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Thermal reaction norms for caterpillar growth depend on diet

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ABSTRACT

Hypothesis: Interactions between diet and temperature affect reaction norms for growth rate in ectotherms.

Organisms: Full-sib families of the small cabbage white butterfly, *Pieris rapae*, derived from field populations near Seattle, WA, USA.

Methods: We used a split sib-family experimental design with two food treatments (collard leaves and artificial diet) and four test temperatures (11, 23, 35 and 40°C), and measured short-term growth rate (mass increase) of fourth-instar caterpillars. The data were analysed using mixed-model analysis of variance.

Results: Temperature, food type, family, and two- and three-way interactions all had significant effects on growth rate. The thermal sensitivity of growth rate was greater on collards than on the artificial diet; mean growth rate was greater on collards than on the artificial diet for temperatures from 11 to 35°C, but this effect was reversed at 40°C. Estimated broad-sense genetic variances were greater on collards than on the artificial diet; the genetic covariance of growth rate at 35 and 40°C was strongly positive on the artificial diet, but weak or negative on collard leaves.

Conclusions: Both the mean and genetic variation in thermal reaction norms for insect growth rate were influenced by food type in this system. Studies of the thermal sensitivity of growth and feeding that utilize artificial diets may not accurately reflect genetic variation or constraints on thermal reaction norms that may occur on natural food resources.

Keywords: diet, insect growth, phenotypic plasticity, *Pieris rapae*, reaction norm, temperature.

INTRODUCTION

Temperature impacts many aspects of the physiology, growth, performance, and fitness of ectothermic animals (Huey and Berrigan, 2001). Temperature effects may be considered as a form of phenotypic plasticity in which the phenotypic trait (e.g. body size, growth or locomotory rate, fitness) expressed by an individual organism or genotype varies as a function of

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environmental state (e.g. temperature). This relationship between phenotype and temperature, termed a 'thermal performance curve' (Huey and Stevenson, 1979) or 'thermal reaction norm' (Angilletta *et al.*, 2003), has been a topic of extensive study by evolutionary physiologists interested in mechanisms and patterns of evolutionary adaptation to different thermal environments (Huey and Kingsolver, 1989; Bennett and Lenski, 1999; Feder *et al.*, 2000). Genetic variation both within and between populations has often been empirically documented (Scheiner, 1993). However, there has been more limited success in demonstrating genetic trade-offs or constraints on performance across different temperatures, and their importance for the evolution of thermal specialization (David *et al.*, 1990; Walton *et al.*, 1995; Gilchrist, 1996; Herron, 1996).

In addition to temperature, diet is an important determinant of growth and fitness. The evolution of diet specialization and its role in adaptation to different food resources has been an area of particular interest to nutritional and evolutionary ecologists. Studies examining the reaction norms for feeding and growth on different host plants in herbivorous insects demonstrate significant genetic variation within and between populations. Some of these studies also provide evidence for genetic trade-offs or constraints on feeding and growth on different hosts (Via, 1984, 1990; Via and Lande, 1985; Futuyma and Philippi, 1987; Futuyma and Moreno, 1988; Fry, 1990; Kause and Morin, 2001).

Variation in nutritional quality within and between individual plants can contribute greatly to variation in feeding and growth of herbivorous insects (Karban and Agrawal, 2002). For example, natural variation in host-plant protein concentration can occur spatially and temporally, producing significant variation in measures of fitness such as growth rates (Broadway and Colvin, 1992). Consequently, laboratory evolution studies with *Drosophila* and other model organisms frequently use artificial diets as a means of minimizing variation due to diet (Huey *et al.*, 1991; Scheiner and Lyman, 1991; Scheiner *et al.*, 1991; Gibert *et al.*, 1998; Kingsolver *et al.*, 2004).

The effects of diet and of temperature may not act independently on feeding and growth in herbivorous insects. For example, several studies with *Manduca* have demonstrated that effects of dietary protein and secondary plant compounds on growth and developmental rates depend on the thermal regime and individual experiences (Stamp and Bowers, 1990; Stamp and Horwath, 1992; Stamp, 1993; Petersen *et al.*, 2000). However, whether reaction norms for diet and temperature interact is unknown.

A recent study with *Pieris rapae* L. (cabbage white) caterpillars feeding on collards estimated genetic variances and covariances for growth rates at temperatures ranging from 8 to 40°C (Kingsolver *et al.*, 2004). The results demonstrated substantial changes in genetic variances across temperatures, and detected a significant trade-off (negative genetic covariance) in growth rate across some temperatures. Here we explore whether changes in food type can alter the patterns of thermal sensitivity of insect growth in *Pieris rapae*. We examine variation in short-term growth rate using two different food types (collard leaves and an artificial diet) and four different temperature treatments. Our results suggest that interactions between diet and temperature can alter our perspectives about plasticity and genetic constraints on insect growth.

MATERIALS AND METHODS

Study system

Pieris rapae L. (Lepidoptera: Pieridae), the small cabbage white butterfly or imported cabbageworm, is native to Europe, and was introduced to eastern North America more than

140 years ago (Scudder, 1887). Populations of *P. rapae* are now found in a variety of open habitats on every continent except Antarctica, and exploit a range of larval host plants in the family Brassicaeae, especially *Brassica*. There are five larval instars, with over 90% of larval growth occurring in the final two instars, and a facultative diapause in the pupal stage that is controlled by photoperiodic cues. The cabbageworm is an agricultural pest on domesticated varieties of *Brassica oleracea*, including collards, cabbage, and broccoli. Furthermore, *Pieris rapae* can be readily maintained on artificial diets in the laboratory with little mortality (Slansky and Feeny, 1977; Slansky, 1978). Its pest status and ease of maintenance in the laboratory have led to numerous studies examining various aspects of the feeding, thermal, and population biology of *P. rapae* (Slansky, 1993). Previous research has demonstrated that both temperature and diet type can affect mean growth and developmental rates of *P. rapae* caterpillars (Slansky and Feeny, 1977; Slansky, 1978; Chen and Su, 1982; Gilbert, 1984a, 1984b; Gilbert and Raworth, 1996; Kingsolver, 2000).

The studies described here were conducted using *P. rapae* from the Puget Sound region near Seattle, WA. It is one of the most common butterflies in the area, where it is abundant in urban gardens and organic farms, and where it feeds primarily on domesticated forms of *Brassica oleracea*. In western Washington, *P. rapae* completes 3–4 generations per year, with adult flight seasons from May to October.

Our studies began with fresh (wing wear classes 1–2) adult female *P. rapae* collected at organic farms in the Puget Sound area and transported overnight to our laboratory in North Carolina. In the field, females almost always mate within a few hours of adult eclosion; after mating, females are refractory for 2–4 days before they re-mate (Wiklund *et al.*, 2001). In addition, there is nearly complete sperm precedence when females re-mate (Wedell and Cook, 1998, 1999a, 1999b; Wiklund *et al.*, 2001). As a result, all offspring of each female in our studies very likely represent full-sibs, although we cannot rule out some possibility for multiple paternity.

Experimental design

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 within each family were assigned at random to one of two food treatment groups: collards or an artificial diet. Caterpillars in the collard treatment were fed freshly picked young collard leaves from plants maintained in the greenhouse; leaves were changed every 2 days (more frequently for larger, more voracious caterpillars). Caterpillars in the artificial diet treatment were fed on a standard artificial diet (Troetschler *et al.*, 1985) for *P. rapae*, which includes 1.6% dried collard leaves by weight; a fresh diet was provided every 2 days. Caterpillars in both food treatments were maintained in petri dishes placed in environmental chambers under a diurnal fluctuating thermal regime (11–35°C) and a light : dark cycle (16 : 8 h) that mimics the thermal conditions experienced by caterpillars during mid-summer in Seattle (Kingsolver, 2000; Kingsolver *et al.*, 2001).

Growth rate measurements began after each caterpillar had newly moulted into the fourth larval instar. Body mass was measured at 10.00 h on the day after moult into the fourth instar, and short-term growth (mass increase) of each caterpillar was then evaluated at a series of four test temperatures: 23°C, 11°C, 35°C, and 40°C for a duration of 6 h at each temperature. Each test temperature was maintained in a different environmental chamber (Percival 36-VL) calibrated with a Wescor TH-65 thermocouple thermometer. Fresh leaf or

artificial diet was used for each test temperature. Caterpillar mass at the start and end of each test period was measured with a Mettler Toledo model AT261 DeltaRange balance (± 0.01 mg). Thus, all four growth rate measurements were completed within ~24 h for each caterpillar. Caterpillars from 30 full-sib families were measured, with 10–12 caterpillars in each food treatment in each family. Caterpillars that were visibly sick, moulting, did not feed during two or more test temperatures, or that died during the experiment were excluded from the analyses. The original data files contained 441 (leaf) and 514 (diet) individuals. After excluding individuals using the above criteria, there were 423 (leaf) and 512 (diet) individuals used in the analyses; excluded individuals were not concentrated in one or a few families. The mean (and median) number of caterpillars per family was 14 (15) in the leaf treatment and 17 (20) in the diet treatment.

We did not randomize the order of temperature exposure (23°C, 11°C, 35°C, and 40°C) in the experiments for two reasons. First, exposure to 40°C may elicit stress responses such as heat shock protein (HSP) expression that could affect growth at subsequent temperatures (Feder and Hofmann, 1999). Thus, 40°C was the final test temperature for each caterpillar, and was preceded by the next highest temperature (35°C). Second, in natural environments (and in the rearing conditions for the experiments) temperatures do not occur in random order but rather in a diurnally patterned sequence; we attempted to maintain this diurnal pattern, within the constraints of the test temperatures used in the study. A previous study of this population that used a different order of test temperatures gave similar results for mean and variation in short-term growth rate (Kingsolver *et al.*, 2004). The thermal sensitivity of short-term growth rate is similar in fourth and fifth instar *P. rapae* (Kingsolver, 2000), so it is unlikely that there were substantial changes in thermal sensitivity within the fourth instar during our studies.

Statistical analyses

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. Relative growth rate represents the proportional increase in mass per unit time. Note that the test duration is approximately 6 h in all of our measurements: thus division by t in the definition of relative growth rate simply rescales the data. Visual inspection of residuals suggested that the distribution of relative growth rate was approximately normal.

The relative growth rate data were analysed using a linear mixed-effects analysis of variance (ANOVA), with temperature and food as fixed effects and family as a random effect (Fry, 1992, 2004). All two- and three-way interaction terms were also included: the two-way interaction between temperature and food was treated as a fixed effect, while all two- and three-way interactions including family were treated as random effects. Temperature was modelled as a repeated measure within individuals, because growth rate for each individual was measured at each temperature (Fry, 2004). Model parameters were estimated with a restricted maximum likelihood procedure in SAS PROC MIXED. We used the Akaike information criterion (AIC) to identify the best model for the data, and likelihood ratio tests to evaluate the statistical significance of the various random effects in the model.

The data were also used to estimate the broad-sense genetic variances and covariances for relative growth rate at each temperature, considering the collard and artificial diet treatment groups as separate samples. Genetic variances and covariances (and associated standard

errors) were estimated with restricted maximum likelihood using DFREML (Meyer and Hill, 1997; Meyer, 1998). Strong negative or positive genetic covariaces are typically used as evidence for trade-offs or constraints on performance in different environments (Via, 1984, 1987; Via and Lande, 1985; Scheiner, 1993). Our goal here is to explore whether patterns of genetic covariances in relative growth rate across temperatures depend on food type. We examined whether the genetic variance–covariance matrices (G-matrices) for the collard and artificial diet treatments showed significant similarity, using the program CPCRAND to perform randomization tests of the Flury hierarchy (Flury, 1988; Phillips and Arnold, 1999). This method provides tests of matrix equality, proportionality, and common principal component structure (Flury, 1988).

RESULTS

Food, temperature, and family effects

Results of the mixed-model ANOVA are summarized in Table 1. The fixed terms in the model (temperature, food, and temperature × food) all had significant effects on relative growth rate. Likelihood ratio tests for the random terms show that including family and each two- and three-way interaction among family, temperature, and food significantly increased the likelihood of the model: the best model for the data (based on the AIC)

Table 1. Results of mixed-model ANOVA of relative growth rate of fourth-instar *Pieris rapae*. Temperature and food type are treated as fixed effects; family and all interactions involving family are treated as random effects; temperature is treated as a repeated measure within individuals.

(a) *F*-tests of fixed effects

Factor	*F*-value	d.f.	*P*-value
Temperature	801.7	3	<0.0001
Food	6.87	1	0.0142
Temperature × food	89.23	3	<0.0001

(b) Likelihood-ratio (LR) tests of each random effect, where L(0) is the likelihood of the base model, and L(1) is the likelihood of the model including the random effect of interest. Each model includes all fixed effects above. λ is the test statistic for the LR test:

$$\lambda = -2\ln\left[\frac{L(0)}{L(1)}\right]$$

L(0) model	L(1) model: added effect	d.f.	λ	*P*-value
No random parameters	Family	1	77.0	$\ll$0.0001
Family	Family × temperature	1	73.7	$\ll$0.0001
Family	Family × food	1	55.0	$\ll$0.0001
Family + family × food	Family × temperature	1	77.0	$\ll$0.0001
Family + family × temperature	Family × food	1	58.3	$\ll$0.0001
Family + family × temperature × food	Family × temperature × food	1	95.0	$\ll$0.0001

included all fixed, random, and two- and three-way interactions. These analyses suggest that temperature, food type, and family interact to influence the growth rate of an individual.

Figure 1 shows thermal reaction norms for the population mean (heavy line) and for each family, considering the collard (Fig. 1a) and artificial diet (Fig. 1b) treatment groups separately. For both food types, population mean relative growth rate increased with increasing temperature, was greatest at 35°C, and then declined at 40°C. There was substantial variation in thermal reaction norms among families in each food treatment group, and the rank order of many families changed across temperatures (i.e. the reaction norms cross). Note that one family had a low mean relative growth rate at all temperatures in the artificial diet group, but not in the collard group (see Fig. 2); omitting this family from the analyses did not change the qualitative results.

Figure 2 shows the dietary reaction norms for each family, considering each test temperature separately (Figs. 2a–d). There was substantial variation in reaction norms among families, especially at the higher temperatures, and the rank order of many families changed between diets at each temperature. This illustrates the interactions between family and food type within temperatures.

Figure 3 shows the mean difference in relative growth rate between the collard and artificial diet groups for each family and for the population (heavy line). Population mean relative growth rate was slightly greater on collard leaves than on the artificial diet at 11, 23 and 35°C (i.e. the difference is positive), but this mean effect was reversed at 40°C (i.e. the difference is negative). There was substantial variation in reaction norms among families, especially at higher temperatures. This variation illustrates the three-way interactions among temperature, food type, and family.

Genetic variances and covariances

Table 2 summarizes broad-sense genetic variances and covariances (with standard errors in parentheses) for relative growth rate for the collard (Table 2a) and artificial diet (Table 2b) treatments separately. For both treatment groups, genetic variance in relative growth rate increased with increasing temperature. Broad-sense heritabilities ranged from 0.18 (at 11°C)

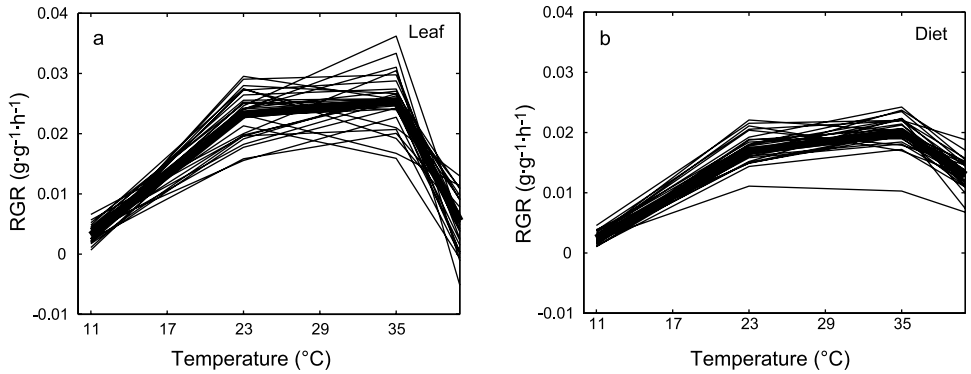

Fig. 1. Reaction norms of mean relative growth rate, *RGR* (in $g \cdot g^{-1} \cdot h^{-1}$), as a function of temperature (°C) for fourth-instar *Pieris rapae* caterpillars from Seattle, WA. The mean reaction norms for the population (heavy line) and for each family are indicated. (a) collard leaf treatment; (b) artificial diet treatment.

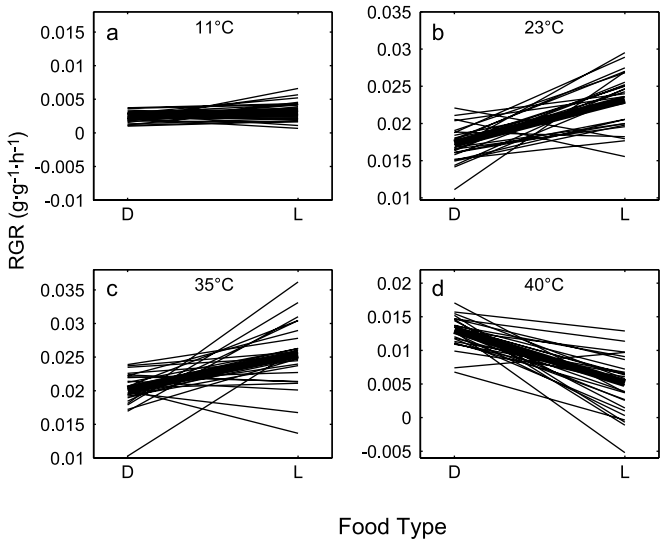

Fig. 2. Reaction norms of mean relative growth rate, *RGR* (in $g \cdot g^{-1} \cdot h^{-1}$), as a function of food type (artificial diet and collard leaf) for fourth-instar *Pieris rapae* caterpillars from Seattle, WA. The mean reaction norms for the population (heavy line) and for each family are indicated. (a) 11°C; (b) 23°C; (c) 35°C; (d) 40°C.

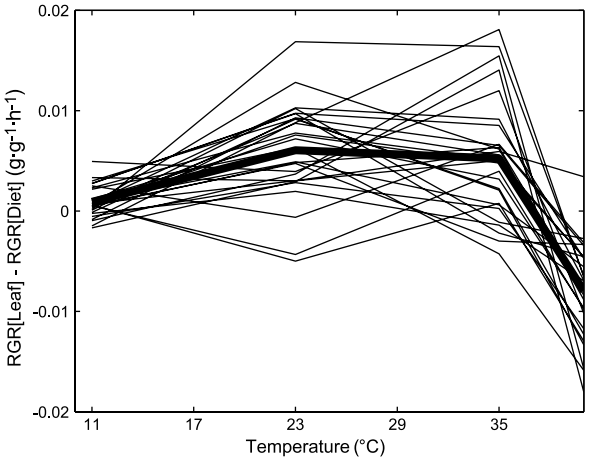

Fig. 3. Reaction norms of the mean difference in relative growth rate, *RGR* (in $g \cdot g^{-1} \cdot h^{-1}$), in the collard leaf and artificial diet treatments as a function of temperature (°C) for fourth-instar *Pieris rapae* caterpillars from Seattle, WA. The reaction norms for the population mean (heavy line) and for each family are indicated.

to 0.42 (40°C) in the collard treatment, and from 0.17 (11°C) to 0.24 (35°C) in the artificial diet treatment. However, the patterns of covariances and correlations in relative growth rate between temperatures appear different for the two food treatments. Genetic variances were two- to four-fold greater at each temperature for relative growth rate on leaves compared with that on the artificial diet. In the artificial diet treatment, all genetic covariance

Table 2. Matrix of broad-sense genetic variances and covariances for relative growth rate across temperatures (in °C) for fourth-instar *P. rapae* caterpillars from Seattle, WA, on two different food types. Standard errors are shown in parentheses; standard errors greater than 1.96 are given in **boldface**

	11°C	23°C	35°C	40°C
(a) *Artificial diet treatment*				
11°C	**0.000725 (0.00032)**			
23°C	0.000944 (0.000674)	**0.006418 (0.002661)**		
35°C	0.000229 (0.000918)	0.004061 (0.002774)	**0.013742 (0.00529)**	
40°C	0.000439 (0.000973)	0.004791 (0.002978)	**0.011531 (0.004537)**	**0.013977 (0.005864)**
(b) *Collard leaf treatment*				
11°C	**0.002163 (0.001101)**			
23°C	−0.00177 (0.00211)	**0.019404 (0.007747)**		
35°C	−0.00082 (0.018069)	0.003621 (0.010261)	**0.062182 (0.023582)**	
40°C	0.005129 (0.004038)	0.003062 (0.010447)	−0.01983 (0.01869)	**0.080189 (0.028257)**

estimates were positive, with a large and significant positive genetic covariance for relative growth rate at 35 and 40°C (genetic correlation $r_g = 0.83$). In contrast in the collard treatment, there was a negative (but not significant) genetic covariance for relative growth rate at 35 and 40°C (genetic correlation $r_g = -0.28$). Similarly, principal components analyses (not shown) of the variance–covariance matrices indicated positive loadings at all temperatures for the first principal component for the artificial diet group, but a mixture of positive and negative loadings across temperatures for the first principal component for the collard group. Randomization tests indicated no significant similarity at any level of the Flury hierarchy between the G-matrices for the collard and artificial diet treatments. Collectively, these results suggest that the patterns of genetic variation and covariation in thermal reaction norms are altered by food type. In contrast, there were no significant among-family correlations between relative growth rate on collard leaves and on the artificial diet at any test temperature, with correlations ranging from −0.234 at 35°C to 0.121 at 11°C.

DISCUSSION

Limitations of the studies

Our studies used measurements of growth rate (mass increase) over short (6 h) time periods rather than chronic exposure to constant temperatures. 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 (Kingsolver, 2000). Indeed, chronic exposure to constant temperatures may have quite different effects on growth and survival than short-term exposure. For example, in *P. rapae* mean short-term (2–6 h) growth rate is greatest at 35°C, but longer exposure (> 48 h) at 35°C yields negative growth rates and increased mortality (Kingsolver, 2000). We note that at these short time-scales, our index of growth rate (net mass increase per time) may

reflect processes in addition to accumulation of new tissue, including transient changes in ingestion, water balance, and gut volume that are averaged out over longer time-scales. Previous studies with *P. rapae* indicate that growth rate and consumption rate are tightly correlated (Kingsolver, 2000). In addition, studies with other herbivorous caterpillars suggest that assimilation rates are not strongly influenced by temperature, except under chronic exposure to extreme temperatures (Kingsolver and Woods, 1997). Because the caterpillars in our study were from a single population, we believe that short-term mass increase is tightly associated with tissue accumulation on each food type, and is thus an appropriate index of growth rate in this system.

The experimental design used in these experiments involved repeated measurements of short-term growth rate for each individual at a series of temperatures, with the same order of temperatures for every individual in a study. As a result, temperature effects are potentially confounded with time, age, and size. We did not randomly order temperatures for three reasons. First, the higher (35 and 40°C) temperatures considered may be stressful; for example, exposure of *P. rapae* caterpillars to 40°C for more than 48 h greatly increases mortality rate, and chronic exposure to 35°C prevents successful metamorphosis (J.G. Kingsolver and J.G. Shlichta, unpublished data). Because short-term exposure to stressful temperatures could affect subsequent growth, measurements at 40°C were always done last, and those at 35°C always next to last. We are unaware of evidence for any insect that short-term exposure to non-stressful temperatures affects subsequent growth. Second, to avoid confounding growth (i.e. mass increase) responses from developmental responses (e.g. moulting), we restrict our measurements to a 48 h period with a single larval instar. Previous data for this population show that the thermal sensitivity of short-term growth rate is very similar for fourth and fifth instar caterpillars (see Kingsolver, 2000, fig. 1), so changes in thermal sensitivity within the fourth instar in our study are unlikely. Third, a previous study successfully used a similar experimental design to estimate genetic variances and covariances for *P. rapae* feeding on collards (Kingsolver *et al.*, 2004; see below). An alternative design would be to measure each individual at only a single (randomly assigned) temperature. Such a design would not only increase the number of individuals required by six-fold, but would also inflate the sampling error, because the only information available for estimating genetic covariances would come from measurements on different (genetically related) individuals (Meyer, 1991).

Temperature, diet, and insect growth

There is abundant documentation of the effects of both temperature and food type on feeding and growth in herbivorous insects (Casey, 1993; Slansky, 1993). A few studies have also documented interactions between food and temperature. For example, the effects of the flavinoid rutin on growth and development in *Manduca sexta* vary with temperature (Stamp and Horwath, 1992; Stamp, 1993). Similarly, effects of dietary protein concentration on growth rates in *Manduca* depend on rearing temperature, and this interaction is strongest at high temperatures (Petersen *et al.*, 2000).

Our present studies with *Pieris rapae* also demonstrate significant interactions between diet and temperature in determining short-term growth rates (Table 1). On both collard leaves and the artificial diet, mean growth rates increase with increasing temperature, are highest at 35°C, and decline at temperatures above 35°C (Fig. 1). This pattern is consistent with previous results in this system (Kingsolver, 2000). However, the mean effects of diet vary with temperature. At temperatures between 23 and 35°C, mean growth rates are 25–35%

greater for caterpillars feeding on collard leaves than those on the artificial diet. At 40°C this effect is reversed: mean growth rates are more than 50% lower on collard leaves than on the artificial diet. The reasons for this reversal at high temperature are unclear, but might result from reduced water content or other changes in leaf quality or mechanical aspects of ingesting or digesting leaves versus diet. Regardless of the causes, one important consequence of this pattern is that the thermal sensitivity of growth rate is greater for *P. rapae* feeding on collard leaves than on artificial diet. If this result holds in other model systems, it would suggest that studies utilizing artificial diets may underestimate the thermal sensitivity of growth and other processes.

Genetic variation in reaction norms

Many studies have documented genetic variation in thermal reaction norms, both for developmentally plastic traits in response to developmental temperatures (Scheiner, 1993), and for performance traits that respond quickly and reversibly to current temperature conditions (Huey and Kingsolver, 1989; Gilchrist, 1996). Similarly, genetic variation in reaction norms for diet has frequently been shown in various insects and other systems (Via, 1990; Via *et al.*, 1995). Of particular interest has been the identification of strong negative or positve genetic covariances that may represent trade-offs or constraints on performance across different diets or temperatures. These trade-offs and constraints are central to understanding the evolution of specialization to different diets or different thermal conditions (Via, 1984; Gilchrist, 1996; Herron, 1996).

A previous study with *P. rapae* caterpillars feeding on collards estimated broad-sense genetic variances and covariances for short-term growth rate at seven temperatures ranging from 8 to 40°C (Kingsolver *et al.*, 2004). These estimates were based on a larger number of full-sib families ($n = 90$) than in the present study ($n = 30$). Genetic variances in growth rate increased strongly with increasing temperatures, as in the present study (Table 2). In addition, the previous study revealed a large and significant negative genetic correlation ($r_g = -0.59$) between short-term growth rate at 35 and at 40°C, suggesting a genetic trade-off in growth rate at these two temperatures (Kingsolver *et al.*, 2004).

Our present studies with *P. rapae* suggest that interactions between diet and temperature can modulate the patterns of genetic covariation in plasticity. The broad-sense genetic variance–covariance matrices for relative growth rate at different temperatures show different patterns for the collard leaf and artificial diet groups, even though these represent the same set of families (Table 2). It is noteworthy that the estimated genetic correlation for relative growth rate between 35 and 40°C is strongly positive (0.83) in the artificial diet treatment but zero or negative (–0.28) in the collard leaf treatment. Although the small number of families in our analyses limit the power of our estimates of genetic covariances, our results suggest that studies of thermal sensitivity that utilize artificial diets may not accurately reflect trade-offs in performance across temperatures occurring on more natural food resources. Studies with *Drosophila* have illustrated that exposure to and selection in novel laboratory environments can alter patterns of genetic covariation within a few generations and initially mask genetic trade-offs among life-history traits (Service and Rose, 1985). Interestingly, we see little change in mortality or fecundity for *P. rapae* maintained on an artificial diet compared with those on collards, but mean growth and developmental rates are slower on the diet than on collards at most temperatures (J.G. Kingsolver and J.G. Shlichta, unpublished data).

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