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Environmental determinants of population divergence in life-history traits for an invasive species: climate, seasonality and natural enemies

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Abstract

Invasive species cope with novel environments through both phenotypic plasticity and evolutionary change. However, the environmental factors that cause evolutionary divergence in invasive species are poorly understood. We developed predictions for how different life-history traits, and plasticity in those traits, may respond to environmental gradients in seasonal temperatures, season length and natural enemies. We then tested these predictions in four geographic populations of the invasive cabbage white butterfly (*Pieris rapae*) from North America. We examined the influence of two rearing temperatures (20 and 26.7 °C) on pupal mass, pupal development time, immune function and fecundity. As predicted, development time was shorter and immune function was greater in populations adapted to longer season length. Also, phenotypic plasticity in development time was greater in regions with shorter growing seasons. Populations differed significantly in mean and plasticity of body mass and fecundity, but these differences were not associated with seasonal temperatures or season length. Our study shows that some life-history traits, such as development time and immune function, can evolve rapidly in response to latitudinal variation in season length and natural enemies, whereas others traits did not. Our results also indicate that phenotypic plasticity in development time can also diverge rapidly in response to environmental conditions for some traits.

Introduction

Temperature and seasonality are important selective forces for all organisms, particularly ectotherms. Populations of the same species may experience radically different climatic regimes at different latitudes (Janzen, 1967). Higher-latitude populations may experience lower mean temperatures, greater annual variability and shorter seasons for active growth and development. Latitude may also generate important differences in biotic interactions, as the abundances of natural enemies, competitors and food resources may vary geographically (Kraaijeveld & Godfray, 2001; Ardia, 2007; McKinnon *et al.*, 2010). This latitudinal variation in climate and other ecological factors can cause evolutionary divergence in phenotypic traits. Numerous reciprocal transplant studies have documented adaptive divergence in phenotype in populations from different parts of a species range (Hereford, 2009). For example, critical photoperiod in insects (the day length required to induce and maintain diapause) varies strongly with latitude in a variety of species (Tauber & Tauber, 1972; Bradshaw & Holzapfel, 2001). Plants show similar latitudinal clines in growth, phenology, physiology and life history (Maron *et al.*, 2007; Colautti *et al.*, 2010a).

Phenotypic plasticity as well as trait value is under selection by latitudinal gradients in climate. Theoretical models predict that for a population to maintain or increase phenotypic plasticity, there must be predictable environmental variation on the temporal scale of a generation (Young & Badyaev, 2007). Populations at high latitudes may experience more extreme and more variable environmental conditions (Janzen, 1967).

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Therefore, plasticity as well as trait value may vary along latitudinal gradients (James *et al.*, 1997).

Although clinal variation in traits is well documented in native species, less is known about how exotic species respond to selection along geographic gradients in climate. Biotic invasions are natural experimental systems for studying the rapid evolution of traits and plasticity because invasive species are exposed to novel environments and, thus, experience strong selection. Exotic species that undergo a rapid range expansion over a latitudinal gradient of temperature and seasonality are particularly useful for studying the evolution of latitudinal clines (Colautti *et al.*, 2010b). Responses to selection along latitudinal gradients can be predictable, and patterns of traits arising in invasive populations can be compared with those in the native range. Exotic species therefore offer a unique opportunity to test whether climatic gradients produce predicted patterns of adaptation and how quickly such adaptation can occur. However, observed latitudinal clines in invasive species may be the result of phenotypically plastic responses to geographic variation in the environment, local adaptation of phenotypes or a combination of both. The rapid evolution of plasticity may be important in explaining the success of introduced species, but has been infrequently investigated empirically (Donohue *et al.*, 2001; Richards *et al.*, 2006; Yeh & Price, 2011). Even less is known about the maintenance or evolution of phenotypic plasticity in invasive species once they have successfully established in a new environment. Although some invasive species lose plasticity in fitness traits after introduction, ultimately reaching an optimum for their new environment, other invasive populations maintain or increase trait plasticity (Gilchrist & Huey, 2004; Aubret & Shine, 2010). In this study, we develop specific predictions about how climatic temperatures, season length and natural enemies may produce geographic differences among populations in life-history traits and plasticity and test these predictions in the invasive species *Pieris rapae*.

Materials and methods

Study system and predictions

We studied reaction norms for body size, development time, potential fecundity and immune function in four populations of the invasive cabbage white butterfly (*Pieris rapae*). *Pieris rapae* was introduced to south-eastern Canada in the 1860s and rapidly colonized most of North America, spreading across the continent. Previous studies of *P. rapae* from North Carolina and Washington indicate that these populations have diverged in thermal reaction norms for body size and development time both from each other and from ancestral populations in Europe (Kingsolver *et al.*, 2007).

Because it is an agricultural pest, *P. rapae* is the target of biological control programmes using the imported European parasitoid wasp, *Cotesia glomerata* (Vos & Vet, 2004). *Cotesia glomerata* is the most important natural enemy of *P. rapae* larvae in many populations, and previous studies have suggested that mortality due to *C. glomerata* may vary with climate and seasonality (Van Driesche, 1988; Ohsaki & Sato, 1994). Rates of parasitism may also increase throughout the summer, as longer growing seasons allow for more generations and greater populations of parasitoids (Ohsaki & Sato, 1994). In this study, we performed a meta-analysis of field studies of *P. rapae* to assess latitudinal patterns in parasitism rates. We then developed and tested predictions about how parasitism may vary by latitude, about how the population means of life-history traits may vary geographically and about how plasticity in these traits may also vary by latitude.

Latitude and selection by parasitoids

To evaluate the relationship between climate and parasitoid prevalence, we conducted a meta-analysis on field studies of parasitism in *P. rapae* from a range of latitudes. We tested for a relationship between parasitoid infection rate and latitude using prevalence values extracted from the literature (see Appendix A). We predicted that *P. rapae* populations from high latitudes would have lower parasitoid prevalence, because the growing season is shorter in colder climates, allowing less time for parasitoid populations to grow.

To test for a relationship between parasitism rates and latitude, we performed a meta-analysis. We compiled data from studies of parasitism rates of *Cotesia glomerata* and its congener *Cotesia rubecula* on *P. rapae* for field sites ranging from 35 to 45° N (see Appendix A) covering most of *P. rapae*'s range in the northern hemisphere. We used the keywords '*Pieris rapae*', 'Parasitoid', '*Cotesia glomerata*', '*Cotesia rubecula*', '*Apanteles glomerata*' (a previous name for *C. glomerata*) and 'natural enemies' as search terms. We located a total of nine studies appropriate for the meta-analysis. A majority of parasitoid surveys were carried out in late summer or early fall; for studies that sampled throughout the growing season, we used data from August. We used a two-level linear model to test for a relationship between latitude and parasitism rate. We used parasitoid prevalence, logit-transformed, as a response variable, latitude as a covariate and study as a random effect; responses were weighted by the square root of sample size for each study. For two studies where sample size was not available, we used mean sample size as a weight.

Predictions for population divergence in trait means

We tested whether mean body size, development time and potential fecundity have diverged in populations from four climatically distinct regions: North Carolina,

Washington, Nova Scotia and Michigan. The latter two populations are at similar latitudes but have very different mean temperatures and growing seasons (see Fig. 1). In general, we predict that mean body size will have a negative relationship with mean summer temperature. We base these predictions on Bergman's rule, which states that populations at higher latitudes (with lower mean temperatures) tend to have larger body sizes (Fig. 1a). Egg production (potential fecundity) should follow a similar pattern, as fecundity and body size are often strongly related in insects (Fig. 1a).

Mean development time can have either a positive or a negative relationship with growing season. In regions where there is no climatic limit to development time, species should optimize early reproduction over body size. In populations where the growing season is limited, some populations may reach reproductive maturity early and produce extra generations, whereas others may best utilize the available frost-free period by delaying maturation until they are larger and have greater fecundity (Roff, 1980; Kivelä *et al.*, 2012). Like most insects, *P. rapae* faces the problem of optimizing either early reproduction (and thus producing more generations per year) or body size (and thus having greater potential reproductive fitness). Adding a second generation may increase fitness for individuals, but they must halve their development time to do so. Therefore, *P. rapae* in regions either with longer growing seasons might develop more slowly because more frost-free days are available for growth or, alternately, with long growing seasons might select for rapid maturity and more generations per year (Fig. 1b; Roff, 1980). However, models predict that early reproduction increases fitness more than body size, and we therefore predict that longer frost-free periods should favour early reproduction (Kingsolver & Huey, 2008).

Selection by parasitoids is hypothesized to be stronger in regions where parasitoid populations have more time available for population growth, and we predict that mean immune function in *P. rapae* will increase with the length of the growing season (Kraaijeveld & Godfray, 2001). Larvae from populations with short growing seasons should have weaker immune systems, because the short frost-free periods of northern populations limit the growth of parasitoid populations (Fig. 1b).

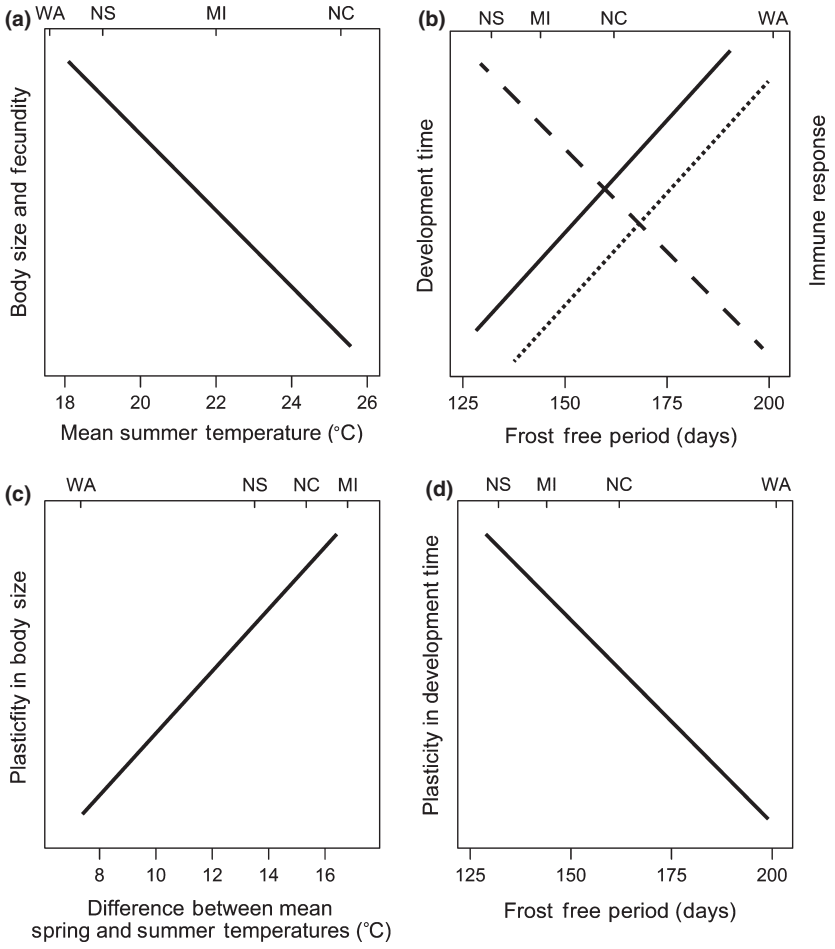

Fig. 1 Predicted relationships of life-history traits and plasticity with seasonal temperatures and growing season length. See text for details and rationale. (a) We predict that both body size and fecundity will decrease in regions with higher mean summer temperatures. (b) We predict that development time may either increase in regions with longer growing seasons (longer frost-free periods) if populations maximize body size (solid line), or decrease with longer growing seasons if early reproduction is favoured to maximize the number of generations in the growing season (dashed line). We also predict that immune response will increase in regions with longer growing seasons (dotted line). (c) We predict that phenotypic plasticity for body size should be greater in populations that experience a larger difference between spring and summer temperatures. (d) We predict that phenotypic plasticity for development time should be greater in regions with shorter frost-free periods.

Predictions for population divergence in plasticity

We also tested whether phenotypic plasticity for genotypes in a population (here defined as the difference in phenotypes between the warm and cool temperature treatments for each full-sib family) varied with climate (Stearns, 1992). Both phenotype and phenotypic range can undergo selection by environmental variation. Theory predicts that plasticity should be more likely to evolve in regions where there is significant environmental variation on the scale of a generation (Roff, 1980). Ectotherms may optimize their growth rate in response to temperature, and variation in growth rate typically translates into variation in body size (Yamahira & Conover, 2002). We therefore predicted that populations that experience greater differences between spring and summer temperatures should have greater plasticity in growth rate and therefore exhibit greater temperature dependent differences in body size (Fig. 1c; Young & Badyaev, 2007; Snell-Rood, 2012).

We predict that plasticity in development time should also vary with climate. *P. rapae* varies in voltinism, ranging from two to three generations in the northern populations of Nova Scotia and Michigan, to more than six in North Carolina. Our predictions for plasticity in development time parallel those for development time themselves. Selection favours early reproduction and more generations in populations with long growing seasons. In populations with long seasonal windows, we predict that there should be less plasticity for development time (Stearns, 1989, 1992; Kingsolver & Huey, 2008). We expect greater plasticity in populations with shorter growing seasons that must negotiate trade-offs between fecundity and development rate (Fig. 1d). We tested these predictions using a series of laboratory experiments to see whether the four populations had diverged in these important life-history traits and whether those differences were in agreement with our predictions based on seasonal temperatures and season length (Fig. 1).

Experimental studies

We collected *P. rapae* females from four populations during the summers of 2010 (Washington, Michigan and Nova Scotia) and 2011 (North Carolina). Washington (WA) females were collected from farms outside Seattle (47.61° N) and from a system of community gardens within the Seattle metropolitan area. Michigan (MI) females were collected from organic farms near Ann Arbor (42.33° N), and Nova Scotia (NS) females were collected in community gardens in Halifax and from an organic farm near Wolfville (45.08° N). Animals were shipped live to the laboratory in Chapel Hill, NC, using an overnight refrigerated shipping service. North Carolina (NC) females were collected from organic farms in Chatham County, NC (35.91° N), and did not require shipping. Females were kept in greenhouse conditions (~24 °C, 60–80% humidity, natural photocycle of 14 light–10 dark) and provided with fresh collard leaves (*Brassica oleracea*) for oviposition. Eggs were collected daily. Collard leaves with eggs were placed in plastic containers and maintained in another environmental chamber (Percival 36-VL: Percival Scientific, Perry, IA, USA) at 25 °C with 14 light–10 dark photoperiod until hatching. During the first instar (24–48 h after hatching), caterpillars were transferred to artificial diet and placed in individual Petri dishes. To quantify thermal reaction norms for growth, development time and immune function, we reared caterpillars from the four populations at two temperatures: 20 and 26.7 °C with a 14 light–10 dark photoperiod. All populations experience these temperatures regularly during the course of a growing season. Our experiment included eight families of *P. rapae* caterpillars from North Carolina ($N = 303$), seven families from Nova Scotia ($N = 336$), five from Michigan ($N = 202$) and nine from Washington ($N = 446$). Caterpillars from each mother (sibship) were then assigned randomly to one of the two experimental temperature treatments. Caterpillars were fed *ad libitum* on artificial diet (following the recipe from Snell-Rood & Papaj, 2009), and diet was changed three times per week to reduce bacterial growth and spoilage. Petri dishes were checked daily for mortality and to identify individuals that had pupated. When an individual reached pupation, it was removed from the Petri dish and weighed using standard gravimetric techniques. Pupae were placed in plastic cups on a piece of damp filter paper, with a moist sponge to reduce desiccation, and returned to their experimental temperature. Cups were checked daily to determine the date of eclosion. Newly eclosed individuals were sexed, massed and placed in a flight cage with individuals from their cohort for 48 h to allow egg maturation. Adults were fed *ad libitum* on a 10% honey–water solution and freeze-killed after 48 h. Abdomens of female butterflies were dissected in a glycerol solution, and mature eggs (fully yolked with a developed chorion) were counted under a dissecting microscope.

Immune assay

We assessed immune function by injecting silica beads into the caterpillars' haemocoel (Sigma Aldrich Corporation, St. Louis, MI, USA). The beads activate cellular encapsulation by hemocytes. This technique of assaying immune response by injection with a foreign body is commonly used in insect immunology and has been shown to have a significant correlation with immune response to real parasites and pathogens (Rantala & Roff, 2005; Smilanich *et al.*, 2009; Diamond & Kingsolver, 2011) Caterpillars were randomly selected for the injection assay at the beginning of the experiment but were reared in the same incubators using exactly the same

methods as those not selected. Injections were administered during the first 24 h of the 5th (final) instar, because immune function declines immediately prior to metamorphosis (Beetz *et al.*, 2008). However, because individuals developed at different rates, caterpillars were at the same physiological stage but not at the same chronological age when the assay was administered.

Our immune assays follow those of Diamond & Kingsolver (2011). We used DEAE Sephadex-A25 silica chromatography beads (Sigma Aldrich Corporation). Beads were dyed with a 0.1 mg mL^{-1} solution Congo red dye and allowed to dry completely before being stored in a freezer to sterilize and prevent contamination. Beads were mixed in a standard solution of 1 g of dyed beads and 0.01 L of sterile Grace's Insect Cell Culture Medium (Sigma Aldrich Corporation) to standardize the number of beads each individual received. Caterpillars were injected with 5 μL of the bead solution to using a Hamilton 7000 series syringe with a 25-gauge tip (Hamilton Company, Reno, Nevada). Caterpillars received an average of 15.24 ± 0.52 beads from the injection. After injection, caterpillars were placed on fresh diet and returned to the appropriate temperature for 24 h; after 24 h, they were freeze-killed. Beads were extracted post-mortem from caterpillars by dissection of the whole caterpillar and mounted on glass slides in a glycerol solution.

We measured encapsulation (area of hemocyte aggregation) as a continuous response variable using both differential interference contrast microscopy (DIC), which detects the cellular encapsulation area and the bead, and fluorescence microscopy, which detects only the dyed bead (Zeiss LSM 510 confocal microscope; Diamond & Kingsolver, 2011). Encapsulation and bead area were measured using the visualization program ImageJ (Abramoff *et al.*, 2004). We used an automated edge selection tool to determine the area of cellular encapsulation on the DIC image and a thresholding tool to select the area of the fluorescent bead from the fluorescence image. We determined the area of encapsulation by subtracting the area of the bead from the fluorescence image from the total area of the bead and encapsulation measured in the DIC image. Of 484 beads, three had a negative encapsulation value (the bead was larger than detected encapsulation area, although encapsulation should be bounded at zero) and these were excluded from the analysis.

Climatic data

We used the 1950–1980 Canadian Climate Normals (Canada Department of the Environment, 1982) to determine the 90% probability frost-free period for Nova Scotia. Because frost normals for Canada were not available after 1980, we also used 90% probability frost-free period from 1950 to 1980 for the US field sites (Koss & Owenby, 1988). To determine the mean monthly temperature for June, July and August, we used current 30-year averages (Environment Canada, 2010; NOAA National Climatic Data Center, 2011). We also confirmed that the current frost-free periods for North Carolina, Michigan and Washington (NOAA National Climatic Data Center, 2011) did not differ substantially from the 1980 data. We calculated mean summer temperature by averaging the mean monthly temperature for June, July and August and mean spring temperature by averaging monthly temperature means for March, April and May.

Statistical analysis

All statistical analyses were performed in R (v. 2.11.0, R Development Core Team, 2008). We ran a separate linear mixed-effects model (using R library nlme) for each of the four life-history traits: pupal mass, pupal development time, egg production (ovariole number) and immune response (cellular encapsulation; Pinheiro *et al.* 2012). For the immune response models, we calculated the mean encapsulation area (log-transformed and corrected for bead size) per individual for use as the response variable in our analyses and included bead size as a covariate in the models. Family (sibship) was included as a random effect in all models. To test our predictions (Fig. 1), we considered models with rearing temperature, sex as fixed effects and a climatic variable as a covariate. Therefore, we included mean summer temperature as the climatic variable in models for body size and egg production and included frost-free period as the climatic variable in models for development time and immune response (Fig. 1a,b). These models allow us to test whether specific climatic variables can explain the differences among populations in mean and plasticity of life-history traits. However, populations might also differ in these traits in ways not predicted by these climatic variables. Therefore, in cases where climate was not significant, we ran a second set of models in which the climatic covariate was replaced with population of origin as a fixed effect. This allowed us to test for possible differences among populations that are not associated with climate.

To test our predictions about differences in plasticity among populations, we calculated plasticity as the difference in mean phenotype between the two temperature treatments for each family in each population (Valladares *et al.*, 2006). Plasticity in both development time and body size was computed for each family; we used family as the experimental unit because we measured individual's phenotypes in the either warm or cool environment and therefore computed plasticity at the genotype level. We then used linear models to test whether plasticity in development time is associated with frost-free period (Fig. 1d) and whether plasticity in body size is associated with the difference between mean temperatures in spring and summer (Fig. 1c).

Because sample size (n) per family varied substantially within and among populations, we weighted our models by sqrt(n).

Results

Meta-analysis

Parasitism by *Cotesia spp*. was significantly greater at lower latitudes ($F_{1,14}$, $F = 5.29$, $P = 0.037$; Fig. 2). As a result, parasitoid prevalence was greater in regions with higher average temperatures and longer growing seasons.

Pupal mass

Contrary to our prediction for pupal size (Fig. 1a), mean summer temperature did not predict mean body size ($F_{1,464} = 0.053$, $P = 0.820$). We therefore ran a second model that included population of origin as a fixed effect (see Methods), and we report results from the population model below. We found that rearing temperature decreased body size ($F_{1,460} = 35.195$, $P \leq 0.0001$) and that males were larger than females ($F_{1,460} = 38.867$, $P \leq 0.0001$). Population had a significant effect on body size ($F_{3,25} = 3.935$, $P = 0.0199$), and there was also a significant interaction between population and rearing temperatures ($F_{3,460} = 7.768$, $P \leq 0.0001$). Michigan, North Carolina and Washington were all smaller at warm temperatures, whereas Nova Scotia was larger at warm temperatures (Fig. 3a,b). Mean body size in Michigan was smaller than in the other populations. There was also a significant interaction between rearing temperature and sex ($F_{1,457} = 6.340$, $P \leq 0.0121$); males were larger over all, and this difference was more pronounced at high temperatures. No other interaction terms were significant.

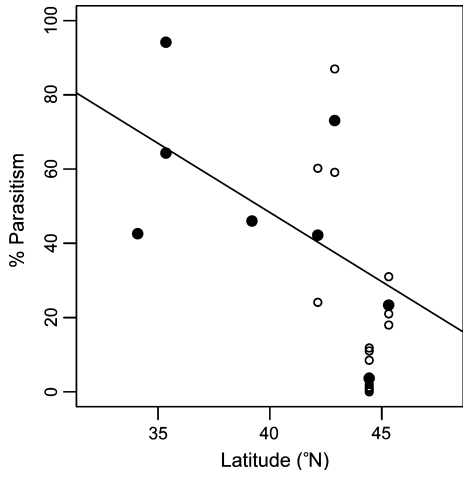

Fig. 2 Rate of parasitism of *Pieris rapae* larvae by *Cotesia glomerata* parasitoids as a function of latitude. Solid circles represent means, and open circles indicate data from multiple years. Parasitism rate declined significantly with increasing latitude. 104 × 122 mm (300 × 300 DPI).

Potential fecundity

There was a significant effect of mean summer temperature on the number of eggs produced by females ($F_{1,24} = 3.826$, $P = 0.0320$). In agreement with our predictions (Fig. 1a), females from populations with higher mean summer temperatures produced fewer eggs (Fig. 3c). However, these effects on fecundity were not mediated by differences in body size. There were no significant effects of pupal mass ($F_{1,88} = 3.2862$, $P = 0.0733$) or rearing temperature ($F_{1,88} = 0.0331$, $P = 0.8561$) on egg production, and no two-way interaction terms were significant.

Development time

Frost-free period had a significant effect on development time ($F_{1,27} = -10.42$, $P < 0.001$). Butterflies developed more quickly in populations with long growing seasons, in agreement with our predictions (Fig. 1b). Warmer rearing temperatures reduced development time ($F_{1,463} = 8519.7$, $P < 0.0001$), but development time did not differ by sex ($F_{1,463} = 1.38$, $P = 0.24$). We compared a model that treated population as a factor and a model that used climatic data as a continuous variable, in this case frost-free period, and included all possible two-way interactions in the models. There was little between-population variation in development time at warm temperatures, but at cool temperatures, Michigan and Nova Scotia developed more slowly than North Carolina and Washington ($F_{1,463} = 99.07$, $P < 0.001$; Fig. 4a). Females developed slightly faster than males ($F_{1,463} = 4.314$, $P < 0.038$).

Immune response

Frost-free period also had a significant effect on cellular encapsulation ($F_{1,29} = 4.378$, $P = 0.0453$). In agreement with our predictions (Fig. 1b), encapsulation response was greater in populations with longer growing seasons (Fig. 4b). Larger beads also produced greater encapsulation responses ($F_{1,60} = 14.181$, $P = 0.0004$). There was no significant effect of rearing temperature on immune response ($F_{1,60} = 0.397$, $P = 0.5310$), nor a significant interaction between temperature and frost-period period.

Phenotypic plasticity

In contrast to our predictions (Fig. 2c), there was no significant effect of seasonal temperature difference (between spring and summer temperatures) on plasticity in body size, so we used a model that included

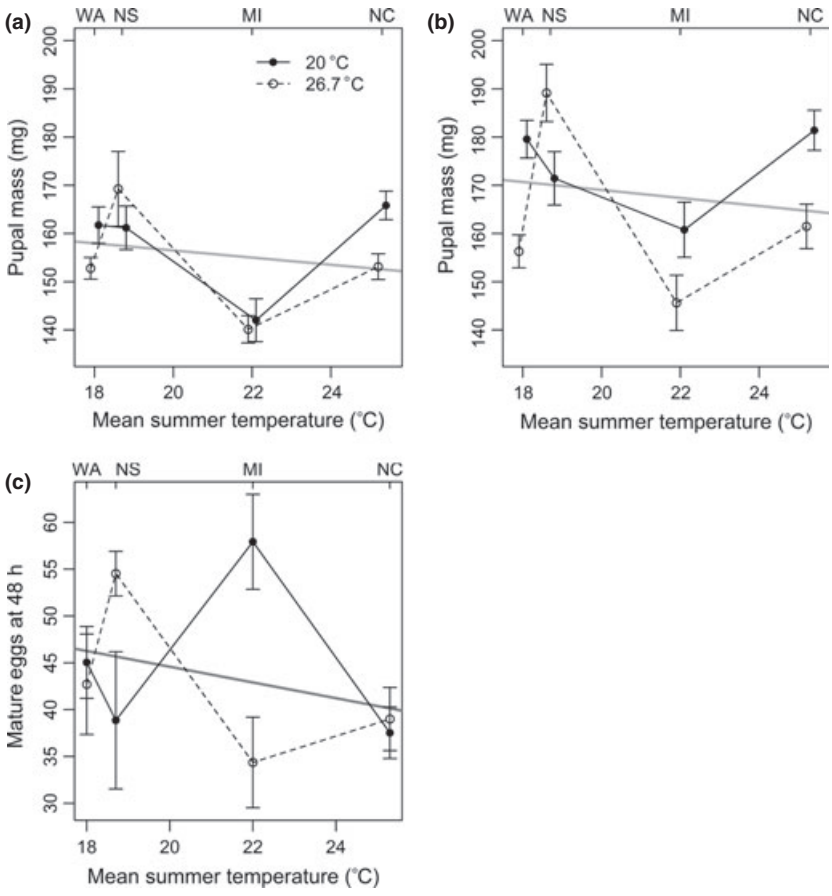

Fig. 3 Body size (a, b) or egg production (c) as a function of mean summer temperature (°C) for four study populations of *Pieris rapae* (MI, Michigan; NC, North Carolina; NS, Nova Scotia; WA, Washington). Open circles indicate individuals reared at cool temperatures; filled circle indicate those reared at warm temperatures. (a) Mean (+1 SE) body mass (in g) of females. (b) Mean (+1 SE) body mass (in g) of males. (c) Mean (+1 SE) number of mature ovarioles at 48 h.

population as a fixed effect. However, population was not significant either ($F_3 = 0.768$, $P = 0.5181$). Males were more plastic than females ($F_1 = 12.372$, $P = 0.0010$). However, plasticity in development time decreased significantly with increasing frost-free period ($F_1 = 32.67$, $P < 0.0000$; Fig. 5b), in agreement with our predictions. The sexes did not differ in plasticity for development time ($F_1 = 0.41$, $P = 0.52$). Families from Washington and North Carolina had lower plasticity in development time, whereas those from Michigan and North Carolina were the most plastic (Fig. 5b). Note that this pattern of plasticity is largely due to differences at the lower rearing temperature (20 °C): mean development times were very similar among populations at the higher rearing temperature (Fig. 4a).

Discussion

Evolution of latitudinal clines in traits

Latitudinal gradients are often thought to produce corresponding patterns in life-history traits, and when invasive species colonize a new region, climatic gradients are expected to select for geographic clines in traits that parallel those in the native range. Some invasive species have re-established predicted patterns in traits after they are introduced; the invasive fruit fly *Drosophila subobscura* shows a strong latitudinal cline in body size in its native range in Europe, and introduced populations in North and South America show the evolution of a parallel latitudinal pattern in mean body size (Gilchrist & Huey, 2001). The fall webworm (*Hyphantria cunea*), a North American moth species, re-established latitudinal clines in mean photoperiod and mean development time <50 years after its introduction to Japan (Gomi, 2007). In both cases, the geographic differences among populations were strongly associated with latitudinal gradients in average temperature and day length.

Our results with *P. rapae* indicate significant evolutionary divergence among populations in mean body size. However, in contrast to our predictions (Fig. 1a), these population differences are not explained by the differences in mean summer temperatures among the populations (Fig. 4a). This suggests that the evolutionary divergence in size is not the result of selection for adaptation to local thermal conditions. Gilbert (1984; Gilbert 1988) studied population differentiation in body size for several European and Australian populations of *P. rapae* and did not detect significant differences in reaction norms or means for body mass between

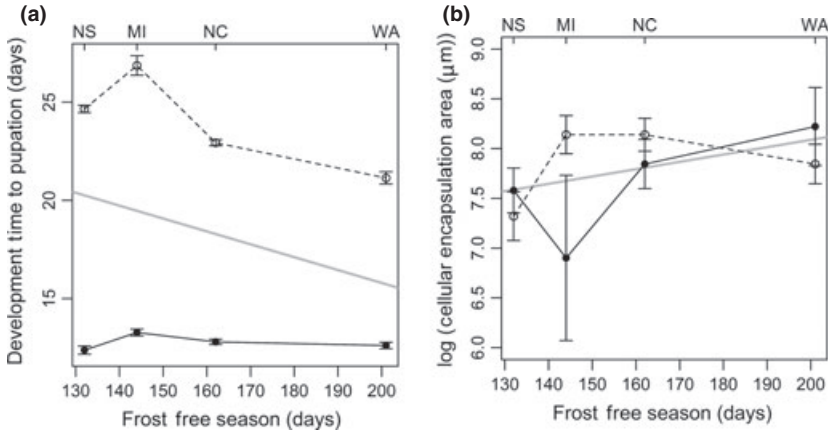

Fig. 4 Development time (a) and immune function (b) as a function of frost-free season length (in days) for four study populations of *Pieris rapae*. Grey lines represent model coefficients. Symbols and abbreviations as in Fig. 3. (a) Mean (+1 SE) development time to pupations (in days). (b) Mean (+1 SE) area of log-transformed cellular encapsulation (in 'm).

populations, although he did note that growth was somewhat slow on his particular diet formula. Population differences in responses to host plant quality and to artificial diets can also influence patterns of growth rate and body size and may explain observed divergence (Kingsolver *et al.*, 2006; and see below). Maternal effects can affect phenotype for both body size and development time (Fox *et al.*, 1995; Mousseau, 1998). However, a previous experiment in North Carolina and Washington *P. rapae* populations compared thermal reaction norms for body size and development time in individuals from wild-caught mothers and from mothers that were reared in the laboratory for a generation, and found only minor differences in development time due to maternal effects. It is therefore likely that maternal effects play a small role in the differences in reaction norms we find between North American populations. We also found that mean egg production decreased with mean summer temperatures (Fig. 3c). Although this pattern is consistent with our prediction (Fig. 1a), it is not mediated by population differences in body size as we anticipated (Fig. 3a,b).

In contrast, population differences in mean immune function were significantly associated with the length of the frost-free period: populations with longer frost-free periods had greater immune function than those with short frost-free periods (Fig. 4b). Our meta-analysis of *P. rapae* populations (Fig. 2) showed a significant decline in the rate of parasitism by *C. glomerata* with increasing latitude. Parasitoid populations typically increase throughout the growing season, and therefore, populations with long frost-free periods have the potential to reach greater peak parasitoid densities (Ohsaki & Sato, 1990). Our results support the hypothesis (Fig. 1b) that greater parasitoid abundance in areas with longer growing seasons has produced evolutionary divergence in immune responses among *P. rapae* populations. Latitudinal clines in immune function have been found in other invertebrate species, particularly those which are under selection by parasitoids. For example, Kraaijeveld and Godfray (1999) found that encapsulation response of European populations of *Drosophila subobscura* covaried geographically with the virulence of the parasitoid wasps *Asobara tabida* and *Lepropolina boulardi*. Local differences in parasite prevalence were also found to be a good predictor of immune function for populations of the amphipod *Gammarus pulex* (Franceschi *et al.*, 2010). Other studies have found latitudinal clines in immune function to be the result of energetic tradeoffs with growth and development. DeBlocke *et al.* (2008) found that immune function in damselflies was negatively correlated with development time and latitude; northern damselfly populations had longer development times and invested more to immune function. Our results do not support trade-offs between immune function and growth, but rather indicate that selection by natural enemies is an important determinant of immunocompetence for *P. rapae*. Theory predicts that invasive species should invest less in immune function because they are released from selection by the natural enemies of their native range. However, our study shows that clines in immune function can re-evolve if invasives are re-introduced to natural enemies (Lee & Klasing, 2004; Llewellyn *et al.*, 2011).

Mean development time was also significantly associated with the length of the frost-free period: populations with longer frost-free periods had shorter development times than those with shorter frost-free periods (Fig. 4). The length of the growing season has both direct and indirect ecological effects on the fitness consequences of life-history traits. First, longer growing seasons allow a greater number of generations per year in *P. rapae*. Our results suggest that shorter development times in regions with longer growing seasons may reflect selection for increasing the number of generations in these regions (Roff, 1980) in agreement with our predictions (Fig. 1). Second, higher rates of parasitism in regions with longer growing seasons (Fig. 2) may also select for more rapid development. Field studies of *P. rapae* in Maryland have shown that rapid development rate reduced the rate of mortality due to

parasitism (Benrey & Denno, 1997); selection for rapid development to avoid parasitism by *Cotesia* wasps has also been demonstrated in *M. sexta* (Kingsolver *et al.*, 2012). Our present results suggest that development time can evolve rapidly in response to growing season, as a consequence of climatic effects, biotic interactions or both.

Latitudinal clines in plasticity

Although invasive species may accommodate novel environments through rapid evolution of traits, they may also cope with variation in their new environment through extant phenotypic plasticity (Donohue *et al.*, 2001; Yeh & Price, 2011). Our results indicate that North American populations of *P. rapae* have evolved significant differences in the slope and direction of reaction norms for body size and development time. Pupae and adults from Michigan, Washington and North Carolina were larger when reared at cooler temperatures, although the magnitude of this relationship varied between populations (Fig. 4). In contrast, Nova Scotia pupae were larger when reared at warmer temperatures and bigger overall (Fig. 4). Our results suggest that both the slope and direction of plasticity can evolve rapidly in natural populations, on time scales similar to those for the evolution of trait means. However, population differences in the mean plasticity of body size were not significantly associated with seasonal temperature differences (Fig. 5). As a result, the evolutionary divergences in mean size and in plasticity of size are unlikely to represent adaptive responses to local thermal fluctuation.

Mean plasticity in development time was significantly associated with frost-free period: as we predicted (Fig. 1d), populations that experienced longer growing seasons had less plasticity in development time (Fig. 5b). This pattern of plasticity is largely due to differences at the lower rearing temperature (20 °C), as mean development times showed little variation at the higher rearing temperature (26.7 °C; Fig. 3). Our results suggest that longer growing seasons may generate selection for and evolution of more rapid development especially at higher temperatures, allowing the completion of more generations per year (Roff, 1980; Taylor, 1981).

The rapid evolution of plasticity may be important in explaining the success of introduced species, but has been infrequently investigated empirically (Richards *et al.*, 2006). Many studies argue that phenotypic plasticity should be beneficial to introduced species because plastic responses allow organisms to thrive in a broader range of environments. Adaptive phenotypic plasticity has been shown to facilitate colonization in a variety of taxa (Donohue *et al.*, 2001; Yeh & Price, 2011), but evidence for the hypothesis that plasticity promotes successful invasion is inconsistent. In a review of 14 studies of invasion success in plants, only seven showed that plasticity was advantageous (Richards *et al.*, 2006). Even less is known about the maintenance or evolution of phenotypic plasticity in invasive species once they have successfully established in a new environment. Plasticity in gape size in snakes was beneficial in populations in the early stages of colonization, as snakes encountered novel prey. However, plasticity declined after the population became established; snakes evolved towards a canalized optimal phenotype matching prey size (Aubret & Shine, 2010). However, there is evidence that latitudinal variation may cause geographic gradients in plasticity in native species: a study of 20 *Drosophila subobscura* populations from Europe and North Africa showed that not only did cold tolerance covary with latitude, temperate populations were more plastic in cold tolerance than tropical ones (James *et al.*, *1997*). Presumably temperate populations experienced greater thermal variation than their tropical counterparts, selecting for increased plasticity. However, empirical evidence for environmental clines in plasticity is still limited for both native and invasive species, and our results provide some of the first evidence that latitude may select for differences in plasticity during colonization.

There is previous evidence for geographic differentiation of life history in *P. rapae* (Kingsolver *et al.*, 2007). By design, we resampled the North Carolina and Washington

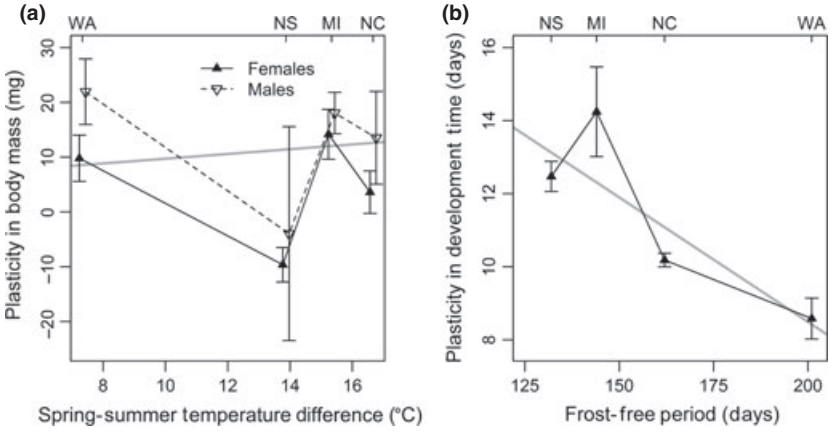

Fig. 5 Plasticity in body size as a function of spring–summer temperature differences (a) and development time as a function of frost-free season length (in days) for four study populations of *Pieris rapae*. (b). Grey lines represent model coefficients. (a) Plasticity in body mass. Open symbols are family plasticities for males, and closed circles are for females. (b) Plasticity in development time for each family. Because there was no significant effect of sex in the model, males and females are pooled. 104 × 61 mm (300 × 300 DPI).

populations and place them in the context of a wider range of climates. However, our results from the resampled North Carolina and Washington populations differ from this previous study in one important regard: experiments in 2003 and 2004 found that in North Carolina *P. rapae* reared at 20 and 26 °C, and body size had a positive relationship with temperature. Our results for this population (based on experiments in 2010 and 2011) show a negative relationship between body size and temperature (Fig. 3). An important difference between the previous and current studies is the nutritional content of the artificial diets: our recent experiments used a diet with more lipids (37% more cholesterol and 67% more linseed oil) than the diet used in previous experiments (following Snell-Rood & Papaj, 2009; Troetschler *et al.*, 1985). Mean growth rates (pupal mass/pupal development time) for both populations were greater in the 2011 than in the 2003 study, especially at the higher rearing temperature, suggesting that higher lipid content increased growth rate. However, the different individual responses of pupal mass and development time in the two studies were complex and depended on population and rearing temperature. Further studies of diet quality and thermal reaction norms are required to better understand the complex interactions between nutrition, temperature and growth rate. Diet affects thermal reaction norms in many insect species. Diamond & Kingsolver (2010) found that reaction norms for body size in *M. sexta* were highly dependent on diet, and our results indicate that adaptation to local host plants may result in variation of thermal reaction norms, which may play an important role in observed body size. Further experiments are needed to better understand the role of diet on adult body size and development time.

Organisms may adapt to environmental variation along latitudinal gradients through genetic adaptation of trait means as well as through the evolution of plasticity. Recent studies with invasive species have documented rapid evolutionary divergence along latitudinal gradients, but have not clearly identified the specific ecological factors underlying these patterns. Our results suggest that geographic differentiation in development time, immune response and plasticity in *P. rapae* is more closely associated with latitudinal variation in season length and natural enemies than with variation in environmental temperatures. Understanding the environmental factors that cause population differentiation along latitudinal gradients is important for understanding patterns of biological diversity and abundance in nature and for predicting evolutionary responses to climate in native species, or in invading exotics.

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Appendix A: Studies used in parasitism and latitude meta-analysis

Study	Years	% Parasitism	Site	Latitude	Parasite
Parker (1970)	1970	42.57	Columbia, MO	34.1	*Cotesia glomerata*
Ohsaki & Sato (1990)	1999	94.16	Kyoto, Japan	35.36	*C. glomerata*
Sato (1978)	1976	64.29	Kyoto, Japan	35.36	*C. glomerata*
Benrey and Denno (1994)	1995	46	Beltsville, MD	39.2	*C. glomerata*
Tanaka *et al.* (2007)	2001, 2004	60.21–24.09	Sapporo, Japan	42.142	*C. glomerata*
Sato (1978)	1976	42.2	Sapporo, Japan	42.142	*C. glomerata*
Van Driesche & Bellows (1988)	1985–1986	86.98–59.13	Amherst, MA	42.9	*C. glomerata*
Wold-burkness *et al.* (2005)	1991–2003	2–11.8	Rosemount, MN	44.44	*C. glomerata*
Godin & Bovin (1998)	1993–1994	18–31	Montreal, QC	45.31	*C. rubecula*

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