Relating Environmental Variation to Selection on Reaction Norms: An Experimental Test

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abstract: Theoretical models predict that selection on reaction norms should depend on the relative frequency of environmental states experienced by a population. We report a laboratory experimental test of this prediction for thermal performance curves of larval growth rate in *Pieris rapae* in relation to their thermal environment. We measured short-term relative growth rate (RGR) for each individual at a series of five temperatures, and then we assigned individuals randomly to warm or cool selection treatments, which differ in the frequency distributions of environmental temperatures. Selection gradient analyses of two independent experiments demonstrated significant positive selection for increasing RGR, primarily through its effects on survival to adulthood and on development rate. In both the warm and cool selection treatments, the magnitude of directional selection on RGR was consistently greater at lower (suboptimal) temperatures than at higher temperatures; differences in selection between the treatments did not match model predictions. The temporal order and duration of environmental conditions may affect patterns of selection on thermal performance curves and other continuous reaction norms, complicating the connections between variation in environment, phenotype, and fitness.

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The notion that organisms with different phenotypic traits are adapted to different environmental conditions is fundamental to evolutionary biology. The values of a phenotypic trait that increase fitness in one environment may decrease fitness in a different environment. Many lab and field studies have documented how the direction and magnitude of selection on phenotypic traits can vary with changes in environmental conditions (Endler 1986). In most cases, however, the quantitative relationship between environment and selection is unknown. Mathematical models specifying the relation of phenotype to fitness in different environments have been developed for a handful of study systems (Schluter 2000), but these are rarely used to predict the patterns and strength of selection on traits in different environments (Etterson and Shaw 2001). Indeed, traditional evolutionary models do not consider environment explicitly at all, incorporating such effects into the selection parameters. Yet quantitative predictions for how selection and evolution will be altered by climatic warming, increased CO_2, eutrophication, and other environmental changes are a major challenge for evolutionary biology today.

Reaction norms and performance curves are a particularly useful class of traits for exploring these issues. For an individual or genotype, the reaction norm represents the phenotypic trait value expressed as a function of environmental conditions. For aspects of organismic performance such as growth, feeding, and locomotion, the performance curve represents the relationship between an individual's performance and some continuous environmental variable such as temperature (Huey and Stevenson 1979). Theoretical models suggest that selection on reaction norms and performance curves should depend on the relative frequency and predictability of different environmental states experienced by a population (Lynch and Gabriel 1987; Moran 1992; Gilchrist 1995, 2000; Sultan and Spencer 2002). Several studies have demonstrated selection

on reaction norms in alternative environments (Weis and Gorman 1990; Kingsolver 1995; Schmitt et al. 2003), but the quantitative relationship between environmental conditions and selection on reaction norms and performance curves has not been explored.

A recent theoretical model for the evolution of continuous reaction norms considers the relation between environmental variation and selection in fine-grained environments, in which all individuals or genotypes experience similar environmental variation within a generation (Kingsolver and Gomulkiewicz 2003). One key prediction of this model is that selection on continuous reaction norms is directly related to the frequency distribution of environmental variation (see "Material and Methods"). In this article, we test this prediction by experimentally manipulating the frequency distribution of thermal environments and estimating the resulting changes in selection on thermal performance curves for growth rate in *Pieris rapae* caterpillars.

Material and Methods

The Model and Predictions

We can consider the thermal performance curve of an individual as a continuous function $z(T)$, where performance varies as a function of the individual's body temperature. If performance at some or all temperatures affects the fitness, then variation in $z(T)$ among individuals within a population may cause variation in fitness; that is, there may be phenotypic selection on performance curves. Selection on performance curves, or similar traits that are continuous functions, is usefully characterized by the directional selection gradient function $\beta(T)$, which represents the strength of directional selection on performance as a function of temperature T (Kirkpatrick and Lofsvold 1988; Kirkpatrick and Heckman 1989; Gomulkiewicz and Kirkpatrick 1992). The selection gradient function $\beta(T)$ is the continuous analogue to the more familiar selection gradient vector $\boldsymbol{\beta}$, which represents directional selection on a set (vector) of quantitative traits $\mathbf{z}$ (Lande 1979; Lande and Arnold 1983).

It is intuitive that selection on thermal performance curves in a population should be related to the thermal environmental conditions experienced during selection. A recent theoretical model explores this relationship for thermal performance curves of relative growth rate (Beder and Gomulkiewicz 1998; Kingsolver and Gomulkiewicz 2003). Consider an episode of selection of duration L for some population of interest, and let $z(T)$ represent the relative growth rate of an individual as a function of temperature T. Suppose that the absolute fitness of an individual is related to its relative growth rate, and assume that relative growth rate and its contributions to fitness at any time are determined only by temperature T, not by the specific time or temporal order at which that temperature occurs. Finally, let $f(T)$ be the frequency distribution (technically, the probability density function) of temperatures experienced by all individuals during the selection episode. With these assumptions, one can show that

$$\beta(T) = Lv(T)f(T), \tag{1}$$

where $v(T)$ is a weighting function that weights the contribution of relative growth rate at temperature T to fitness, and L and $v(T)$ are independent of $f(T)$ (Kingsolver and Gomulkiewicz 2003). This theoretical result predicts that the form of the selection gradient function on thermal performance curves, $\beta(T)$, is related to the frequency distribution of temperatures experienced during selection, $f(T)$ (Kingsolver and Gomulkiewicz 2003).

The model provides a means to predict patterns of selection in altered environmental conditions. For example, suppose that for some selective environment C (cool), we estimate the frequency distribution $f_C(T)$ and selection gradient $\beta_C(T)$. We can rearrange equation (1) to show that $Lv(T) = \beta_C(T)/f_C(T)$. Now consider some new selective environment W (warm) with frequency distribution $f_W(T)$. The model predicts that $Lv(T) = \beta_W(T)/f_W(T)$. Equating these two expressions for $Lv(T)$ and rearranging yields a prediction for $\beta_W(T)$ in terms of $\beta_C(T)$, $f_C(T)$, and $f_W(T)$:

$$\beta_{W_{\text{predicted}}}(T) = \beta_C(T) \times \frac{f_W(T)}{f_C(T)}. \tag{2}$$

This provides a straightforward and testable prediction of the model: experimentally shifting $f(T)$ should shift $\beta(T)$ by the ratio of the environmental temperature distributions. The lab experiments reported here test this prediction.

Study System

Pieris rapae L. (Lepidoptera: Pieridae) is native to Europe and was introduced to eastern North America more than 140 years ago (Scudder 1887). Populations of *P. rapae* now occur on every continent except Antarctica and exploit a variety of larval host plants in the family Brassicaceae. There are five larval instars, with more than 90% of larval growth occurring in the last two instars. In many areas, *P. rapae* is an agricultural pest on domesticated varieties of *Brassica oleracea*, including collards, cabbage, and broccoli. *Pieris rapae* can also be readily maintained on artificial diets in the laboratory (Slansky and Feeny 1977; Slansky 1978). Under appropriate temperature and food condi-

tions, *P. rapae* will feed and grow in daytime and nighttime, in both the light and the dark. The studies described here were conducted using *P. rapae* collected in the Piedmont region of central North Carolina. Here, it is one of the most common butterflies in the area, where it is abundant in urban gardens and organic farms and feeds primarily on domesticated forms of *B. oleracea*. In this region, *P. rapae* completes five or more generations per year, with adult flight seasons from March to October.

Thermal Biology of Pieris rapae

Pieris rapae caterpillars are cryptically colored, spend much of their time on the shady sides of leaves, and do not actively regulate body temperature except at extremely high temperatures (Jones 1977; A. Nagle and J. G. Kingsolver, unpublished manuscript). As a result, we have successfully used physical models to assay variation in body temperature in the field (Bakken et al. 1985; Kingsolver 2000). Field measurements in collard gardens in Washington and North Carolina show that *P. rapae* caterpillars experience a wide range of body temperatures on a daily basis, with temperatures often varying by 20°–25°C over a 12-h time period (Kingsolver 2000; Kingsolver et al. 2004*a*). The frequency distribution of mean operative temperatures for July–August 2001 at our study site in Chapel Hill, North Carolina, shows that caterpillars spend much of their time between 18° and 25°C, with a peak near 23°C, reflecting nighttime and morning conditions; however, there is a long right tail to the distribution, reflecting hot midday conditions, with mean temperatures sometimes exceeding 38°C (fig. 1, *top*). By comparison, the frequency distribution for our study site in Seattle, Washington, is similar in overall shape to that for North Carolina, but it shifted to lower temperatures by 6°–8°C (fig. 1, *top*).

As with most larval insects, temperature has strong effects on growth rates, developmental rates, and mortality of *P. rapae* caterpillars (Slansky and Feeny 1977; Slansky 1978; Chen and Su 1982; Gilbert 1984*a*, 1984*b*; Gilbert and Raworth 1996; Kingsolver 2000). For example, mean short-term (6 h) growth rates for *P. rapae* increase with increasing temperatures between 8° and 35°C, are maximized near 35°C, and decline rapidly at temperatures above 35°C (Kingsolver 2000). However, the timescale of thermal exposure has important effects on growth rate responses. For example, at 35°C, relative growth rate is rapid at 6 h, but it declines by 50% after 24–30 h exposure; after 48 h exposure, mortality rate increases significantly (J. G. Kingsolver and J. G. Shlichta, unpublished manuscript). As a result, both the shape and location of the maximum for thermal performance curves for growth rate change over timescales from 6 to 54 h (J. G. Kingsolver and J. G. Shlichta, unpublished manuscript). Because cat-

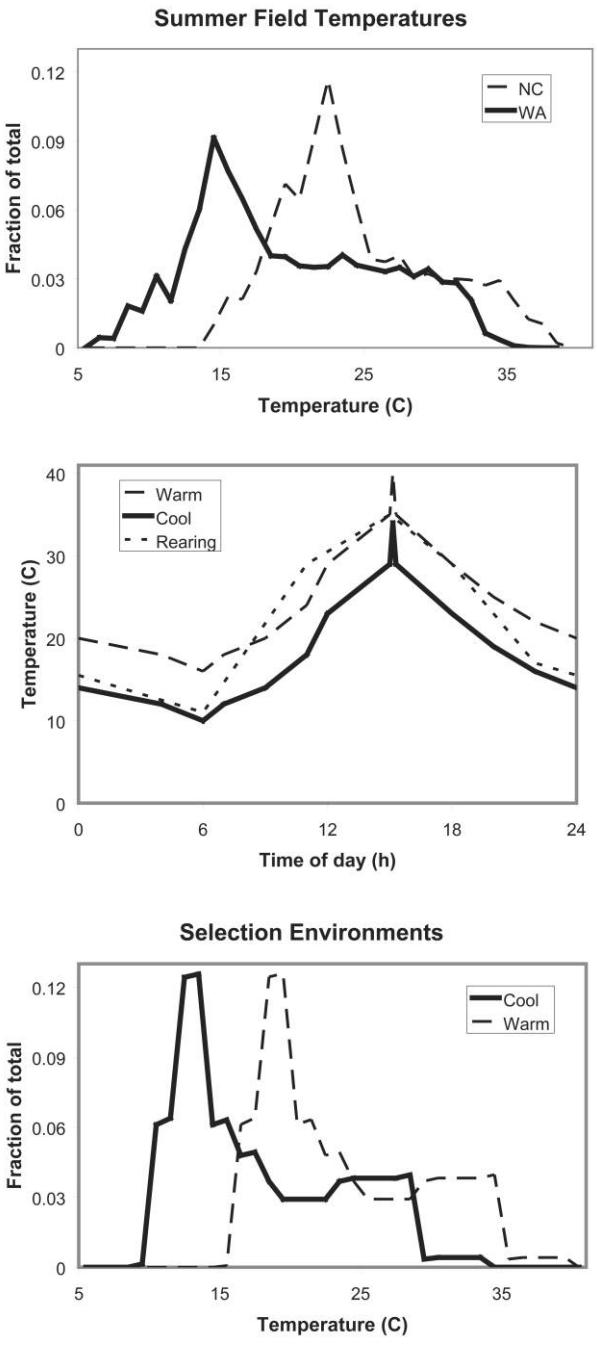

Figure 1: *Top*, frequency distribution of mean operative temperatures for model caterpillars of *Pieris rapae* in collard gardens in Chapel Hill, North Carolina (*dashed line*), and Seattle, Washington (*solid line*), for July–August 2000 and 2001. Data from Kingsolver (2000) and Kingsolver et al. (2004*a*). *Middle*, diurnal temperature cycles used in the experiments for the rearing conditions (*dotted line*) and the warm (*dashed line*) and cool (*solid line*) selection treatments. *Bottom*, frequency distribution of temperatures for the warm (*dashed line*) and cool (*solid line*) selection treatments used in the experiments.

erpillar body temperatures change substantially at hourly timescales under natural field conditions (Kingsolver 2000), we believe that short-term assays of growth rate are essential for understanding adaptive aspects of thermal performance curves in our system. There is substantial phenotypic and genetic variation in short-term growth rate at temperatures from 8° to 40°C, with phenotypic and genetic variation generally increasing with increasing temperature (Kingsolver et al. 2004*b*).

Experimental Design

Our studies begin with fresh (wing wear classes 1–2) adult female *P. rapae* collected from organic farms in Orange County, North Carolina, and transported directly to our laboratory in Chapel Hill. In the field, females almost always mate within a few hours of adult eclosion; after mating, there is a refractory period of 2–4 days before remating by a female occurs (Wiklund et al. 2001). In addition, there is nearly complete sperm precedence when females remate (Wedell and Cook 1998, 1999*a*, 1999*b*; Wiklund et al. 2001). As a result, all offspring of each female in our studies very likely represent full sibs, though we cannot rule out some possibility for multiple paternity. Our studies employed a split sib-family design. Each female was allowed to lay eggs on a young collard plant (*B. oleracea* var. collard: CO350 Champion variety) for 48 h. Newly hatched caterpillars were maintained in petri dishes placed in environmental chambers under a diurnal fluctuating thermal regime (11°–35°C) and light : dark cycle (16L : 8D) that mimics midsummer thermal conditions (fig. 1, *middle*; Kingsolver 2000; Kingsolver et al. 2001*b*). Caterpillars were fed on a standard artificial diet (Troetschler et al. 1985) for *P. rapae* that includes 1.6% dried collard leaves by weight; fresh diet was provided every 2 days.

The phenotypic measurements began when caterpillars were newly molted into the fourth instar. In the morning following molt, the growth rate (mass increase/time) of each caterpillar was measured at a series of five constant temperatures such that all measurements on an individual were completed within a 36-h period. Each test temperature was maintained in a different environmental chamber (Percival 36-VL) calibrated with a Wescor TH-65 thermocouple thermometer. All mass measurements were made with a Mettler Toledo electronic microbalance (± 0.01 mg) interfaced to a notebook computer. The duration and time-of-day of exposure to each measurement temperature were chosen to reflect thermal conditions typical of diurnal conditions in the field: higher temperatures involved shorter measurement durations during daytime, whereas lower temperatures involved longer measurement durations during nighttime (Kingsolver et al. 2004*b*). No acclimation period was used before or between test measurements. Two independent experiments were performed, in 2002 and 2004, each beginning with field-caught females. The 2002 experiment measured growth rates for temperatures between 11° and 35°C; the 2004 experiment measured growth rates for temperatures between 13° and 35°C. The temporal orders (and durations) of the growth rate measurements for the 2002 experiment were 23°C (4–5 h), 29°C (4 h), 11°C (12–14 h), 17°C (7 h), and 35°C (2–3 h) and for the 2004 experiment were 25°C (4 h), 30°C (4 h), 13°C (12–14 h), 19°C (7 h), and 35°C (2–3 h). In this design, all caterpillars experience the same order of temperature measurement rather than some random order; we believe it was more important to maintain a natural ordering of temperature exposure (see Kingsolver et al. 2004*b* and "Discussion"). Fresh artificial diet was used at each test temperature. In the 2002 experiment, 248 caterpillars from 15 sib families were measured, and in the 2004 experiment, 304 caterpillars from 40 sib families were measured.

More than 80% of all larval growth occurs during the late fourth instar and the fifth instar, after the growth rate measurements were completed. Following the growth rate measurements, individuals within each family were randomly assigned to one of two selection treatments: warm or cool (fig. 1, *middle*). These treatments involved diurnally cycling temperatures with a 16L : 8D photocycle that mimicked typical summer conditions in North Carolina and Washington, respectively (fig. 1, *top*). The shape of the frequency distribution of temperatures, $f(T)$, was identical in the two treatments but with the distribution shifted by 6°C (fig. 1, *bottom*). Each selection treatment was maintained in a different environmental chamber (Percival 36-VL) calibrated with a Wescor TH-65 thermocouple thermometer. Because of limited chambers, the treatments were not replicated, but different chambers were used for the two selection treatments in 2002 and 2004. Caterpillars were maintained in individual petri dishes, provided new food every 2 days, and checked daily. For each caterpillar we recorded the following correlates of fitness: survival from larva to adult (adult survival), time from larva to adult (development time), adult mass, and female fecundity. On eclosion, adult females were placed in a large mating cage with multiple males for 2 days; then each female was put in a small oviposition cage with a collard plant. The total number of eggs laid in 48 h was used as an index of fecundity.

Statistical Analyses

The main effects of selection treatment on the response variables were evaluated with MANOVA. Mean and standard error were estimated for each response variable for

each treatment. Short-term growth rate of each individual at each temperature was quantified as relative growth rate (RGR), defined as RGR $= [\ln(m_f) - \ln(m_i)]/t$, where m_i and m_f are initial and final masses, respectively, and t is the duration of the test period. The RGR represents the proportional increase in mass per unit time, with units of h^{-1} (see "The Model and Predictions").

A major goal for the analyses is to determine how RGR at different temperatures affects aspects of fitness and whether the effects of RGR on fitness differ between selection treatments. We focused attention on four correlates of fitness: survival to adulthood, development time, adult mass, and fecundity. For each fitness component, we estimated the full linear model, including terms for the treatment, RGR at each of the five temperatures, and the interaction between treatment and RGR at each temperature. We then dropped terms from this full model and use the Akaike Information Critierion (AIC) to identify the best model for the data. The AIC represents a balance between the likelihood explained by the model and the number of model parameters; the best model minimizes AIC.

Finally, we estimated directional selection gradients (β) for RGR separately in each selection treatment. Because methods for estimating selection gradient functions are not available, we estimated the multivariate selection gradients by considering RGR at each measurement temperature as a distinct (but correlated) trait (Lande and Arnold 1983; Via and Lande 1985). Because development time is expected to be inversely associated with fitness, through its effects on generation time, we use development rate (1/development time) as a fitness correlate in the analyses. Relative fitness values (i.e., standardized for the mean within each treatment) are used as the response variable for each fitness correlate. Note that the selection gradients in equations (1) and (2) are not standardized by variance or mean of the phenotypic trait values—these do not represent standardized selection gradients (Lande and Arnold 1983; Arnold and Wade 1984; Kingsolver et al. 2001*a*; Hereford et al. 2004). Gradients for each fitness correlate (survival to adulthood, development rate, adult mass, and female fecundity) were estimated separately. In addition, the product of survival, development rate, and adult mass was used as a metric of combined fitness for each individual, and selection gradients for combined relative fitness were also estimated (Arnold and Wade 1984). Note that RGR has units of mg/mg/h = 1/h, so that our estimates of β have units of h.

Our warm and cool selection treatments have frequency distributions that are similar in shape, but $f_W(T)$ is shifted along the temperature axis by 6°C relative to $f_C(T)$ (fig. 1, *bottom*). As a result, the ratio $f_W(T)/f_C(T)$ changes in a complex way as a function of temperature T. For example, at $T = 25$°C, $f_W(25)/f_C(25) \approx 1$, whereas at $T = 13$°C, $f_W(13)/f_C(13) \sim 0$ (fig. 1, *bottom*). By equation (2), our model therefore predicts that $\beta_W(25)/\beta_C(25) \sim 1$ and that $\beta_W(13)/\beta_C(13) \sim 0$. To test our model, we use the measurements of $f_W(T)$ and $f_C(T)$ and our estimates of $\beta_C(T)$ to predict $\beta_W(T)$ and compare this to the estimated values. Note that for temperatures above 34°C, the ratio $f_W(T)/f_C(T)$ approaches infinity, making the model predictions very sensitive to estimation error above this temperature.

Results

Main Effects

The MANOVA showed that there were significant effects of the selection treatment on the response variables in both experiments ($P < .0001$). Mean development time was greater in the cool treatment than in the warm treatment in both experiments (table 1). In the 2002 experiment, adult survival and fecundity were greater in the cool treatment than in the warm treatment (table 1).

Population mean RGR generally increased with temperature across this temperature range, but there was substantial phenotypic variation in thermal performance curves among individuals (fig. 2). The estimated phenotypic variance-covariance matrix for short-term RGR at each temperature shows that phenotypic variance increased markedly at higher temperatures in both experiments (table 2). Phenotypic covariances in RGR across temperatures were modest, with phenotypic correlations ranging from −0.21 to 0.23 in the 2002 experiment and from 0.13 to 0.52 in the 2004 experiment. Thus, there was substantial phenotypic variation in RGR at different temperatures on which selection could have operated.

Table 1: Mean (1 SE) response variables in the warm and cool treatments

Response	Cool	Warm
2002 experiment:		
Adult survival	.926 (.024)	.873 (.030)
Development time (days)	25.76 (.18)	18.19 (.15)
Adult mass (mg)	95.12 (2.62)	91.95 (2.74)
Fecundity	69.52 (7.21)	47.66 (6.79)
2004 experiment:		
Adult survival	.967 (.014)	.947 (.018)
Development time (days)	26.55 (.23)	19.04 (.15)
Adult mass (mg)	99.74 (2.21)	101.19 (2.05)
Fecundity	96.31 (7.39)	83.24 (6.40)

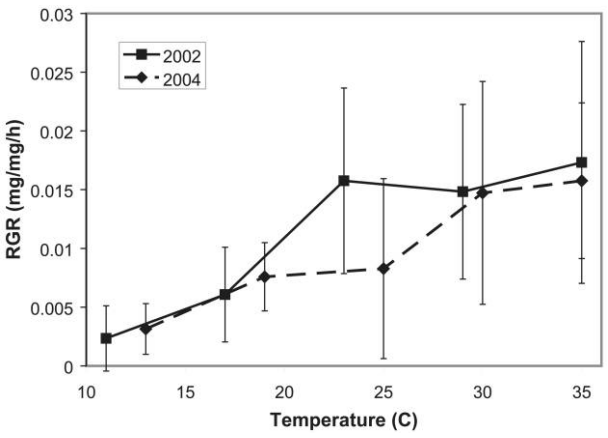

Figure 2: Short-term relative growth rate (RGR, in mg/mg/h) as a function of temperature for *Pieris rapae* from Chapel Hill, North Carolina, for the 2002 (*solid line*, *squares*) and 2004 (*dashed line*, *diamonds*) experiments. Mean ± 1 SD is indicated.

Model Fitting

Stepwise model fitting identified the best model for each of the four fitness components: survival to adulthood, development time, adult mass, and fecundity (table 3). In both experiments, the best model for each fitness component included effects of the selection treatment and/or of RGR for at least some temperatures. In the 2002 experiment, the best models for survival and development time did not include interactions between treatment and RGR; however, the best models for adult mass and fecundity included interactions between treatment and RGR for at least some temperatures. In the 2004 experiment, the best model for adult mass did not include interactions between treatment and RGR; however, the best models for survival, development time, and fecundity included interactions between treatment and RGR for at least some temperatures. These analyses suggest that the selection treatment environment altered the relationship between thermal performance curves for RGR and fitness for some fitness components but not for others.

Selection Gradient Analyses

Estimated directional selection gradients (β) for RGR at different temperatures in each selection environment for the 2002 experiment are illustrated in figure 3. For survival to adulthood, there was significant directional selection on RGR in both the warm ($P = .0004$) and cool ($P < .0001$) selection treatments. For survival, there was selection favoring increased RGR at all temperatures, with stronger selection at the lower (11° and 17°C) temperatures (fig. 3, *top*); the pattern of selection for survival was similar in both warm and cool selection treatments. For development rate, there was significant directional selection on RGR in the warm ($P = .0018$) but not in the cool ($P = .0911$) selection treatment. In the warm treatment, there was selection favoring increased RGR, with stronger selection at the lower temperatures (fig. 3, *middle*). For adult mass, there was again significant directional selection on RGR in the warm ($P = .0014$) but not in the cool ($P = .58$) selection treatment. In the warm treatment, there was positive selection for increased RGR at 17° and at 35°C but not at other temperatures (fig. 3, *bottom*). For fecundity, there was no significant directional selection on RGR in either the warm ($P = .758$) or the cool ($P = .1164$) selection treatment.

Estimated directional selection gradients (β) for RGR at different temperatures in each selection environment for the 2004 experiment are illustrated in figure 4. For survival, there was significant directional selection on RGR in the cool ($P = .0001$) but not in the warm ($P = .1242$) selection treatment. In the cool treatment, there was positive selection favoring increased RGR at 19° and at 35°C but not at the other temperatures (fig. 4, *top*). For development rate, there was significant directional selection on RGR in both the warm ($P < .0001$) and the cool ($P < .0001$) selection treatments. In both selection treatments, there was positive selection favoring increased RGR, with stronger selection at the lowest temperature (13°C; fig. 4, *bottom*). For adult mass, there was no significant directional selection on RGR in either the warm ($P = .727$) or the cool ($P = .525$) selection treatment. Similarly for fecundity, there was no significant directional

Table 2: Phenotypic variance-covariance matrices for short-term relative growth rate (RGR) at different temperatures for fourth instar *Pieris rapae*

	2002 experiment				
	RGR11	RGR17	RGR23	RGR29	RGR35
RGR11	7.741	−1.304	3.021	−4.423	−6.032
RGR17	−.116	16.25	1.005	2.834	−5.806
RGR23	.137	.032	62.49	−3.765	6.655
RGR29	−.214	.095	−.064	55.287	17.966
RGR35	−.211	−.14	.082	.235	105.993
	2004 experiment				
	RGR13	RGR19	RGR25	RGR30	RGR35
RGR13	4.654	2.682	8.661	8.583	2.259
RGR19	.428	8.431	9.048	9.312	6.388
RGR25	.524	.407	58.657	34.625	6.795
RGR30	.419	.338	.477	89.98	18.154
RGR35	.158	.332	.134	.289	43.862

Note: For example, RGR11 refers to the relative growth rate at 11°C. Variances and covariances (in mg/mg/h × 10^6) are along and above the diagonal; correlations are below the diagonal.

Table 3: Best models (based on Akaike Information Criterion) for response variables

Response	TRT	SEX	RGR11	RGR17	RGR23	RGR29	RGR35	RGR11 × TRT	RGR17 × TRT	RGR23 × TRT	RGR29 × TRT	RGR35 × TRT
2002 experiment:												
Survival	Y	NA	Y	Y	Y	Y	Y					
Development time	Y		Y	Y	Y	Y	Y					
Adult mass	Y			Y	Y		Y		Y			Y
Fecundity	Y	NA	Y	Y	Y			Y	Y	Y		
2004 experiment:												
Survival	Y	NA		Y		Y	Y				Y	Y
Development time	Y		Y		Y	Y	Y				Y	
Adult mass		Y		Y								
Fecundity	Y	NA		Y			Y		Y			

Note: Y = term included in best model; NA = term not available.

selection on RGR in either the warm ($P = .142$) or the cool ($P = .3179$) selection treatment.

Our combined fitness metric integrates the responses of survival, development rate, and adult mass (see "Material and Methods"). In the 2002 experiment, there was significant selection on RGR in the warm ($P = .0003$) but not in the cool ($P = .7653$) selection treatment. In the warm treatment, there was positive selection favoring increased RGR at intermediate (17°–23°C) and high (35°C) temperatures, with the strongest selection at 17°C (fig. 5, *top*). In the 2004 experiment, there was marginally significant selection on RGR in the warm ($P = .057$) and significant selection in the cool ($P = .0017$) selection treatment. In both warm and cool treatments, there was positive selection favoring increased RGR at the lowest temperature (13°C; fig. 5, *bottom*).

Discussion

Patterns of Selection on Growth Rate

Several general patterns emerge from our experimental analyses of selection on RGR. Both of our experiments detected positive directional selection on RGR through its effects on survival. Because mean survival to adulthood was quite high in the two selection treatments for both experiments (ranging from 87%–97%), the opportunity for selection via survival differences was rather limited. Our results suggest that those individuals who died during selection had relatively low growth rates, perhaps reflecting an effect of general vigor. In the field, where larval mortality rates for *Pieris rapae* may exceed 90%, the opportunity for selection via variation in survival is probably much greater.

There was also positive selection on RGR through its effects on development rate in both experiments, occurring in both the warm and cool selection treatments. Selection for growth rate and other traits that increase development rate and decrease generation time may be particularly important for *P. rapae* and other species that have a variable number of generations per year. In central North Carolina, *P. rapae* overwinter in a pupal diapause and may have as many as six complete generations per year during the active season. In this population, the first one to two adult flight seasons in the spring are highly synchronous, but during summer and fall, adult emergence is more or less continuous, reflecting a mixture of different generations. In the field, rapid development rates can also increase survival to adulthood. For example, Benrey and Denno (1997) showed that more rapid growth was associated with reduced parasitism and mortality rates of *P. rapae* in the field.

By contrast, we detected significant selection on RGR through its effects on adult body size only in the warm treatment in the 2002 study. While there is substantial phenotypic and genetic variation in adult size in *P. rapae* (J. G. Kingsolver, K. R. Massie, G. J. Ragland, and M. H. Smith, unpublished manuscript), the endocrine processes during molting and metamorphosis reduce the association between larval growth rate and adult body size (Davidowitz and Nijhout 2004). Similarly, we detected no significant selection on RGR through effects on fecundity in either experiment. Previous studies have demonstrated a strong correlation between adult body size and fecundity in *P. rapae* (Jones et al. 1982), but these are not strongly connected to larval growth rate in this and perhaps other holometabolous insect species.

Our analyses indicate positive directional selection for growth rate, but we found no significant negative selection for growth rate at any temperature in either experiment: in no case was higher growth rate correlated with reduced fitness. Many authors have suggested the possibility of fitness costs associated with rapid growth, but our selection analyses did not detect such costs (Arendt 1997; Roff 2002;

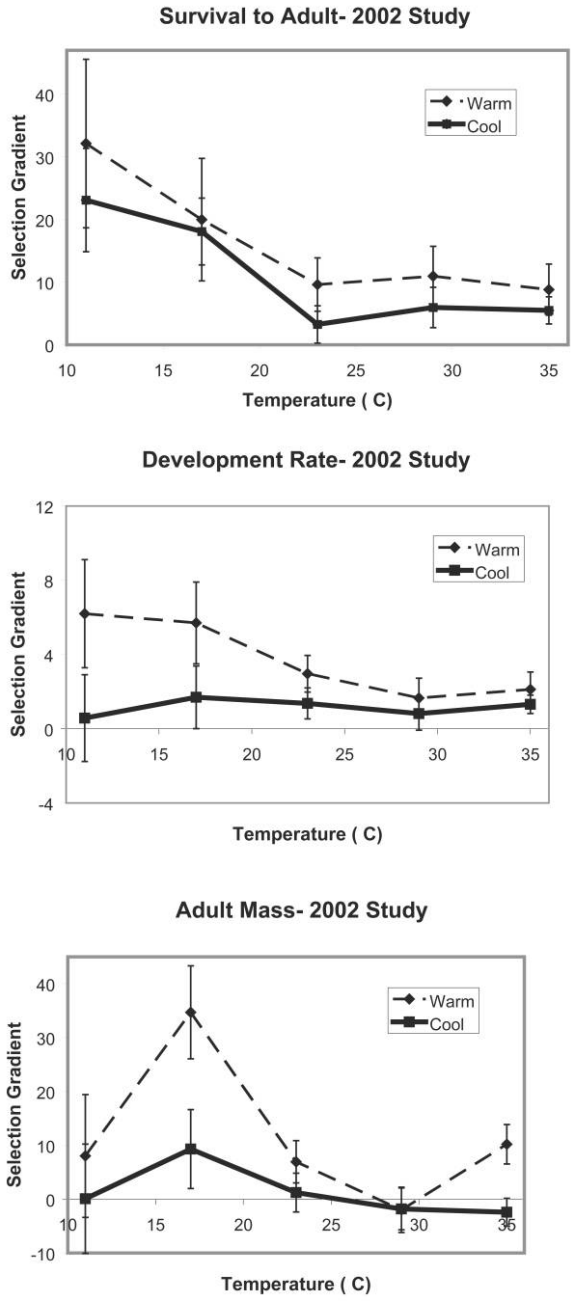

Figure 3: Directional selection gradients β (± 1 SE) for relative growth rate as a function of temperature for the 2002 experiment for the warm (*dashed lines*) and cool (*solid lines*) selection treatments. *Top*, adult survival; *middle*, development rate; *bottom*, adult mass.

Angilletta et al. 2003). Of course, such fitness costs might only emerge in the presence of predators, parasitoids, or other factors that were excluded from our laboratory studies.

Environmental Variation and Selection on Thermal Performance Curves

The major motivation for our experiments was to explore the relationship between patterns of environmental variation (in this case, temperature) and patterns of phenotypic selection (here, on thermal performance curves for growth rate). In particular, we tested a specific prediction from a theoretical model for selection on performance curves: that a shift in the frequency distribution of environmental temperatures during selection (fig. 1) should generate a predictable change in the selection gradient function for thermal performance curves (eq. [2]; Kingsolver and Gomulkiewicz 2003).

Our results provide several lines of evidence that shift in selective environments can alter temperature-specific patterns of selection on RGR. For example, the best statistical models for our data include interactions between the treatment (shifts in environmental temperatures) and RGR at some temperatures. For the 2002 experiment, selection gradient analyses indicated that selection on RGR was generally stronger in the warm than in the cool treatment for survival, development, and adult mass (fig. 3). The analyses also suggest stronger selection on RGR at some temperatures than at others. For most fitness components in both studies, selection gradients indicated stronger selection at lower temperatures (11°–19°C) than at higher temperatures (25°–35°C). The analyses of combined fitness suggested the strongest selection at 17°C in the 2002 experiment and at 13°C in the 2004 experiment.

As described above (see "Material and Methods"), we can use our estimates of β and $f(T)$ in the cool treatment to predict the selection gradient for the warm treatment (eq. [2]). We computed these predictions for combined fitness in each study, using $f(T)$ binned at 5°–6°C intervals to match the temperatures at which RGR was measured. (Further analyses indicated that alternative binning did not alter our qualitative conclusions.) These calculations (fig. 6) reveal a poor match between the predicted and estimated values of β at most temperatures in both studies, with predicted values often more than 1–2 standard errors outside the estimated values. The predicted and estimated patterns of selection were particularly divergent in the 2004 study. Therefore, our results do not support the predictions of equations (1) and (2).

There are several possible explanations for the failure of the model. A key assumption of the model leading to equations (1) and (2) is that the duration or temporal ordering of exposure to different temperatures does not affect growth rate or its relationship to fitness (Kingsolver and Gomulkiewicz 2003). For example, many physiological studies with insects and other ectotherms show that duration of exposure to high temperature can affect sub-

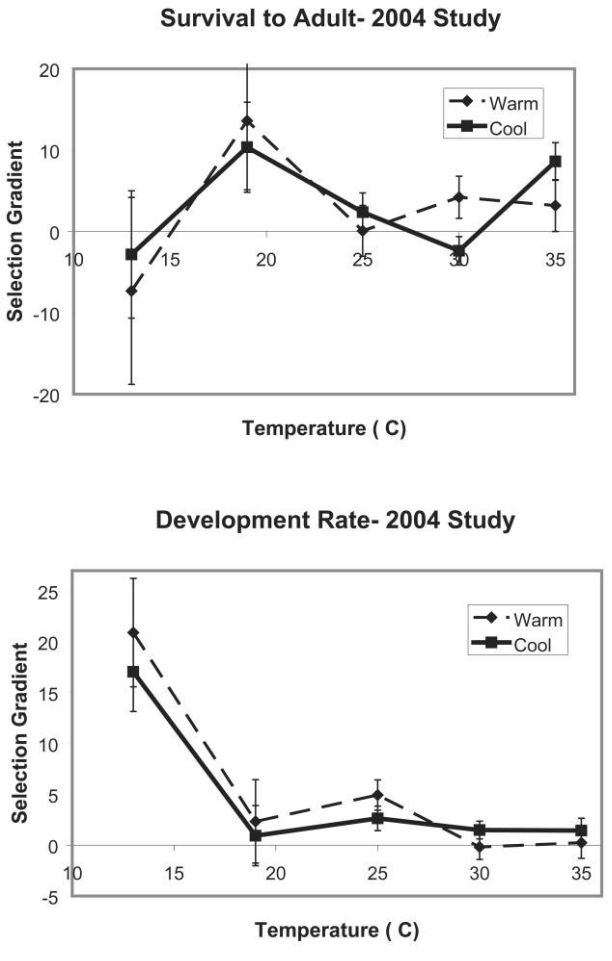

Figure 4: Directional selection gradients β (± 1 SE) for relative growth rate as a function of temperature for the 2004 experiment for the warm (*dashed lines*) and cool (*solid lines*) selection treatments. *Top*, adult survival; *bottom*, development rate.

sequent growth and fitness (Hoffmann 1999; Feder et al. 2000). This may be an issue in the warm treatment, in which temperatures range between 35° and 40°C for ~30 min each day; chronic exposure of *P. rapae* to 40°C leads to reductions in growth rate and increased mortality after 24–48 h (J. G. Kingsolver and J. G. Schlichta, unpublished manuscript). Note, however, that in central North Carolina, *P. rapae* caterpillars routinely approach body temperatures of 40°C during midday in July–August (Kingsolver et al. 2004*a*).

Another possibility is that feeding and growth at specific times of day may contribute disproportionately to survival, development rate, or other fitness components. For example, our selection treatments used diurnally fluctuating conditions in which the coolest temperatures always occur overnight, as in natural thermal environments. If growth or related processes occurring overnight have a disproportionate impact on fitness, this would generate stronger selection on growth rate at lower temperatures, as observed in our studies (figs. 3, 4). Similarly, a common feature of natural thermal environments in temperate regions is that the modal (most common) temperature is lower than the median or mean daily temperature (fig. 1, *top*); this is also the case for our selection treatments. If duration of exposure to the modal temperature affects growth and fitness, this would also generate stronger selection on growth rate at lower (modal) temperatures.

A final possibility is that our measured phenotypic traits (RGR at each temperature) are correlated with other unmeasured traits associated with fitness. This is a common and difficult problem for analyses of selection that rely on natural variation in phenotypes (Mitchell-Olds and Shaw

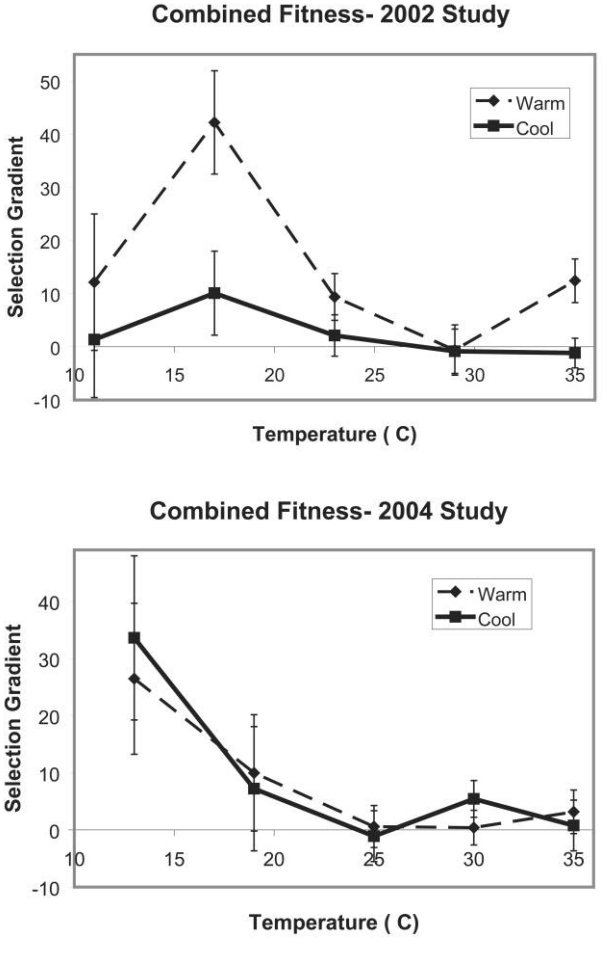

Figure 5: Directional selection gradients β (± 1 SE) for relative growth rate as a function of temperature through its effects on combined fitness for the warm (*dashed lines*) and cool (*solid lines*) selection treatments. *Top*, 2002 experiment; *bottom*, 2004 experiment.

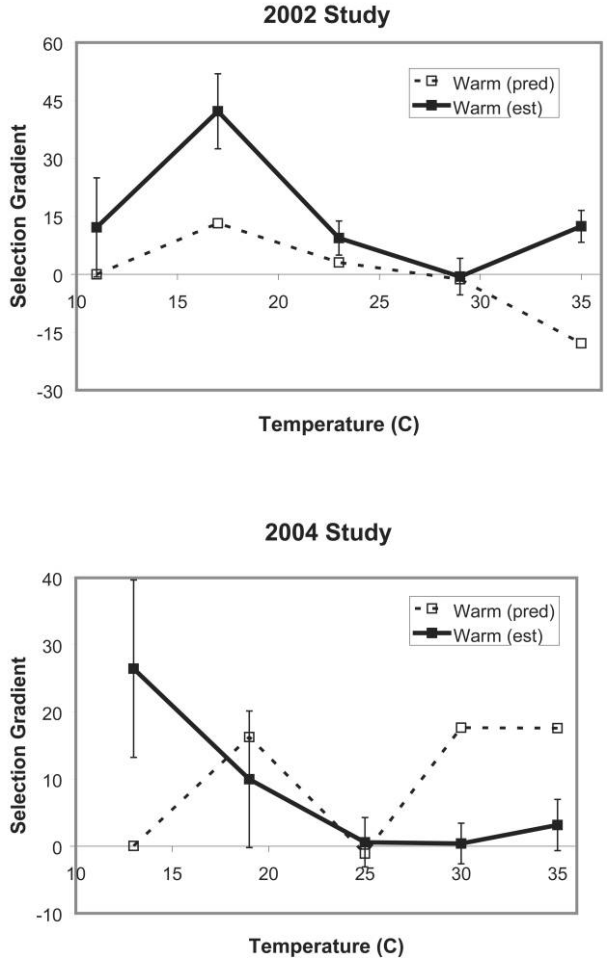

Figure 6: Estimated (*solid lines*) and predicted (*dashed lines*) directional selection gradients β (± 1 SE) for relative growth rate as a function of temperature through its effects on combined fitness for the warm selection treatments. *Top*, 2002 experiment; *bottom*, 2004 experiment.

1987). Our finding of selection on RGR at 11° and 13°C via development rate in the warm treatment, in which selection temperatures never fall below 16°C, suggests this interpretation.

It is worth noting an important special case of the model (eq. [1]) in which absolute fitness is proportional to total growth or size increase during the selection episode (or equivalently, the rate at which a final size is achieved; Kingsolver and Gomulkiewicz 2003). In this case equation (1) simplifies to

$$\beta(T) = Lf(T). \tag{3}$$

For this special case, the model predicts that the frequency distribution of temperatures experienced during selection, $f(T)$, directly determines the selection gradient function on thermal performance curves, $\beta(T)$ (Kingsolver and Gomulkiewicz 2003). In contrast, our experimental results show that the shape or position of $f(T)$ (fig. 1) is not similar to the shape or position of the resulting selection gradients (figs. 3–5). This suggests that the variation in thermal performance curves of growth rate must contribute to fitness in ways not accounted for by total cumulative growth during selection.

Despite the failure of the model predictions, our studies do suggest several important results about selection on growth and thermal performance curves. First, we have demonstrated significant positive selection for growth rate, primarily through its effect on survival and development rate. The positive fitness consequences of rapid growth are frequently assumed but rarely demonstrated (Arendt 1997). Conversely, our results detect no fitness costs of rapid growth in the absence of natural enemies. Second, although short-term growth rate is greatest at relatively high temperatures, near 35°C (fig. 2; Kingsolver 2000), we observed selection on RGR predominately at much lower temperatures (11°–19°C). For larval growth rate, we found little evidence for the notion of strong selection on peak performance capacity (Hertz et al. 1983, 1988): instead, those individuals that grew relatively rapidly at the more common, suboptimal conditions had greater fitness. Such selection at lower, suboptimal temperatures may be relevant to the evolution of the shape of thermal performance curves (Heinrich 1977; Huey and Kingsolver 1989). It is interesting to note that in many insects, growth rate tends to increase more or less linearly with increasing temperature until approaching maximal growth rate at the optimal temperature; this linearity is the basis for the success of degree-day models for insect growth (Worner 1992). The cause of this linear relationship, rather than the exponential relationship that might be expected based on Q_{10} effects of temperature, has puzzled entomologists (Gilbert 1984*a*, 1984*b*). Consistent directional selection on growth rate at lower temperatures could potentially alter the shape of the mean thermal performance curve in this manner; however, the generality of our results for other insects remains to be established.

Limitations of the Study

To our knowledge, our studies with *P. rapae* represent the first to estimate the strength of selection on thermal performance curves. One probable reason for this is that estimating phenotypic selection on thermal performance curves requires performance measurements at a series of temperatures for each individual, for a large sample of individuals from a population. This involves a number of challenges and compromises that may affect the power and interpretation of such studies.

First, we determined the thermal sensitivity of growth rate of an individual using measurements of mass increase over short (2–14 h) time periods at a series of fixed temperatures during <36 h of a single larval instar. We believe that short-term measurements more accurately reflect conditions in natural field conditions, where individuals routinely experience body temperatures ranging over 20°C during a single diurnal cycle and where caterpillars consistently experience higher temperatures for shorter time periods during daytime and lower temperatures for longer periods during nighttime (Kingsolver 2000; see Kingsolver et al. 2004*b* for a detailed discussion of the rationale and implications of this approach to measuring thermal performance curves).

Second, because of handling constraints, we cannot measure thermal performance curves in *P. rapae* until the start of the fourth larval instar. As a result, the selection episode in each experiment only involves the late fourth instar, fifth instar, and pupal stages rather than the entire larval period. We note that more than 80% of all larval growth (mass increase) occurs during the late fourth instar and the fifth instar, so that the selection episode does encompass most of the time interval relevant to the fitness consequences of variation in growth rate.

Third, the statistical power of our estimates of selection is strongly affected by sample size. The sample sizes in the 2002 and 2004 experiments (248 and 304 individuals, respectively) are substantially larger than in most studies of phenotypic selection (Kingsolver et al. 2001*a*), but they do result in rather large standard errors on the estimated selection gradients (figs. 3–5) and limit the power of our analyses to reject the null hypothesis of no selection (Hersch and Phillips 2004).

Fourth, estimates of phenotypic selection may be biased by environmental covariation, if individuals experience different microenvironmental conditions that impact both phenotypes and fitness (Rausher 1992). Our experimental design minimizes this problem by rearing all individuals in common, standard conditions, measuring their phenotypes, then assigning individuals at random to different selection treatments. The alternative approach, genotypic selection analysis (Rausher 1992), reduces the effective sample size to the number of clones or families (e.g., to 15 and 40 families in our experiments).

Finally, our analyses of selection estimate selection gradient vectors, treating RGR at different temperatures as separate (but potentially correlated) traits. This analysis does not take advantage of the fact that RGR is a continuous function of temperature, and it ignores the natural ordering of RGR along the temperature axis (Kirkpatrick and Heckman 1989). Unfortunately, formal statistical methods for estimating and comparing selection gradient functions (eqq. [1], [2]) have not yet been developed.

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