

## Environmental Variation and Selection on Performance Curves<sup>1</sup>

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**SYNOPSIS.** Many aspects of physiological and organismal performance vary with some continuous environmental variable: *e.g.*, photosynthetic rate as a function of light intensity; growth rate or sprint speed as a function of temperature. For such ‘performance curves’, the environment plays two roles: it affects both the levels of performance expressed, and the relationship between performance and fitness. How does environmental variation within a generation determine natural selection on performance curves? We describe an approach to this question that has three components. First, we quantify natural environmental variation and assess its impact on performance in the field. Second, we develop a simple theoretical model that predicts how fine-grained environmental variation determines selection on performance curves. Third, we describe how directional selection on performance curves may be estimated and compared to theoretical predictions. We illustrate these steps using data on performance curves of short-term growth rate as a function of temperature (thermal performance curves) in *Pieris* caterpillars. We use this approach to explore whether selection acts primarily on growth rate at specific temperatures, or on more integrated aspects of growth.

### INTRODUCTION

The level of performance of some key function—photosynthesis, locomotion, nutrient uptake, growth—by an organism is frequently viewed as a characteristic or trait of an individual organism. For example, Arnold’s (1983) classic analysis explored how the performance of an individual is influenced by its morphological traits, and how performance in turn influences individual fitness. However, many aspects of individual performance are not fixed, but rather may vary continuously and reversibly as a function of environmental conditions: photosynthetic rate as a function of light intensity, climbing rate as a function of slope, or growth rate as a function of temperature. As an individual experiences different environmental conditions, its performance may also change. In such cases, the performance of an individual is not a single trait (or a set of distinct traits) but is a curve or mathematical function, which we will call a performance curve (Huey and Stevenson, 1979).

Thermal performance curves, in which individual performance varies as a function of temperatures, have been widely studied by evolutionary physiologists and physiological ecologists (Huey and Stevenson, 1979; Huey and Kingsolver, 1989, 1993; Bennett and Huey, 1990; Bennett and Lenski, 1993; Gilchrist, 1995). Thermal performance curves often have a characteristic shape, in which performance increases with increasing temperature, reaches a maximum, and then declines rapidly with further increases in temperature (Fig. 1). There is now a substantial literature on the physiological bases, genetic variation, phylogenetic patterns, and experimental laboratory evolution of per-

formance curves (Garland and Carter, 1994; Huey and Berrigan, 1996; Bennett and Lenski, 1999; Feder, 1999). However, few studies have explored selection and microevolution of performance curves in nature, because of the difficulties of measuring performance curves on large numbers of individuals, and the lack of analytical tools for estimating selection on performance curves (Kingsolver *et al.*, 2001a).

The environment impacts performance curves in two ways. First, environment ( $T$ ) determines the level of performance ( $z$ ) expressed by an individual; as a result, the trait or phenotype of an individual—the performance curve—is a function of environment,  $z(T)$ . Second, the environment may affect the relationship of performance  $z(T)$  to fitness ( $W$ ) for individuals in a population; as a result, environment may affect selection on performance curves. Extending the framework proposed by Arnold (1983) to consider performance curves, environment impacts both performance and selection on performance (Irshick, 2003; Schmitt *et al.*, 2003).

Here we explore this basic issue: how does environmental variation determine variation in performance and selection on performance curves in nature? First, we quantify natural environmental variation and its impact on performance. Second, we present a theoretical framework describing variation, selection and evolution of performance curves, and discuss simple models that incorporate environmental variation into this framework. Third, we estimate directional selection on performance curves from lab and field data, and relate selection to patterns of environmental variation in the field. We will illustrate these points using data from our studies of thermal performance curves for growth rates in Cabbage White (*Pieris rapae*) caterpillars.

### ENVIRONMENTAL VARIATION AND PERFORMANCE

To measure the performance curve of an individual, we must measure performance at a series of defined

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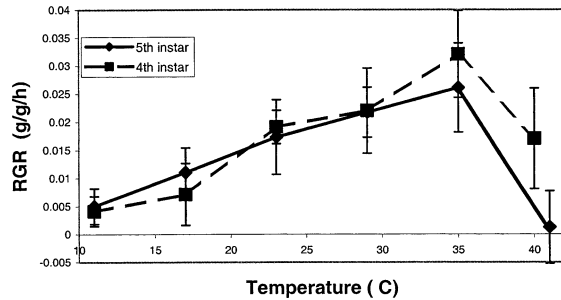


FIG. 1. Short-term (6 hr) relative growth rate (RGR, in g/g/hr) as a function of temperature (in °C) for *Pieris rapae* caterpillars from Seattle WA. Means ( $\pm 1$  SD) are indicated for early 4th (squares, dashed line) and 5th (diamonds, solid line) instar caterpillars. From Kingsolver (2000).

environmental conditions, typically under laboratory conditions. To relate such lab measurements to performance in the field, we must also quantify the patterns of environmental variation experienced by individuals in the field. If an individual organism experiences a range of environments during its life—*i.e.*, the environment is fine-grained (Levins, 1968)—then it will also express a range of performance values. We will illustrate how environmental variation may affect performance in the field by exploring variation in the thermal environment and its consequences for growth rate and growth in *P. rapae* caterpillars.

The Cabbage White Butterfly *Pieris rapae* L. (Lepidoptera: Pieridae) is native to Europe, and was introduced to eastern North America over 140 years ago (Scudder, 1887). It is now found on every continent except Antarctica, and occurs in a variety of open habitats and on a range of larval hostplants in the family Brassicaceae, especially on *Brassica*. In many regions it is an agricultural pest on domesticated varieties of *Brassica oleracea*, including cabbage, broccoli and collards. Because of its pest status and ease of maintenance in the lab, many aspects of the feeding, thermal and population biology of *P. rapae* have been studied.

In many ectothermic animals, temperature has strong effects on feeding, growth and development. Caterpillars are impressive eating and growing machines (Casey, 1993; Stamp, 1993): For example under optimal conditions of food and temperature, a *P. rapae* caterpillar can grow from a newly hatched first instar to its final (5th instar) larval size—a 10,000-fold increase in mass—in about three weeks. We have quantified short-term (6 hr) growth rates of *P. rapae* caterpillars at different temperatures in terms of the relative growth rate (RGR), defined as  $RGR = [\ln(m_f/m_i)]/(t_f - t_i)$ , where  $m_i$  is initial mass at time  $t_i$ , and  $m_f$  is final mass at time  $t_f$ . Figure 1 shows short-term RGR as a function of temperature for 4th and 5th instar *P. rapae* from Seattle WA (Kingsolver, 2000).

Thermal effects on growth in insect larvae can impact three important correlates of fitness: larval survival, larval development time, and pupal and adult body mass. First, extreme low or high temperatures

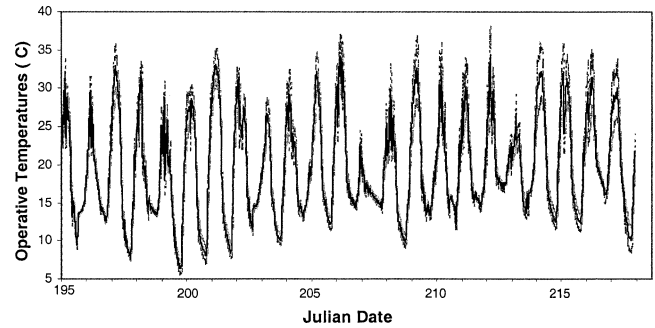


FIG. 2. Mean ( $\pm 1$  SD) operative caterpillar temperature (in °C) as a function of time [ $T(t)$ , Julian date] for 20 *P. rapae* model caterpillars in an experimental collard garden in July–Aug 2000, in Seattle, WA.

can directly reduce larval survival in *P. rapae* and other insects (Feder and Hofmann, 1999; Kingsolver, 2000). In addition, in at least some *P. rapae* populations slower growth rates result in higher mortality by parasitoids, by increasing the time of exposure (Benrey and Denno, 1997). Second, increased growth and development rates at higher temperatures reduce the generation time, increasing Malthusian fitness (intrinsic rate of increase) for multivoltine (multiple generations/year) species such as *P. rapae* (Taylor, 1981; Huey and Berrigan, 2001). Third, pupal mass is affected by growth rate and temperature, and is strongly and linearly correlated with total egg production in *P. rapae* (Jones *et al.*, 1982). As a result, variation in the thermal environment will affect growth and fitness, and may generate selection on thermal performance curves for growth rate.

How do we characterize the thermal environment of a caterpillar? Many insect larvae, including *P. rapae* caterpillars, are thermoconformers: they do not behaviorally orient to solar radiation or wind direction (except to avoid deleteriously high body temperatures), and do not use evaporative cooling or metabolic heat production to regulate body temperature (Casey, 1993). *P. rapae* caterpillars are cryptic green, and usually rest on the shady undersides of leaves, moving to the margins of the leaves to feed (Jones, 1977). As a result, physical models of caterpillars can mimic the body temperatures of early 5th instar *P. rapae* caterpillars within 1–2°C, and these provide a useful means of quantifying the thermal environment of caterpillars in the field (Bakken *et al.*, 1985; Kingsolver, 2000). For example, Figure 2 summarizes (model) caterpillar temperature data in an experimental collard garden in Seattle WA in July–August 2000. Caterpillar temperatures frequently vary by 20–25°C over a 12 hr period, covering nearly the entire temperature range allowing effective growth: individual caterpillars routinely experience a wide temperature range on a daily basis. Temporal (primarily diurnal) variation is much greater than spatial variation, although operative temperatures do vary spatially during midday. Between-day variation in thermal conditions is substantial, especially in the frequency of higher temperatures during daytime.

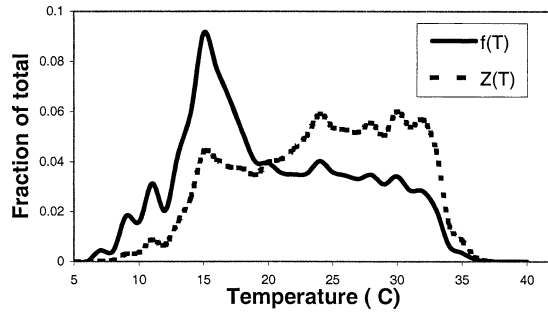


FIG. 3. Frequency distribution of operative temperatures (solid line),  $f(T)$ , and total growth (dashed line),  $Z(T)$ , as a function of temperature, for the time period indicated in Figure 2. See text for explanation.

Clearly, individuals experience a wide range of body temperatures  $T$ , and therefore express a range of growth rates  $z(T)$ , in the field.

An alternative view of these data is to quantify the frequency distribution of body temperatures,  $f(T)$ , experienced by caterpillars in the field (Fig. 3, solid line). These temperature distributions are typically bimodal, with a higher 'nighttime' peak at lower temperatures, and a less distinct 'midday' peak at higher temperatures. Importantly, under natural summer conditions in Seattle, caterpillars sometimes experience short periods of time each day at temperatures allowing rapid short-term growth (30–35°C), but spend the majority of their time at lower temperatures (<15–20°C) where growth is much slower (Fig. 3).

How does growth rate at different temperatures contribute to the total amount of growth that occurs during some time period in the field? We can address this question by combining data on the TPC for growth rate,  $z(T)$  (e.g., Fig. 1), with data on the frequency distribution of operative temperatures in the field,  $f(T)$  (Fig. 3, solid line) (Huey and Slatkin, 1976). For simplicity, let us assume that our metric of growth rate (and more generally, performance) does not depend directly on age, size, time, or prior environmental experience, but depends only the environment state  $T$  during some time period of interest (e.g., the duration of total larval development or a larval instar, or of an episode of selection). Then  $Z(T)$  is the proportion of total growth that results from growth at a particular temperature, and is simply the weighted average of growth rate at temperature  $T$  and the fraction of time spent at that temperature,  $f(T)$ :

$$Z(T) = \frac{f(T)z(T)}{\sum_T f(T)z(T)}.$$

Figure 3 (dashed line) plots  $Z(T)$  for *P. rapae* for the summer conditions in Seattle shown in Figure 2. An important result is that growth rates at higher (>28°C) temperatures can contribute substantially—up to 50%—towards total growth, even though such temperatures typically represent less than 20% of the total time. This is a simple but important consequence of

the much higher growth rates at higher temperatures. Conversely, the low growth rates that occur at lower temperatures (<18°C) can contribute substantially to total growth, because these temperatures predominate most of the time. More generally, whenever performance represents some instantaneous rate of performance (such as growth rate, locomotory rate, uptake rate, etc.) and performance rate varies with environmental conditions, rate of performance in some conditions may contribute disproportionately to total (integrated) performance over some time period that experiences fluctuating environmental conditions.

These results raise two interesting issues. First, if growth rate (or performance rate) at certain temperatures (say,  $z(T_1)$ ) contributes disproportionately to total growth, does this imply that selection on growth rate at temperature  $T_1$  is stronger than selection on growth rate at other temperatures? Second, does selection act directly on growth rate (or performance) at specific temperatures, or on more integrated measures of performance such as total growth over some time period? For example, if some aspect of performance (such as sprint speed) is important for escape from predators, selection might act primarily on performance at those temperatures during which predation risks are greatest. Alternatively, if performance (such as relative growth rate) is important for feeding, growth or development, selection might act primarily on total growth or development over some time interval (Beder and Gomulkiewicz, 1998). Can we distinguish between selection on rate of performance in a specific range of environments, and selection on more integrated aspects of overall performance? To address these questions we must consider in more detail the nature of selection on performance curves.

#### SELECTION ON PERFORMANCE CURVES

##### Models of selection

Suppose that performance is a quantitative trait,  $z$ , that may affect the fitness  $W$  of an individual, and there is variation in  $z$  and  $W$  within a population. One standard way to quantify the strength of directional selection on a quantitative trait  $z$  (including performance) is in terms of the linear selection gradient ( $\beta$ ), which relates variation in the trait (in units of standard deviation of the trait) to variation in relative fitness  $w$  (where mean fitness in a population or sample is defined to equal 1) (Lande, 1979):

$$w = 1 + \beta(z - \bar{z}) \quad (1a)$$

For a set of traits,  $z = \{z_1, z_2, \dots, z_n\}$ , the directional selection gradient is a vector  $\beta = \{\beta_1, \beta_2, \dots, \beta_n\}$  (Lande and Arnold, 1983). The components of the selection gradient vector represent the direct strength of selection on each trait, adjusting for the phenotypic correlations among the traits. It is related to relative fitness via

$$w = 1 + \underline{\beta}(\underline{z} - \underline{\bar{z}})' = 1 + \sum_{i=1}^n \beta_i(z_i - \bar{z}_i); \quad (1b)$$

the prime indicates vector transpose. One advantage of this approach is that selection gradients can be readily estimated using partial regression analysis, by regressing relative fitness onto trait values for a set of individuals in a population (Lande and Arnold, 1983; Arnold and Wade, 1984a, b). In addition, the selection gradient provides a standardized metric of directional selection ( $\beta$  represents changes in relative fitness per phenotypic standard deviation of the trait from its mean value) that facilitates comparisons among different traits and study systems (Kingsolver *et al.*, 2001b). Importantly, the selection gradients are directly relevant to models for the evolution of quantitative traits, so that estimates of selection gradients can be used to predict evolutionary responses to selection (Lande, 1979; Lande and Arnold 1983 #1535; Arnold and Wade, 1984b).

This multivariate approach has been used to estimate selection on phenotypic plasticity by considering an individual's (or genotype's) phenotype in different environments ( $T$ ) as distinct, correlated traits—*i.e.*, as a vector of distinct, correlated measures for each value  $T_i$ :  $\{z(T_1), z(T_2), \dots, z(T_n)\}$  (Via and Lande, 1985). If we view performance curves as a type of plasticity, in which the trait (performance) varies continuously, rapidly and reversibly with environmental state ( $T$ ), we can use this same multivariate approach to quantifying variation, selection and evolution of performance curves (Walton *et al.*, 1995; Gilchrist, 1996). The resulting selection gradient vector  $\beta$  thus represents the strength of directional selection on performance at each environmental state  $T_i$ .

However, the multivariate approach ignores the important fact that for a performance curve, temperature (and therefore performance) may take on an infinite number of values—*i.e.*, a performance curve is a continuous function (Kirkpatrick and Lofsvold, 1992). Fortunately, the multivariate model has been extended to the case where the trait of interest is a mathematical function,  $z(T)$  (Kirkpatrick and Heckman, 1989; Gomuikiewicz and Kirkpatrick, 1992). In this “function-valued” framework, equations (1) and (2) still apply, but  $z$  and  $\beta$  now are represented by functions rather than vectors or matrices. Performance as a function of environmental state ( $T$ ) is represented as a continuous function  $z(T)$ . In particular, selection is now represented by a selection of gradient function,  $\beta(T)$ , that indicates the strength of directional selection on the performance curve  $z(T)$  over the continuous range of environments  $T$  (Gomuikiewicz and Kirkpatrick, 1992; Kirkpatrick and Lofsvold, 1992). Using this extension of the multivariate model, estimates of selection and genetic variances and covariances can allow predictions of the evolutionary response to selection for performance curves and other function-valued traits (Kingsolver *et al.*, 2001a). In particular, the relationship between the selection gradient function and relative fitness analogous to (1a) & (1b) is

$$w = 1 + \int \beta(T)[z(T) - \bar{z}(T)] dT, \quad (1c)$$

where integration is over the range of environmental states  $T$ .

For any particular temperature  $T_i$ , the value of  $\beta(T_i)$  indicates the strength of directional selection on performance at temperature  $T_i$ , holding performance at all other temperatures constant. The strength of directional selection on performance may change with temperature  $T$ : thus the selection gradient is a function of temperature.

Note that the selection gradient function simply represents the relationship between variation in the performance curve and variation in fitness—between phenotype and fitness (eq. 1c). As first proposed by Arnold (1983), selection is estimated by measuring performance of each individual under controlled conditions in the lab, and by measuring fitness (more commonly, components or correlates of fitness) of each individual in the field. As a result, estimates of  $\beta(T)$  for performance curves do not directly involve data about the environmental conditions that occur during selection. But clearly we expect that environmental conditions should impact the strength and pattern of selection on performance curves. What is the relation between selection on performance curves and the pattern of environmental variation?

#### *Relating environmental variation to selection gradient functions*

Here we will describe several simple theoretical models that relate performance curves, fitness, and environmental variation to selection on performance (Gilchrist, 1995, 1996, 2000). We develop the models in terms of growth rate and temperature, but the results apply directly to other aspects of performance and of environment. We are interested in selection in thermal performance curves for growth rate in relation to environmental temperature. 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 at time  $t$ . Suppose that the absolute fitness,  $W$ , of an individual is related to its relative growth rate, such that  $W = k \exp[\int_0^L c(t)z(t) dt]$ , where the function  $c(t)$  weights the contribution of relative growth rate to fitness at time  $t$  and  $k$  is a positive constant. Let  $T(t)$  be the trajectory of temperatures over time experienced during the selection episode (*e.g.*, Fig. 2). We would expect the the weighting function  $c(t)$  will somehow relate to the trajectory of temperatures  $T(t)$ .

One useful simplification is to assume that relative growth rate and its contributions to fitness at any time are determined only by temperature  $T$ , not by the specific times at which that temperature occurs. Then  $z(t) = z[T(t)] = z(T)$ ,  $c(t) = c[T(t)] = c(T)$ , and  $W = k \exp[\int_0^L c[T(t)]z[T(t)] dt]$ . Note that now  $z(T)$  is the thermal performance curve for relative growth rate, and  $c(T)$  represents the weighting function that

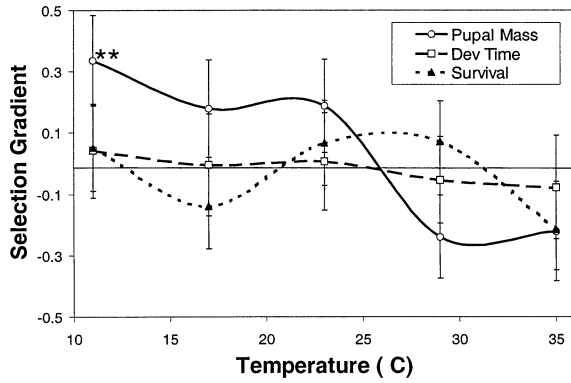


FIG. 4. Estimated directional selection gradient functions ( $\pm 1$  SE) for short-term RGR as a function of temperature, for *P. rapae* caterpillars in an experimental collard garden in Seattle, WA, 28 Jul to 5 Aug 1999. Survival to pupation (dotted line), time to pupation (dashed line) and pupal mass are used as components of fitness. Based on Kingsolver *et al.* (2001).

reflects the contributions of growth rate at temperature  $T$  to fitness. Let  $f(T)$  be the probability density function (*e.g.*, Fig. 3, solid line) of the trajectory of temperatures over time during selection,  $T(t)$ . Then we can show (see the Appendix for the details) that:

$$\beta(T) = Lc(T)f(T) \quad (2)$$

There are several important consequences of this simple theoretical result. First, the selection gradient function,  $\beta(T)$ , is directly connected to the distribution of environmental temperatures,  $f(T)$ , experienced during selection. The quantitative relationship between selection and environmental distribution has been previously noted in the simulation models of Gilchrist (Gilchrist, 1995, 1996, 2000). Second, the intensity of selection on relative growth rate is completely independent of the state of the population in a given generation: a unit increase in relative growth always has the same adaptive value no matter what the mean growth rate function,  $\bar{z}(T)$ .

Let us consider an important special case of this model. Suppose that the absolute fitness of an individual is proportional to its total growth—its total increase in size—over the time period  $L$ . In this case, relative growth rates at particular temperatures are not important, except as they contribute to size at the end of the time period: *i.e.*, selection is acting on final size. Then  $W(z) = k \exp[\int_0^L z[T(t)] dt]$ . The selection gradient in this case is (see the Appendix for details):

$$\beta(T) = Lf(T) \quad (3)$$

where  $f(T)$  is the probability density for temperatures  $T$ .

In this case, the weighting function  $c(T) = 1$  for all temperatures  $T$ : in other words, an extra bit of growth at one temperature has the same effect on total growth, and therefore on fitness, as an extra bit at any other temperature. This analysis suggests a remarkably simple prediction: When selection acts on total relative growth or final size at the end of the selection episode, the form of selection gradient function will precisely

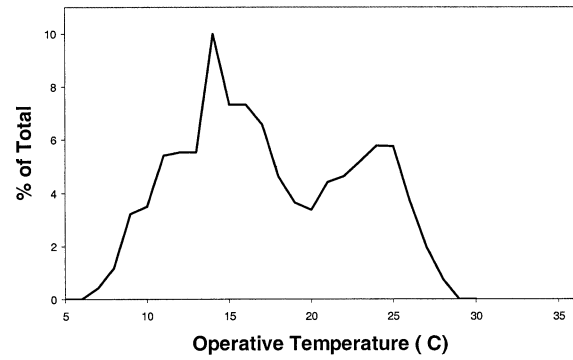


FIG. 5. Frequency distribution of mean operative caterpillar temperatures in the field during the selection episode represented in Figure 4.

mirror the distribution of environmental temperatures during selection. Note that these results hold regardless of how the mean or variance in growth rate changes with temperature. Even though growth rate at higher temperatures can contribute disproportionately to total growth (Fig. 3), this does not necessarily result in stronger selection (as indexed by the selection gradient) on growth rate at higher temperatures. As illustrated in the Appendix, qualitatively similar theoretical results are obtained when we consider absolute rather than relative growth rates.

These simple theoretical predictions have several interesting consequences. First, selection on integrated performance (eq. 3) can yield selection gradient functions that are quite bumpy for natural environments. For example, for thermal performance curves of growth rate in caterpillars and other terrestrial ectotherms, eq. 3 predicts that selection will be strongest at lower temperatures, but with a second, lower peak at higher temperatures (*e.g.*, Fig. 3). Second, the model provides a quantitative prediction for the selection gradient function, based solely on the distribution of environmental conditions experienced during selection (Schmitt *et al.*, 2003). By comparing the selection gradient function [ $\beta(T)$ ] with the distribution of environmental conditions [ $f(T)$ ] in the field for some natural population, we can evaluate whether the pattern is consistent with selection on integrated performance, or requires a more complex interpretation. Third, observed differences between  $\beta(T)$  and  $f(T)$ —*i.e.*, the failure of equation (3) to describe observed data—may indicate that selection does not act simply on total growth or integrated performance, but instead acts disproportionately on growth rate at particular temperatures. In terms of our models, this implies that the weighting function  $c(T)$  is not a constant (as in eqn. 3, but varies with temperature (eq. 2)). Thus, observed differences in the forms of  $\beta(T)$  and  $f(T)$  provide quantitative information about the weighting function  $c(T)$  that relates growth rate to fitness.

#### Measuring selection in the field

Phenotypic selection on performance curves may be estimated with the same basic methods used in other

studies of selection on quantitative traits: by measuring the trait values (the performance curve) of each individual under controlled conditions in the lab, and by measuring fitness (more commonly, components or correlates of fitness) of each individual in the field (Arnold, 1983). To illustrate the approach, we have conducted preliminary studies of selection on thermal performance curves for short-term growth rate in *P. rapae* caterpillars from Seattle WA (Kingsolver *et al.*, 2001a). In each study, we raised 200–300 *P. rapae* caterpillars from egg through 3rd instar on collard (*Brassica oleracea* var. collards) leaves in a fluctuating (10–30°C) temperature regime in an environmental chamber. Following molt into the 4th instar, we estimated short-term (2–12 hr) relative growth rate (RGR) of each caterpillar at a series of 5 temperatures between 11 and 35°C. We then placed the caterpillars (now late 4th-instars) on individual collard plants in an experimental collard garden in Seattle WA, and monitored survival and time to pupation and pupal mass for each caterpillar. The collard garden was covered with coarse-mesh, bridal veil netting to exclude social wasps and other large predators.

We can use these data to estimate the selection gradient functions that relate variation in TPCs of relative growth rate, as measured in the lab, to variation in survival, time to pupation, and pupal mass measured in the field. Selection gradients for studies in August 1999 indicated no significant relationship between the TPCs for growth rate and larval survival or development time, but detected significant directional selection on TPCs for growth via effects on pupal mass. The estimated selection gradient function indicated positive selection at lower temperatures and negative selection at higher temperatures (Fig. 4); hypothesis testing detected significant selection at low temperatures (11°C), but not at higher temperatures. These preliminary results suggest that caterpillars with relatively higher growth rates at low temperatures (11–17°C) had relatively greater pupal masses, whereas caterpillars with relatively higher growth rates at higher (29–35°C) temperatures had similar or relatively smaller pupal masses.

How does this pattern of selection compare to variation in the thermal environment? By monitoring sets of physical models of caterpillars in the garden (see above), we can quantify the frequency distribution of operative temperatures experienced by the caterpillars in the field (Fig. 5). During this episode of selection, the distribution of temperatures was bimodal, with modes near 15 and 25°C, mean operative temperatures ranged from 7 to only 29°C, and temperatures were below 23°C during 75% of the time period of selection. Thus during this selection episode, positive directional selection for increased growth rate appeared only in the range of temperatures (below ~23°C) that dominated during selection in the field (Fig. 4), but suggests no selection or negative directional selection at higher temperatures.

Note that, even though both involve temperature  $T$ ,

the data used to estimate  $\beta(T)$  (Fig. 4) and  $f(T)$  (Fig. 5) are independent:  $\beta(T)$  represents the regression of fitness components measured in the field on performance curves measured in the lab;  $f(T)$  represents the distribution of operative temperatures in the field. Our simple model above (eqn 4) predicts that, if selection acts on total growth during the selection episode, then the pattern of selection  $\beta(T)$  should be directly proportional to the distribution of field temperature  $f(T)$ . There is some qualitative similarity between our preliminary estimates of  $\beta(T)$  and  $f(T)$  (Fig. 7):  $\beta(T)$  is positive at temperatures which dominated during the selection episode (11–23°C), and is negative (though not significantly so) at temperatures which were infrequent or absent (29–35°C). This qualitative similarity is consistent with the hypothesis that selection acts primarily on total growth occurring at these temperatures. The lack of quantitative agreement could occur for a variety of reasons. First, because growth rate was only measured at 6°C intervals, the ‘bumpiness’ of the resulting selection gradient function is limited relative to that for  $f(T)$ . Indeed, applying a moving-average filter with a 6°C bandwidth to  $f(T)$  substantially increases the quantitative similarity between  $\beta(T)$  and  $f(T)$  at lower temperatures. Second, one or more assumptions of the model may be invalid in this temperature range. For example, selection at higher temperatures may act on rates of growth in ways that do not simply reflect their contributions to total growth (see, *e.g.*, Fig. 3). Alternatively, selection on growth rate may depend not only on the frequency distribution of temperatures, but also on the temporal pattern of temperature change (Fig. 2). Third, individuals with relatively higher growth rates at higher temperatures may pupate at a smaller body mass (Atkinson, 1993; Berrigan and Charnov, 1994; Atkinson and Sibly, 1997; Huey and Berrigan, 2001).

We emphasize that these data and analyses on *P. rapae* are preliminary: We present them here to illustrate the concept, estimation, and interpretation of selection gradient functions. These considerations suggest a three-step approach to understanding phenotypic selection on performance curves. First, selection gradient functions for performance curves may be estimated using a straightforward extension of Arnold’s (1983) approach: by regressing variation in fitness in the field onto variation in performance curves measured in the lab. Second, by quantifying the distribution of environmental conditions experienced during selection and comparing this to the selection gradient function, we can test predictions from a model that assumes that selection acts on total or integrated performance. Third, deviations from these predictions may suggest more complex determinants of selection—specifically, that there is selection on the rate of performance within narrow ranges of environmental conditions. In principle, this approach provides a means of distinguishing selection on overall performance from selection on rate of performance in specific environments.

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## APPENDIX

*Selection on relative growth rates*

Suppose that the fitness of an individual over an episode of selection of length  $L$  is  $W = k \exp[\int_0^L c(t)z(t) dt]$  where  $z(t)$  is the organism's relative growth rate and  $c(t)$  weights the contribution to fitness of relative growth at time  $t$ . If both relative growth and the contribution to fitness depend only on the temperature at time  $t$ , and if  $T(t)$  is the trajectory of temperatures over time, then  $W = k \exp[\int_0^L c(T(t))z(T(t)) dt]$ .

Under these assumptions, individual fitness can be computed entirely in terms of temperature  $T$ , without referring to specific times  $t$ . To do this, let  $f(T)$  be the frequency distribution (technically, probability density function) of temperatures over the selection episode (e.g., Fig. 4). Then  $\int_0^L c[T(t)]z[T(t)] dt = \int_{T_{\min}}^{T_{\max}} c(T)z(T)f(T) dT$ , where the integral on the right-hand side is computed over the range of possible temperatures  $T$ . This equation holds because (a) total contributions to (log) fitness can be computed by summing contributions to fitness at each temperature [the product  $c(T)z(T)$ ] weighted by the amount of time spent at that temperature (since  $c(T)$  and  $z(T)$  depend only on temperature, by assumption) and (b) the amount of time the temperature falls in the interval  $(T, T + dT)$  is  $Lf(T) dT$ . This gives an equivalent way to write the fitness of an individual with performance curve  $z(T)$  without referring to specific times  $t$ :  $W = k \exp[\int_{T_{\min}}^{T_{\max}} c(T)z(T)[Lf(T)] dT]$ . According to Beder and Gomulkiewicz (1998, p. 309), the selection gradient for a fitness function of this form is

$$\beta(T) = Lc(T)f(T). \quad (A1)$$

Eq. 3 in the text is a special case with  $c(T) = 1$  for all temperatures  $T$ .

An informal justification of (A1) is as follows. Suppose there are just  $n$  distinct temperatures,  $T_1, T_2, \dots, T_n$ . Let  $z_i, c_i,$  and  $f_i$  be the relative growth rate, weighting, and proportion of time, respectively, at temperature  $T_i$ . Define the vectors  $\mathbf{c} = (c_1, c_2, \dots, c_n)$ ,  $\mathbf{z} = (z_1, z_2, \dots, z_n)$ , and  $\bar{\mathbf{z}} = (\bar{z}_1, \bar{z}_2, \dots, \bar{z}_n)$ , where  $\bar{z}_i$  is the mean growth rate at temperature  $T_i$ . The fitness of an individual with multivariate phenotype  $\mathbf{z}$  is  $W(\mathbf{z}) = k \exp L \sum_{i=1}^n c_i z_i f_i$ .

Lande (1979) showed that, if  $\mathbf{z}$  has a multivariate normal distribution and fitnesses are frequency independent, the  $i$ th component of a selection gradient vector (see eq. 1b) is equal to the partial derivative of the log mean population fitness,  $\bar{W}(\bar{\mathbf{z}})$ , with respect to  $\bar{z}_i$ . In the present case, it can be shown that  $\bar{W}(\bar{\mathbf{z}}) = A \exp(L \sum_{i=1}^n c_i \bar{z}_i f_i)$  where the factor  $A$  is independent of  $\bar{\mathbf{z}}$ . The  $i$ th selection gradient component is then  $\beta_i = \partial \ln \bar{W}(\bar{\mathbf{z}}) / \partial \bar{z}_i = \partial (\ln A + L \sum_{i=1}^n c_i \bar{z}_i f_i) / \partial \bar{z}_i = Lc_i f_i$ . The analogy to (A1) should be clear.

*Selection on absolute growth rates*

For certain modular organisms, new modules are added in response to prevailing conditions regardless of the organism's current size. In these cases, it may be more realistic to assume that *absolute* growth rates are thermally sensitive. Suppose that  $\zeta(T)$  is an organism's absolute growth rate at temperature  $T$  and that the absolute fitness of an individual with thermal performance curve  $\zeta(T)$  is  $W(\zeta) = L \int_{T_{\min}}^{T_{\max}} C(T)\zeta(T)f(T) dT$ . Here  $f(T)$  is again the probability density of temperatures over a growth period of length  $L$ . Analogous to  $c(T)$ , the function  $C(T)$  weights the contribution of absolute growth to fitness at temperature  $T$ .

To find the selection gradient, one could apply the method that was used to derive (A1) or use equation (1c) directly. To use (1c), first subtract and add from  $W(\zeta)$  the mean absolute fitness,  $\bar{W} = E[W] = L \int_{T_{\min}}^{T_{\max}} C(T)\bar{\zeta}(T)f(T) dT$ ;  $\bar{\zeta}(T)$  is the mean absolute growth rate at temperature  $T$ . Re-arranging the result gives  $W(\zeta) = \bar{W} + L$

$\int C(T)[\zeta(T) - \bar{\zeta}(T)]f(T) dT$ . Then the relative fitness of  $\zeta$  is  $W(\zeta)/\bar{W} = 1 + \int_{T_{\min}}^{T_{\max}} [C(T)f(T)/\bar{W}][\zeta(T) - \bar{\zeta}(T)] dT$ . Comparing this with (1c) shows that the selection gradient function in this case is the first term in brackets:

$$\beta(T) = \frac{LC(T)f(T)}{\bar{W}} = \frac{C(T)f(T)}{\int_{T_{\min}}^{T_{\max}} C(T)\bar{\zeta}(T)f(T) dT}. \quad (A2)$$

Note that, unlike for relative growth rates, the intensity of selection on absolute growth rates is inversely proportional to the mean size of individuals; for example as mean size increases over generations, the adaptive value of a unit absolute increase would decline.

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