POPULATION ECOLOGY

Phenotypic plasticity in a complex world: interactive effects of food and temperature on fitness components of a seed beetle

R. Craig Stillwell · William G. Wallin · Lisa J. Hitchcock · Charles W. Fox

Abstract Most studies of phenotypic plasticity investigate the effects of an individual environmental factor on organism phenotypes. However, organisms exist in an ecologically complex world where multiple environmental factors can interact to affect growth, development and life histories. Here, using a multifactorial experimental design, we examine the separate and interactive effects of two environmental factors, rearing host species (*Vigna radiata*, *Vigna angularis* and *Vigna unguiculata*) and temperature (20, 25, 30 and 35°C), on growth and life history traits in two populations [Burkina Faso (BF) and South India (SI)] of the seed beetle, *Callosobruchus maculatus*. The two study populations of beetles responded differently to both rearing host and temperature. We also found a significant interaction between rearing host and temperature for body size, growth rate and female lifetime fecundity but not larval development time or larval survivorship. The interaction was most apparent for growth rate; the variance in growth rate among hosts increased with increasing temperature. However, the details of host differences differed between our two study populations; the degree to which *V. unguiculata* was a better host than *V. angularis* or *V. radiata* increased at higher temperatures for BF beetles, whereas the degree to which *V. unguiculata* was the worst host increased at higher temperatures for SI beetles. We also found that the heritabilities of body mass, growth rate and fecundity were similar among rearing hosts and

temperatures, and that the cross-temperature genetic correlation was not affected by rearing host, suggesting that genetic architecture is generally stable across rearing conditions. The most important finding of our study is that multiple environmental factors can interact to affect organism growth, but the degree of interaction, and thus the degree of complexity of phenotypic plasticity, varies among traits and between populations.

Keywords Plasticity · Reaction norm · Genetic architecture · *Callosobruchus maculatus*

Introduction

A change in an organism's phenotype in response to the environment (phenotypic plasticity) is a universal characteristic of all organisms (West-Eberhard 2003). While numerous studies have now investigated phenotypic plasticity, nearly all of these have examined how a single environmental factor impacts an organism's phenotype (Pigliucci 2001). Yet, organisms exist in ecologically complex worlds, simultaneously experiencing variation in many environmental factors that can have interactive effects on growth, development and life histories (Sultan et al. 1998; Sultan 2001; Relyea 2004; Ris et al. 2004; Relyea and Auld 2005). To predict evolutionary responses to selection in nature it is necessary to understand how interactions between multiple environmental factors affect reaction norm shape.

Two of the most important environmental factors affecting the growth and development of ectotherms, particularly insects, are diet and temperature. Both variables induce substantial plasticity in a number of traits. Animals fed lower quality diets generally have lower survivorship,

Communicated by Jay Rosenheim.

R. C. Stillwell (✉) · W. G. Wallin · L. J. Hitchcock · C. W. Fox
Department of Entomology, University of Kentucky,
S225 Agricultural Science Center North,
Lexington, KY 40546-0091, USA
e-mail: rstil2@uky.edu

increased development time (Nylin and Gotthard 1998), mature at a smaller adult body size (Berrigan and Charnov 1994), have slower growth rates (Atkinson and Sibly 1997), lower fecundity (Awmack and Leather 2002) and produce smaller eggs/offspring (Fox and Czesak 2000). Animals reared at lower temperature generally have higher survivorship (Angilletta et al. 2004; Kozłowski et al. 2004), longer development time (Atkinson 1994), mature at a larger adult size (Atkinson 1994; Angilletta and Dunham 2003), have reduced growth rate (Atkinson and Sibly 1997), lower fecundity (Ernsting and Isaaks 2000; Stillwell and Fox 2005) and produce larger eggs/offspring (Fox and Czesak 2000). Though diet and temperature effects on growth and life history traits are commonly investigated, the interactions between them are rarely examined (Kingsolver et al. 2006). Those studies that have simultaneously examined the effect of both of these variables have generally found interactive effects on growth and development (Stamp and Bowers 1990; Gresens 1997; Sultan et al. 1998; Petersen et al. 2000; Sultan 2001; Relyea 2004; Ris et al. 2004; Relyea and Auld 2005; Kingsolver et al. 2006), suggesting that the interactive effect of these two variables is likely to be important. Although adaptation to local environmental conditions is ubiquitous in nature, and populations frequently evolve differences in reaction norms, few of these studies have examined how interactions among multiple environmental variables vary among populations.

Patterns of phenotypic plasticity can also vary substantially among traits. For example, temperature reaction norms vary considerably among morphological traits of the cricket, *Gryllus firmus* (Bégin et al. 2004), and among life history traits of the seed beetle, *Stator limbatus* (Stillwell and Fox 2005). Likewise, plasticity in response to host species varies among traits in seed beetles (Fox 1993; Fox et al. 1994, 1996). A more realistic understanding of the evolution of phenotypic plasticity can thus only be achieved by simultaneously examining the responses of several traits to multiple environmental factors.

A complete understanding of the evolution of phenotypic plasticity also requires knowledge of the genetic architecture underlying phenotypically plastic traits and how this genetic architecture changes with environmental conditions. However, the genetic basis of plasticity is still poorly understood (Scheiner 1993; Promislow 2005; Czesak et al. 2006). Plasticity is often studied by measuring the heritability of traits in each environment, quantifying genotype-by-environment interactions and measuring cross-environment genetic correlations (r_G). The cross-environment r_G measures the extent to which a trait is correlated among environments, and thus how independent trait evolution is across environments (Scheiner 1993; Via 1994). Empirical studies that measure the genetic basis of plasticity across multiple environments are lacking due to the difficultly in estimating these genetic parameters in complex experiments. However, studies that measure genetic architecture are needed to provide insight into the evolution of plasticity in nature where environmental complexity is the norm.

Here we explore the separate and interactive effects of larval diet (host species) and rearing temperature on growth and life history traits in two populations of the seed beetle *Callosobruchus maculatus*. Several prior studies have shown that rearing host species (Wasserman and Futuyma 1981; Chandrakantha and Mathavan 1986; Chandrakantha et al. 1987; Credland 1987; Fox 1993; Kawecki 1995; Timms 1998; van Huis and de Rooy 1998; Boeke et al. 2004; Messina 2004a, b; Vamosi 2005) and temperature (Chandrakantha and Mathavan 1986; Chandrakantha et al. 1987; Giga and Smith 1987; Guntrip et al. 1997; Lale and Vidal 2000, 2003a, b; Mbata et al. 2005) have large effects on a variety of traits of *C. maculatus*, but how these factors interact to affect reaction norm shape is poorly understood. Using a factorial experimental design, we examine the separate and interactive effects of rearing host and temperature on egg-to-adult survivorship, egg-to-adult development time, adult body mass, growth rate and female lifetime fecundity of *C. maculatus*. Also, because an understanding of the evolution of plasticity requires knowledge of the underlying genetic architecture, we explore how rearing host and temperature influence genetic variances and heritabilities of body mass, growth rate and fecundity, and how cross-temperature r_Gs change with rearing host.

Materials and methods

Natural history and study populations

The seed beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae), is a generalist seed herbivore of storage crops, but uses primarily species in the genus *Vigna* in nature. Its life cycle revolves around seeds. Females cement their eggs directly onto the seeds of their host plant. Eggs hatch and larvae burrow directly underneath the egg into the seed. Larval growth and pupation take place entirely within a single seed. Upon emergence from the seed, adults mate and females begin to lay eggs within hours. *C. maculatus* needs only the resources inside of a single seed to complete development and reproduce; additional food and water are not necessary (Fox et al. 2004a, b). Because of its ease of laboratory rearing, *C. maculatus* is a widely used model system for life history, behavior and genetic studies (Bieri and Kawecki 2003; Fox et al. 2004b; Messina 2004a, b; Arnqvist et al. 2005; Vamosi 2005).

We examine the separate and interactive effects of rearing host and temperature in two populations of *C. maculatus*

that are adapted to different species of *Vigna*. The South India (SI) population was collected in 1979 from infested pods of mung bean, *Vigna radiata* (L.) Wilczek, and the closely related black gram, *Vigna mungo* (L.) Hepper, in Tirunelveli, India (Mitchell 1991). The Burkina Faso (BF) population was collected in 1989 from infested pods of cowpea, *Vigna unguiculata* (L.) Walp., in Ouagadougou, Burkina Faso (Messina 1993). These two populations differ in a large number of traits including body size, adult lifespan, larval competitiveness, oviposition behavior and the amount of paternal investment into reproduction (Savalli et al. 2000; Fox et al. 2004a, b, c), many of which have likely evolved due to differences in the properties of their host species (Messina and Karren 2003; Messina 2004b). Both populations were maintained in laboratory growth chambers on seeds of *V. radiata* (SI) or *V. unguiculata* (BF) at >1,000 adults per generation for >100 generations (BF) or >200 generations (SI) prior to this experiment.

Experimental design

We used a completely randomized design with a multifactorial treatment arrangement to examine the effects of host species and temperature on egg-to-adult survivorship, egg-to-adult development time, adult body mass, growth rate and female lifetime fecundity in both populations of *C. maculatus*. In short, larvae of full-sib families were reared on three host plants (mung, *V. radiata*; azuki, *V. angularis*; and cowpea, *V. unguiculata*) and at four rearing temperatures (20, 25, 30 and 35°C; all at 15:9 h, light:dark) yielding 12 treatment combinations for each population. Offspring of each full-sib family were reared on only one host (i.e., no split-brood design) but siblings were divided equally among the rearing temperature treatments creating a spilt-brood design for rearing temperature.

Cowpea and mung are the native hosts for the BF and SI populations, respectively, and azuki is an alternate host to which neither is adapted. We thus expected that these populations would exhibit better responses to rearing on their native hosts compared to non-native hosts. The temperatures we used are within the normal range of temperatures at which *C. maculatus* can develop and reproduce (Chandrakantha and Mathavan 1986; Chandrakantha et al. 1987; Mbata et al. 2005). However, because the native climates of the BF and SI populations are very similar (mean temperature difference between sites is ~0.4°C; National Climatic Data Center's Global Surface Summary of Day, Asheville, N.C.) and because these populations have been maintained in laboratory colonies for more than 100 generations under benign and identical conditions, we did not expect them to show different responses to temperature.

To create families, seeds bearing eggs were randomly selected from our laboratory colonies and isolated in

35-mm Petri dishes (one seed per dish, one egg per seed). Adults emerging from these seeds were used as parents to generate full-sib families by randomly pairing virgin males and virgin females within each population. Each pair was randomly assigned to one of three rearing hosts (60-mm dishes containing 30 seeds of cowpea, 35-mm dishes containing 40 seeds of mung or 35-mm dishes containing 30 seeds of azuki) and placed in a growth chamber to lay eggs (25°C; 15:9 h, light:dark). Dishes were checked for eggs twice per day until females laid eggs on ~32 seeds (seeds bearing eggs were replaced at each check) after which adults were discarded. Seeds containing eggs were scraped to one egg per seed (to eliminate larval competition) and placed individually in 35-mm Petri dishes. Egg bearing seeds were randomly assigned to one of the four rearing temperature treatments within 12 h of being laid, such that offspring from each family were divided evenly among the four treatments (split-brood design), with approximately eight offspring per treatment. All offspring were reared in Petri dishes inside temperature-controlled Percival reach-in growth chambers. Developing larvae were rotated daily to control for spatial variation within growth chambers.

Emerging adult beetles were collected twice daily. Subsamples of six offspring per family per rearing temperature were weighed on an electronic balance (AT261 Delta Range; Mettler Toledo, Columbus, Ohio) to the nearest 0.1 mg. These six offspring were randomly selected at the egg stage by marking their dish. After beetles were weighed, females were paired with a randomly chosen male (within each population-by-host-by-temperature treatment combination) and placed in 60-mm Petri dishes containing ~170 mung seeds. Pairs were allowed to lay eggs until death at 27.5°C (15:9 h, light:dark) after which every seed was examined and all eggs were counted to estimate female lifetime fecundity. Oviposition host and temperature will certainly affect female egg-laying behavior and lifetime fecundity (Messina and Karren 2003; Stillwell and Fox 2005) but our focus here is on the effect of the larval rearing environment and not the adult oviposition environment. We thus use a common oviposition environment for all egg-laying females.

We estimated growth rate as log(mass)/larval development time. However, because our original measure of development time also includes the duration of the pupal period, in which growth is not taking place, we first subtracted the average duration of the pupal period for each temperature treatment (rearing host does not affect the length of the pupal period; Chandrakantha and Mathavan 1986). Average pupal intervals were obtained from a previous study that used identical rearing temperatures (Chandrakantha and Mathavan 1986). Though it is possible that the length of the pupal period in this study differs from that

found in Chandrakantha and Mathavan (1986), analysis of the data not correcting for the duration of the pupal period [i.e., log(mass)/egg-to-adult development time] gave qualitatively identical results, indicating that the correction does not bias our conclusions.

Because a single growth chamber was used for each temperature treatment in our study, other sources of environmental variation among growth chambers are potentially confounded with temperature. These effects are likely to be small relative to the large effect of temperature observed for all traits (see Results), but are nonetheless confounded with temperature. However, our focus here is on the degree to which variation along different environmental axes interact to influence the phenotype of organisms, and the degree to which these interactions vary among multiple traits. Thus, while variation among chambers affects the specific interpretation of the temperature main effect, this variation among chambers (temperature + unknown environmental variation) nonetheless reflects an environmental axis completely separate from the other environmental axis (diet) that is manipulated in our study. Thus, chamber effects do not limit our ability to address our original question.

In total, 9,785 adults from 387 full-sib families were reared to adult. 7,658 of these were weighed. Lifetime fecundity was recorded for 2,851 females.

Statistical analyses

All statistical analyses were done with SAS 9.1 (SAS Institute, Cary, N.C.) using ANOVA (PROC GLM). Normal probability plots revealed that all data were approximately normally distributed, except egg-to-adult survivorship. However, development time was log-transformed prior to analysis to stabilize variances among temperature treatments. For our ANOVAs we included population, host, temperature and family as main effects. The family effect is included because of the non-independence of siblings within treatments. Also, because families are unique to each population-by-host combination, the family effect was nested within populations and hosts. Consequently, we can test for a family-by-rearing temperature interaction but not a family-by-host interaction. The family effect is used as the denominator mean square for hypothesis tests for all main effects and their interactions.

For egg-to-adult survivorship we first calculated the proportion of surviving offspring for each family-by-rearing temperature combination. We then used these means (arcsine-square root transformed to meet the assumptions of normality) for analysis. Using family means prevented us from including a family-by-rearing temperature interaction term in the model.

The main focus of this study is on interactions between rearing host and temperature. However, because interactions between factors in an ANOVA measure changes in the linear distance between treatment means, they are dependent on scale. Because of the large effect of temperature on many of the traits we examined (especially development time and growth rate), host-by-temperature interactions, and other interactions involving temperature, may be detected when no interactions exist. Conversely, significant interactions can be masked due to changes in scale. We thus performed our analyses as a two-step process. First, we examined main effects. We then created relative trait values (individual trait value/mean in each temperature treatment) to remove the large temperature effect. These relative trait values were used for testing for interactions between/among variables that were affected by temperature (Stanton and Thiede 2005).

All ANOVAs were first performed using the full model with all possible interaction terms present; non-significant three-way and higher order interaction terms were dropped from all final models. Although we initially included population as a main effect, we subsequently conducted separate analyses for each population due to large interactions involving the population effect for several traits.

Genetic variances (V_G), broad-sense heritabilities (H^2) and cross-temperature genetic correlations (r_G) were estimated using the variance component procedure in SAS (PROC VARCOMP, REML estimation; Fry 1992; Astles et al. 2006). V_G was calculated as twice the phenotypic variance (V_P) among full-sib families (the variance was obtained via the family variance component in PROC VARCOMP) and H^2 was calculated as V_G/V_P (Falconer and Mackay 1996). r_G was calculated for each pair of temperatures as: $\sigma^2_{temperature1,\,2}/\sigma_{temperature1}\sigma_{temperature2}$, where $\sigma^2_{temperature1,\,2}$ is the family variance component for the mixed model with data for the two temperatures pooled (the covariance between temperatures), and $\sigma_{temperature1}$ and $\sigma_{temperature2}$ are square roots of the family variance component for the two reduced models, one for each temperature (the variance within temperatures; Fry 1992; Astles et al. 2006). SEs of genetic parameters were obtained by jackknifing families (Roff and Preziosi 1994; Windig 1997) via a routine created by the authors in SAS. We conducted an ANOVA of the pseudovalues of V_G and H^2 that were generated from the jackknifing routine to explore the impact of population, sex, rearing host and temperature on genetic variation. We also used multivariate ANOVA (MANOVA) to examine the overall effect of population, sex and rearing host on r_G, and subsequently used ANOVA to tease apart which specific r_Gs were affected by the rearing hosts. This method was originally developed by Roff (2002) for the analysis of genetic variance/covariance matrices. The sampling distributions of the pseudovalues created by the jackknifing were all approximately normally distributed and were thus not transformed prior to analysis. Genetic param-

eters were not calculated for development time due to extremely low H^2 and large maternal effects (Fox 1994).

Results

Population, host and population-by-host effects

Because previous comparisons between the BF and SI populations of *C. maculatus* revealed genetic differentiation between populations in a large number of traits (Savalli et al. 2000; Fox et al. 2004a, b, c) we predicted that we would find differences between the populations in all of the traits that we examined in this experiment. As expected, BF beetles had higher egg-to-adult survivorship ($F_{1,385} = 177$, $P < 0.0001$), slightly longer development time ($F_{1,381} = 150$, $P < 0.0001$), were smaller ($F_{1,381} = 224$, $P < 0.0001$), grew slower ($F_{1,380} = 191$, $P < 0.0001$) and had higher fecundity ($F_{1,380} = 469$, $P < 0.001$) than SI beetles (Figs. 1, 2, 3, 4, 5). The effect of rearing host on development time, body mass and growth rate differed between the populations (highly significant population-by-host interaction—development time, $F_{2,381} = 134$, $P < 0.0001$; body mass, $F_{2,381} = 35.8$, $P < 0.0001$; growth rate, $F_{2,380} = 107$, $P < 0.0001$). BF beetles generally had the shortest development time (Fig. 1a, b; highly significant

rearing host effect in Table 1; $P < 0.001$; see figure legends for pair-wise comparisons), were the largest ($P < 0.001$; Fig. 2a, b; Table 1) and grew fastest ($P < 0.001$; Fig. 3a, b; Table 1) when reared on cowpea (except body mass at 20°C). In contrast, SI beetles generally had the shortest development time ($P < 0.001$; Fig. 1a, b; Table 1), were the largest ($P < 0.001$; Fig. 2a, b; Table 1) and grew fastest ($P < 0.001$; Fig. 3a, b; Table 1) when reared on azuki and mung. In addition, the fecundity of SI females (but not BF females; Table 1) was affected by rearing host; SI females laid the fewest eggs when reared on cowpea, though the effect was small ($P < 0.01$; Fig. 4a, b; Table 1). There was also a highly significant population-by-host interaction for egg-to-adult survivorship ($F_{2,385} = 15.3$, $P < 0.0001$); both populations had high survivorship when reared on azuki and mung, and the lowest survivorship on cowpea, but the host effect was much larger for SI than BF beetles ($P < 0.001$; Fig. 5; Table 1).

Temperature and population-by-temperature effects

Development time decreased substantially with increasing rearing temperature ($P < 0.001$; Fig. 1a, b; Table 1). The temperature effect on development time differed slightly between populations ($F_{3,381} = 4.45$, $P = 0.004$; Fig. 1c, d). As expected, both populations decreased significantly in
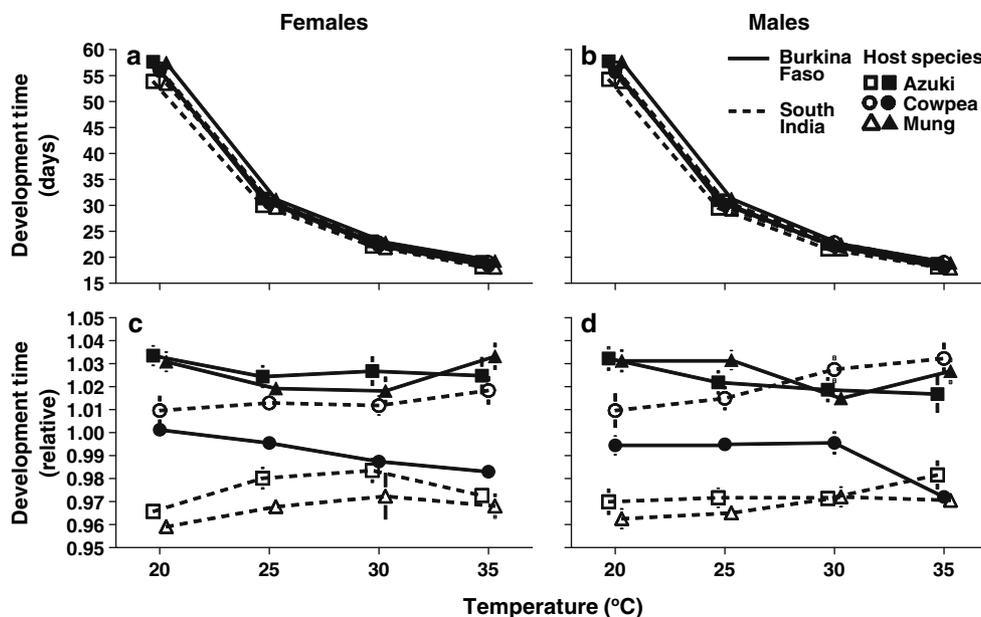
Fig. 1 Egg-to-adult development time of **a**, **c** females and **b**, **d** males of the Burkina Faso (*solid lines*) and South India (*dashed lines*) populations of *Callosobruchus maculatus* in response to rearing on azuki (*squares*), cowpea (*circles*) and mung (*triangles*) at different temperatures (20, 25, 30, 35°C). **c**, **d** Development time (relative) are the means after removing the large temperature effect [individual development time/mean development time for each temperature treatment, follow-

ing Stanton and Thiede (2005)]. SEs are included, but are smaller than the symbols for some experimental treatments. Pair-wise comparisons for Burkina Faso—Tukey's test for cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P < 0.05$; azuki versus mung, $P > 0.05$; South India—cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P < 0.05$; azuki versus mung, $P > 0.05$
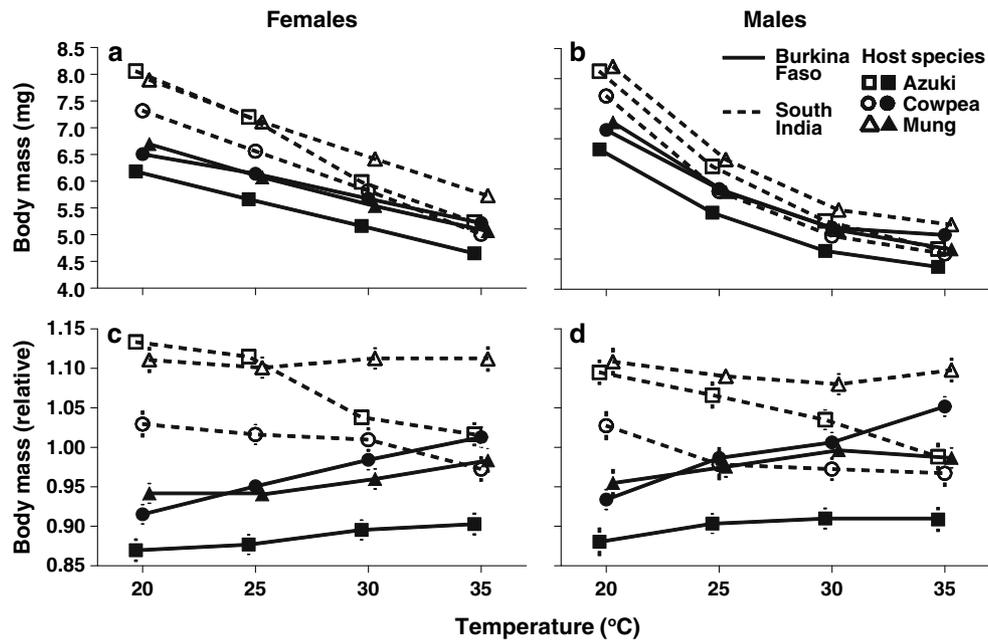
Fig. 2 Adult body mass of **a**, **c** females and **b**, **d** males of the Burkina Faso (*solid lines*) and South India (*dashed lines*) populations of *C. maculatus* in response to rearing on azuki (*squares*), cowpea (*circles*) and mung (*triangles*) at different temperatures (20, 25, 30, 35°C). **c**, **d** Body mass (relative) are the means after removing the large temperature effect [individual body mass/mean body mass for each temperature treatment, following Stanton and Thiede (2005)]. SEs are included, but are smaller than the symbols for some experimental treatments. Pairwise comparisons for Burkina Faso—Tukey's test for cowpea versus mung, $P > 0.05$; cowpea versus azuki, $P < 0.05$; azuki versus mung, $P < 0.05$; South India—cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P < 0.05$; azuki versus mung, $P > 0.05$
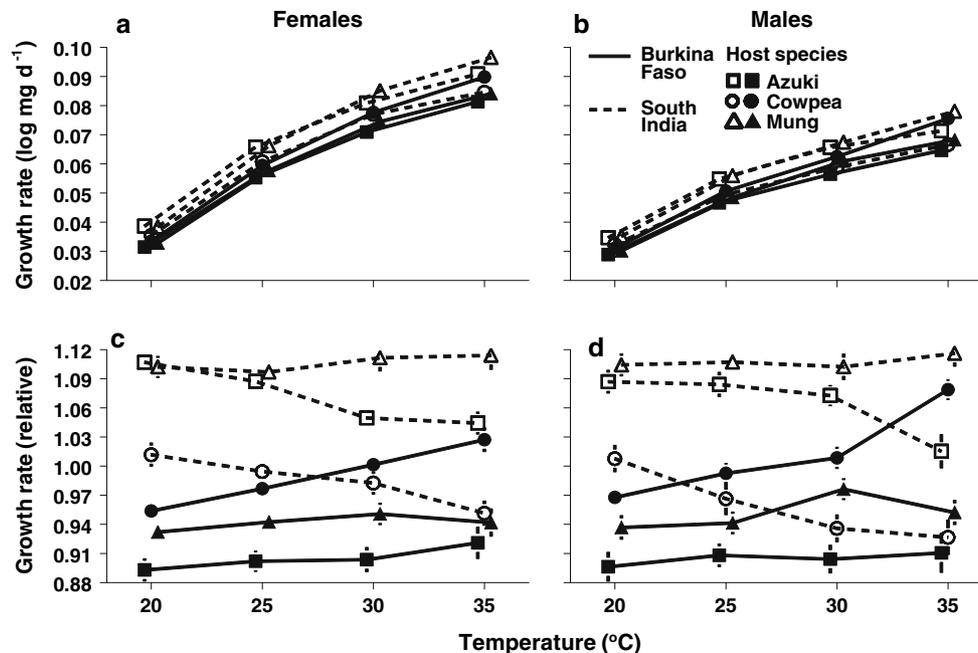
Fig. 3 Growth rate [log(body mass)/larval development time] of **a**, **c** females and **b**, **d** males of the Burkina Faso (*solid lines*) and South India (*dashed lines*) populations of *C. maculatus* in response to rearing on azuki (*squares*), cowpea (*circles*) and mung (*triangles*) at different temperatures (20, 25, 30, 35°C). **c**, **d** Growth rate (relative) are the means after removing the large temperature effect [individual growth rate/mean growth rate for each temperature treatment, following Stanton and Thiede (2005)]. SEs are included, but are smaller than the symbols for some experimental treatments. Pair-wise comparisons for Burkina Faso—Tukey's test for cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P < 0.05$; azuki versus mung, $P < 0.05$; South India—cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P < 0.05$; azuki versus mung, $P > 0.05$
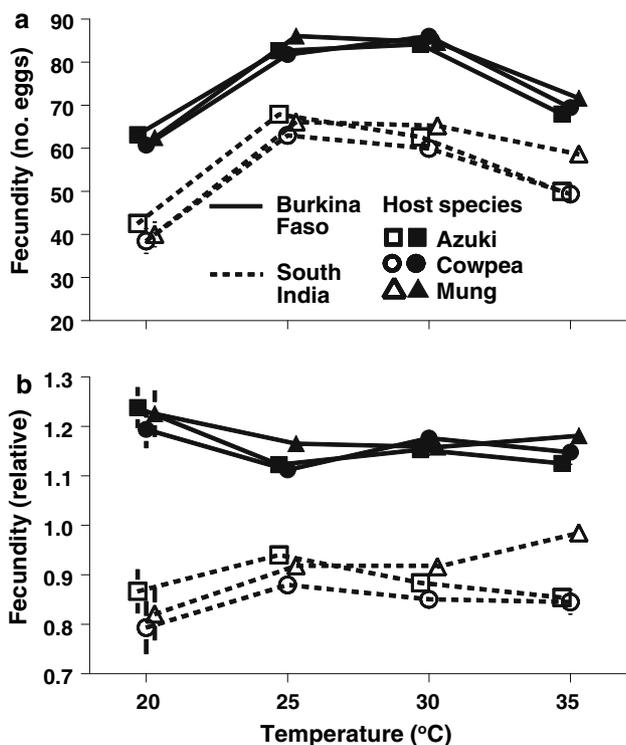
Fig. 4 a Lifetime fecundity of females of the Burkina Faso (*solid lines*) and South India (*dashed lines*) populations of *C. maculatus* in response to rearing on azuki (*squares*), cowpea (*circles*) and mung (*triangles*) at different temperatures (20, 25, 30, 35°C). **b** Fecundity (relative) are the means after removing the large temperature effect [individual fecundity/mean fecundity for each temperature treatment, following Stanton and Thiede (2005)]. SEs are included, but are smaller than the symbols for some experimental treatments. Pair-wise comparisons for Burkina Faso—Tukey's test for cowpea versus mung, $P > 0.05$; cowpea versus azuki, $P > 0.05$; azuki versus mung, $P > 0.05$; South India—cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P > 0.05$; azuki versus mung, $P > 0.05$

Fig. 5 Egg-to-adult survivorship of the Burkina Faso (*solid lines*) and South India (*dashed lines*) populations of *C. maculatus* in response to rearing on azuki (*squares*), cowpea (*circles*) and mung (*triangles*) at different temperatures (20, 25, 30, 35°C). SEs are included, but are smaller than the symbols for some experimental treatments. Pair-wise comparisons for BF—cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P < 0.05$; azuki versus mung, $P < 0.05$; South India—cowpea versus mung, $P < 0.05$; cowpea versus azuki, $P > 0.05$; azuki versus mung, $P > 0.05$

body mass with increasing rearing temperature ($P < 0.001$; Fig. 2a, b; Table 1), but SI beetles decreased in mass considerably more than did BF beetles (evident as convergence of BF and SI lines at higher temperatures; significant population-by-temperature interaction; $F_{3,381} = 10.5$, $P < 0.0001$; Fig. 2c, d). Growth rate increased substantially with increasing rearing temperature ($P < 0.001$; Fig. 3a, b; Table 1), though the effect differed between populations (highly significant populations-by-temperature interaction; $F_{3,380} = 8.24$, $P < 0.0001$) with growth rate of BF beetles increasing with rearing temperature faster than growth rate of SI beetles (evident as convergence of BF and SI lines at higher temperatures; Fig. 3c, d).

The relationship between female lifetime fecundity and temperature was not monotonic; females laid the most eggs when reared at the intermediate temperatures (25 and 30°C) and laid the fewest when reared at either extreme (20 and 35°C, $P < 0.001$; Fig. 4a; Table 1). This pattern was differ-

ent between populations (significant population-by-temperature interaction; $F_{3,380} = 7.87$, $P < 0.0001$); the populations responded similarly to temperature between 25 and 35°C, but fecundity of SI females increased more dramatically between 20 and 25°C than did fecundity of BF beetles (evident as convergence of BF and SI lines at 25°C; Fig. 4b). Egg-to-adult survivorship was highest when beetles were reared at the intermediate temperatures (25 and 30°C) and lowest when reared at the extremes, but the effect was small (20 and 35°C; $P < 0.01$; Fig. 5; Table 1). This effect was similar in both populations (non-significant population-by-temperature interaction; $F_{3,385} = 0.94$, $P = 0.42$).

Host-by-temperature effects

We found significant interactions between rearing host and temperature for three of the five traits we examined (body mass, growth rate and fecundity; Figs. 2,3,4; Table 1). This was most evident for growth rate; the variance in growth rate among hosts increased with increasing temperature (Fig. 3c, d; Table 1). However, this effect differed between populations (significant population-by-host-by-temperature interaction; $F_{6,380} = 2.33$, $P = 0.03$); the degree to which cowpea was a better host than azuki or mung increased at higher temperature for BF beetles, whereas the degree to which cowpea was the worst host increased at higher temperature for SI beetles (Fig. 3c, d).

Genetic variances and heritabilities

Overall, the genetic variance and heritability of body mass was similar for males (mean ± SEM; $V_G = 0.25 \pm 0.11$;

Table 1 ANOVA (type III sums of squares) for the effects of rearing host and temperature on egg-to-adult development time, adult body mass, growth rate, female lifetime fecundity and egg-to-adult survivorship in the Burkina Faso (*BF*) and South India (*SI*) populations of *Callosobruchus maculatus*

	BF		SI	
	df	F	df	F
Egg-to-adult development time				
Females				
Temperature	3	21,588.02***	3	18,722.20***
Host	2	40.10***	2	59.47***
Family (host)	199	1.75***	180	2.24***
Temperature × host[a]	6	1.22	6	1.05
Temperature × family (host)[a]	579	0.98	505	1.23**
Error	1,860		1,422	
Males				
Temperature	3	16,757.64***	3	18,815.90***
Host	2	32.80***	2	88.76***
Family (host)	199	2.30***	181	1.73***
Temperature × host[a]	6	1.53	6	1.26
Temperature × family (host)[a]	582	1.00	503	1.09
Error	2,066		1,461	
Mass				
Females				
Temperature	3	101.59***	3	190.66***
Host	2	22.22***	2	22.09***
Family (host)	200	2.56***	180	3.55***
Temperature × host[a]	6	2.45*	6	3.07**
Temperature × family (host)[a]	559	1.04	485	1.03
Error	1,238		959	
Males				
Temperature	3	196.60***	3	360.54***
Host	2	17.50***	2	19.51***
Family (host)	199	3.72***	180	3.36***
Temperature × host[a]	6	1.84	6	1.85
Temperature × family (host)[a]	561	0.93	487	1.15*
Error	1,462		1,100	
Growth rate				
Females				
Temperature	3	2,115.01***	3	2,252.26***
Host	2	30.23***	2	60.44***
Family (host)	199	1.96***	180	2.44***
Temperature × host[a]	6	1.86	6	2.58*
Temperature × family (host)[a]	559	1.04	484	1.09
Error	1,236		959	
Males				
Temperature	3	1,260.13***	3	1,128.30***
Host	2	34.52***	2	61.12***
Family (host)	199	2.12***	180	2.20***
Temperature × host[a]	6	2.19*	6	2.23*
Temperature × family (host)[a]	561	0.88	487	1.05
Error	1,461		1,099	

Table 1 continued		BF		SI	
		df	F	df	F
	Fecundity				
	Temperature	3	76.32***	3	75.85***
	Host	2	1.11	2	5.19**
	Family (host)	200	1.79***	180	2.12***
	Temperature × host[a]	6	1.53	6	2.42*
	Temperature × family (host)[a]	522	1.08	420	1.29**
	Error	913		592	
	Egg-to-adult survivorship				
* $P < 0.05$, ** $P < 0.01$,	Temperature	3	4.79**	3	5.14**
*** $P < 0.001$	Host	2	16.95***	2	36.18***
[a] Interactions from analyses on	Family (host)	202	1.69***	183	2.37***
relative trait values (individual	Temperature × host	6	1.22	6	0.93
value/mean for each temperature treatment)	Error	597		542	

$H^2 = 0.44 \pm 0.2$) and females ($V_G = 0.31 \pm 0.15$; $H^2 = 0.47 \pm 0.22$; $F < 2.66$, $P > 0.10$). Rearing temperature had a large effect on V_G for mass due to a substantial increase in V_G at 20°C (35°C, 0.21 ± 0.10; 30°C, 0.22 ± 0.10; 25°C, 0.25 ± 0.12; 20°C, 0.43 ± 0.22; $F_{3,2850} = 5.29$, $P = 0.001$). However, this was concordant with an increase in the phenotypic variance with temperature such that there was no effect of temperature on the H^2 for body mass (35°C, 0.46 ± 0.21; 30°C, 0.46 ± 0.21; 25°C, 0.46 ± 0.20; 20°C, 0.45 ± 0.23; $F_{3,2850} = 0.01$, $P = 1$). The genetic variance in body mass of BF females was similar to that of BF males when they were reared on cowpea (females, 0.04 ± 0.02; males, 0.03 ± 0.02) and mung (females, 0.04 ± 0.04; males, 0.02 ± 0.02), but lower when they were reared on azuki (females, 0.03 ± 0.03; males, 0.09 ± 0.04; host-by-sex interaction; $F_{2,1524} = 3.39$, $P = 0.03$). However, this pattern was not observed for heritabilities (host-by-sex interaction; $F_{2,1524} = 0.12$, $P = 0.88$). There was no difference between populations ($F < 2.47$, $P > 0.12$) or among rearing hosts ($F < 0.98$, $P > 0.37$) for either V_G or H^2 of body mass, nor were any of the interaction terms significant ($F < 1.36$, $P > 0.26$).

The genetic variance and heritability of growth rate was not significantly different between males ($V_G = 0.045 \pm 0.025$; $H^2 = 0.33 \pm 0.19$) and females ($V_G = 0.042 \pm 0.027$; $H^2 = 0.32 \pm 0.20$; $F < 0.46$, $P > 0.5$). There was a large effect of temperature on V_G for growth rate (35°C, 0.098 ± 0.058; 30°C, 0.048 ± 0.031; 25°C, 0.021 ± 0.011; 20°C, 0.007 ± 0.005; $F_{3,2873} = 10.82$, $P < 0.0001$): this was concordant with an increase in the phenotypic variance with increasing temperature such that H^2 did not change with temperature (35°C, 0.29 ± 0.18; 30°C, 0.3 ± 0.18; 25°C, 0.33 ± 0.17; 20°C, 0.39 ± 0.25; $F_{3,2873} = 0.76$, $P = 0.52$). There was a marginal host-by-temperature interaction on the genetic variance in growth rate (V_G,

$F_{6,2873} = 2.62$, $P = 0.02$; H^2, $F_{6,2873} = 1.81$, $P = 0.09$); both V_G and H^2 were highest on azuki at 35°C (V_G—azuki, 0.15 ± 0.07; cowpea, 0.06 ± 0.04; mung, 0.08 ± 0.06; H^2—azuki, 0.37 ± 0.17; cowpea, 0.23 ± 0.17; mung, 0.27 ± 0.19), but V_G was similar for all three hosts at 20°C (azuki, 0.008 ± 0.005; cowpea, 0.0055 ± 0.0037; mung, 0.0086 ± 0.0051) while H^2 was highest on mung at 20°C (azuki, 0.32 ± 0.2; cowpea, 0.33 ± 0.22; mung, 0.5 ± 0.3). The genetic variance and heritability for growth rate of SI females was approximately twice that of SI males when they were reared on mung (V_G—SI females, 0.08 ± 0.03; SI males, 0.03 ± 0.02; H^2—SI females, 0.64 ± 0.28; SI males, 0.29 ± 0.23), but lower than that of males when they were reared on azuki and cowpea (V_G—SI females reared on azuki, 0.04 ± 0.02; SI males reared on azuki, 0.06 ± 0.03; H^2—SI females reared on azuki, 0.34 ± 0.18; SI males reared on azuki, 0.47 ± 0.19; V_G—SI females reared on cowpea, 0.03 ± 0.03; SI males reared on cowpea, 0.06 ± 0.04; H^2—SI females reared on cowpea, 0.23 ± 0.22; SI males reared on cowpea, 0.27 ± 0.17; host-by-sex interaction; V_G, $F_{2,1338} = 3.90$, $P = 0.02$; H^2, $F_{2,1338} = 2.95$, $P = 0.05$). In contrast, the genetic variance and heritability of growth rate did not change with temperature (temperature-by-sex interaction; $F < 0.59$, $P > 0.62$). There was no effect of population ($F < 2.3$, $P > 0.13$), rearing host ($F < 1.28$, $P > 0.28$) or any of the other interactions ($F < 0.94$, $P > 0.39$) on V_G or H^2 of growth rate.

V_G for fecundity of SI females (113 ± 68) was double that of BF females (63 ± 50; $F_{1,1331} = 4.33$, $P = 0.04$). This was not due to a scale effect; H^2 of fecundity of SI females was twice as high as that of BF females (SI, 0.51 ± 0.31; BF, 0.23 ± 0.17; $F_{1,1331} = 9.21$, $P = 0.003$). There was no effect of rearing host ($F < 0.36$, $P > 0.7$), temperature ($F < 1.48$, $P > 0.22$) or any of the interactions ($F < 3.00$, $P > 0.05$) on V_G and H of fecundity.

Cross-temperature r_Gs

The overall average cross-temperature r_G for mass ($r_G = 0.94 \pm 0.09$), growth rate ($r_G = 0.92 \pm 0.17$) and fecundity ($r_G = 0.78 \pm 0.27$) were near 1.0. The cross-temperature r_Gs were not different between populations or among hosts, nor were there any significant population-by-host interactions for any traits (MANOVA, $F < 1.22$, $P > 0.3$; ANOVA of all pair-wise r_Gs, $F < 2.55$, $P > 0.11$) with one exception—there was a significant effect of rearing host on r_G for growth rate (MANOVA, Wilks' $\lambda = 0.93$, $F_{12,972} = 2.79$, $P = 0.0009$). However, this was due mainly to a large host effect on r_G at 35 and 25°C (ANOVA, $F_{2,491} = 5.37$, $P = 0.005$); the correlation was lowest on cowpea ($r_G = 0.69 \pm 0.54$) and highest on mung (0.94 ± 0.08). Also, there was a significant sex effect on the cross-temperature r_Gs for growth rate (MANOVA, Wilks' $\lambda = 0.95$, $F_{6,486} = 3.91$, $P = 0.0008$) due to a large difference between the sexes in r_G at 35 and 25°C (ANOVA, $F_{1,491} = 11.16$, $P = 0.0009$); the average cross-temperature r_G for males (0.99 ± 0.02) was larger than was the cross-temperature r_G for females (0.69 ± 0.36). There were no sex-by-population or sex-by-host effects on cross-temperature r_Gs for either body mass or growth rate (MANOVA, $F < 1.22$, $P > 0.3$; ANOVA of all pair-wise r_Gs, $F < 4.52$, $P > 0.03$).

Discussion

In this study we investigated the interactive effects of rearing diet (i.e., host species) and temperature on growth and life history traits in two populations of the seed beetle, *C. maculatus*. We detected a significant host-by-temperature interaction for three of the five traits we examined. The host-by-temperature interaction for growth rate differed in magnitude between our two study populations. This indicates that the effects of multiple environments on beetle traits can be either simple (reaction norm shape along one environmental axis is not affected by other environmental axes) or complex (reaction norm shape along one environmental axis is affected by other environmental axes) depending on the trait and the population examined. In addition, though estimates of genetic variation varied with rearing environment, the heritability estimates for body mass, growth rate and fecundity (which are estimates of the proportion of total variance that is due to genetic variance, and thus removes scale effects) were generally similar among rearing hosts and temperatures. Cross-temperature r_Gs were near 1.0 for all traits and did not differ among hosts suggesting that the genetic architecture underlying temperature-induced phenotypic plasticity is stable and not affected by rearing host. Finally, we found that these popu-

lations of beetles responded differently to both rearing host and temperature.

Simple versus complex patterns of phenotypic plasticity

Although organisms grow and develop in complex environments where they are exposed simultaneously to multiple environmental factors, most empirical assessments of plasticity have manipulated a single environmental factor. Recent studies have demonstrated that environmental factors can have interactive effects on phenotypes, generating complex reaction norms (Stamp and Bowers 1990; Gresens 1997; Sultan et al. 1998; Petersen et al. 2000; Sultan 2001; Relyea 2004; Ris et al. 2004; Relyea and Auld 2005; Kingsolver et al. 2006). For instance, the effects of dietary protein concentration on the growth rate of the caterpillar, *Manduca sexta*, is highly dependent on rearing temperature (Petersen et al. 2000). Likewise, the growth rate of the midge, *Pseudochironomous richardsoni*, is always higher on a detritus diet relative to a diatom diet, but this difference is significantly larger at high rearing temperatures (Gresens 1997). In this study, we found that rearing host and temperature had interactive effects on body mass, growth rate and fecundity of *C. maculatus*. Thus, the shape of reaction norms was complex. This was most obvious for growth rate; the variance in growth rate among hosts increased with increasing temperature. However, this effect differed between our two study populations; the degree to which cowpea was a better host than azuki or mung increased at higher temperatures for BF beetles, whereas the degree to which cowpea was the worst host increased at higher temperatures for SI beetles. Whereas numerous studies have previously demonstrated that reaction norm shape can vary among populations our study shows that interactions between variables can likewise vary among populations within species. Consequently, future studies should include several populations to fully understand how these interactions affect patterns and the evolution of phenotypic plasticity. In contrast, we did not find any significant interactions between host and temperature for larval survivorship or development time, suggesting that the effects of host and temperature are independent for these traits.

Are reaction norms likely to be simple or more complex in environments where organisms are exposed to many variables? Recent empirical investigations have found both complex (Stamp and Bowers 1990; Gresens 1997; Sultan et al. 1998; Petersen et al. 2000; Sultan 2001; Relyea 2004; Ris et al. 2004; Relyea and Auld 2005; Kingsolver et al. 2006) and simple (Teplitsky et al. 2004; Hoverman et al. 2005) patterns of plasticity, but most of these studies have examined only one or a few traits and only a single population within a species. It is difficult to generalize because few studies have explored plasticity of multiple traits and

populations under more complex environmental scenarios. Studying multiple levels of each of several environmental factors requires complicated experimental designs and very large sample sizes, making such studies impractical for most organisms. Nevertheless, we need to understand reaction norm shape in more complex environments to have a better understanding of how plasticity evolves, because many environmental factors vary simultaneously both spatially and temporally in the natural environments in which organisms develop and experience selection.

Genetic architecture in complex environments

A complete understanding of the evolution of phenotypic plasticity requires knowledge of the genetic architecture underlying phenotypically plastic traits and how this genetic architecture changes with environmental conditions. However, virtually nothing is known about the genetics of plasticity in environments that vary along multiple environmental axes. The evolution of plasticity will be slowed when $r_G = 1.0$ because selection on the trait in one environment will result in a similar correlated response in the other environment, though how similar depends on environmental effects on genetic variation (Cheverud et al. 1985). In this study, we examined the cross-temperature r_G for body mass, growth rate and fecundity, and investigated whether rearing host influenced the cross-temperature r_G. The overall average cross-temperature r_G was not significantly different from 1.0 and there was little evidence for a genotype-by-temperature (family-by-temperature) interaction for any of the measured traits (Table 1). This suggests that the evolution of temperature-induced plasticity will be very slow in *C. maculatus*. Also, the cross-temperature r_G was not generally affected by rearing host, indicating that the cross-temperature r_G is stable with respect to rearing conditions. Interestingly, even when reared in common conditions for >100 generations some genetically distinct populations of *C. maculatus* retain distinct genetic architectures underlying growth and life history traits (Bieri and Kawecki 2003) suggesting that genetic architecture may be evolutionarily stable in addition to being stable across environments.

Although a very high r_G will limit the rate of the evolution of plasticity (Via and Lande 1985), plasticity can evolve even with $r_G = 1.0$ if the heritability of a trait differs between environments, a point originally made by Cheverud et al. (1985) in the context of the between-sex r_G. Though the heritability for fecundity differed between populations, we have no evidence that the heritability for body size, growth rate and fecundity varies with rearing host or temperature. Thus, the evolution of plasticity in *C. maculatus* will probably be hindered by both very high r_Gs and similar heritabilities in the different environments.

However, our estimates of the genetic parameters must be interpreted with caution; despite the large number of beetles reared in this experiment, the large number of treatments led to large SEs on individual parameters. Future studies that attempt to investigate genetic architecture in complex environments will likewise be problematic because of the number of treatments required to address this complexity. Nevertheless, exploring the genetic basis of plasticity using experiments that more realistically reflect the complex environments found in nature are required to fully understand the evolution of plasticity.

Adaptation of populations to host and temperature

Adaptation of insect populations to their native host plants is common in nature. Genetic differentiation in growth and life history traits between the BF and SI populations of *C. maculatus* is well documented and is probably due both to adaptation to their indigenous hosts and long-term rearing on these hosts in culture (Savalli et al. 2000; Fox et al. 2004a, b, c), so we expected that BF beetles would generally perform better on cowpea and SI beetles would perform better on mung. We did find that BF beetles generally had a shorter development time, were larger in size (except when reared at the lowest temperature) and had a faster growth rate when they were reared on their native host (cowpea) while SI beetles had a shorter development time, were larger and had a faster growth rate when reared on their native host (mung) and the alternate host (azuki), but the effects were small. In addition, fecundity of females from both populations was generally unaffected by rearing host and both populations had the highest egg-to-adult survivorship on mung and azuki seeds.

Divergence between populations in thermal reaction norms for growth traits occur in several species of insects (Norry et al. 2001; Bochdanovits and De Jong 2003; Stillwell and Fox 2005). Genetic differentiation in population-level thermal reaction norms is often attributed to temperature-mediated natural selection when the reaction norms match differences in climate between collection localities of populations (Stillwell and Fox 2005). Here, we found that the BF and SI populations responded differently to rearing temperature; the relative difference in development time, body size and growth rate between the two populations decreased with increasing rearing temperature. Likewise, the relative difference in fecundity between the BF and SI populations decreased from 20 to 25°C. However, this is not likely a consequence of adaptation to different temperatures because the BF and SI populations originated from tropical locations that have very similar climates (see Materials and methods). Moreover, these populations have been maintained in laboratory colonies for more than 100 generations under benign and identical climates. Consequently, it is unlikely that the differ-

ences in responses we observed are caused by adaptation to temperature, but further work is needed to reveal why these populations respond differently to temperature.

Conclusions

The most important implication of our study is that phenotypic plasticity can be complex for some traits, but simple for others, and that populations can vary in the magnitude of this complexity. Understanding the degree of complexity in plasticity and why traits differ in the degree to which environmental effects are independent versus interactive requires studies that measure a multitude of traits in several populations of a species. Also, though our study found that genetic architecture was stable across environments, future studies should investigate how the genetic architecture of plasticity is affected in complex environments so that more accurate predictions can be made regarding responses to selection in nature. Our data demonstrate that studying plastic responses along one environment axis or for only one or a few traits, will miss much of the complexity of reaction norm shape that occurs in nature. Quantifying plasticity along multiple axes will certainly be difficult because multifactorial experimental designs require large sample sizes to have adequate power to distinguish real from random variation. However, understanding this complexity will yield new and exciting insights into how plasticity evolves in ecologically complex worlds.

References

Angilletta MJ, Dunham AE (2003) The temperature–size rule in ectotherms: simple evolutionary explanations may not be general. Am Nat 162:332–342

Angilletta MJ, Niewiarowski PH, Dunham AE, Leache AD, Porter WP (2004) Bergmann's clines in ectotherms: illustrating a life-history perspective with sceloporine lizards. Am Nat 164:E168–E183

Arnqvist G, Nilsson T, Katvala M (2005) Mating rate and fitness in female bean weevils. Behav Ecol 16:123–127

Astles PA, Moore AJ, Preziosi RF (2006) A comparison of methods to estimate cross-environment genetic correlations. J Evol Biol 19:114–122

Atkinson D (1994) Temperature and organism size—a biological law for ectotherms? Adv Ecol Res 25:1–58

Atkinson D, Sibly RM (1997) Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends Ecol Evol 12:235–239

Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. Annu Rev Entomol 47:817–844

Bégin M, Roff DA, Debat V (2004) The effect of temperature and wing morphology on quantitative genetic variation in the cricket *Gryllus firmus*, with an appendix examining the statistical properties of the Jackknife-MANOVA method of matrix comparison. J Evol Biol 17:1255–1267

Berrigan D, Charnov EL (1994) Reaction norms for age and size at maturity in response to temperature: a puzzle for life historians. Oikos 70:474–478

Bieri J, Kawecki TJ (2003) Genetic architecture of differences between populations of cowpea weevil (*Callosobruchus maculatus*) evolved in the same environment. Evolution 57:274–287

Bochdanovits Z, De Jong G (2003) Temperature dependence of fitness components in geographical populations of *Drosophila melanogaster*: changing the association between size and fitness. Biol J Linn Soc 80:717–725

Boeke SJ, van Loon JJA, van Huis A, Dicke M (2004) Host preference of *Callosobruchus maculatus*: a comparison of life history characteristics for three strains of beetles on two varieties of cowpea. J Appl Entomol 128:390–396

Chandrakantha J, Mathavan S (1986) Changes in developmental rates and biomass energy in *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) reared on different foods and temperatures. J Stored Prod Res 22:71–75

Chandrakantha J, Muthukrishnan J, Mathavan S (1987) Effect of temperature and host seed species on the fecundity of *Callosobruchus maculatus* (F.). Proc Indian Acad Sci Anim Sci 96:221–227

Cheverud JM, Dow MM, Leutenegger W (1985) The quantitative assessment of phylogenetic constraints in comparative analyses: sexual dimorphism in body weight among primates. Evolution 39:1335–1351

Credland PF (1987) Effects of host change on the fecundity and development of an unusual strain of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). J Stored Prod Res 23:91–98

Czesak ME, Fox CW, Wolf JB (2006) Experimental evolution of phenotypic plasticity: how predictive are cross-environment genetic correlations? Am Nat 168:323–335

Ernsting G, Isaaks A (2000) Ectotherms, temperature, and trade-offs: size and number of eggs in a carabid beetle. Am Nat 155:804–813

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman, Essex

Fox CW (1993) A quantitative genetic analysis of oviposition preference and larval performance on two hosts in the bruchid beetle, *Callosobruchus maculatus*. Evolution 47:166–175

Fox CW (1994) Maternal and genetic influences on egg size and larval performance in a seed beetle (*Callosobruchus maculatus*): multigenerational transmission of a maternal effect? Heredity 73:509–517

Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. Annu Rev Entomol 45:341–369

Fox CW, Waddell KJ, Mousseau TA (1994) Host associated fitness variation in a seed beetle (Coleoptera: Bruchidae): evidence for local adaptation to a poor quality host. Oecologia 99:329–336

Fox CW, Harbin AD, Mousseau TA (1996) Suitability of a non-host palo verde for development of *Stator limbatus* (Horn) (Coleoptera: Bruchidae) larvae. Pan Pac Entomol 72:31–36

Fox CW, Bush ML, Roff DA, Wallin WG (2004a) Evolutionary genetics of lifespan and mortality rates in two populations of the seed beetle, *Callosobruchus maculatus*. Heredity 92:170–181

Fox CW, Czesak ME, Wallin WG (2004b) Complex genetic architecture of population differences in adult lifespan of a beetle: nonadditive inheritance, gender differences, body size and a large maternal effect. J Evol Biol 17:1007–1017

Fox CW, Stillwell RC, Amarillo AR, Czesak ME, Messina FJ (2004c) Genetic architecture of population differences in oviposition behaviour of the seed beetle *Callosobruchus maculatus*. J Evol Biol 17:1141–1151

Fry JD (1992) The mixed model analysis of variance applied to quantitative genetics: biological meaning of the parameters. Evolution 46:540–550

Giga DP, Smith RH (1987) Egg production and development of *Callosobruchus rhodesianus* (Pic.) and *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) on several commodities at two different temperatures. J Stored Prod Res 23:9–15

Gresens SE (1997) Interactive effects of diet and thermal regime on growth of the midge *Pseudochironomus richardsoni* Malloch. Freshwater Biol 38:365–373

Guntrip J, Sibly RM, Holloway GJ (1997) The effect of novel environment and sex on the additive genetic variation and covariation in and between emergence body weight and development period in the cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Heredity 78:158–165

Hoverman JT, Auld JR, Relyea RA (2005) Putting prey back together again: integrating predator-induced behavior, morphology, and life history. Oecologia 144:481–491

Kawecki TJ (1995) Expression of genetic and environmental variation for life history characters on the usual and novel hosts in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Heredity 75:70–76

Kingsolver JG, Shlichta JG, Ragland GJ, Massie KR (2006) Thermal reaction norms for caterpillar growth depend on diet. Evol Ecol Res 8:703–715

Kozłowski J, Czarnoleski M, Danko M (2004) Can optimal resource allocation models explain why ectotherms grow larger in cold? Integr Comp Biol 44:480–493

Lale NES, Vidal S (2000) Mortality of different developmental stages of *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic.) (Coleoptera: Bruchidae) in bambara groundnut *Vigna subterranea* (L.) Verdc. seeds exposed to simulated solar heat. J Plant Dis Prot 107:553–559

Lale NES, Vidal S (2003a) Effect of constant temperature and humidity on oviposition and development of *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic.) on bambara groundnut *Vigna subterranea* (L.) Verdcourt. J Stored Prod Res 39:459–470

Lale NES, Vidal S (2003b) Simulation studies on the effects of solar heat on egg-laying, development and survival of *Callosobruchus maculatus* (F.) and *Callosobruchus subinnotatus* (Pic.) in stored bambara groundnut *Vigna subterranea* (L.) Verdcourt. J Stored Prod Res 39:447–458

Mbata GN, Johnson M, Phillips TW, Payton M (2005) Mortality of life stages of cowpea weevil (Coleoptera: Bruchidae) exposed to low pressure at different temperatures. J Econ Entomol 98:1070–1075

Messina FJ (1993) Heritability and evolvability of fitness components in *Callosobruchus maculatus*. Heredity 71:623–629

Messina FJ (2004a) How labile are the egg-laying preferences of seed beetles? Ecol Entomol 29:318–326

Messina FJ (2004b) Predictable modification of body size and competitive ability following a host shift by a seed beetle. Evolution 58:2788–2797

Messina FJ, Karren ME (2003) Adaptation to a novel host modifies host discrimination by the seed beetle *Callosobruchus maculatus*. Anim Behav 65:501–507

Mitchell R (1991) The traits of a biotype of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) from South India. J Stored Prod Res 27:221–224

Norry FM, Bubliy OA, Loeschcke V (2001) Developmental time, body size and wing loading in *Drosophila buzzatii* from lowland and highland populations in Argentina. Hereditas 135:35–40

Nylin S, Gotthard K (1998) Plasticity in life history traits. Annu Rev Entomol 43:63–83

Petersen C, Woods HA, Kingsolver JG (2000) Stage-specific effects of temperature and dietary protein on growth and survival of *Manduca sexta* caterpillars. Physiol Entomol 25:35–40

Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore, Md.

Promislow D (2005) A regulatory network analysis of phenotypic plasticity in yeast. Am Nat 165:515–523

Relyea RA (2004) Fine-tuned phenotypes: tadpole plasticity under 16 combinations of predators and competitors. Ecology 85:172–179

Relyea RA, Auld JR (2005) Predator- and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. Ecology 86:1723–1729

Ris N, Allemand R, Fouillet P, Fleury F (2004) The joint effect of temperature and host species induce complex genotype-by-environment interactions in the larval parasitoid of *Drosophila*, *Leptopilina heterotoma* (Hymenoptera: Figitidae). Oikos 106:451–456

Roff DA (2002) Comparing G matrices: a MANOVA approach. Evolution 56:1286–1291

Roff DA, Preziosi R (1994) The estimation of the genetic correlation: the use of the jackknife. Heredity 73:544–548

Savalli UM, Czesak ME, Fox CW (2000) Paternal investment in the seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae): variation among populations. Ann Entomol Soc Am 93:1173–1178

Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. Annu Rev Ecol Syst 24:35–68

Stamp NE, Bowers MD (1990) Variation in food quality and temperature constrain foraging of gregarious caterpillars. Ecology 71:1031–1039

Stanton ML, Thiede DA (2005) Statistical convenience vs biological insight: consequences of data transformation for the analysis of fitness variation in heterogeneous environments. New Phytol 166:319–338

Stillwell RC, Fox CW (2005) Complex patterns of phenotypic plasticity: interactive effects of temperature during rearing and oviposition. Ecology 86:924–934

Sultan SE (2001) Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. Ecology 82:328–343

Sultan SE, Wilczek AM, Bell DL, Hand G (1998) Physiological response to complex environments in annual *Polygonum* species of contrasting ecological breadth. Oecologia 115:564–578

Teplitsky C, Plenet S, Joly P (2004) Hierarchical responses of tadpoles to multiple predators. Ecology 85:2888–2894

Timms R (1998) Size-independent effects of larval host on adult fitness in *Callosobruchus maculatus*. Ecol Entomol 23:480–483

Vamosi SM (2005) Interactive effects of larval host and competition on adult fitness: an experimental test with seed beetles (Coleoptera: Bruchidae). Funct Ecol 19:859–864

van Huis A, de Rooy M (1998) The effect of leguminous plant species on *Callosobruchus maculatus* (Coleoptera: Bruchidae) and its egg parasitoid *Uscana lariophaga* (Hymenoptera: Trichogrammatidae). Bull Entomol Res 88:93–99

Via S (1994) The evolution of phenotypic plasticity: what do we really know? In: Real LA (ed) Ecological genetics. Princeton University Press, Princeton, N.J., pp 35–57

Via S, Lande R (1985) Genotype-environment interaction and the evolution of phenotypic plasticity. Evolution 39:505–522

Wasserman SS, Futuyma DJ (1981) Evolution of host plant utilization in laboratory populations of the southern cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae). Evolution 35:605–617

West-Eberhard MJ (2003) Developmental plasticity and evolution. Oxford University Press, New York

Windig JJ (1997) The calculation and significance testing of genetic correlations across environments. J Evol Biol 10:853–874