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VARIATION IN THERMAL PERFORMANCE AND REACTION NORMS AMONG POPULATIONS OF *DROSOPHILA MELANOGASTER*

Peter Klepsatel,[1] Martina Gáliková,[1] Nicola De Maio,[1] Christian D. Huber,[1,2] Christian Schlötterer,[1] and Thomas Flatt[1,3,4]

[1]*Institut für Populationsgenetik, Vetmeduni Vienna, Veterinärplatz 1, A-1210 Vienna, Austria*

[2]*Max F. Perutz Laboratories, University of Vienna, Campus Vienna Biocenter 5, Dr. Bohr Gasse 9, A-1030 Vienna, Austria*

[3]*Current address: Department of Ecology and Evolution, University of Lausanne, UNIL Sorge, Biophore, CH-1015 Lausanne, Switzerland*

[4]*E-mail: thomas.flatt@unil.ch*

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The major goal of evolutionary thermal biology is to understand how variation in temperature shapes phenotypic evolution. Comparing thermal reaction norms among populations from different thermal environments allows us to gain insights into the evolutionary mechanisms underlying thermal adaptation. Here, we have examined thermal adaptation in six wild populations of the fruit fly (*Drosophila melanogaster*) from markedly different natural environments by analyzing thermal reaction norms for fecundity, thorax length, wing area, and ovariole number under ecologically realistic fluctuating temperature regimes in the laboratory. Contrary to expectation, we found only minor differences in the thermal optima for fecundity among populations. Differentiation among populations was mainly due to differences in absolute (and partly also relative) thermal fecundity performance. Despite significant variation among populations in the absolute values of morphological traits, we observed only minor differentiation in their reaction norms. Overall, the thermal reaction norms for all traits examined were remarkably similar among different populations. Our results therefore suggest that thermal adaptation in *D. melanogaster* predominantly involves evolutionary changes in absolute trait values rather than in aspects of thermal reaction norms.

KEY WORDS: **Fecundity, life history, phenotypic plasticity, temperature, thermal adaptation.**

Temperature is a crucial environmental factor that affects all biological processes and has major effects on physiology and fitness of ectotherms. Insights into how organisms adapt to different thermal environments are particularly important for a better understanding of life-history variation in ectotherms (Clarke 1993; Angilletta 2009). The effects of temperature on performance are usually and most completely described by thermal performance curves, a type of thermal reaction norm (Huey and Stevenson 1979; Fig. S1). Thermal performance curves are defined by several biologically important parameters: the temperature at which performance is maximal, the optimal temperature (T_{opt}); the breadth of the range over which performance is above some arbitrary level, called performance breadth (B, i.e., a measure of spread of the curve); and the critical thermal limits that permit performance (CT_{min} and CT_{max}; Angilletta et al. 2002). Thermal performance curves are usually asymmetrically bell shaped, with a gradual increase from the lowest temperature up to the optimal temperature where performance is maximal, followed by a steep decline in performance for temperatures higher than the optimal temperature (Dewitt and Friedmann 1979). Physiologically, two phases

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can be distinguished (Logan et al. 1976). A first phase of increasing performance is determined by the exponential relation between rates of biological activity and temperature (Van't Hoff–Arrhenius relation; Gillooly et al. 2001, 2002), whereas during the second phase of decreasing performance the negative effects of high temperature prevail. Deleterious effects of high temperature can be attributed to several factors, including protein and nucleic acid denaturation, and damage to cell membranes and mitochondria (Neven 2000).

Although organisms can exist across a broad range of temperatures, most species have a limited thermal range over which they are able to live and reproduce, and an even more narrow range over which they can achieve maximal performance. Hertz et al. (1983) formulated two alternative views of how thermal performance might evolve. According to the "labile" view, thermal physiology is thought to evolve readily in response to selection. For fitness to be maximized, optimal performance temperature should vary with the temperature at which the performance occurs naturally (Angilletta et al. 2002). Alternatively, the "conservative" view postulates that thermal physiology does not evolve readily because the underlying evolutionary changes are either too costly or otherwise constrained. Under this model, species or populations that inhabit different thermal environments should not differ in their performance curves. Which of these two patterns describes the evolution of thermal performance best is subject to debate (Angilletta et al. 2002). In fact, the two views represent the endpoints of a continuum of possibilities (Hertz et al. 1983). Angilletta et al. (2002), for example, hypothesize that the evolution of thermal physiology might be highly taxon specific, with performance being more constrained in some taxa than in others.

Two major models have been put forward to account for constraints upon the evolution of thermal physiology: the "hotter is better" and the "jack-of-all-temperatures" hypothesis (Huey and Kingsolver 1989). According to the "hotter is better" hypothesis, maximal thermal performance should be higher in organisms with higher optimal temperatures. The slower rate of biological processes imposed by lower temperatures cannot be overcome by adaptation, so that cold-adapted species are expected to have lower maximal rates than warm-adapted species. Tests of this hypothesis based on comparative studies have given mixed results (Carriere and Boivin 1997; Izem and Kingsolver 2005; Frazier et al. 2006; Knies et al. 2009). The "jack-of-all-temperatures" hypothesis posits that thermal constraints evolve due to trade-offs between performance breadth and maximal performance (Huey and Kingsolver 1989). This hypothesis rests on the notion that enzymes are usually efficient only over a narrow range of temperatures (Hochachka and Somero 2002), suggesting that obtaining and maintaining a high efficiency over a broad temperature range might be energetically too costly. Empirical tests of this hypothesis have yielded mixed results as well (Huey and Hertz 1984; Gilchrist 1996; Palaima and Spitze 2004).

Studies of thermal adaptation are further complicated by the fact that ectotherms experience daily and seasonal thermal fluctuations. The issue of how thermal heterogeneity affects the evolution of thermal physiology has been addressed in several studies. The model by Lynch and Gabriel (1987), for example, predicts that temporal environmental heterogeneity selects for more broadly adapted individuals, with within-generation variance being more important than among-generation variance. Another model, developed by Gilchrist (1995), predicts that constant environments, or environments with substantial within-generation and among-generation variance, favor thermal specialists with narrow performance breadth, whereas environments with significant among-generation variance but little within-generation variance favor generalists with broad thermal ranges. Under this model, increased performance during optimal conditions increases fitness more than increased performance breadth (Gilchrist 1995). Thus, both models predict that optimal thermal performance should evolve so as to enable maximal performance at the most frequently experienced body temperature. Genotypes from hot environments should thus have thermal optima at higher temperatures than genotypes from cold environments (Angilletta 2009).

In small ectotherms such as *Drosophila*, body temperature is identical with surrounding ambient temperature (Stevenson 1985). However, fruit flies can thermoregulate behaviorally, at least to some extent, by avoiding thermal stress via microhabitat selection (Feder et al. 2000). Because *Drosophila melanogaster* is cosmopolitan, different populations experience markedly different thermal conditions across geography, both in terms of average temperature and seasonal range of temperatures. For example, geographical clines have been identified for many morphological and life-history traits in *D. melanogaster* (De Jong and Bochdanovits 2003), with temperature being considered the major underlying selective agent (Stalker and Carson 1947; David et al. 1977; Partridge et al. 1994). This makes *D. melanogaster* an excellent system for studying thermal adaptation at the intraspecific level. In particular, because fruit flies occur across a wide range of thermal environments in both space and time, the thermal heterogeneity encountered by this species might have led to major differentiation in thermal performance and reaction norms among populations.

To examine variation in thermal adaptation, we investigated thermal performance and reaction norms for fecundity and morphological traits in six outbred wild populations of *D. melanogaster* originating from markedly different thermal environments (Fig. S2 and Tables 1, S2). We examined three tropical (two low-altitude, one high-altitude), two temperate, and one intermediate population. This geographically broad sampling of populations enabled us to (1) maximize differences among

Table 1. Geographic and climatic information on the populations examined in this study, as well as parameter estimates of thermal performance curves for fecundity. T_{opt}, estimated optimal temperature; u_{max}, estimated maximal fecundity at optimal temperature; B_{75}, 75% performance breadth; B_{50}, 50% performance breadth. Values not connected by the same letter are significantly different ($P < 0.05$; see Table S7). For further details, see text and Table S1.

Population	Locality	Map reference	Altitude (m)	T_{annual} (°C)	T_{season} (°C)	Seasonality (annual/seasonal)	Seasonal thermal range (°C)	T_{opt} (°C)	u_{max} (eggs)	B_{75}	B_{50}
Ethiopia (low altitude)	Gambela	8.25°N, 34.58°E	525	27.7 ± 0.4	27.7 ± 0.4	1.32/1.32	26.5 – 30.5	26.57 ± 0.49 (A)	441.78 ± 8.48 (D)	7.51 ± 0.31 (A)	10.18 ± 0.22 (A)
Ethiopia (high altitude)	Fiche	9.8°N, 38.73°E	3050	16.9 ± 0.3	16.9 ± 0.3	1.03/1.03	16.0 – 18.5	26.52 ± 0.51 (A)	492.19 ± 12.69 (C)	7.16 ± 0.51 (A)	10.21 ± 0.4 (A)
Zambia	Siavonga	16.53°S, 28.72°E	578	24.5 ± 1.0	24.3 ± 1.0	3.57 / 3.57	18.7 – 29.2	26.09 ± 0.53 (A)	563.14 ± 13.48 (B)	7.03 ± 0.44 (A)	9.98 ± 0.63 (A, B)
South Africa	Phalaborwa	23.93°S, 31.12°E	420	22.5 ± 1.0	22.5 ± 1.0	3.34/3.34	17.5 – 26.5	26.12 ± 0.39 (A)	518.65 ± 10.16 (C)	7.33 ± 0.36 (A)	10.07 ± 0.36 (A)
Switzerland	Zürich	47.37°N, 8.55 °E	408	8.8 ± 1.9	15.6 ± 1.0	6.73 / 2.17	12.0 – 17.9	25.27 ± 0.58 (A, B)	507.25 ± 15.83 (C)	7.3 ± 0.68 (A)	10.4 ± 0.72 (A)
Austria	Kahlenberg	48.28°N, 16.33°E	484	10.6 ± 2.2	17.6 ± 1.1	7.46/2.35	12.0 – 20.5	25.00 ± 0.19 (B)	604.04 ± 9.41 (A)	6.37 ± 0.28 (B)	8.94 ± 0.24 (B)

thermal environments-of-origin and (2) perform a preliminary yet systematic comparison of thermal performance among populations from significantly different thermal environments. We predicted that these diverse populations would differ substantially in thermal adaptation, in particular in their thermal reaction norms. For example, one might expect that adaptations to high latitude mirror adaptations to high altitude (Lencioni 2004; Pitchers et al. 2013). Similarly, one might predict that flies from tropical lowland habitats would be less thermally plastic than flies from temperate/seasonal environments that are subject to a greater amount of thermal fluctuations. Unlike most previous studies which used constant experimental temperatures (Delpuech et al. 1995; David et al. 1997, 2006; Trotta et al. 2006), we attempted to implement ecologically realistic conditions by exposing flies to daily temperature fluctuations. This is important because for a variety of traits fluctuating temperatures can yield different results than measurements at constant temperatures (Siddiqui and Barlow 1972; Bozinovic et al. 2011; Vanin et al. 2012).

Our study had two specific aims. First, we aimed to investigate the phenotypic response of populations to a range of fluctuating temperatures. Because our populations originated from significantly different thermal environments, we expected them to have been shaped by different thermal selection pressures. To determine thermal differentiation among populations, we analyzed thermal performance curves for female fecundity across a range of temperatures. Fecundity is a major, temperature-sensitive fitness component and thus likely a direct target of thermal selection. Previous studies suggest that *Drosophila* species vary substantially in oviposition temperature (Schnebel and Grossfield 1986; Junge-Berberovic 1996). In *D. melanogaster*, for instance, fecundity increases linearily between 12°C and 20°C, with maximum egg production continuing through 25°C to 28°C and then declining and reaching zero around 32.5°C to 34°C (Schnebel and Grossfield 1986). However, to our knowledge, no systematic comparison of thermal fecundity performance among *D. melanogaster* populations of different climatic origin has been performed so far; the extent of differentiation in thermal fecundity performance among populations remains therefore unknown. We predicted that different populations would vary in performance maxima according to the most frequently experienced temperature of their environment-of-origin. Our second aim was to examine among-population variation in thermal reaction norms for two proxies of body size (thorax length, wing area) and ovariole number (a fitness-related morphological trait which sets an upper limit for maximum daily fecundity; David 1970). Differences in thermal reaction norms among populations might be attributed to geographic variation in thermal adaptation. Indeed, latitudinal clines—presumably caused by differences in temperature across latitude—have been well documented for these and others traits (David and Bocquet 1975; Coyne and Beecham 1987;

Capy et al. 1993; James et al. 1995); however, whether thermal *reaction norms for these traits* themselves differ as a function of climatic origin remains unknown.

Materials and Methods

FLY POPULATIONS AND MAINTENANCE

We used six outbred wild populations of *D. melanogaster* of different climatic origin (Fig. S2 and Tables 1, S1, S2). All populations (except Austria) were obtained as isofemale lines and outbred for four generations before experiments. To establish outbred populations for each set of lines, 10 females and 10 males from each isofemale line were introduced into a population cage (390 × 280 × 280 mm); after 5 days, we collected eggs to initiate the F1 generation. Upon eclosion, we placed F1 adults (approx. 400) into a new cage (discarding the parents) and after 7 days allowed them to oviposit for 2 hours, using the eggs to start the F2. Subsequent generations were initiated using the same procedure but allowing for overlapping generations (without discarding adults) to avoid inadvertent selection for early life history. The Austrian population was established with approx. 200 freshly collected females and males. All populations were kept as mass-bred populations at a population size of approx. 1500 to 2000 adults; flies were maintained on standard cornmeal-agar-yeast medium in bottles (8 oz round bottles; 6.03 cm diameter and 13.02 cm height) with overlapping generations (generation time 2–3 weeks) at room temperature (∼25°C). Except for the Swiss population, which had been kept in the laboratory for 4 years before our assays (and might thus have been subject to laboratory adaptation), all other populations were kept in the laboratory for no longer than 6 months before experiments. Note that for logistic reasons we could not assay all populations simultaneously; both Ethiopian populations were measured 14 months after the other populations were assayed.

THERMAL PERFORMANCE AND REACTION NORMS

Overall experimental design and thermal treatments

To initiate experimental populations, we placed four Petri dishes containing standard cornmeal-agar-yeast medium with active yeast into the cages and allowed females to oviposit for 1 hour at room temperature (∼25°C). Eggs were placed into vials (20–30 vials per population; 50 eggs per vial) and reared at a given thermal regime. To initiate treatment groups, egg collections were timed so that all eggs were placed into given temperature/light regime during the first hour of the scotophase, that is the dark phase during which oviposition is maximal.

Most previous experiments on the thermal biology of *D. melanogaster* have been performed across a range of constant temperatures; yet, it is clear that under natural conditions temperatures fluctuate: generally, temperature is at its daily minimum at dawn and reaches its maximum 3–4 hours after solar noon (Petersen et al. 2011). In our experiments, we attempted to mimic ecologically realistic thermal regimes by exposing flies to a range of fluctuating temperatures. Each population was exposed to seven temperature treatments (average [range]) throughout development *and* adulthood: 14°C (9–19°C), 18°C (13–23°C), 22°C (17–27°C), 24°C (19–29°C), 26°C (21–31°C), 28°C (23–33°C), and 30°C (25–35°C), all at a 12:12 light:dark light regime. In each treatment, the minimum temperature was set to the beginning of the photophase and the maximum temperature according to the following formula: $(t_{light}/2) + 4$ hours, where t_{light} is the length of the photophase (12 hours). The daily temperature range in each thermal treatment was 10°C, with temperature changes being gradual (using the incubators' ramping program). The photophase started and ended with a 30-minute transition period, during which light intensity was reduced to 50%. Temperature experiments were performed in Percival incubators (Percival DR-36VL Controlled Environment Chamber; Percival Scientific Inc., Perry, IA). Deviations from the programmed thermal regime did not exceed the allowed ±0.5°C range; temperatures were double checked daily at random times with a digital thermometer (TFA, Dostmann, Wertheim, Germany). Relative air humidity was kept in the range of 60–70%.

To allow for a direct comparison with both Ethiopian populations, which were measured later than the other populations, and to account for possible differences caused by laboratory adaptation (Partridge et al. 1995; Hoffmann et al. 2001; Sgró and Partridge 2001), three populations (Zambia, South Africa, Austria) were remeasured at the end of experiment at an intermediate (24°C [19–29°C]) and an extreme temperature (30°C [25–35°C]). This experiment did not reveal any significant deviations from our previous results (data not shown).

Phenotypic measurements

For phenotyping, we placed adults (40 females, 40 males), all enclosed within a 24-hour period, into large (390 × 280 × 280 mm) population cages. Before assays, flies were reared at a given temperature throughout development, at a standard density of 50 eggs per vial. For each population, we used a minimum of two and a maximum of five replicate cages (Table S3). Cages were supplied daily with two fresh Petri plates containing standard medium (cornmeal, agar, 2% yeast) with active dry yeast sprinkled on top.

We first examined daily female fecundity at each thermal regime over the first 10 days of adulthood. Absolute fecundity (i.e., absolute performance) was defined as the mean cumulative number of eggs laid per female during the first 10 days; relative fecundity was defined as a percentage of maximum absolute fecundity (set to 100%). For each cage, we adjusted fecundity

estimates for female mortality. Next, we examined ovariole number, thorax length, and wing area of 10–15 randomly chosen females from each treatment and replicate (20–30 females per population per temperature; the Swiss population was not measured) on days 11–12 of adulthood. Ovaries were dissected in water and the number of ovarioles was counted using a stereo-dissecting microscope. Ovariole number was defined as the sum of the number of ovarioles in both ovaries. To describe the relationship between body size and ovariole number, we estimated an "ovariole index" for each individual, that is the ratio between ovariole number and body size (∼cube of thorax length); flies with a higher ovariole index have a relatively higher number of ovarioles per unit body size than flies with a smaller index.

Because *D. melanogaster* is known to undergo reproductive diapause at temperatures ≤12–13°C (Emerson et al. 2009), we also examined the proportion of diapausing females in our low temperature treatments (14°C [9–19°C]; 18°C [13–23°C]). A female was considered to be in diapause if all egg chambers in all ovarioles were previtellogenic (< stage 8; King 1970).

Thorax length and wing area were examined using a stereo-dissecting microscope (Leica M205FA; Leica Microsystems GmbH, Wetzlar, Germany), with a digital camera (DFC 300 FX) attached to it. Thorax length was measured from the base of the most anterior humeral bristle to the posterior tip of the scutellum on the left side of the fly (French et al. 1998). For wing area measurements, the left wing was removed and mounted between two microscope slides. Wing contours were traced (Fig. S3) and wing areas measured using the Leica Application Software. Wing loading was defined as (thorax length)^3/(wing area) (Starmer and Wolf 1989).

Analysis of thermal performance curves and reaction norms

To analyze thermal performance curves (a type of thermal reaction norm; Fig. S1) for fecundity, we fitted different functions (second- and third-degree polynomials; Gaussian; functions 6 and 10 in Logan et al. 1976) to our data. Based on the Bayesian information criterion (BIC; Schwarz 1978; Table S4), we found that thermal fecundity performance (F) was best described by equation 10 of Logan et al. (1976):

$$F(T) = \alpha([1 + k\exp(-\rho T)]^{-1} - \exp(-\tau)).$$

Here, α, k, and ρ denote free parameters and τ is defined as:

$$\tau = (T_M - T)/\Delta T,$$

where T_M is the lethal, maximum temperature; T denotes a given experimental temperature;

ΔT is the width of the high-temperature boundary layer ($T_M - T_A$), where T_M and T_A are additional free parameters. Temperatures are expressed in degrees above base temperature (=14°C). For further details, see Logan et al. (1976). We estimated parameters by minimizing the squared error in R (vs. 2.12.2) using the function nls() with the Gauss–Newton algorithm. From these estimates we calculated optimal temperature (T_{opt}), maximum fecundity (u_{max}), and 75% (B_{75}) and 50% (B_{50}) performance breadth (i.e., the breadth of the range over which relative performance is above 75% and 50%, respectively; see Fig. S1). Parameters were calculated from the estimated function by evaluating the function output for a grid of input temperatures, with a density of 1000 points per °C and a range from 14°C to 35°C. The optimal temperature and the lower and upper temperatures of 75% and 50% relative performance were evaluated numerically; note that this led to a maximum error of 0.001°C in the estimates. To estimate *mean population* thermal reaction norms (i.e., population-level reaction norms that represent the *average* reaction norm across all genotypes present in a population) for morphological traits (thorax length, wing area, wing loading, ovariole number, ovariole index), we fitted polynomial functions (first to fourth degree polynomial) to the data, thus relating population trait means to temperature. Functions with minimal BIC were selected as best-fitting models. Reaction norms for thorax length, wing area, and ovariole number were best described by a second-degree (quadratic) polynomial. Reaction norms for wing loading and ovariole index were best described by a first-degree (linear) polynomial. For all calculations, temperatures were standardized to the lowest temperature (base temperature = 14°C), which we set to zero to obtain biologically meaningful values for intercepts.

Climate data

To relate our data to thermal conditions experienced by the populations in their natural settings, we obtained climate data (for all locations-of-origin [±10 km]) from the World Meteorological Organization (WMO; worldweather.wmo.int) for the WMO-defined, 30-year climate reference period from 1961 to 1990 (Table S2). For the Ethiopian high-altitude population from Fiche, no climate data were available, so that we used data for Addis Ababa, located approximately 40 km away. From the climate data we calculated mean annual temperature (T_{annual}), mean seasonal temperature (T_{season}), seasonality (standard deviation of monthly mean temperatures; Deutsch et al. 2008), and the seasonal thermal range (minimum to maximum of mean monthly temperature of the season). We defined "season" as the period during which the average monthly temperature did not fall below 12°C; we used this thermal limit because development of *D. melanogaster* is not possible below 12°C (David and Clavel 1966; Cohet et al. 1980).

STATISTICAL ANALYSIS

We first analyzed the effects of population and temperature on absolute and relative fecundity by using fully factorial, two-way fixed-effects analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc tests. In addition, we also analyzed absolute fecundity using analysis of covariance (ANCOVA), with "population" and "temperature" as fixed factors and thorax length as the covariate. Second, we investigated thermal plasticity for fecundity by analyzing thermal fecundity performance curves based on equation (10) of Logan et al. (1976). *P*-values and confidence intervals for performance curve parameters were estimated by parametric bootstrapping: we (1) used parameter estimates obtained from fitting equation (10) to simulate fecundity data for each population (standard deviations estimated from data; residuals assumed to be normal); (2) calculated T_{opt}, u_{max}, and B_{75} and B_{50} (i.e., two measures of spread of the thermal performance curves) from these data; and (3) repeated this process 1000 times to obtain *P*-values and confidence intervals. *P*-values from pairwise comparisons were corrected for multiple testing using the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995). Third, we analyzed the effects of population and temperature on morphological traits, again using two-way ANOVA, followed by Tukey's HSD post hoc tests. Fourth, to investigate thermal plasticity for morphological traits we used multiple regression: for traits with a quadratic reaction norm (thorax length, wing area, ovariole number) we performed multiple nonlinear regressions with one categorical ("population") and two continuous (temperature, [temperature]^2) factors, including two-way interactions (population × temperature; population × [temperature]^2); for traits with linear reaction norms (wing load, ovariole index) we performed multiple linear regressions with one categorical ("population") and one continuous (temperature) factor, including the population × temperature interaction. Finally, we used analysis of means (ANOM) to analyze reaction norm parameter estimates from regression analysis. ANOM is a multiple comparison procedure that represents an alternative to fixed-effects ANOVA; it constructs simultaneous confidence intervals for contrasts of population means versus the overall (grand) mean (Nelson et al. 2005). Importantly, unlike ANOVA, which determines whether there are differences among treatment means, ANOM identifies those treatment means that are significantly lower or higher than the overall mean. All analyses were performed in JMP version 10.0.0 (SAS, Raleigh, NC), assuming a significance threshold of $\alpha = 0.05$. Normality and homoscedasticity of residuals were checked using Shapiro–Wilk and Brown–Forsythe tests, respectively. In some cases, variances were not homogeneous; however, we obtained qualitatively identical results when analyzing data using Welch one-way ANOVAs (which relax the assumption of homoscedasticity), suggesting that variance heterogeneity was not a problem. Raw data are available in the Dryad data repository (doi:10.5061/dryad.vc1q1).

Results

THERMAL FECUNDITY PERFORMANCE

Thermal regime (but not population) had a significant effect on both absolute and relative fecundity (ANOVA), yet different populations showed different responses to temperature (absolute fecundity: population: $F_{5,81} = 0.4$, $P = 0.84$, temperature: $F_{6,81} = 981.6$, $P < 0.0001$, population × temperature: $F_{30,81} = 7.6$, $P < 0.0001$; relative fecundity: population: $F_{5,81} = 0.4$, $P = 0.82$, temperature: $F_{6,81} = 1008.0$, $P < 0.0001$, population × temperature: $F_{30,81} = 4.7$, $P < 0.0001$). Temperate flies (Austria, Switzerland), for example, had higher absolute fecundity (at 24°C [19–29°C]) than all other populations (Tables S5). Tropical flies, however, had higher relative fecundity at higher temperatures (30°C [25–35°C]) (Table S6). We also examined the effects of body size on absolute fecundity using ANCOVA: both population and thermal regime significantly affected fecundity, but body size did not (population: $F_{4,82} = 9.9$, $P < 0.0001$, temperature: $F_{6,82} = 200.7$, $P < 0.0001$, thorax length: $F_{1,82} = 0.68$, $P = 0.41$; homogeneity of slopes tests: population × thorax length: $F_{4,82} = 1.5$, $P = 0.20$, and temperature × thorax length: $F_{6,82} = 0.64$, $P = 0.70$; note that, due to insufficient power, we were not able to estimate all interaction terms).

Optimal temperature

Analysis of parameter estimates for fecundity performance curves (T_{opt}, u_{max}) showed that populations were differentiated in their thermal responses (Fig. 1A, B and Tables 1, S7). Although European temperate-zone populations did not differ in optimal temperature for fecundity (T_{opt}; Austria: 25.00 ± 0.16°C; Switzerland: 25.27 ± 0.33°C), tropical African populations had slightly but overall significantly higher thermal optima than temperate populations (Zambia: 26.09 ± 0.30°C; Ethiopia, low altitude: 26.57 ± 0.40°C; Fig. 2A and Table S7). (Note, however, that differences in optimal temperature between Swiss and African populations were not significant.) South African flies did not differ from tropical flies in optimal temperature for fecundity (26.12 ± 0.23°C; Fig. 2A and Tables 1, S7). This might be due to the fact that, although the South African population belongs to the temperate zone, the mean seasonal temperature for this population was more similar to that of the tropical Zambian population than to that of temperate European populations (Table 1). Strikingly, despite profound differences in altitude (~2500 m) and thermal environment, Ethiopian high-altitude versus low-altitude populations did not differ in optimal temperature (26.52 ± 0.41°C vs. 26.57 ± 0.40°C; Fig. 2A and Tables 1, S7).

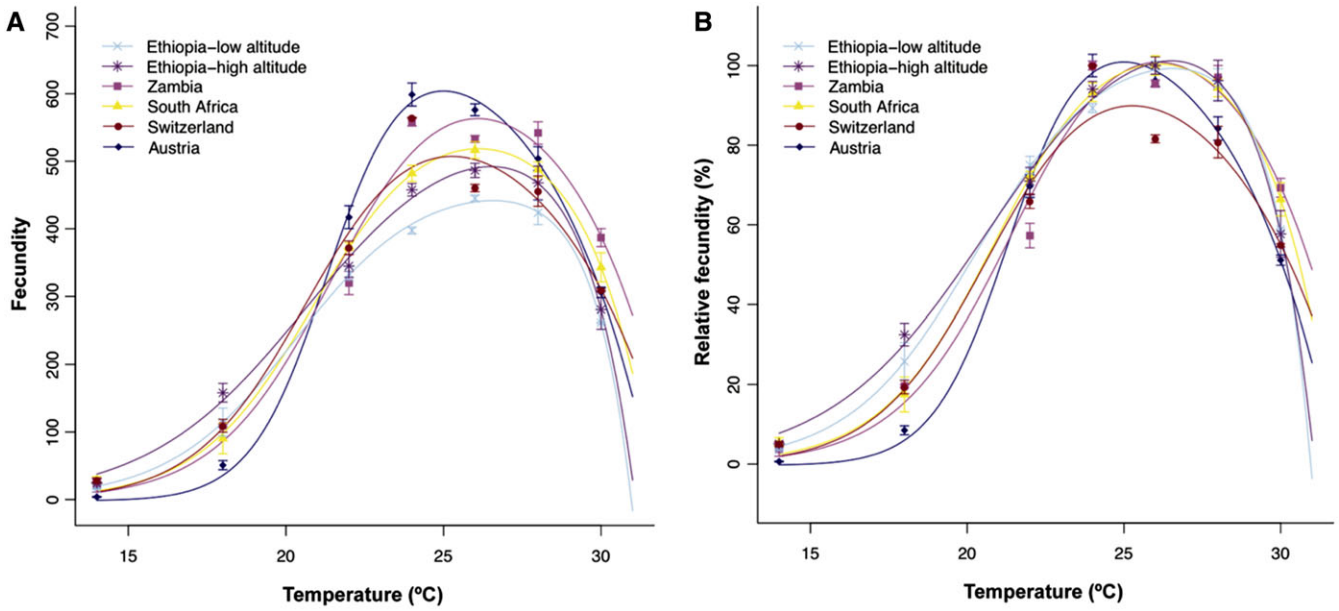

Figure 1. **Thermal performance curves for fecundity (estimates based oneq. 10 from Logan et al. 1976). Shown areperformance curves for absolute fecundity (left) and relativefecundity (right). Temperatures are expressed as meantemperatures. Error bars represent standard errors of themean.**

When comparing optimal temperatures for fecundity (T_{opt}) with the mean thermal range of a given environment and mean seasonal temperature (T_{season}; Table 1), we found that the optimal temperature for temperate populations and the Ethiopian high-altitude population was outside (above) the mean thermal range, whereas for tropical populations the optimal temperature was within the mean range of the natural environment. Overall, thermal optima for fecundity were relatively invariant among populations, despite their markedly different climatic origins.

Maximum fecundity

Populations also differed in maximum fecundity (u_{max}; Fig. 2B and Tables 1, S7). Contrary to the prediction of the "hotter is better" hypothesis, we did not find higher maximum fecundity for tropical as compared to temperate populations. Interestingly, Ethiopian high-altitude flies had significantly higher maximum fecundity than Ethiopian low-altitude flies (Ethiopia, high altitude: 492.19 ± 10.34 eggs; Ethiopia, low altitude: 441.78 ± 7.14 eggs; Fig. 2B and Tables 1, S7).

Thermal performance breadth

We also investigated variation among populations in the breadth of performance curves, that is the range over which performance is above a certain threshold (thus, a measure of the spread or variance of performance curves). Most populations did not differ in 75% (B_{75}) and 50% (B_{50}) performance breadth (Tables 1, S7). Only the Austrian population showed a lower B_{75} as compared to South African and Ethiopian low-altitude populations; moreover, it had a lower B_{50} than all other populations except Zambia (Tables 1, S7). The decreased performance at low temperatures and the narrower performance breadth of Austrian flies was likely caused by a proportion of these females being in reproductive diapause, which we did not observe in any other population except South Africa. At 18°C (13–23°C), 6.1% of Austrian females (4 of 66 flies) and 7.0% of South African females (5 of 71) were in diapause, whereas at 14°C (9–19°C) 45.5% of Austrian females (40 of 88) and 18% of South African females (14 of 78) were diapausing.

THERMAL RESPONSES OF MORPHOLOGICAL TRAITS

Trait values for thorax length, wing area, wing loading, ovariole number, and ovariole index were significantly affected by population, temperature, and their interaction (ANOVA; Tables S8, S9). We additionally analyzed thermal reaction norms using multiple linear or nonlinear (quadratic) regression and ANOM (Fig. 3 and Tables S10–S21). Both population and temperature (or [temperature]^2) had significant main effects on all traits (Fig. 3 and Tables S10–S15). For thorax length and ovariole number, temperature had qualitatively identical effects across populations (Fig. 3; nonsignificant interactions in Table S10), suggesting that reaction norms for these traits were parallel and their shapes did not differ among populations. Consistent with this, ANOM revealed that populations only varied in terms of intercept and maximum but not in slope or quadratic coefficient (Tables S16, S17, S19). For wing area, wing loading, and ovariole index, significant temperature by population interactions indicated that

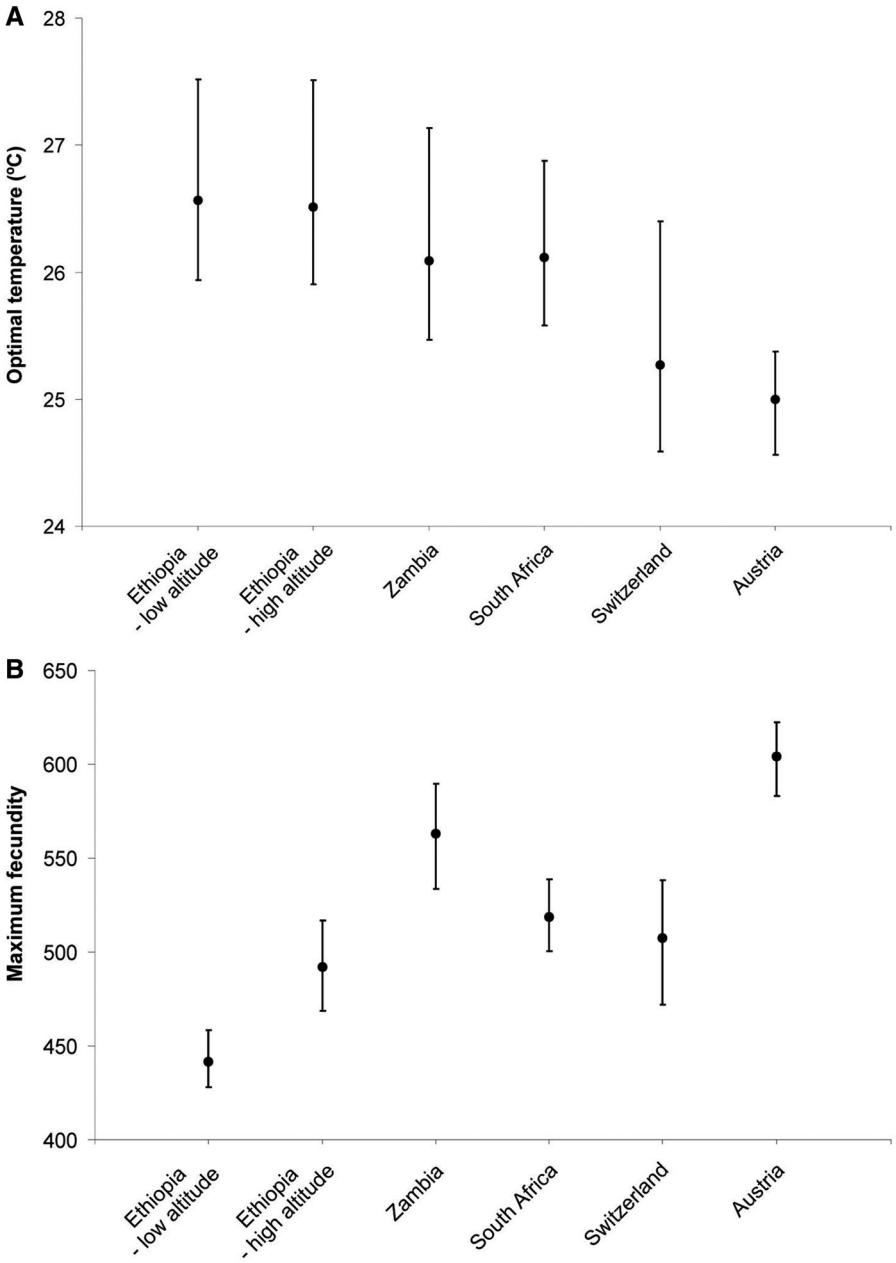

Figure 2. **Parameter estimates for reproductive performance. (A, top) Differences among populations in estimated optimal temperature for fecundity. (B, bottom) Differences in estimated maximum fecundity among populations. Error bars represent 95% confidence intervals.**

reaction norms vary slightly among populations (Fig. 3; interactions in Table S10; Tables S18, 20, 21). For wing area, this pattern was driven by a single population (Zambia), which differed from the overall mean across populations in slope and quadratic coefficient (Fig. 3 and Table S18). For wing loading, populations differed slightly in slope (Fig. 3 and Table S20); again, this pattern was driven by a single population (South Africa), which had a steeper slope than other populations. For ovariole index, the Zambian population had a steeper, whereas the Ethiopian (low-altitude) population had a shallower slope as compared to the mean of populations (Fig. 3 and Table S21).

Interestingly, although population reaction norms for ovariole number showed the well-known parabolic shape, reaction norms for ovariole index were linear with a positive slope (Fig. 3), indicating that the number of ovarioles per unit body size increases with increasing temperature. This implies that the typical parabolic shape of the reaction norm for ovariole number is a consequence of differences in body size; in contrast, when ovariole number is standardized by body size, the reaction norm becomes linear. Thus, overall, differences in reaction norms among populations tended to be very minor and mainly driven by only one or two populations (Fig. 3).

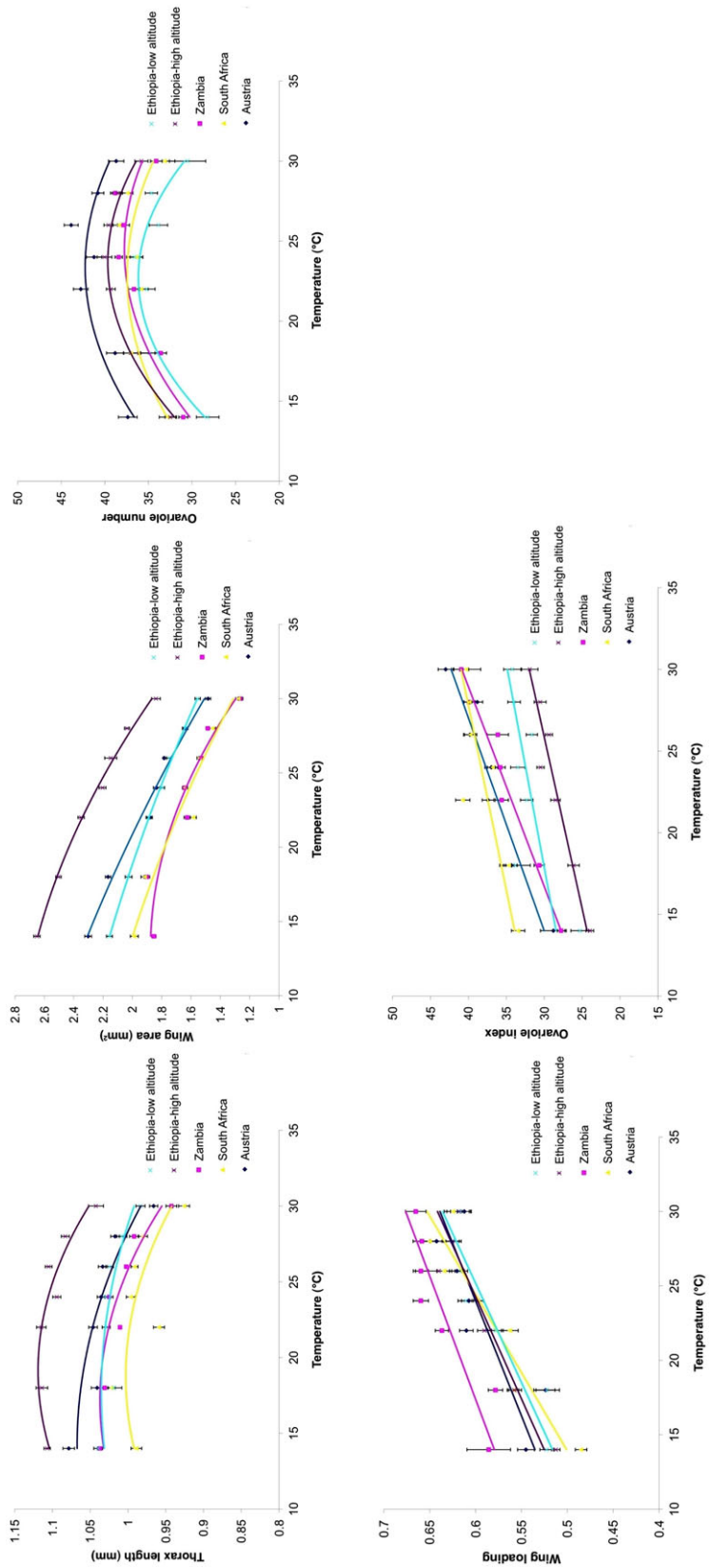

Figure 3. Thermal reaction norms for morphological traits. Shown are quadratic reaction norms for thorax length, wing area, and ovariole number, and linear reaction norms for wing loading and ovariole index. Temperatures are expressed as mean temperatures. Error bars represent standard errors of the mean.

Discussion

Here we have studied thermal performance and reaction norms of six wild populations of *D. melanogaster* from substantially different thermal environments using ecologically realistic fluctuating temperatures in the laboratory. Our results suggest that thermal adaptation in *D. melanogaster* mostly involves changes in absolute trait values rather than in other major aspects of thermal reaction norms or performance curves.

THERMAL OPTIMA ARE REMARKABLY INVARIANT AMONG POPULATIONS

Although populations differed substantially in fecundity and morphology, thermal optima for fecundity were remarkably similar across populations and largely independent of climatic origin. These minor differences in optimal temperature (1–1.5°C) were particularly striking in view of the large differences in mean natural field temperatures between the habitats of these populations. For temperate and tropical high-altitude populations, estimated optimal temperatures for fecundity were higher than mean seasonal temperatures and in fact outside the thermal range of their natural environment, whereas for tropical populations the difference between estimated optimal temperatures for fecundity and seasonal temperatures was considerably smaller and within the range of the natural environment.

Several explanations might account for this relative invariance of optimal temperature among populations. The evolution of more profound intraspecific differences in thermal fecundity optima might be constrained by gene flow, which would hinder adaptation to local conditions. However, because our populations differed strongly in morphological and life-history traits, this seems quite unlikely. A second, biologically more plausible explanation might be that temperate flies tend to select warm microhabitats (Jones et al. 1987), or that they restrict reproduction to warm periods (Gilchrist 1995), two effects that would minimize differences between temperate and tropical populations. However, *Drosophila subobscura* flies exhibit lower body temperature at high than at low latitude and thus seem to be unable to compensate for latitudinal differences in temperature (Huey and Pascual 2009). Thus, it remains unclear whether this second explanation can account for our observations.

For the Ethiopian high-altitude population, the difference between optimal and seasonal temperature was so pronounced that it seems unlikely that these flies are able to restrict reproduction to a warmer period, especially because the temperature of their habitat is relatively stable, with only minor monthly changes in mean temperature; yet, we cannot fully exclude the possibility of microhabitat selection (Mani 1968). The fact that Ethiopian high-altitude flies had higher absolute fecundity than low-altitude flies might suggest that absolute performance at a given temperature is more important in determining reproductive output than optimal temperature. In microevolutionary terms, it might be easier for selection to increase performance by acting on morphological traits correlated with fecundity (body size, ovariole number) than by shifting optimal temperature for fecundity, which might require changes in structural and kinetic properties of many enzymes, or which might be constrained by other traits. The higher fecundity of flies from colder environments might thus be driven, for example, by their genetically larger body size and their higher ovariole number, two traits that are known to be positively correlated with fecundity (e.g., Robertson 1957; Tantawy and Rakha 1964; David 1970; Partridge et al. 1986; Honek 1993; Blanckenhorn 2000; Kingsolver and Huey 2008; Klepsatel et al. 2013). (Note, however, that we failed to find an effect of body size on absolute fecundity, perhaps due to insufficient power.) If so, cold-adapted flies might have been selected for increased fecundity at lower temperatures, with the increase at other temperatures being a byproduct or a correlate of increased absolute performance at lower temperatures; their higher fecundity might thus represent a case of imperfect temperature compensation (Pörtner et al. 2006), whereby an increase in fecundity partially compensates for a temperature-driven decrease in performance.

In agreement with our data, previous studies of optimal temperature for walking speed in *D. melanogaster* have also found only very minor or no differentiation in thermal optima among populations. Gilchrist et al. (1997) observed only minor differences (0.3–1.6°C) in optimal temperatures for walking speed among *D. melanogaster* lines evolved for at least 100 generations under different thermal conditions in the laboratory. Similarly, comparisons of populations from Congo and France did not reveal differences in thermal sensitivity for locomotory performance (Gibert et al. 2001). These invariant intraspecific patterns contrast with findings from interspecific comparisons (Angilletta et al. 2002; Angilletta 2009). For instance, when measuring daily fecundity in response to temperature in sympatric *D. subobscura* (which is more cold-adapted) and *D. melanogaster* (which is more warm-adapted), *D. subobscura* had a lower mean thermal optimum for fecundity than *D. melanogaster* (Junge-Berberovic 1996). As in *D. melanogaster*, however, the thermal optimum of *D. subobscura* is higher than its mean habitat temperature (Junge-Berberovic 1996). This indicates that the intraspecific potential for thermal adaptation by evolutionary changes in optimal temperature may be relatively limited or constrained.

Similar to our findings for fecundity, a recent study of thermal performance across several insect species found that differences between optimal temperatures for population growth and habitat temperatures are larger for temperate species, with tropical species having thermal optima much closer to their habitat temperatures (Deutsch et al. 2008). This suggests that differences between thermal optima for fecundity and mean seasonal

temperatures found in our study might represent a general pattern rather than reflecting imperfect thermal adaptation due to the relatively recent colonization of temperate zones by *D. melanogaster*. If we consider the thermal optimum to be the point where the positive and negative effects of high temperature on a trait are at equilibrium (Logan et al. 1976), then the position of the optimum might be a consequence of heat resistance rather than a result of direct selection for a specific value. If so, the thermal optimum might depend more strongly on the critical thermal maximum (CT_{max}) than on mean habitat temperature. CT_{max} decreases only slightly with latitude in insects (Addo-Bediako et al. 2000; Deutsch et al. 2008), and the same pattern has been observed for altitudinal gradients (Gaston and Chown 1999b). This is due to the fact that annual mean daily temperature decreases more rapidly with latitude (or altitude) than absolute maximum temperature (Gaston and Chown 1999a). The relative stability of CT_{max} might thus be responsible for the larger difference between thermal optima and habitat temperature in temperate (and high altitude) as compared to tropical populations or species. This implies that cold-adapted populations or species might have lower thermal optima not because they live in environments with lower mean seasonal temperatures, but because they exhibit lower heat resistance (lower CT_{max}).

POTENTIAL CAUSES OF VARIATION IN FECUNDITY PERFORMANCE

Variation in reproductive performance among populations in our experiment was mainly due to variation in absolute fecundity, with temperate populations having higher fecundity at 24°C [19–29°C] than tropical populations, even when controlling for differences in body size (see Results and Discussion sections). In contrast, tropical flies showed higher relative (and partly also absolute) fecundity at high temperature (30°C [25–35°C]). Our data are consistent with findings by Trotta et al. (2006), who found that at 31.2°C the productivity of tropical *D. melanogaster* was significantly higher than that of temperate flies. Similarly, our finding that temperate *D. melanogaster* have higher fecundity at intermediate temperatures agrees well with previous results by Bouletreau-Merle et al. (1982), who found that temperate flies are more fecund than tropical flies. A comparison of temperate and subtropical flies from North America also suggests that temperate flies have higher fecundity (Cooper et al. 2010; but see Schmidt and Paaby 2008). Given the positive relation between body size, ovariole number, and fecundity in fruit flies and other insects (Robertson 1957; Tantawy and Rakha 1964; David 1970; Partridge et al. 1986; Honek 1993; Klepsatel et al. 2013), this pattern is consistent with latitudinal clines observed for body size (David and Bocquet 1975; Coyne and Beecham, 1987; James et al. 1995) and ovariole number (David and Bocquet 1975; Capy et al. 1993). However, we failed to find an effect of body size on fecundity in our data, presumably due to a lack of sufficient power. Yet, however, even though Austrian and Zambian flies were significantly smaller than Ethiopian high-altitude flies in our experiment, they had higher maximum fecundity, perhaps suggesting that the higher fecundity in these two populations is driven by factors other than body size (e.g., ovariole number, higher metabolic rate; also see McCabe and Partridge 1997). Thus, although it would clearly be interesting to examine the interrelationship between body size, ovariole number and fecundity for multiple populations across a range of experimental thermal regimes, such a detailed analysis would require larger sample sizes than in our study.

Latitudinal clines are typically explained by temperature gradients, but other important variables that are correlated with latitude might also contribute to such clines (Blackburn et al. 1999). A higher reproductive output of temperate populations has been observed in multiple taxa, including insects (Huston and Wolverton 2009). Huston and Wolverton (2009) explain these patterns by higher short-term rates in net primary productivity in temperate regions, which are able to support greater biomass at all trophic levels. In line with this, Hawaiian Drosophilids that occupy ecological niches with limited food supply have fewer ovarioles and lower fecundity than species who occupy niches rich in larval nutrition and who have evolved more ovarioles (Kambysellis and Heed 1971). Consequently, the higher fecundity of temperate flies might be a consequence of lower average habitat temperature and higher seasonal resource availability (Bouletreau-Merle et al. 1982; Reznick et al. 2002).

The physiological mechanisms underlying the differences in fecundity performance among populations we have observed remain unclear. Two possible explanations might be differences among populations in diapause incidence or in heat resistance. For example, the decreased performance of Austrian flies at lower temperatures and their more narrow performance breadth was clearly due to their diapause-induced decrease in fecundity. The fecundity of the Swiss population is consistent with this idea: flies from this population, which had been kept in the laboratory for several years, never entered diapause (and hence did not exhibit decreased fecundity) at low temperatures. Interestingly, the ability of *D. melanogaster* to undergo diapause can be rapidly lost under laboratory culture conditions (Schmidt and Conde 2006). The lack of diapause in Swiss flies is therefore likely secondary because in the wild they are expected to cope with similar conditions as the Austrian flies. Differences among populations in heat resistance might be another cause. Hoffmann et al. (2002) found that temperate populations along the Australian latitudinal cline have lower heat resistance than tropical populations. Even though we did not quantify heat resistance, this compares quite well with our observation that Austrian flies suffered

from higher mortality at 28°C (23–33°C) than tropical flies from Zambia (unpublished data). Lower resistance to heat stress might thus shift the mean optimal temperature for fecundity to lower values.

THERMAL REACTION NORMS FOR MORPHOLOGICAL TRAITS ARE RELATIVELY INVARIANT AMONG POPULATIONS

Similar to the minor differences we observed among populations in thermal fecundity performance, populations differed primarily in absolute values of morphological traits, but overall much less in their reaction norms. For thorax length and ovariole number we could not detect any variation in slope or shape of population reaction norms; for wing area, wing loading, and ovariole index we identified rather minor differences in slope or shape, but these patterns were mainly driven by one or two populations only. These findings are broadly consistent with work by Delpuech et al. (1995) who also failed to find significant differentiation among populations in the reaction norm for ovariole number. A few studies have identified variation in reaction norms, with variation among species often being larger than among populations within species. David et al. (1997), for instance, detected differences in reaction norms for wing size among several *Drosophila* species, and Morin et al. (1999) reported variation in reaction norm parameters for wing and thorax length among tropical and temperate populations of both *D. melanogaster* and *Drosophila simulans*. Moreteau et al. (1997) found that the temperatures that maximize trait values for wing length, thorax length, and ovariole number were shifted to lower temperatures in cold adapted *D. subobscura* as compared to warm-adapted *D. melanogaster*. Interestingly, these authors speculate that selection might be more efficient, at least in the short run, in changing trait values rather than reaction norms. Because variation in reaction norms is commonly observed at the interspecific level (Moreteau et al. 1997), and occasionally—but to a lesser extent—at the intraspecific level (Morin et al. 1999; Liefting et al. 2009), changes in trait values might represent the first step of thermal adaptation, with long-term changes eventually leading to changes in reaction norms themselves. Alternatively, differences among species or populations in the temperature that maximizes trait values might reflect differences in thermal tolerance. In *D. subobscura*, for example, the temperatures that maximize the trait values of thorax length or wing size might be lower than in *D. melanogaster* due to higher cold resistance in this species (David et al. 2003). If so, higher cold tolerance in *D. subobscura* might allow for a temperature-driven increase in body and wing size, even under thermal conditions that represent the lower thermal limit for development in *D. melanogaster* (Moreteau et al. 1997).

Conclusions

In summary, our study suggests that thermal adaptation in *D. melanogaster* predominantly involves evolutionary changes in absolute trait values rather than in major aspects of thermal reaction norms. Although the reasons for this pattern must remain largely unclear, our results demonstrate that thermal reaction norms are overall surprisingly invariant among different populations. Even though we did not analyze populations along well-defined clines, our geographically and climatically broad comparison of populations suggests that any differences in thermal performance along clinal gradients are likely to be even more subtle than the patterns we have reported here. The fact that thermal reaction norms are surprisingly invariant among populations of substantially different climatic origin suggests that the rewiring of the genetic and physiological architecture underlying thermal plasticity may be strongly constrained.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Example of a thermal performance curve.
Figure S2. Mean temperatures for all locations-of-origin (± 10–40 km) for the populations examined in this study.
Figure S3. The area of the wing used for wing area measurements.
Table S1. Populations, collection dates, collectors, and the number of isofemale lines for each of the populations used in this study.
Table S2. Climate data.
Table S3. Number of replicate cages used in fecundity experiments.
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