

Latitudinal and voltinism compensation shape thermal reaction norms for growth rate

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Abstract

Latitudinal variation in thermal reaction norms of key fitness traits may inform about the response of populations to climate warming, yet their adaptive nature and evolutionary potential are poorly known. We assessed the contribution of quantitative genetic, neutral genetic and environmental effects to thermal reaction norms of growth rate for populations of the damselfly *Ischnura elegans*. Among populations, reaction norms differed primarily in elevation, suggesting that time constraints associated with shorter growth seasons in univoltine, high-latitude as well as multivoltine, low-latitude populations selected for faster growth rates. Phenotypic divergence among populations is consistent with selection rather than drift as Q_{ST} was greater than F_{ST} in all cases. Q_{ST} estimates increased with experimental temperature and were influenced by genotype by environment interactions. Substantial additive genetic variation for growth rate in all populations suggests that evolution of trait means in different environments is not constrained. Heritability of growth rates was higher at high temperature, driven by increased genetic rather than environmental variance. While environment-specific nonadditive effects also may contribute to heritability differences among temperatures, maternal effects did not play a significant role (where these could be accounted for). Genotype by environment interactions strongly influenced the adaptive potential of populations, and our results suggest the potential for microevolution of thermal reaction norms in each of the studied populations. In summary, the observed latitudinal pattern in growth rates is adaptive and results from a combination of latitudinal and voltinism compensation. Combined with the evolutionary potential of thermal reaction norms, this may affect populations' ability to respond to future climate warming.

Keywords: evolutionary potential, genetic correlation, heritability, life history plasticity, Q_{ST} vs. F_{ST} , time constraints

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Introduction

Thermal reaction norms of key life history traits (such as growth rate) play a central role in our understanding of the extent to which populations can persist locally or

may be replaced by southern populations under anthropogenic temperature changes (Walther *et al.* 2002; Davis *et al.* 2005; Parmesan 2006). Two aspects of thermal reaction norms of particular interest in this context are the adaptiveness of their pattern of latitudinal population differentiation (i.e. latitudinal compensation) and their evolutionary potential (Bradshaw & Holzapfel 2006; Angilletta 2009).

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Species typically show latitudinal compensation whereby growth rates in cold, high-latitude populations equal those of warm, low-latitude populations (Conover & Present 1990). Both low temperature and strong seasonal time constraints can select for latitudinal compensation and will result in temperature adaptation or countergradient variation, respectively (Conover & Schultz 1995; Yamahira & Conover 2002; Yamahira *et al.* 2007). Temperature adaptation occurs when thermal reaction norms in northern populations are horizontally shifted along a temperature axis towards lower temperatures than southern populations (Levinton 1983; Yamahira *et al.* 2007). Countergradient variation occurs when genetic and environmental influences on phenotypes are in opposition across an environmental gradient, i.e. faster growing genotypes occur in northern populations (Levins 1969; Conover & Schultz 1995). Seasonal time constraints have been found to be the driving environmental factor for countergradient variation of growth rates for several taxa (overview in Angilletta 2009). In these cases, growth occurs over the same range of temperatures for both northern and southern populations, but reaction norms differ in elevation such that high-latitude populations grow faster across all temperatures to compensate for the shorter growth season (Conover & Schultz 1995; Yamahira & Conover 2002). Although largely overlooked, the degree of voltinism (number of generations per year) at lower latitudes is also associated with seasonal time constraints and can potentially impose stronger time constraints than those of shorter favourable thermal periods at higher latitudes (Roff 1980). Evidence for this is scarce, yet in a recent study, low-latitude, time constrained bivoltine butterflies grew faster than higher latitude, univoltine individuals when reared in a common environment (Nygren *et al.* 2008).

The study of interpopulation latitudinal variation in thermal reaction norms is considered to be one of the best ways to infer the environmental factors driving adaptive evolution of latitudinal compensation (Yamahira *et al.* 2007). Yet, this approach can be misleading as stochastic processes such as genetic drift can produce similar patterns (Endler 1977; Merilä & Crnokrak 2001). Therefore, to explicitly assess the adaptive nature of latitudinal evolution of thermal reaction norms, population-level comparisons of genetic divergence in quantitative traits (Q_{ST}) and neutral DNA markers (F_{ST}) are needed. Greater divergence in quantitative traits relative to neutral markers (i.e. $Q_{ST} > F_{ST}$) is indicative of an adaptive pattern in response to directional selection (Merilä & Crnokrak 2001). Importantly, this conclusion may critically depend upon temperature: Q_{ST} estimates may be sensitive to the environment and genotype by environment interactions (G×E) because the underlying quantitative genetic parameters

often vary with the environmental conditions under which they are estimated (Hoffmann & Merilä 1999; Merilä & Crnokrak 2001; Palo *et al.* 2003).

Identifying (adaptive) latitudinal differences in the pattern of thermal reaction norms only reveals past selection events. To predict further thermal adaptation, evolutionary potential and possible genetic constraints need to be evaluated. This has been identified as a major gap in our current knowledge to predict effects of global warming (Angilletta 2009; but see Palo *et al.* 2003; Yamahira *et al.* 2007; Nygren *et al.* 2008). Assessing the potential of thermal reaction norms to evolve is very relevant to understand how species can deal with rapid climate change. Here, the discrimination between microevolutionary (genetic) adaptations and environmentally induced plasticity is paramount to derive correct predictions of a population's adaptive potential (Gienapp *et al.* 2008).

The combined objectives of this study were to unravel the pattern of latitudinal population differentiation of thermal reaction norms of growth rate, its adaptive nature and evolutionary potential using the damselfly *Ischnura elegans* (Order: Odonata) as a model system. This ectotherm encounters a wide range of environmental conditions across its distribution (Dijkstra & Lewington 2006), and selection for local differentiation among populations harbours components of thermal adaptation as well as time constraints, which is reflected in the range of voltinism seen across a latitudinal cline. Our integrated study addresses these issues using two complementary approaches: (i) by quantifying thermal reaction norms of growth rate and their evolutionary potential and (ii) by determining the relative importance of selection vs. drift in shaping patterns of population divergence in growth rate (Q_{ST} vs. F_{ST}), and the extent to which Q_{ST} estimates may be influenced by the environment and G×E.

Methods

Species and populations

The damselfly *Ischnura elegans* (Vander Linden 1820) has a broad latitudinal distribution across Europe, with a range extending from Sweden to Spain (Dijkstra & Lewington 2006). Populations are univoltine in the north, bivoltine in the centre of its area and multivoltine (>2 generations per year) in the south (Corbet *et al.* 2006; Dijkstra & Lewington 2006). Eggs hatch within 2–3 weeks after oviposition followed by an aquatic larval stage ranging from 2 months (multivoltine) up to 11 months (univoltine).

During the peak of the local flight season in 2007, copulating pairs were collected from three populations

spanning nearly the complete latitudinal range (i.e. 1400 km). Specifically, damselflies were collected from a univoltine northern population (Sweden: 55°39'N, 13°32'E), a bivoltine central population (Belgium: 50°50'N, 4°39'E) and a multivoltine southern population (France: 43°30'N, 4°40'E). Because of the large size of the experiment (more than 3000 experimental units), we chose to rear one population per latitude. Given that four other rearing experiments of this species using replicated populations per latitude consistently found that interpopulation differences in life history traits at given latitudes were much smaller than interlatitude differences (Stoks & De Block 2011; De Block & Stoks in preparation; Stoks & De Block in preparation; Swillen *et al.* in preparation), we feel confident that any population effects in the current study would truly reflect latitude effects.

Egg clutches were collected by placing mated females on wet filter paper that was used as an oviposition substrate. Filter papers with eggs were kept in aged tap water at 20 °C until hatching. Prior to larval hatching, a subsample of eggs ($n = 10$) from each clutch was removed from the filter paper and photographed for digital analyses of egg size. Egg size (mm^3) was estimated as the volume of an ellipsoid. Dimensions of 14 day old eggs from each family were measured to the nearest 0.001 mm in IMAGE-PRO PLUS 5.0 (Media Cybernetics, Inc., Bethesda, MD, USA). Mean egg size per family was then used as a covariate in statistical analyses to control for potential egg size-mediated maternal effects (see e.g. Laugen *et al.* 2002). We produced 30 and 35 full-sibling egg clutches for Sweden and France populations, respectively. Within the Belgium population, 19 males from initial copulating pairs were used for subsequent matings with new field-collected females resulting in 38 paternal half-sibling egg clutches. The use of half-sibling families allowed for better estimates of additive genetic variance corrected for maternal effects (Lynch & Walsh 1998).

Common garden experiment

Hatchlings were randomly divided among 21 heated water baths set to one of the three rearing temperatures: 18, 21 and 24 °C. This temperature gradient has been shown to generate clear thermal reaction norms in closely related species (Van Doorslaer & Stoks 2005a,b) and spans the natural temperature regime of the populations of this species during the largest part of the growth season (see also Laugen *et al.* 2003; Van Doorslaer & Stoks 2005b). For the first 14 days, hatchlings from each family were held in groups of *c.* 40 individuals in 200 mL plastic dishes at each of the three temperatures ($n = c.$ 120 hatchlings per family in total). On day

15, larvae were assigned individually to plastic cups containing 100 mL of aged tap water. Ten randomly chosen offspring from each family were reared individually at each of the three temperatures in a full factorial randomized design (total $n = 3090$ larvae). Individuals were randomly shuffled among water baths of the same temperature on several occasions during the experiment. Larvae were fed twice daily *ad libitum* with laboratory-reared brine shrimp nauplii (334.1 ± 14.6 *Artemia* sp. per feeding). Photoperiod was maintained at 16 h L:8 h D throughout the experiment. For logistic reasons, we ended the experiment when larvae reached 65 days of age or the final instar, whichever occurred first. At this point, larvae were towel-dried and weighed to the nearest 0.001 mg. In most cases (87.4%), larvae were measured at age 65 days. Twelve individuals (1.3%) from the Belgium population and 36 individuals (6.3%) from the Sweden population reached the final instar, whereas 251 individuals (34.6%) from the France population reached the final instar (mostly from 24 °C). Excluding those individuals that reached the final instar did not change the qualitative outcome of statistical analyses; therefore, we retained all individuals to maintain more balanced sample sizes in all treatments. Growth rate was calculated as $\ln \text{mass}/\text{age}$ (see e.g. Johansson *et al.* 2001; Richter-Boix *et al.* 2010).

Statistical analyses

Survival (arcsine-transformed proportions) of larvae was tested by ANOVA using population, temperature and their interaction as fixed factors. Egg size differences among populations were tested by ANOVA using family mean egg sizes. Differences among families in egg size were tested by ANOVA using individual eggs and for each population separately.

Inter- and intrapopulation variation of thermal reaction norms for growth rate were tested using generalized linear mixed models using a Bayesian approach with an animal model implemented in the MCMCGLMM package (Hadfield 2010) in the R statistical environment (R Development Core Team 2007). All analyses used proper but weak priors, partitioning observed variance equally between random effects and residuals. Chains were run for 500 000 iterations, and posterior distributions were estimated after removing a burn-in of 200 000 iterations and taking every 100th sample to optimize chain mixing. Results are expressed as posterior modes from these distributions with 95% confidence intervals. Convergence of MCMC chains was estimated with the *coda* package by evaluating autocorrelation between consecutive samples from the posterior distribution. Autocorrelation coefficients of <0.05 were considered to represent well-mixed chains.

In a first approach focussing on among-population variation, the effects of population, experimental temperature, family (nested within population) and egg size on growth rates were analysed using family means. To find the best-fitting model, we sequentially increased model complexity by inclusion of additional factors starting with the random effect family, followed by the fixed effects population, temperature and egg size. We treated population as a fixed factor because they were chosen from latitudes selected a priori based on voltinism (Underwood 1997), and temperature was considered as discrete and not continuous. Model terms were evaluated by the change in deviance information criterion (DIC), assuming that a DIC value change of greater than two represents a significantly better model fit (Spiegelhalter *et al.* 2002). Fixed effects were additionally tested by evaluating whether parameter estimates from the MCMC samples of the posterior distribution overlapped zero.

In a second approach focusing on within-population variation, genetic/additive variance (V_G) and heritability (h^2) of growth rates at the three experimental temperatures and genetic correlations between temperatures were calculated using a character-state multivariate animal model treating growth rates at different temperatures as separate traits (Falconer 1952; Via & Lande 1985). As growth rates at different temperatures were measured in different individuals, we fixed the within-individual covariance to zero and only estimated genetic correlations (r_G) among families. Significance of genetic correlations was tested by evaluating whether parameter estimates from the MCMC samples of the posterior distribution overlapped zero (Wilson *et al.* 2010). An r_G differing from 1 represents a G×E (Windig 1997). As all r_G estimates are confined to a maximum of 1, we tested significance of G×E by a resampling procedure as advocated by Windig (1997). Specifically, we evaluated the proportion of samples from the posterior distribution of genetic correlations across character states (i.e. growth rates at the different temperatures) overlapping with the minimum estimate of both genetic correlations within a single character state (e.g. r_G between 18 and 21 °C was compared with lowest estimate obtained for r_G within 18 and 21 °C). Theoretically, the within-character-state values should be 1, but sampling from the posterior distribution will provide a sampling error distribution and thereby generate a lower confidence limit, which we used as a cut-off value to determine significance. Empirically, these values ranged from 0.93 to 0.99.

Heritabilities at each experimental temperature and genetic correlations based on full-sibling analyses were estimated for all populations. As the animal model imposes an expectation of the genetic covariance among individuals based on their additive relationship matrix,

these estimates reflect additive (narrow-sense) estimates under the assumption of no nonadditive effects. Given the full-sibling structure and potential maternal effects, these estimates may therefore be upwardly biased. In the Belgium population, heritabilities were calculated using 19 families, i.e. only one family from each sire. Additionally, we used all 38 families to calculate estimates based on half-sibling families, which enabled us to also estimate the maternal contribution by adding 'dam' as an additional random factor in the model.

Population genetic analyses

Population-level divergence in neutral marker genes (as measured by F_{ST}) was determined by multilocus genotyping of 36–40 individuals per population using 10 polymorphic microsatellite loci (see Appendix SI, Supporting information for PCR protocols). Fragments were analysed on a 3130 genetic analyser (Applied Biosystems). Data collection and allele scoring were performed using GeneMapper v3.7. All loci were checked for the presence of null alleles using MICROCHECKER (Van Oosterhout *et al.* 2004). Of the 10 loci, two contained significant null alleles (I015 and I053). Therefore, when estimating overall and pairwise population F_{ST} , we used FreeNA (Chapuis & Estoup 2007) to obtain unbiased F_{ST} values in the presence of null alleles. All measures of within-population genetic diversity were estimated using eight loci, i.e. without I015 and I053. Genetic diversity of populations was estimated from the mean number of alleles, as well as observed and expected heterozygosity using GENETIX (Belkhir *et al.* 1998). Deviations from Hardy–Weinberg equilibrium (estimated as F_{IS}) were calculated using FSTAT v2.9.3.2 (Goudet 1995).

Q_{ST} vs. F_{ST} (quantitative vs. neutral genetic divergence)

Divergence in quantitative traits can be expressed as (Spitze 1993)

$$Q_{ST} = \frac{\sigma_B^2}{\sigma_B^2 + 2\sigma_W^2}$$

where σ_B^2 and σ_W^2 are the respective between- and within-population components of (additive) genetic variation for the trait under consideration. Analogous to the variance/covariance estimation mentioned above, we also estimated the genetic variance components within and among populations for Q_{ST} calculations using a character-state approach with an animal model implemented in the MCMCGLMM package (Hadfield 2010) using the *animal* variance component as a

measure of within-population variance. Confidence intervals of Q_{ST} were obtained by sampling parameter estimates from the posterior distribution. Point estimates of Q_{ST} were calculated as posterior modes with 95% confidence intervals (Hall *et al.* 2007). The influence of environment (temperature alone) on Q_{ST} estimates was assessed by fitting between- and within-population components of genetic variation in a univariate model pooled across all temperatures and comparing the model fit to a multivariate character-state model separating growth rates at each temperature. The effect of G×E (population × temperature) was then assessed with the multivariate character-state model, fitting interaction terms between growth rates at each temperature and population as random effects. This was done for each pairwise population combination as well as for an overall estimate including all populations.

Results

Survival differed among populations and temperatures (population $F_{2,296} = 20.17$, temperature $F_{2,296} = 48.70$, population × temperature $F_{4,296} = 4.68$; all $P \leq 0.001$). Survival was highest in Belgium larvae at all temperatures (Fig. S1, Supporting information). In general, survival (%) decreased with increasing experimental temperature (85.0, 75.7 and 60.9 at 18, 21 and 24 °C, respectively). The significant population × temperature interaction was mainly driven by lower survival of France larvae at 21 °C (Fig. S1, Supporting information). Mean egg size differed among populations ($F_{2,95} = 35.88$, $P < 0.001$). Largest mean egg sizes (mm^3) were from the Belgium population (0.188 ± 0.003), followed by Sweden (0.174 ± 0.004), and the smallest eggs were from the France population (0.151 ± 0.003). Egg size also differed significantly among families within each population ($P < 0.001$ for all populations), but did not show any interaction with temperature on growth rates (egg size × temperature interaction $F_{2,189} = 0.39$; $P = 0.676$), and no significant egg size × temperature × population interaction ($F_{6,227} = 0.17$; $P = 0.986$). Mean masses of larvae (mg) at the end of the experiment (65 days) were 8.02 (range: 1.09–34.54), 12.95 (1.43–31.56) and 11.34 (0.94–29.81) for Belgium, France and Sweden, respectively.

Interpopulation variation in thermal reaction norms for growth rate

The best-fitting model in the interpopulation analysis contained the random factor family as well as the fixed factors population and temperature with the respective interaction term (Table 1). Growth rates were highest for France larvae, intermediate for Sweden larvae and

Table 1 Interpopulation variation of larval growth rates and their thermal sensitivity in *Ischnura elegans* analysed by generalized linear mixed models and Markov chain Monte Carlo sampling of the posterior distribution. Model fits were evaluated as deviance information criterion (DIC) after sequential inclusion of family, population, temperature and egg size as effects on growth rate

	DIC	Δ DIC	<i>P</i>
(Intercept)	1045.47	–	
<i>Random effects</i>			
Family (population)	1030.86	–14.61	
<i>Fixed effects</i>			
Population	946.00	–84.86	<0.0001
Temperature	578.79	–367.21	<0.0001
Population × temperature	387.62	–191.17	<0.0001
Egg size	387.61	–0.01	0.889

Note: *Family* depicts family means, not individuals. Egg size (mm^3) based on family means using $n = 10$ eggs/family. Significance was estimated by the change in DIC. Additionally, significance of fixed effects was tested by sampling the parameter estimates resulting from the posterior distribution, and *P*-values give the proportion of samples smaller than zero.

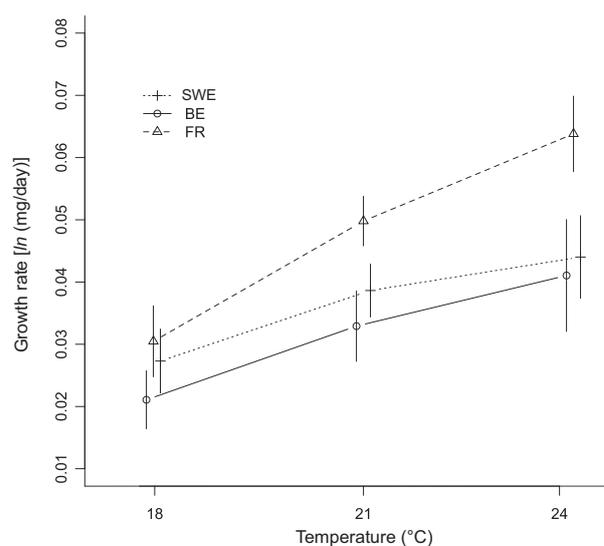


Fig. 1 Population-level thermal reaction norms of larval growth rate for *Ischnura elegans* from a univoltine northern population (Sweden), a bivoltine central population (Belgium) and a multivoltine southern population (France). Means are given \pm SE.

lowest for Belgium larvae (Fig. 1). All populations showed an increase in growth rate with increasing temperature (temperature main effect; Table 1), and differences among populations were more pronounced at the highest temperature (Fig. 1). Sweden and Belgium larvae reacted similarly to increasing temperatures, whereas France larvae reacted with a much stronger

increase in growth rate (population \times temperature interaction; Table 1, Fig. 1).

Intrapopulation variation and heritability of growth rates

The multivariate animal model revealed that populations of *Ischnura elegans* harboured substantial within-population variation of growth rate at all experimental temperatures. A large proportion of this variation was attributable to genetic effects (null model only fitting intercept DIC = 2164.75 vs. model containing 'animal' as random effect DIC = 1683.75). Full-sibling heritabilities tended to be lower in France (0.33–0.68) when compared with Belgium (0.68–0.80) or Sweden (0.71–0.88; Table 2). Models evaluating heritabilities separately per character state (growth rate per temperature) showed a significantly better fit than those fitting a single value averaged over all temperatures. Changes in DIC favouring temperature-specific heritabilities were 163.6, 224.0 and 243.4 for France, Belgium and Sweden, respectively, indicating that heritability estimates were sensitive to environmental conditions with a tendency to increase with increasing temperature (Table 2). Half-sibling heritabilities were somewhat lower than full-sibling estimates in the Belgium population (Table 2b), especially at higher temperatures (21 and 24 °C), revealing the potential bias introduced by nonadditive genetic effects when estimating heritability based on full-sibling families. However, this bias does not appear to be caused by maternal effects as a model including dam as an additional random effect showed a substantially weaker fit than the model containing only genetic effects (DIC with additive effects: 1683.76 vs. DIC with additive and maternal effects: 1797.81). The lack of maternal effects bias is further supported by low dam variance components ranging from 0.08 (CI: 0.05–0.21) at 18 °C to 0.11 (CI: 0.06–0.27) at 21 °C and 0.11 (CI: 0.05–0.29) at 24 °C.

In all three populations, temperature-specific character states of growth rates showed significant positive genetic correlations (r_G) in most cases (Table 2). It is worth noting that in the Sweden population, r_G did not differ significantly from zero between 18 and 21 °C nor between 18 and 24 °C, showing that G \times E are particularly strong in the northernmost population. More general, all estimates of r_G were significantly lower than within-character-state estimates, also those based on half-siblings in the Belgium population, indicating the presence of G \times E in all populations (Table 2).

Neutral genetic variation and differentiation

The 10 microsatellite loci surveyed were highly polymorphic; a total of 95 different alleles were found in the

three *I. elegans* populations (see Table S1, Supporting information for genetic diversity of loci). Within populations, the mean number of alleles (based on eight loci without null alleles) ranged from 7.000 to 7.875 (Table 3). Observed heterozygosity ranged from 0.606 in the Sweden population to 0.625 in the Belgium population. Only the Sweden population showed a deviation from Hardy–Weinberg equilibrium (HWE) as evidenced by the significant F_{IS} value observed (Table 3). Overall unbiased F_{ST} was low, but significant ($F_{ST} = 0.0096$, 95% CI: 0.0025–0.0174), which also was the case for all pairwise measures of unbiased F_{ST} (Table 3).

Quantitative vs. neutral genetic differentiation

Population-level differentiation in the quantitative trait growth rate (Q_{ST}) was significantly greater than differentiation at neutral markers (F_{ST}) as evidenced by the nonoverlapping 95% confidence intervals for all Q_{ST} vs. F_{ST} comparisons (Fig. 2). Posterior modes for overall Q_{ST} were 0.43 at 18 °C, 0.71 at 21 °C and 0.75 at 24 °C. Pairwise population estimates of Q_{ST} ranged between 0.31 and 0.92. Models assessing Q_{ST} values using a multivariate character-state model showed a significantly better fit than univariate models for the overall estimates (Δ DIC in favour of character state: 1789.6), as well as for pairwise estimates (Δ DIC_{BE-FR} = 1116.6, Δ DIC_{BE-SW} = 558.1, Δ DIC_{FR-SW} = 1362.2), indicating that Q_{ST} estimates are sensitive to environmental conditions. In line with our heritability estimates, quantitative genetic differentiation increased with increasing temperatures (Fig. 3). Fitting population \times temperature interactions further improved the model fit (Δ DIC_{overall} = 478.9, Δ DIC_{BE-FR} = 265.1, Δ DIC_{BE-SW} = 99.8, Δ DIC_{FR-SW} = 279.0), showing that Q_{ST} estimates were sensitive not only to temperature but also to G \times E. This interaction was mainly driven by the France population at higher temperatures where quantitative genetic differentiation greatly exceeded that of Q_{ST} between the Belgium and Sweden population (Fig. 3).

Discussion

Our study illustrates the involvement of diversifying natural selection imposed by time constraints associated with latitude and voltinism rather than neutral processes in shaping the latitudinal pattern of larval growth rates in *Ischnura elegans*, and this is despite high gene flow among populations. Our data suggest the potential for microevolution of thermal reaction norms and underscore the need to evaluate the evolutionary potential of populations under expected higher temperatures to assess their ability to deal *in situ* with climate warming.

Table 2 Genetic variance–covariance matrices of intrapopulation variation in larval growth rates of *Ischnura elegans* analysed by multivariate animal models taking temperature-specific growth rates (character states) as response variables: (a) full-sibling families and (b) Belgium half-sibling families

	18 °C	21 °C	24 °C
<i>(a) Full-sibling families</i>			
Sweden			
18 °C	V_A 0.38 (0.26–0.63) V_E 0.13 (0.03–0.22) h^2 0.71 (0.49–0.92)	CoV 0.09 (–0.03 to 0.23) r_G 0.32 ^{ns/***} (–0.07 to 0.65)	CoV 0.16 (–0.12 to 0.35) r_G 0.34 ^{ns/***} (–0.25 to 0.60)
21 °C		V_A 0.23 (0.15–0.35) V_E 0.07 (0.02–0.13) h^2 0.80 (0.58–0.95)	CoV 0.23 (0.07–0.33) r_G 0.61 ^{***/**} (0.27–0.83)
24 °C			V_A 0.63 (0.38–0.84) V_E 0.09 (0.04–0.22) h^2 0.88 (0.68–0.96)
Belgium			
18 °C	V_A 0.35 (0.16–0.61) V_E 0.19 (0.06–0.30) h^2 0.68 (0.40–0.90)	CoV 0.19 (0.03–0.41) r_G 0.55 ^{*/***} (0.15–0.77)	CoV 0.27 (0.10–0.48) r_G 0.72 ^{***/**} (0.24–0.86)
21 °C		V_A 0.50 (0.26–0.74) V_E 0.13 (0.23–0.46) h^2 0.80 (0.52–0.94)	CoV 0.27 (0.16–0.59) r_G 0.79 ^{***/**} (0.44–0.91)
24 °C			V_A 0.61 (0.34–0.80) V_E 0.18 (0.05–0.29) h^2 0.79 (0.54–0.94)
France			
18 °C	V_A 0.63 (0.28–0.99) V_E 0.40 (0.20–0.62) h^2 0.60 (0.38–0.83)	CoV 0.16 (0.02–0.42) r_G 0.56 ^{*/***} (0.11–0.85)	CoV 0.33 (0.06–0.62) r_G 0.68 ^{***/**} (0.20–0.80)
21 °C		V_A 0.19 (0.09–0.40) V_E 0.37 (0.23–0.46) h^2 0.33 (0.19–0.64)	CoV 0.13 (–0.03 to 0.36) r_G 0.54 ^{*/***} (0.06–0.82)
24 °C			V_A 0.70 (0.31–1.03) V_E 0.36 (0.18–0.62) h^2 0.68 (0.38–0.88)
<i>(b) Belgium half-siblings</i>			
18 °C	V_A 0.36 (0.18–0.54) V_E 0.23 (0.13–0.35) h^2 0.60 (0.37–0.80)	CoV 0.20 (0.04–0.38) r_G 0.59 ^{*/***} (0.20–0.77)	CoV 0.22 (0.04–0.39) r_G 0.54 ^{***/**} (0.15–0.81)
21 °C		V_A 0.44 (0.27–0.74) V_E 0.21 (0.09–0.35) h^2 0.66 (0.43–0.88)	CoV 0.29 (0.14–0.57) r_G 0.74 ^{***/**} (0.47–0.90)
24 °C			V_A 0.39 (0.23–0.76) V_E 0.33 (0.15–0.47) h^2 0.60 (0.36–0.83)

Elements on the diagonal give estimated variance components for V_{animal} and V_E plus the resulting heritability with 95% CI. Off-diagonal elements give genetic covariances and genetic correlations (r_G) between character states. Significance of genetic correlations was tested in two directions: (i) as the proportion of posterior values overlapping zero to demonstrate genetic correlation and (ii) as proportion overlapping r_G estimates for each character state with itself to test for G×E (see Methods).

* $P < 0.05$, ** $P < 0.01$, *** $P > 0.001$.

Selection vs. drift in shaping population divergence

Populations of *I. elegans* differed in growth rates across a latitudinal cline. Such geographical differentiation is often the result of spatially varying selection pressures; however, among-population differences also can arise by genetic drift alone (Endler 1977). Hence, only signifi-

cantly larger differentiation at quantitative traits (Q_{ST}) than at neutral genetic markers (F_{ST}) can be interpreted as a sign of diversifying natural selection (Merilä & Crnokrak 2001; McKay & Latta 2002; Leinonen *et al.* 2007). Populations from France, Belgium and Sweden were differentiated from one another at putatively neutral microsatellite markers by about 1%, suggesting

Table 3 Genetic composition and pairwise measures of genetic differentiation (F_{ST}) of three *Ischnura elegans* populations spanning a latitudinal gradient across Europe. Unbiased estimates of F_{ST} are shown below the diagonal with significant pairwise comparisons depicted by *. Confidence intervals (95%) of pairwise F_{ST} values are shown above the diagonal

Population	n	A	H_o	H_e	F_{IS}	Sweden	Belgium	France
Sweden	36	7.125	0.606	0.683	0.127*	–	0.0027–0.0185	0.0009–0.0304
Belgium	40	7.875	0.625	0.675	0.082	0.0106*	–	0.0014–0.0108
France	40	7.000	0.619	0.668	0.086	0.0127*	0.0058*	–

Unbiased estimates of F_{ST} in the presence of null alleles were calculated based on 10 loci using FreeNA (Chapuis & Estoup 2007). n , Sample sizes; A, mean number of alleles; H_o/H_e , observed/expected heterozygosity; F_{IS} , deviations from HWE (averaged over eight loci).

*Significance after Bonferroni correction.

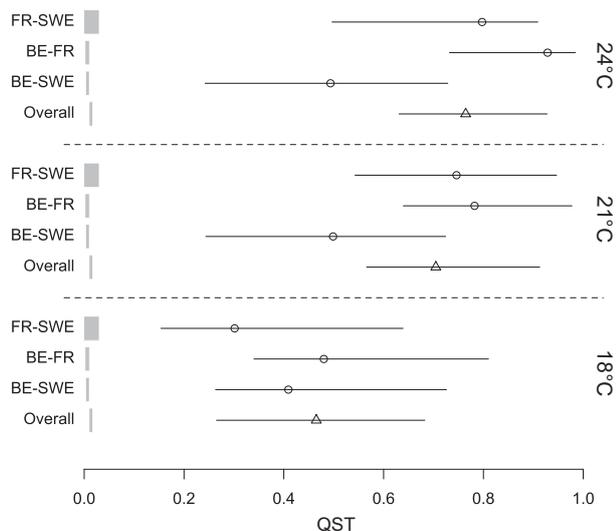


Fig. 2 Genetic differentiation in growth rate (Q_{ST}) at three experimental temperatures for *Ischnura elegans* populations from Sweden (SWE), Belgium (BE) and France (FR). Triangles depict overall Q_{ST} estimates, and circles represent pairwise population comparisons; 95% highest posterior densities are shown around the point estimates (posterior modes), and 95% confidence intervals for differentiation at neutral markers (F_{ST}) are shown as grey boxes.

widespread gene flow across Europe. A similar pattern of F_{ST} across Europe was recently shown for yellow dung flies, with the authors concluding that the lack of population structure probably resulted from high gene flow due to mobility in combination with large population sizes (Demont *et al.* 2008). Here, we found that Q_{ST} of growth rate was greater than F_{ST} in all cases, suggesting a strong effect of selection rather than drift in shaping patterns of *I. elegans* population divergence of this trait. To date, few studies have explicitly assessed the adaptive nature of latitudinal evolution of thermal reaction norms by comparing Q_{ST} vs. F_{ST} (but see Palo *et al.* 2003; Demont *et al.* 2008). Our findings are in agreement with these studies, as well as the majority of Q_{ST} vs. F_{ST} research that has shown that selection rather than genetic drift is the more likely causal explanation

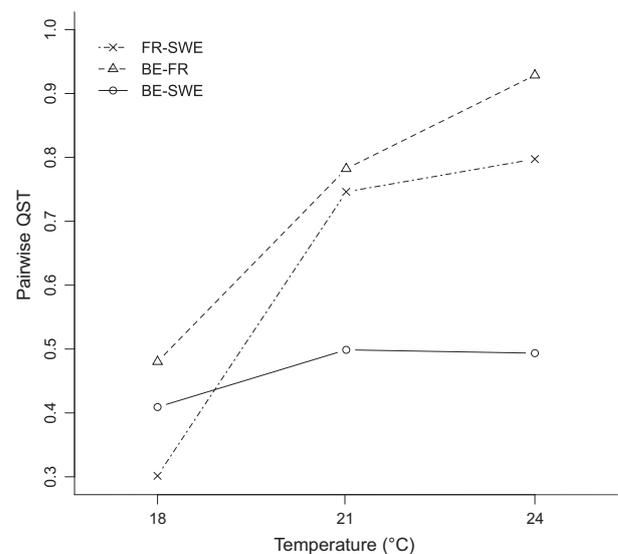


Fig. 3 Thermal reaction norms of pairwise Q_{ST} estimates (posterior modes) showing an increase in adaptive differentiation with increasing temperature and genotype by environment interactions.

for population divergence in quantitative traits (Merilä & Crnokrak 2001; McKay & Latta 2002; Leinonen *et al.* 2007; Richter-Boix *et al.* 2010). Moreover, our results contribute to the growing body of evidence, indicating that in some systems, adaptive divergence of populations can be maintained by natural selection in spite of high gene flow (see e.g. Endler 1977; Garant *et al.* 2007).

We also found that overall Q_{ST} differed among experimental temperatures, lending support to the hypothesis that Q_{ST} estimates are sensitive to environmental conditions (Merilä & Crnokrak 2001; Palo *et al.* 2003). The observed increase in overall Q_{ST} with increasing temperature is probably linked to the underlying higher pairwise estimates involving the France population. Thermal reaction norms of Q_{ST} show nonparallel slopes, the steepest of which (France–Sweden) being mostly

driven by high growth rates at 24 °C in the France population. This suggests that our Q_{ST} estimates may be sensitive not only to environment but also to G×E, which can complicate evolutionary inferences (Palo *et al.* 2003). Note that the increase in Q_{ST} with temperature also could be driven by bias stemming from population-specific nonadditive effects (Merilä & Crnokrak 2001). Life history traits such as growth rate are known to harbour substantial nonadditive genetic effects compared with, for example, morphological traits (Roff 1997). Although a half-sibling breeding design cannot partition out dominance or epistatic effects, at least in the Belgium population where we could estimate maternal effects, these did not have a significant influence on genetic variance. Note also that the order of population mean growth rates (Belgium < Sweden < France) is opposite to the order for population mean egg size, indicating that egg size-mediated maternal effects are unlikely to contribute to population divergence in growth rates.

Survival differed among populations and temperatures; however, these differences are likely not driving latitude or Q_{ST} patterns. Higher survival of Belgium larvae at all temperatures may be due to their overall lower growth rates: high growth rate has been shown to be traded off against other functions including energy storage in damselfly larvae (Stoks *et al.* 2006a). Accordingly, lower survival at higher temperatures seen in all populations is probably the result of higher (relative) growth rates. Furthermore, a temperature-mediated growth increase was most pronounced in France larvae between 18 and 21 °C and matches the marked reduction in survival under the same conditions (giving rise to the population by temperature interaction for survival). Differential survival is also unlikely to bias patterns in our Q_{ST} estimates. For example, at 21 °C survival was highest in Belgium larvae and equally low in France and Sweden larvae, yet the pairwise Q_{ST} estimate for Belgium–France was much higher than that of Belgium–Sweden. Moreover, the former was similar to the Q_{ST} estimate for Sweden–France. Also, at 24 °C, the pattern in Q_{ST} estimates does not seem to reflect the pattern in survival. While survival was equally low for all three latitudes at 24 °C, pairwise Q_{ST} estimates differed widely at this temperature. Furthermore, the Q_{ST} estimate for Belgium–Sweden was essentially the same at 21 and 24 °C, while at both latitudes, survival decreased considerably from 21 to 24 °C.

Latitudinal variation in thermal reaction norms of growth rate

Thermal reaction norms among populations differed primarily in elevation, suggesting that time constraints associated with seasonality rather than temperature is

the main environmental factor shaping growth rates in the wild. Time constraints have been shown to be an important factor causing accelerated growth rates in damselflies (e.g. De Block & Stoks 2004, 2005; Stoks *et al.* 2006b). Interestingly, the nature of the time constraint, i.e. the underlying reason for shorter larval growth seasons, differs in function with latitude: multivoltinism at low latitude (France) and inhospitable climate conditions at high latitude (Sweden). Higher growth rates of the France population relative to Sweden indicate that, in our case, time constraints imposed by multivoltinism are stronger than those imposed by latitude. Additionally, there is also some evidence for thermal adaptation playing a role as indicated by the steeper thermal reaction norm of the France population. In other words, we observe a mixed strategy where reaction norms are shifted both vertically and horizontally relative to the importance of seasonality vs. temperature (*sensu* Yamahira *et al.* 2007). Note that the studied temperature range, while ecologically relevant, was rather narrow (only 6 °C) and that studying thermal reaction norms across a broader temperature range may likely identify a more important role of variation in the temperature dependence of growth (Kingsolver *et al.* 2004).

The identified latitudinal growth rate pattern is therefore a combination of latitudinal and voltinism compensation. Several studies have demonstrated growth rate adaptation to seasonality in high-latitude populations (overview in Angilletta 2009), but few have shown adaptation to voltinism, despite its intuitive appeal (but see Nygren *et al.* 2008; Ragland & Kingsolver 2007 for development rate; Karl *et al.* 2008 for voltinism compensation across an altitudinal gradient). Yet, none so far considered their combination. As pure patterns of latitudinal compensation can no longer be expected when populations of mixed voltinism are compared and changes in voltinism are widely documented along latitudinal gradients (Corbet *et al.* 2006; Altermatt 2010), such mixed patterns are likely to be very common. Rather than a monotonous increase in growth rates with increasing latitude, a U-shaped or a more complex saw-tooth pattern reflecting abrupt changes in voltinism along a latitudinal transect may then be expected as documented in our study. This recalls the similar pattern for body size predicted by Roff (1980) based on the same combination of latitudinal and voltinism compensation, and which has been shown in some studies (Johansson 2003; De Block *et al.* 2008; Nygren *et al.* 2008). Note, however, that Roff (1980) did not explicitly consider growth rate and assumed the size pattern to be proximately driven by development rate, while growth rate may vary independently from development rate (e.g. Johansson & Rowe 1999;

Johansson *et al.* 2001), and potentially compensates for changes in development time (Abrams *et al.* 1996).

The latitudinal pattern in growth rate shown here has relevance for northward range shifts under global warming, which have been shown to be particularly strong in odonates (Hickling *et al.* 2006). As growth rate is a key trait linked to fitness and often used as a metric to predict competitive outcome (Lindgren & Laurila 2010), our results suggest (all else being equal) that France animals that invade Belgium will outcompete the local populations, while this may not be the case when Belgium animals invade Sweden. Admittedly, this prediction critically depends on the existence of trade-offs between growth rate and other fitness-related traits (reviewed by Angilletta *et al.* 2003), yet it provides a testable hypothesis. It is worth noting that a recent study showed that northern, fast-growing tadpoles outcompeted southern tadpoles in a competition experiment (Lindgren & Laurila 2010). Such competitive superiority of fast-growing genotypes has the potential to modify not only intraspecific but also interspecific interactions with possible cascading effects on the ecosystem if the climate changes.

The adaptiveness of the here-reported latitudinal pattern in growth rate needs to be considered in the larger context of overall life history variation across latitudes, where besides growth rate, age and mass at emergence are key determinants of adult fitness. Higher growth rates of France larvae are associated with much higher development rates, hence shorter development times, and the lowest mass at emergence (Stoks & De Block 2011; Swillen *et al.* in preparation). This is consistent with the compound interest hypothesis stating that in populations with relatively short generation times as compared to season length, fitness will be maximized by increasing the number of generations, even at the expense of being small (Partridge & French 1996; Fischer & Fiedler 2002). In contrast, Sweden larvae have the longest development times and highest mass at emergence, while Belgium larvae show intermediate values for both (Stoks & De Block 2011; Swillen *et al.* in preparation). Conditions for growth in Sweden do not allow an extra generation, and in such univoltine populations, selection for large size at maturity to maximize adult fitness should be particularly strong (Partridge & French 1996; Fischer & Fiedler 2002).

Evolutionary potential of thermal reaction norms of growth rate

Each of the populations of *I. elegans* showed heritable (additive) genetic variation for growth rate, indicating that adaptive evolution of thermal reaction norms to environmental change does not seem to be constrained

by the amount of standing genetic variation. Comparable results have been found in latitudinal populations of several taxa (Palo *et al.* 2003; Blanckenhorn & Demont 2004; Van Doorslaer & Stoks 2005b; Yamahira *et al.* 2007; Nygren *et al.* 2008). Heritabilities of growth rate also were sensitive to environmental conditions with a pattern of higher heritability at higher temperature. Stressful, unfavourable and/or novel conditions are known to contribute to changes in heritability across environments (reviewed by Hoffmann & Merilä 1999). Mechanisms include changes in additive genetic variance, environmental variance, nonadditive effects including maternal effects, and G×E (Charmantier & Garant 2005; Kruuk *et al.* 2008). In our study, changes in heritability are more influenced by higher (additive) genetic variances at 24 °C than by changing environmental variances (which were similar among temperatures for each population). Note that heritabilities based on half-sibling families in the Belgium population were lower than those estimated from full-siblings and were similar at each temperature, suggesting that changes in full-sibling genetic variances across temperatures also include some form of environment-specific nonadditive effect. Although we could rule out maternal effects in the Belgium population, these still may be present in the Sweden and France populations.

Changes in heritability across experimental temperatures were influenced by G×E. Particularly in the northernmost population (Sweden), low (nonsignificant) genetic correlations between temperatures indicate that the underlying genetic architecture differs at high vs. low temperature (Via & Lande 1985; Hoffmann & Merilä 1999). Moreover, in all populations, significant G×E (genetic correlations less than 1) suggest that thermal reaction norms for growth rate have the potential to evolve (Via 1993; Charmantier & Garant 2005), although there may be differences among populations in the magnitude of G×E, i.e. some populations are more plastic than others or have a higher heritability of plasticity (Roff 1997; Visser 2008).

Adaptive plasticity of thermal reaction norms in response to changing environmental temperatures could allow populations to rapidly track the moving selective optimum and result in partial or full maintenance of fitness in the new environment (Gienapp *et al.* 2008). Our results are in contrast with recent studies of fish (*Oryzias* sp.) that found that within-population reaction norms were more or less parallel across temperatures, with the authors concluding that this represented a constraint in the evolution of latitudinal compensation (Yamahira *et al.* 2007). Here, the adaptive pattern of latitudinal compensation in growth rate that results from a combination of latitudinal and voltinism compensation has the potential to evolve. Evolution of

growth rate in response to climate warming in local populations may buffer against immigration of southern genotypes with currently higher growth rates. Our study illustrates the value of an integrated approach where an evaluation of the adaptiveness of current latitudinal patterns (that reveal past selection events) is combined with identifying the potential for further evolution of thermal reaction norms *in situ*. Especially, the latter information is rarely available yet crucial to predict effects of climate change (Angilletta 2009).

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Data accessibility

Data deposited at Dryad: doi:10.5061/dryad.hs727.

Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Survival of *I. elegans* larvae from three populations (Sweden, Belgium, France) exposed to three experimental rearing temperatures.

Table S1 Genetic diversity of the 10 microsatellite loci used to genotype *Ischnura elegans* from three populations across a latitudinal gradient.

Appendix SI Polymerase chain reaction (PCR) protocols for 10 microsatellite loci amplified using DNA extracted from *I. elegans*.

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