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Rapid population divergence in thermal reaction norms for an invading species: breaking the temperature–size rule

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Abstract

The temperature–size rule is a common pattern of phenotypic plasticity in which higher temperature during development results in a smaller adult body size (i.e. a thermal reaction norm with negative slope). Examples and exceptions to the rule are known in multiple groups of organisms, but rapid population differentiation in the temperature–size rule has not been explored. Here we examine the genetic and parental contributions to population differentiation in thermal reaction norms for size, development time and survival in the Cabbage White Butterfly *Pieris rapae*, for two geographical populations that have likely diverged within the past 150 years. We used split-sibship experiments with two temperature treatments (warm and cool) for *P. rapae* from Chapel Hill, NC, and from Seattle, WA. Mixed-effect model analyses demonstrate significant genetic differences between NC and WA populations for adult size and for thermal reaction norms for size. Mean adult mass was 12–24% greater in NC than in WA populations for both temperature treatments; mean size was unaffected or decreased with temperature (the temperature–size rule) for the WA population, but size increased with temperature for the NC population. Our study shows that the temperature–size rule and related thermal reaction norms can evolve rapidly within species in natural field conditions. Rapid evolutionary divergence argues against the existence of a simple, general mechanistic constraint as the underlying cause of the temperature–size rule.

Introduction

One of the most common forms of phenotypic plasticity is the relationship between adult (final) size and environmental temperature. In most ectotherms, higher temperature during development increases growth and development rates, but decreases adult size (size at maturity). This pattern, known as the temperature–size rule, has been observed in >80% of ectothermic species studied, and occurs in diverse organisms including animals, plants, protozoa and bacteria (Atkinson, 1994). Like most empirical generalizations in biology, this rule has many exceptions (Mousseau & Roff, 1989; Atkinson, 1995; van der Have & de Jong, 1996; Moreteau *et al.*, 1997). Both adaptive and mechanistic models have been proposed to explain the temperature–size rule (Berrigan & Charnov, 1994; Atkinson & Sibly, 1997; Partridge & Coyne, 1997; Davidowitz & Nijhout, 2004), but a single general explanation for the rule and its exceptions remains elusive (Angilletta & Dunham, 2003).

The relationship between phenotypic trait value (e.g. adult size) and an environmental variable (e.g. temperature) for a genotype is called a reaction norm. In this sense, the temperature–size rule describes a reaction norm with negative slope. Population differentiation in thermal reaction norms for size has been described in several insect species (Morin *et al.*, 1999; Gilchrist & Huey, 2004; Stillwell & Fox, 2005), but rapid evolutionary changes in the temperature–size rule in natural field populations have not been documented.

Here we explore population differentiation in thermal reaction norms in an invading species, the Small Cabbage White Butterfly *Pieris rapae* L. Within its native range in

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Europe, *P. rapae* appears to follow the temperature–size rule of decreased body size with increased temperature (Baker, 1968). We consider two North American populations of *P. rapae* that experience different climatic conditions, and that have likely diverged within the past 150 years. We evaluate parental and genetic (maternal and paternal) contributions to population divergence in survival, development time and adult size. Our results suggest rapid genetic divergence in the direction of the temperature–size relationship between these populations. They also indicate strong parental contributions to survival and size responses to temperature in these populations.

Materials and methods

The study system

The Small Cabbage White Butterfly or Imported Cabbageworm, *Pieris rapae* L. (Lepidoptera: Pieridae), is native to Europe and Northern Africa (see below). The larvae feed on a variety of host plants in the mustard family (Brassicaceae). In many areas it is a minor agricultural pest on domesticated forms of *Brassica oleracea*, including cabbage, collards, kale, broccoli and cauliflower. The adults can disperse quite widely, so that there is little genetic differentiation at local to regional scales (Vawter, 1977).

Like many agricultural insect pests, *P. rapae* has dramatically expanded its geographical range in the past several centuries. Scudder (1887) provided a detailed summary of the introduction and spread of *P. rapae* in North America, based on museum records, his own observations, published literature and correspondence with more than 200 collectors. *Pieris rapae* first appeared in Quebec City in 1860, probably transported as larvae or pupae on cabbages brought by European immigrants from Britain (Scudder, 1887; Guppy, 1969). This initial population expanded rapidly, and by 1868 it was abundant throughout southern Quebec, Maine, Vermont and New Hampshire. There was possibly a second introduction from a European source in 1868 by a German collector near New York, NY, in 1868, which expanded into northern New Jersey and eastern New York. These populations had thoroughly intermingled by 1871; by the next year *P. rapae* was found throughout New England, New York, Pennsylvania and southern Quebec and Ontario. This southern and western expansion continued rapidly, and by 1876 it was common throughout Virginia, West Virginia and North Carolina; it reached Nebraska by 1881. By 1886, the species occupied nearly all of the US and southern Canada east of the Rocky Mountains, from Florida, Maine and Nova Scotia in the east to Colorado, Montana and Alberta in the west. Transport of cabbage and other cole crops via train lines was probably the key to its rapid western spread in both the northern US and southern Canada (Dodge, 1882; Guppy & Shepard, 2001). It was first recorded in southeast British Columbia in 1899, and had achieved pest status throughout southern British Columbia by 1901 (Guppy & Shepard, 2001). There is no evidence for hybridization of *P. rapae* with other North American congeners (e.g. *P. napi* and *P. virginiensis*). This detailed historic record indicates that the divergence between the NC and WA populations of *P. rapae* studied here has occurred since the North American introductions in the 1860s, within the past 150 years (see Discussion).

We studied two geographical populations in the US, one in the Puget Sound region near Seattle, WA, and the other in the central Piedmont region near Chapel Hill, NC. The animals used in the current studies were collected from organic vegetable farms in the two regions. The WA population has three to four complete generations per year, whereas the NC population has five to six generations per year; animals in both populations overwinter in a pupal diapause. Not surprisingly, the two populations experience quite different climatic conditions. Because *P. rapae* caterpillars do not thermoregulate behaviourally or physiologically (except to avoid exposure to extreme high body temperatures), we have successfully used physical models to quantify patterns of body temperature variation experienced by caterpillars in the field (Kingsolver, 2000). For example, field measurements during mid-summer (July–August) indicate that mean daily body temperatures are 6 °C higher in the NC population than in the WA population (Kingsolver *et al.*, 2004). Because of their small size, individual caterpillars routinely experience body temperature fluctuations of 20 °C or more over a single diurnal cycle, and body temperatures may vary over a 30 °C range during their lifetime (Kingsolver, 2000; Kingsolver *et al.*, 2004). We use this field information in designing relevant temperature treatments in our experiments (see below).

Experimental design

We used a split sib-family design in our experiments, which were conducted in our laboratory at the University of North Carolina (UNC). Mated females were individually marked and caged in the greenhouse and allowed to oviposit on a young collard plant (*Brassica oleraceaea* var. collard: CO350 Champion variety) for 2 days. Upon hatching, first-instar caterpillars from each female were randomly assigned to one of two diurnally fluctuating temperature treatments: cool (10–34 °C) or warm (16–40 °C) (Fig. 1). These treatments were designed to reflect typical mid-summer thermal conditions for caterpillars in Seattle, WA, and Chapel Hill, NC respectively (Kingsolver *et al.*, 2004). The shape of the frequency distribution of temperatures is identical for the two treatments, but they differ in mean temperature by 6 °C (mean of 20 and 26 °C for the cool and warm treatments respectively). Each temperature treatment was

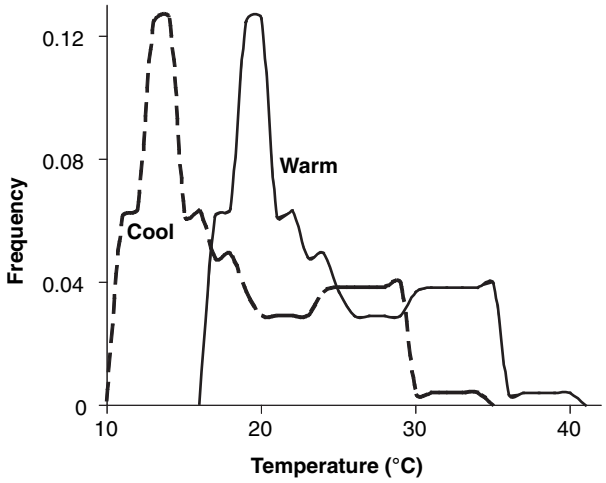

Fig. 1 Frequency distribution (fraction of total time) of temperature (°C) for the diurnally cycling warm and cool treatments used in the experiments. The warm and cool distributions mimic mid-summer temperature conditions experienced by *Pieris rapae* caterpillars in the field in Chapel Hill, NC, and Seattle WA respectively.

maintained in a different environmental chamber (Percival 36-VL: Percival Scientific, Perry, IA, USA) calibrated with a Wescor TH-65 (Wescor Inc., Logan, UT, USA) thermocouple thermometer; a temperature datalogger (ibutton DS1921L: Dallas Semiconductor Maxim, Dallas, TX, USA) was placed in each chamber to confirm the accuracy of the temperature treatments. A 16 : 8 h light : dark cycle was used in both treatments. We were not able to replicate the treatments in multiple chambers in an experiment because of limited chambers; however, we reversed the assignment of treatments to chambers in the two experiments (see below). Caterpillars were reared in individual Petri dishes and fed on a standard artificial diet (Troetschler *et al.*, 1985) for *P. rapae*, which includes 1.6% dried collard leaves by weight; fresh diet was provided every 2 days. Time and survival to eclosion (adult) were monitored daily; newly eclosed adults were placed in glassine envelopes after their wings were fully dried and hardened, and weighed to ±0.01 mg with an electronic balance (Mettler Toledo model AT261 Delta-Range: Mettler Toledo Inc., Columbus, OH, USA). The three response variables in our experiments were survival to adult stage, development time to adult stage, and adult body mass.

As in many pierid butterflies, *P. rapae* females already possess a full complement of oocytes at eclosion, which develop during the first few days of adult life. Following mating, sperm is stored in the female's spermatheca; fertilization does not occur until the developed eggs move through the oviduct during oviposition. As a result, environmental conditions of the parents may influence thermal responses of their offspring. To distinguish potential parental and genetic effects on thermal reaction norms, we conducted two independent experiments in 2003 and 2004. In the 2003 experiment, adult mated female *P. rapae* were collected from our field sites in Washington and North Carolina, and transported overnight to our laboratory at UNC. Eggs obtained from these females were used in the experimental design as described above. As a result, both genetic and parental factors could potentially contribute to observed population differences. There were 588 individuals from 30 families in the WA population, and 687 individuals from 30 families in the NC population in this experiment.

In the 2004 experiment, adult mated females were collected from both field populations; the offspring from these females were reared for one generation in our standard laboratory conditions (11–35 °C diurnally cycling temperature conditions; 16 : 8 h light : dark cycle; artificial diet). Newly eclosed females were mated in large mating cages in the greenhouse, and offspring from these females were used in the experiment. As a result, only genetic factors contributed to observed population differences. There were 368 individuals from eight families in the WA population, and 372 individuals from eight families in the NC population in this experiment. Sex was recorded in the 2003 experiment, but not in the 2004 experiment.

Laboratory and field studies show that *P. rapae* females almost always mate within a few hours of adult eclosion; after mating there is a refractory period of 2–4 days before re-mating by a female occurs (Wiklund *et al.*, 2001). In addition, there is nearly complete sperm precedence when females re-mate (Wedell & Cook, 1998, 1999a,b; Wiklund *et al.*, 2001). As a result, all offspring of each female in our studies very likely represent full-sibs, though we cannot rule out some possibility of multiple paternity.

Statistical analyses

Results of each experiment were analysed using linear mixed-effects models (Pinheiro & Bates, 2000). Temperature and population were treated as fixed effects; we were particularly interested in the temperature by population interaction. Family within population and family by temperature interactions within population were treated as random effects. We assumed a common between-family variance and family by temperature interaction variance for both populations, and estimated these variance components in the model.

Development time and adult mass were approximately normally distributed in these experiments. As a result, we applied a standard linear mixed-effects model and estimated model parameters with restricted maximum likelihood using SAS (Procedure Proc Mixed). Type 3 sums of squares were used to test for the fixed effects in the full model, and likelihood ratios (LR) were used to test for the random effects. Because families within populations did not have identical sample sizes, degrees of freedom were computed

using the Satterthwaite approximation (Satterthwaite, 1946; Kuehl, 1994). *A priori* contrasts were used to test whether the slope of the thermal reaction norm for adult mass for each population differed from zero. We did not adjust reported *P*-values for multiple tests (Quinn & Keough, 2002; Garcia, 2003, 2004; Moran, 2003; Gotelli & Ellison, 2004).

Survival to adulthood of an individual is binomially distributed (dead or alive). As a result, we applied a generalized linear mixed-effects model for the survival data, using generalized estimating equations based on a quasi-likelihood approach (Hardin & Hilbe, 2003). This model is implemented in S-Plus with the glme function in the CorrelatedData library, using a logit link function. Model parameters were estimated with restricted quasi-likelihood (REPQL); LR were used to test for both fixed and random effects (Frees, 2004).

Results

Adult size

In both the 2003 and 2004 experiments, there were significant effects of population and population by temperature interaction on adult body mass, but no significant effects of temperature (Tables 1a and 2a). (Omitting the interaction term from the model does not alter the test results for the main effects.) In both experiments, mean size was greater in the NC than the WA population for both warm and cool treatments (Figs 2 and 3, top). *A priori* contrasts for the 2003 experiment revealed that the slope of the thermal reaction norm for size was significantly positive for the NC population ($F_{1,41} = 9.59$, $P = 0.0035$), but not significantly different from zero for the WA population ($F_{1,50.2} = 0.63$, $P = 0.4295$) (Fig. 2, top). For the 2004 experiment, the slope of the thermal reaction norm for size was significantly positive for the NC population ($F_{1,576} = 3.99$, $P = 0.0462$), and significantly negative for the WA population ($F_{1,576} = 4.10$, $P = 0.0434$) (Fig. 3, top).

Including random effects of between-family variation significantly increased the model likelihood for adult mass in both the 2003 ($\chi^2_1 = 154.9$, $P < 0.001$) and 2004 ($\chi^2_1 = 32.2$, $P < 0.001$) experiments. Including random effects of family by temperature interactions significantly increased the model likelihood for adult mass for the 2003 experiment ($\chi^2_1 = 9.5$, $P < 0.001$), but not for the 2004 experiment ($\chi^2_1 = 2.00$, $P > 0.1$). For the NC population most families had reaction norms for adult mass with positive slopes (i.e. larger mass at higher temperature) (Figs 4 and 5, top); whereas for the WA population families exhibited a mix of negative, positive, and zero slopes (Figs 4 and 5, bottom).

Table 1 Statistical tests for fixed effects in the 2003 experiment. For adult mass (a) and development time (b), *F*-tests using type 3 SS are presented. For survival, results of likelihood ratio tests are presented.

(a) Adult mass

Effect	Num. d.f.	Denom. d.f.	*F*-value	*P*-value
Temperature	1	45.7	2.26	0.1397
Population	1	47	17.91	0.0001
Temp * popn	1	45.7	7.17	0.0102
Sex	1	1051	18.99	<0.0001

(b) Development time

Effect	Num. d.f.	Denom. d.f.	*F*-value	*P*-value
Temperature	1	35.7	552.50	<0.0001
Population	1	34.8	16.45	0.0003
Temp * popn	1	35.7	0.44	0.5112
Sex	1	992	2.72	0.0995

(c) Survival

Model	d.f.	−ln(*L*)	χ^2	*P*-value
Full	7	3781.04		
No temp * popn	6	3764.14	33.80	<0.0001
No popn	5	3754.11	20.07	<0.0001
No temp	5	3749.83	28.62	<0.0001

Table 2 Statistical tests for fixed effects in the 2004 experiment. For adult mass (a) and development time (b), *F*-tests using type 3 SS are presented. For survival, results of likelihood ratio tests are presented.

(a) Adult mass

Effect	Num. d.f.	Denom. d.f.	*F*-value	*P*-value
Temperature	1	14.4	0.01	0.9444
Population	1	13.6	16.14	0.0013
Temp * popn	1	14.4	4.89	0.0437

(b) Development time

Effect	Num. d.f.	Denom. d.f.	*F*-value	*P*-value
Temperature	1	14.1	874.63	<0.0001
Population	1	13.9	1.81	0.2003
Temp * popn	1	14.1	8.08	0.0130

(c) Survival

Model	d.f.	−ln(*L*)	χ^2	*P*-value
Full	7	1751.82		
No temp * popn	6	1751.48	0.67	0.414
No popn	5	1751.22	0.53	0.467
No temp	5	1751.29	0.38	0.539

Development time

Development time was significantly smaller in the warm than the cool temperature treatment in both experiments (Tables 1b and 2b; Figs 2 and 3, middle). In the 2003 experiment there was a significant population effect (Table 1b); mean development time was greater for the

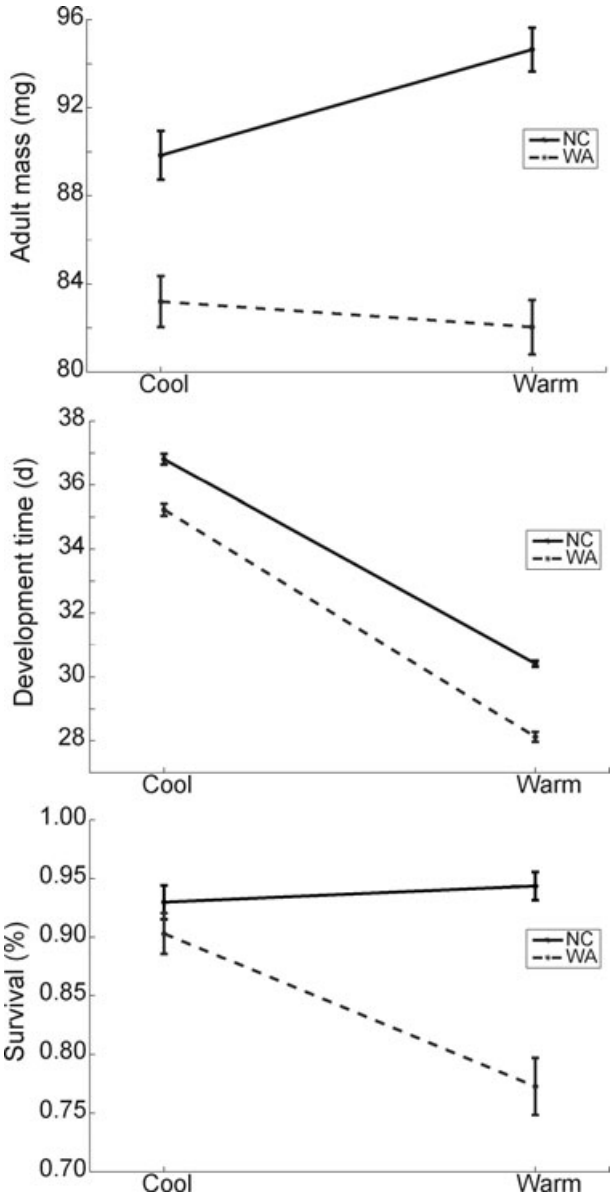

Fig. 2 Mean (±1 SE) thermal reaction norms for adult mass (top), development time (middle) and survival (bottom) for NC (solid line) and WA (dashed line) populations of *Pieris rapae* in the 2003 experiment.

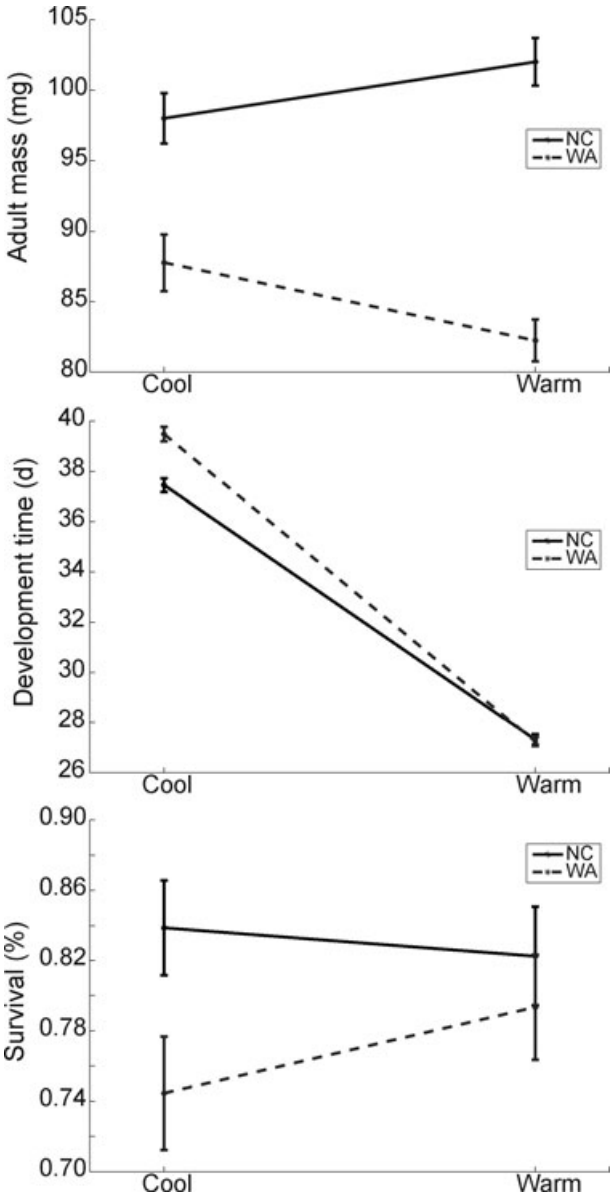

Fig. 3 Mean (±1 SE) thermal reaction norms for adult mass (top), development time (middle) and survival (bottom) for NC (solid line) and WA (dashed line) populations of *Pieris rapae* in the 2004 experiment.

NC than the WA population in both temperature treatments (Fig. 2, middle). In the 2004 experiment there was no significant population effect, but a significant population by temperature interaction (Table 2b); mean development time was smaller for the NC than the WA population in the cool treatment, but similar in the warm treatment (Fig. 3, middle). Omitting the interaction term from the model does not alter the test results for the main effects.

Including random effects of between-family variation significantly increased the model likelihood for development time in both the 2003 ($\chi^2_1 = 193.7$, $P < 0.001$) and 2004 ($\chi^2_1 = 20.4$, $P < 0.001$) experiments. Similarly, including random effects of family by temperature interactions significantly increased the model likelihood for development time for both the 2003 ($\chi^2_1 = 92.8$, $P < 0.001$) and 2004 ($\chi^2_1 = 5.6$, $P < 0.01$) experiments.

Survival

In the 2003 experiment, survival was significantly affected by the interaction of temperature and population;

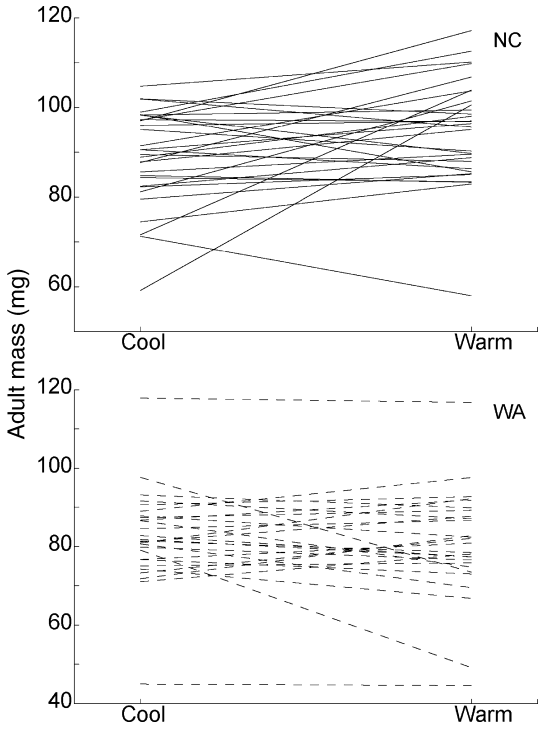

Fig. 4 Mean thermal reactions for adult mass for full-sib families of *Pieris rapae* in the 2003 experiment. Top panel, NC population; bottom panel, WA population.

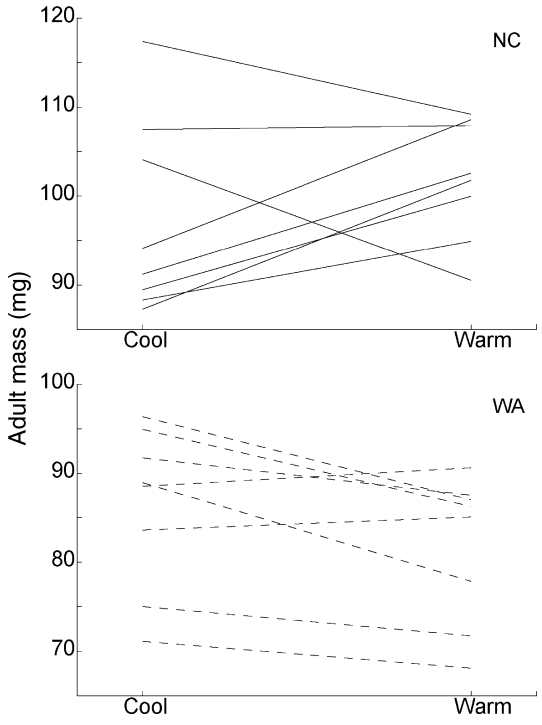

Fig. 5 Mean thermal reactions for adult mass for full-sib families of *Pieris rapae* in the 2004 experiment. Top panel, NC population; bottom panel, WA population.

omitting temperature or population from the model (in the absence of the interaction term) also significantly reduces the likelihood of the model (Table 1c). Mean survival was quite high (90–95%) for the NC population in both temperature treatments and for the WA population in the cool treatment, but mean survival for the WA population was lower (78%) in the warm treatment (Fig. 2, bottom). Survival in the 2004 experiment was not significantly affected by population, temperature or their interaction (Table 2c). Survival was generally higher in 2003 (Fig. 2, bottom) than in 2004 (Fig. 3, bottom).

Including random effects of between-family variation significantly increased the model likelihood for survival in both the 2003 ($\chi^2_1 = 8.25$, $P < 0.01$) and 2004 ($\chi^2_1 = 7.25$, $P < 0.05$) experiments. Including random effects of family by temperature interactions significantly increased the model likelihood for development time for the 2003 ($\chi^2_1 = 10.25$, $P < 0.005$) experiment, but not for the 2004 ($\chi^2_1 = 5.127$, $P > 0.05$) experiment.

Discussion

Rapid population differentiation in *P. rapae*

As described in the Introduction, the historic record for *P. rapae* indicates that the divergence between the NC and WA populations studied here has occurred since the North American introductions in the 1860s, within the past 150 years. The available genetic data for *P. rapae* are also consistent with a recent population divergence in North America. For example, allozyme studies of four polymorphic loci for US populations (including western Oregon and central North Carolina) showed high levels of heterozygosity and little variation in allozyme frequencies among eastern populations (Vawter, 1977). West coast populations had lower heterozygosity, and there were slight differences in allozyme frequencies (but no fixed differences) between eastern and western populations (Vawter, 1977). These considerations all suggest that the genetic differences among these US populations are small in magnitude and recent in origin.

Despite this recent divergence, our studies provide strong evidence for significant population differentiation in body size between NC and WA populations of *P. rapae*. For example, mean adult body mass was 12–24% greater in the NC than in the WA population in the two temperature treatments in both experiments (Figs 2 and 3). Interestingly, this geographical difference in body size is the reverse of that described in most insects and other ectotherms (Bergman's rule), in which populations or species from warmer environments or lower latitudes tend to have smaller adult body size (Angilletta & Dunham, 2003; Angilletta *et al.*, 2003).

Gilbert (1984b, 1988) studied population differentiation in body size for several European (Britain, France and Italy) and Australian (Canberra) populations of *P. rapae*. His analyses did not detect significant differences in pupal mass among populations reared at either 19 or 25 °C. However, Gilbert noted that the mean pupal weights in this study were 10–15% lower than in previous studies (Gilbert, 1984a) 'because of high temperatures or poor nutrition' (Gilbert, 1988, p. 399).

Our results about population differentiation in survival and development time present a more complex picture. Recall that the 2003 experiment includes both genetic and potential parental effects, whereas the 2004 experiment includes only genetic effects (see Materials and methods). The 2004 experiment showed no significant effects of temperature, population or their interaction on adult survival, suggesting no detectable genetic divergence in survival. By contrast, the 2003 experiment showed a significant temperature by population interaction effect on survival, reflecting a substantial reduction in mean survival for the WA population in the warm temperature treatment (Fig. 2). Similarly, mean development time was longer for the NC than the WA population in the 2003 experiment, but this difference disappeared (warm treatment) or was reversed (cool treatment) in the 2004 experiment. These results suggest that parental effects may contribute to the population differences in survival and development observed in this study (Gilchrist & Huey, 2001; Stillwell & Fox, 2005).

Breaking the temperature–size rule

The temperature–size rule – reduced body size with increasing developmental temperature – occurs in more than 80% of the species studied to date, and is common in many different groups of organisms (Atkinson, 1994; Angilletta & Dunham, 2003). Interestingly, most of the known exceptions to the rule are for insects, and the exceptions are found in several different insect orders (including Lepidoptera) (Atkinson, 1994). Of the 67 insect studies summarized by Atkinson (1994), 75% showed significant reductions in size with increased temperature (the temperature–size rule), 18% showed significant size increases, and 7% showed size was maximized at some intermediate temperature (see below for further discussion).

Several previous studies have examined the temperature–size rule in *P. rapae* populations. For example, pupal weight decreased significantly with increasing temperatures from 13 to 25 °C for populations of *P. rapae* from southern England, within the native range of this species (Baker, 1968). A similar relationship is seen in populations from Pakistan and Australia (Jones *et al.*, 1982). Although the ancestral condition for *P. rapae* is unknown, it is parsimonious to suggest that the initial *P. rapae* colonists of North America in the 1860s followed the temperature–size rule.

Our results indicate that significant divergence in the thermal reaction norms for adult size has occurred between some geographical populations of *P. rapae* (Figs 2 and 3, top). Mean adult size was similar to (2003) or smaller (2004) at higher temperature for the WA population, consistent with previous results on British populations. In contrast, for the NC population, the mean size was larger at higher temperature in both experiments. Notably, this population divergence in thermal reaction norms – and the evolutionary reversal in the temperature–size rule – has probably occurred within the past 100–150 years. This divergence can be interpreted as an adaptive response to selection for increasing body size during warmer conditions in the NC population. Because warmer conditions predominant in North Carolina, we would expect stronger selection for increased size under warm than under cool conditions, leading to a reversal of the slope of the thermal reaction norm (Via & Lande, 1985).

Patterns of between-family (genetic and parental) variation in thermal reaction norms within populations are also informative (Figs 3 and 5). For the NC population, mean thermal reaction norms for size had positive slopes (i.e. increased size with increasing temperature) for most families in both experiments. By contrast, mean reaction norms for families in the WA population exhibited a mix of positive, flat and negative slopes in the two experiments. The between-family variation in reaction norm slopes represents the variation in plasticity – the temperature–size rule – on which selection may operate (Via & Lande, 1985; Falconer & MacKay, 1996). Many previous studies have documented quantitative genetic variation in thermal reaction norms for size within populations (Scheiner & Lyman, 1991; Scheiner, 1993).

Several recent studies with *Drosophila* suggest that thermal reaction norms for size are often nonmonotonic (van der Have & de Jong, 1996). For example, in *Drosophila*, components of body size are frequently maximized at intermediate temperatures, with reduced size at both lower and higher temperatures; typically the temperature at which size is maximized is towards the lower end of the temperature range allowing successful development and survival (David *et al.*, 1997; Moreteau *et al.*, 1997; Gibert *et al.*, 2004; Gilchrist & Huey, 2004). Reduced size at extreme low temperatures has been interpreted as a stress response to cold conditions (van der Have & de Jong, 1996). Detailed studies by David *et al.* (1997) have documented differences in the location of the thermal maximum for size between tropical and temperate *Drosophila* species and between tropical and temperate populations of *D. melanogaster* (Moreteau *et al.*, 1997; Morin *et al.*, 1999); the tropical species and populations were smaller than the temperate ones at each temperature. Large shifts in the location of the thermal maximum for size could, in principle, generate an apparent reversal of the temperature–size rule, as

observed in our study (Figs 2 and 4). This interpretation would imply that the cool treatment was stressful for the NC population; however, we did not detect any reduction in survival in cool conditions for this population. We also note that, in contrast to the patterns for *Drosophila*, mean size in *P. rapae* was larger in the southern (NC) than in the northern (WA) population at each temperature.

Most studies of thermal reaction norms and the temperature–size rule have used constant temperatures, in contrast to the fluctuating conditions used in our study. We have argued that diurnally fluctuating temperatures are more relevant to understanding thermal adaptation to natural conditions (Worner, 1992; Kingsolver, 2000; Petavy *et al.*, 2001; Kingsolver & Gomulkiewicz, 2003). One consequence of our fluctuating temperature treatments is that more extreme temperature conditions occur briefly but regularly. For example, it is possible that brief, daily exposure to 10 °C in our cool treatment may be stressful for the NC population, thus affecting the thermal reaction norm for size; however, mean survival was not reduced by the treatment. To our knowledge, the consequences of constant vs. fluctuating conditions for thermal reaction norms for size have not been systematically explored.

The observation of rapid population divergence in thermal reaction norms argues against the existence of a simple, general mechanistic constraint as the underlying cause of the temperature–size rule (Angilletta & Dunham, 2003). However, this does not diminish the utility of mechanistic approaches to understanding evolution of the rule. Rather, exceptions to the temperature–size rule of the sort described in the current study may provide useful definitive tests of mechanistic models to explain the temperature–size relationship (Davidowitz & Nijhout, 2004). Because *P. rapae* has independently invaded both North America and Australia during the past two centuries, further comparisons of the temperature–size rule in both native and nonnative populations of *P. rapae* may prove valuable.

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