climate, then activity T_b would seem an unlikely agent of clinal evolution.

Monitoring thermoregulatory behavior and T_b of tiny ectotherms such as flies is challenging (Jones et al. 1987, Feder et al. 1997b, Gibbs et al. 2003). Therefore, we first developed techniques to approximate T_b and to score behavioral patterns of adult flies in nature. We then monitored *D. subobscura* over all seasons at five sites that span most of the latitudinal range of this species in North America (37° to 49° N vs. 34° to 50° N) and evaluated potential shifts in thermoregulatory behavior and T_b of active flies. We found that these flies do exhibit compensatory behavioral shifts, but nonetheless they still show marked geographic and seasonal variation in T_b even while active.

METHODS

Field sites are shown in Fig. 1. Pascual et al. (1993) studied activity patterns of *D. subobscura* at Gilroy (California, 37°0'21" N), Davis (California, 38°31'01" N), and Eureka (California, 40°46'41" N) and sampled each site at two-month intervals (1988–1989). We reanalyze these data here and add comparable data for two northern sites, Bushes Pasture Park, Salem (Oregon, 44°55'49" N), and Sehome Arboretum, Bellingham (Washington, 48°43'43" N). Both sites are forested city parks. Collecting dates for Eureka, Davis, and Gilroy are in Pascual et al. (1993). Dates for Salem were: 15 February 1999, 8 March 1996, 29 April 1995, 26 May 1996, 24 June 1995, 25 July 1996, 14 September 1995, 18 September 1996, and 22 November 1996. Dates for Bellingham were: 14 January 1998, 11 February 1996, 28 April 1996, 20 June 1998, 21 July 1996, and 24 September 1995.

Collecting procedures generally follow Pascual et al. (1993). During each visit we collected flies throughout most of their daily activity cycle. Baits ($N = 13$, each with mashed bananas and yeast) were placed haphazardly and were visited at intervals. Netted flies were identified, counted, and sexed. The number of flies collected during two-hour intervals serves as a crude estimate of relative activity.

Because heat exchange in tiny organisms is dominated by convection, a fly's T_b must approximate ambient temperature (T_a) even in full sun (Stevenson 1985a). Thus to estimate T_b , we counted the number of flies captured during two-hour intervals and assigned to each fly a T_b equal to the local T_a during that interval. For the California localities, T_a was recorded on site. For Salem and Bellingham, hourly T_a was obtained from local weather stations.

To evaluate thermoregulatory effectiveness of flies (see Hertz et al. 1993), we measured the thermal preference (T_p) of these flies in a laboratory gradient. Flies were collected in Woodinville, Washington, in August 2007, maintained for one generation in an

incubator (~22°C), and then tested in a darkened thermal gradient (42 × 66 cm; aluminum base plate, no food) with temperatures ranging from 8° to 40°C. Adults were introduced and given 1 h to adjust, and then their positions were photographed. Because the temperature at each position had been mapped, we digitized each fly's position and then estimated its T_p . We ran three trials simultaneously (in separate lanes): one replicate had been reared at controlled and low density, and two had been reared at uncontrolled densities. We use the central 50% of records to delimit the T_p range (Hertz et al. 1993).

For latitudinal weather data, we extracted maximum daily temperatures from multiple sources (National Climatic Data Service, Weatherbase). For Salem in midsummer, we also approximated the drying power of air, which is proportional to the difference in vapor density between the air and the organism (Kingsolver 1979).

RESULTS

Seasonal and latitudinal variation in ambient temperature and fly activity

Davis and Gilroy (Fig. 1) generally have the warmest maximum daily temperatures (averaged by month), and Bellingham is coldest (see Appendix A). Davis, which is the most inland site, has the most variable temperatures over the year. Eureka, which is coastal, has the least variation.

We use the average number of flies collected per visit at all seasons (Appendix A) as a crude index of relative yearly activity. Average activity at the two high-latitude (thus cool) sites is roughly an order of magnitude higher than at the two southern sites (Appendix A). Average number of flies is positively correlated with latitude (Spearman $\rho = 0.9$, $P = 0.037$) and negatively (but not significantly correlated) with mean maximum temperature over the year ($\rho = -0.736$, $P = 0.156$). (Note: with only five sites, these tests have little power.)

In most sites *D. subobscura* are primarily active during late winter and spring (Fig. 1), and greatly reduce activity in summer. Flies in the most northern site (Bellingham) maintain high activity even during midsummer (Fig. 1), probably because temperatures there remain mild (Appendix A). Flies at southern sites are rarely caught in traps in summer and/or in autumn (Fig. 1). Curiously, flies in Eureka (a coastal site) also show a summer drop in activity, even though temperatures there are not hot (Appendix A). Flies reduce activity in early to midwinter at most sites (Fig. 1). However, a few flies were active in midwinter even at the two high-latitude sites, though only on relatively warm days.

Daily shifts in activity and T_b

Shifting time of activity (seasonally or latitudinally) is an effective way that ectotherms can thermoregulate (Stevenson 1985b, Huey et al. 2003). In winter, *D. subobscura* were active only from early to mid-afternoon

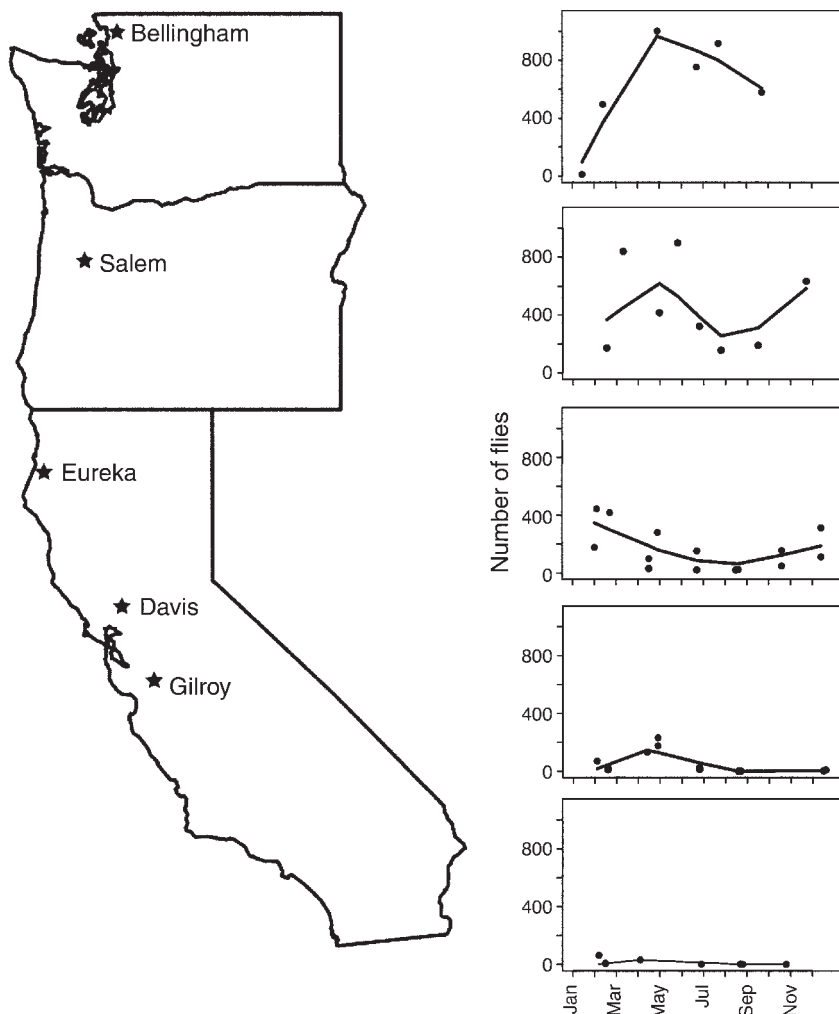


FIG. 1. Map of the western United States showing collecting sites. Panels at right show the number of flies (*Drosophila subobscura*) collected per day over the year at sites, which are arranged (top to bottom) from high to low latitude. More flies are collected per day at the high-latitude sites and during the spring at most sites.

(Fig. 2A). In summer, flies were collected only early in the morning or late in the day, but not at midday. Body temperature estimated at the two-hour interval of maximum activity for each visit is generally between 10° and 23°C (Fig. 2B), except in summer, when higher T_b were observed at a few localities.

Thermal preference in the laboratory

Differences in median thermal preference (T_p) among three replicate trials was minor and not significant (23.5° to 24.0°C; $P = 0.72$). The pooled median T_p was 23.7°C and the central 50% of records ($N = 354$) were bounded by 21.2° to 25.9°C, which is the preferred temperature range (Hertz et al. 1993).

Is thermoregulation effective?

The above behavioral evidence (e.g., regulation of activity time) suggests that these flies are thermoregulating. To evaluate thermoregulatory effectiveness, we

computed the mean T_b of active flies during each season at each site and regressed it against the mean maximum daily temperature for each season and site. (Note: this technique is appropriate for flies because their heat balance is dominated by convection.) If the flies are perfect thermoregulators, mean T_b should show limited variation from day to day, the slope of mean T_b on T_{max} should be ~ 0 (Huey and Slatkin 1976), and mean T_b should approximate 24°C, the preferred body temperature. In fact, mean T_b of active flies on different days varies by 21°C (from 8° to 29°C) and is infrequently in the T_p range. Moreover, the slope is not flat but shows a complex relationship with T_{max} and latitude (Appendix B). Overall, mean T_b during activity is strongly and positively correlated with maximum T_a at any time during the day ($\rho = 0.858$, $P < 0.001$). For the three northern sites, mean T_b parallel (but are $\sim 2^\circ\text{C}$ below) the maximum temperature on that day (Appendix B: mean $T_b = -1.99 + 0.96 \times (\text{maximum } T_a) \pm 0.059$, $P < 0.001$).

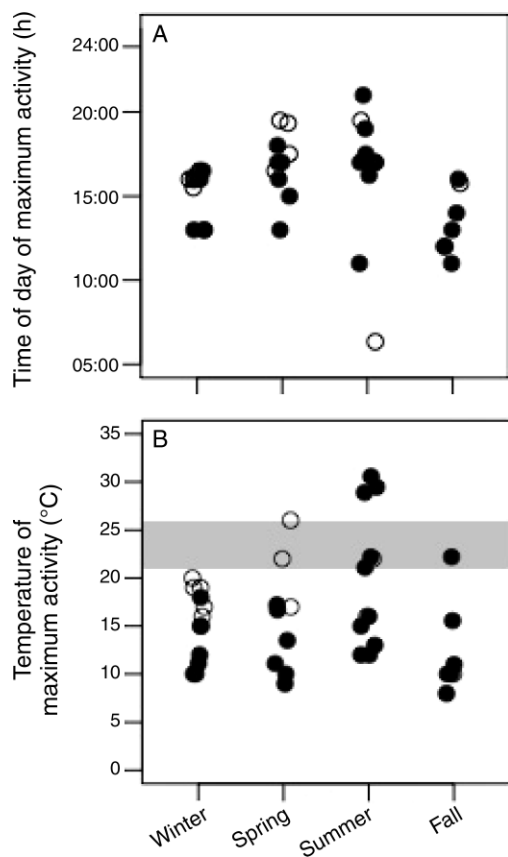


FIG. 2. (A) Time of maximum activity of flies at different seasons. Solid circles are northern sites (Eureka to Bellingham), and open circles are southern sites (Davis, Gilroy). (B) Ambient temperature (equaling body temperature of flies) at the time of maximum activity in different seasons; site symbols are as in panel (A). The preferred temperature range is shown in gray. Points are jittered for clarity.

For the two southern sites, mean T_b is much less related to maximum temperature (slope = 0.27 ± 0.101). Thus, mean activity T_b is far from constant (Appendix B).

We next focused only on the spring season, when fly activity is usually greatest (Fig. 1). Mean T_b increases with average daily maximum temperature for the spring ($\rho = 0.90$, $P = 0.041$) and not with latitude ($\rho = 0.80$, $P = 0.133$, two-tailed test), and it is well below T_p at all sites (Appendix A). Thus even during the season of maximal fly activity, mean T_b varies positively with local climate and differs between sites by as much as 10.5°C (Appendix A).

To determine which environmental factors influence the daily activity pattern of flies, we examined the temporal activity of flies, ambient temperature, and the drying power of air during a warm day in summer in Salem, Oregon. If temperature regulation dominates activity patterns, flies should be most active at times of day ($\sim 08:00$ to $11:00$ hours) during which flies can achieve T_b that approximate their T_p (Hertz et al. 1993): this was not the case as fly activity was very low all day

until near sunset, when activity exploded ($\sim 20:00$ hours, Fig. 3). If, however, minimizing desiccation dominates activity patterns (Kyriacou et al. 2007), flies should be most active at times of day (early morning) when the drying power of air is weakest: this is not the case (Fig. 3) as fly activity was maximal just around sunset, when ambient temperatures were still well above 30°C and the drying power of air still above morning levels.

DISCUSSION

Organisms distributed over broad latitudinal gradients encounter substantial variation in climate. If climate variation influences the actual T_b of organisms and thus their physiological performance, then temperature could serve as a selective agent and drive the evolution of clines in genetic and phenotypic traits. However, if behavioral thermoregulation is fully compensatory, then T_b will not covary with the thermal environment and may have limited impact as a selective agent (Huey et al. 2003).

Seasonal and latitudinal differences in activity are striking (Fig. 1) and suggest that temperature extremes could well be selective agents. For example, in winter in the north, flies are active only on the warmest days and probably have reduced reproduction due to cold (Junge-Berberovic 1996). In summer, flies also have reduced activity at most sites, especially in the south (Fig. 1).

We find evidence of behavioral compensation (Stevenson 1985b), especially as regards daily time of activity (Fig. 2A). In cool seasons, these flies are active primarily at midday, when ambient temperatures are relatively warm (for that season). In warm seasons, they are active primarily very early in the morning or especially just before dark (Noor 1998).

We next evaluated whether behavioral shifts were sufficient to enable flies to maintain relatively constant T_b , independent of season and latitude. Appendices A and B show clearly that these shifts do not compensate for shifts in T_a : mean activity T_b varies strongly with maximal ambient temperatures and usually differs strikingly from the T_p range ($21^\circ\text{--}26^\circ\text{C}$).

We also investigated whether activity patterns of flies on a warm day in summer match expectations based on thermoregulatory or water-balance considerations. If flies time their activity to achieve T_b similar to preferred levels (T_p), then maximal activity would be greatest at mid-morning ($\sim 10:00$ hours), when ambient temperatures are within the T_p range. However, if they time activity to minimize rates of water loss (Kyriacou et al. 2007), they should be active very early in the day. In fact, *D. subobscura* were most active around sunset, when ambient temperatures were still much warmer than the T_p range ($21.2^\circ\text{--}25.9^\circ\text{C}$) and when the desiccating power of air is still strong (Fig. 3). A similar burst of activity near sunset in summer is seen in other populations of this and other species (see Noor 1998).

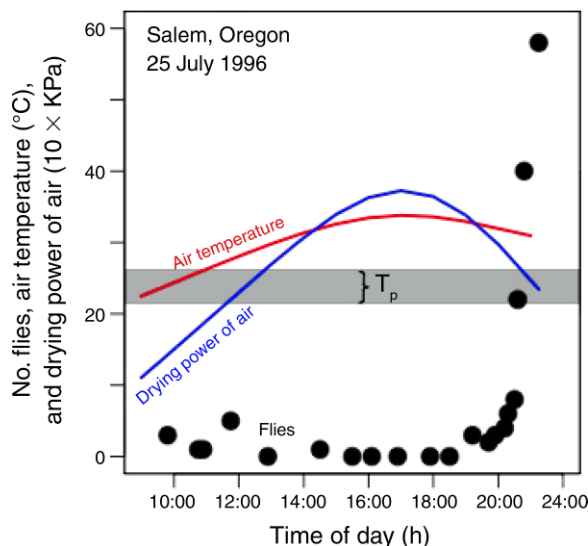


FIG. 3. Number of *D. subobscura* (solid circles) collected per census during the day (25 July 1996) in Salem, Oregon, mean ambient temperature (red line), drying power of air (blue line; numbers have been multiplied by 10), and the preferred temperature range, T_p (gray horizontal bar) for *D. subobscura*. Activity is greatest around sunset (~20:53 hours), and does not coincide with times of day at which ambient temperatures are within the T_p range for the flies or when desiccation risk is least.

Perhaps activity patterns might actually be governed by thermoregulatory considerations of offspring rather than of the adults themselves. Welte et al. (1993) found that embryos of *D. melanogaster* achieve maximal heat tolerance beginning ~10 h after laying (at 25°C). If this same ontogenetic pattern holds for *D. subobscura* and if a female laid her eggs at mid-morning, when $T_a \sim T_p$ (Fig. 3), then her embryos might encounter high or potentially lethal temperatures within six hours. If, however, she laid her eggs near sunset, her embryos would have ~19 hours before experiencing the highest daytime temperatures. Soon thereafter they would probably hatch, and larvae could potentially move to evade high temperatures (Wang et al. 2008). This hypothesis proposes that activity times of flies in warm seasons are driven by thermoregulatory concerns of offspring and is testable experimentally by determining whether eggs put out in mid-morning in summer have lower survival than those put out at sunset.

Conclusions

We have analyzed whether thermoregulatory behaviors enable invasive populations of *D. subobscura* to compensate for seasonal and latitudinal variation in climate. We found clear evidence of thermoregulatory shifts (e.g., seasonal and latitudinal shifts in time of activity). Even so, T_b of these flies varies systematically and dramatically with climate. Consequently, the observed T_b variation could have been a selective agent driving the rapid evolution of clines in *D. subobscura*

although not necessarily the only one (Santos et al. 2005, Bradshaw and Holzapfel 2006). On the other hand, if T_b of active flies was independent of local climates and thus always approximated T_p , we would have concluded that active T_b is unlikely to be a selective agent.

The next step in evaluating the physiological, ecological, and selective role of temperature will be to derive ways of observing its selective impact on active flies (and perhaps their eggs, larvae, and pupae) in nature. Outdoor cage experiments (Junge-Berberovic 1996, Hoffmann et al. 2003) or field releases (Kristensen et al. 2006, Loeschcke and Hoffmann 2007) may provide the tools necessary for evaluating selective roles of temperature in nature.

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APPENDIX A

A table of ambient temperatures and body temperatures of active flies from collecting sites in different seasons (*Ecological Archives* E090-120-A1).

APPENDIX B

A figure showing mean body temperatures of active flies vs. maximum ambient temperatures (*Ecological Archives* E090-120-A2).