E-ARTICLE

Gene Flow Limits Adaptation along Steep Environmental Gradients

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ABSTRACT: When environmental variation is spatially continuous, dispersing individuals move among nearby sites with similar habitat conditions. But as an environmental gradient becomes steeper, gene flow may connect more divergent habitats, and this is predicted to reduce the slope of the adaptive cline that evolves. We compared quantitative genetic divergence of *Rana temporaria* frog populations along a 2,000-m elevational gradient in eastern Switzerland (new experimental results) with divergence along a 1,550-km latitudinal gradient in Fennoscandia (previously published results). Both studies found significant countergradient variation in larval development rate (i.e., animals from cold climates developed more rapidly). The cline was weaker with elevation than with latitude. Animals collected on both gradients were genotyped at ~2,000 single-nucleotide polymorphism markers, revealing that dispersal distance was 30% farther on the latitudinal gradient but 3.9 times greater with respect to environmental conditions on the elevational gradient. A meta-analysis of 19 experimental studies of anuran populations spanning temperature gradients revealed that countergradient variation in larval development, while significant overall, was weaker when measured on steeper gradients. These findings support the prediction that adaptive population divergence is less pronounced, and maladaptation more pervasive, on steep environmental gradients.

Keywords: amphibian, climate gradient, countergradient variation, gene flow, isolation-by-distance method, maladaptation.

Introduction

Classic theory of adaptation under environmental heterogeneity holds that genetic divergence among populations arises from a balance between the homogenizing effect of gene flow and the diversifying effect of selection (Felsenstein 1976; Slatkin 1987; reviewed in Lenormand 2002). The early models of so-called protected polymorphism in two habitats attempted to understand this balance (Haldane 1930; Wright 1931; Levene 1953; Bulmer 1972; reviewed in Felsenstein 1976; Spichtig and Kawecki 2004). These models made simplifying assumptions about the environment and the genetic basis of adaptation, and they highlighted specific thresholds such that adaptation to local conditions occurs when selection is sufficiently strong relative to gene flow but not otherwise. The expectation is different when environmental variation is spatially explicit, with populations arranged along a geographic gradient presenting a continuously changing selection regime. Here too there are threshold conditions stating that precise local adaptation at each point along the gradient can evolve if the spatial change in the environment is sufficiently gradual relative to gene dispersal distance (Haldane 1948; Slatkin 1973; Endler 1977; Pease et al. 1989; Kirkpatrick and Barton 1997; Alleaume-Benharira et al. 2006). But spatially explicit theory predicts that a clinal genetic pattern will evolve even if the rate of spatial change in the environment exceeds the threshold criterion. The equilibrium slope of the cline will simply be lower than the slope of the optimal trait value. If the phenotypic optimum is equated with the condition of the environment itself, then these models predict that increasing gene flow will reduce the slope of a cline, when measured against a given environmental gradient (Slatkin 1973; Pease et al. 1989; Lenormand 2002). Steep gradients should therefore exhibit some degree of maladaptation across much of the gradient. Our study tests this prediction by comparing amphibian populations occurring along geographic gradients in climate.

There is strong evidence that gene flow counteracts adaptation in spatially structured populations. Many studies observe a negative correlation between the degree of local adaptation and the spatial isolation of populations or

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their inferred immigration rates, consistent with the idea that diversifying selection is more effective in populations that are at least partially shielded from immigration (May et al. 1975; Stearns and Sage 1980; King and Lawson 1995; Storfer and Sih 1998; Hendry and Taylor 2004; Nosil and Crespi 2004; Tack and Roslin 2010; Kalske et al. 2016). Similar analyses of adaptation along spatial environmental gradients are less frequent, but two studies of insects observed that clinal variation in quantitative traits along elevational gradients was more pronounced under conditions expected to reduce gene flow (longer gradient: Bridle et al. 2009; species with wingless adults: Levy and Nufio 2015). Paul et al. (2011) discovered that the latitudinal cline in growth rate of a plant was shallower than predicted based on the environmental gradient and that the mismatch between phenotype and environment was greatest in populations receiving many immigrants.

Here, we evaluate the role of gene flow by comparing the extent of adaptive population divergence along climate gradients that differ in steepness, which we define as the rate of change in the environment with geographic distance. The rationale for our comparison is as follows. Suppose that dispersal distance is independent of the steepness of the environmental gradient and that dispersing gametes or progeny do not select habitats that match their natal environment. In this case, immigrants at any particular site will originate from a broader range of natal environments if the gradient is steeper, thus importing more locally maladapted genotypes and reducing the frequency of locally adapted genotypes. Hence, gene flow should more strongly oppose local selection and reduce the slope of a cline on steep environmental gradients (Slatkin 1973; Endler 1977; Keller et al. 2013).

To test this hypothesis empirically, one must first characterize the adaptive cline. This is a simple matter in our study system of amphibian populations occupying gradients of temperature and seasonality. The development rate of larval amphibians has evolved to be faster in populations originating from cold environments (Berven et al. 1979; Berven 1982; Skelly 2004; Muir et al. 2014; Luquet et al. 2019). This is known as countergradient variation because quantitative genetic divergence along the climate gradient is opposed by environmental effects on the phenotype acting in the opposite direction (see fig. 1). Countergradient variation in development rate is interpreted as an adaptation that allows the larval portion of the life cycle to be completed even under an abbreviated growing season in cold regions. The argument is that rapid development rate evolves to compensate for the suppressive influence of cold temperature on development (Levins 1969; Berven 1982; Hodkinson 2005; Conover et al. 2009; Keller et al. 2013). There is much evidence supporting this argument

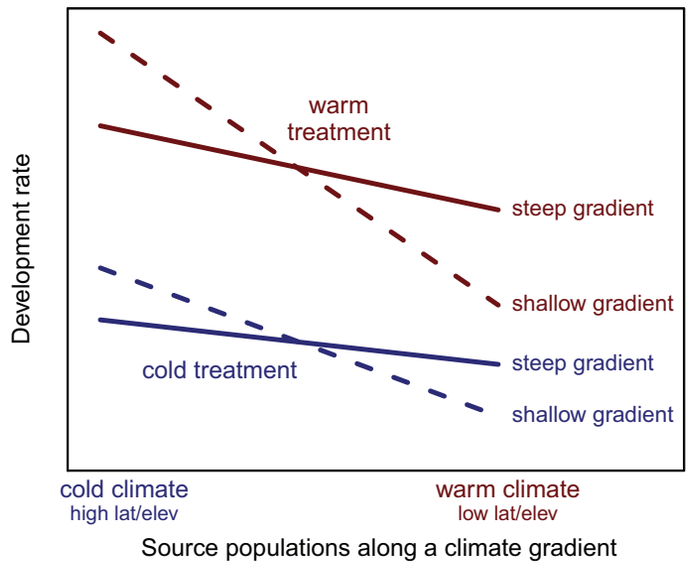

Figure 1: Expected results of a comparison between steep and shallow climate gradients. Populations spanning the gradients are reared under two temperatures in a common-garden experiment. The steepness of the climate gradient refers to the rate of change in the environment with respect to geographic distance. The adaptive cline is less pronounced on a steep climate gradient, as predicted if gene flow opposes adaptation. In this example, the phenotype shows countergradient variation because populations from warmer climates have lower trait values while warmer experimental temperatures increase the trait. Here, we also depicted steeper countergradient variation in the warm treatment because the trait is larger and has greater scope for response. In anuran tadpoles, the diagram corresponds to development rate because that trait is expected to show adaptive countergradient variation. The expected pattern is uncertain for traits without a clear adaptive cline along the gradient.

(Arendt 1997; Conover et al. 2009). For example, individuals from cold-adapted populations of poikilothermic taxa usually develop rapidly, as mentioned above, but not when they can escape time constraints (e.g., species with flexible life histories or in nonseasonal environments; De Block et al. 2008; Eckerstrom-Liedholm et al. 2017). The hypothesis that countergradient variation is adaptive implies a cost of rapid development, and such costs have been identified in fish, insects, and amphibians (Arendt 1997; Billerbeck et al. 2001; Stoks et al. 2005; Dmitriew 2011). Although changes in natural selection on development along climate gradients have not been measured directly, there is evidence from Q_{SF}/F_{ST} comparisons in amphibians that development rate and body size undergo divergent selection associated with elevation and latitude (Palo et al. 2003; Muir et al. 2014; Luquet et al. 2015). All of these findings suggest that countergradient variation in development rate—and perhaps also growth rate—is likely to be adaptive. This leads to the prediction that amphibians will exhibit weaker countergradient variation in development rate along steeper climate gradients where gene flow connects populations occupying distinct environments (fig. 1).

There is less consensus on the adaptive direction of clinal genetic variation in body size—and to some extent growth rate—with temperature or seasonality (see "Discussion"; Abrams et al. 1996; Hodkinson 2005; Dmitriew 2011; Keller et al. 2013). Furthermore, we did not expect phenotypic plasticity in development or growth to vary adaptively along climate gradients because, apart from acclimation (Bacigalupe et al. 2018), there is little evidence that temperature-induced plasticity in development or growth of ectotherms is adaptive (van der Have and de Jong 1996; Angilletta and Dunham 2003; Overgaard et al. 2011). In summary, the pattern illustrated in figure 1 is expected for development rate, but for other traits and plasticities there is no clear prediction.

We used two approaches to compare adaptation along steep and shallow climate gradients. First, we performed a common-garden experiment on tadpoles of the frog *Rana temporaria* originating from populations spanning a relatively short and steep elevational gradient. Studies such as this can demonstrate adaptation only indirectly because they do not reveal the fitness consequences of population divergence within natural settings. But common-garden experiments can be convincing when they evaluate relationships between functionally relevant characters and the environment and when populations originating from a range of environments are reared under treatments that manipulate appropriate features of the environment, thereby testing for adaptation to specific factors (Kawecki and Ebert 2004). In this study, the feature of the environment we manipulated was temperature, and the functionally relevant character was development rate (fig. 1). Our new experimental results were then compared directly with those from a similar experiment on the same species measuring adaptation to a long latitudinal gradient (Laugen et al. 2003, 2005). Gene flow on both gradients was inferred using neutral markers. We predicted that gene flow with respect to environmental conditions would be greater on the elevational gradient, and as a consequence adaptive population divergence should be weaker. The second approach was a meta-analysis of many studies comparing the larval performance of amphibians under controlled temperatures in the laboratory. These studies sampled populations from multiple source locations that differed in their environmental temperature. We predicted that adaptive clinal variation would be most pronounced in studies that sampled very shallow gradients. The results of both comparisons demonstrated that gene flow can reduce population divergence by 70%–90% on the steepest climate gradients, strongly supporting models of adaptation with gene flow along spatial environmental gradients.

Methods

Common-Garden Experiment

The experiment included *Rana temporaria* collected from 12 breeding ponds in eastern Switzerland, ranging in elevation from 445 to 2,542 m (fig. 2*A*; table A1; tables A1–A6 are available online). There were no strong elevational trends in the surface area of source ponds, depth, or the number of egg clutches. Although the most distant ponds were located 102 km apart, this region has a steep elevational gradient: distances from the four lowest elevation sites to the nearest known high-elevation population of *R. temporaria* (>2,000 m) were never greater than 8.5 km. Populations above 1,000 m were situated on three different mountains separated by low valleys, which suggests that these higher sites were colonized independently from lower elevations after glacial retreat. Therefore, although the experiment includes just a single "replicate" elevational transect, potentially independent instances of colonization may confer some degree of generality.

The laboratory experiment exposed tadpoles from the 12 source populations to a 2 × 2 complete factorial design, with 10 families per population and two replicate tadpoles per family and treatment. The experimental factors were temperature and the presence or absence of predator cues. We chose these factors because they were expected to vary across the elevational gradient in the Alps. Indeed, air temperature during the larval period of *R. temporaria* averages about 5°C lower at an elevation of 2,500 m than at 500 m (fig. A1*G*; figs. A1, A2 are available online). We used data loggers to measure water temperature in 23 ponds over the same elevational range and found a decline of 4.2°C

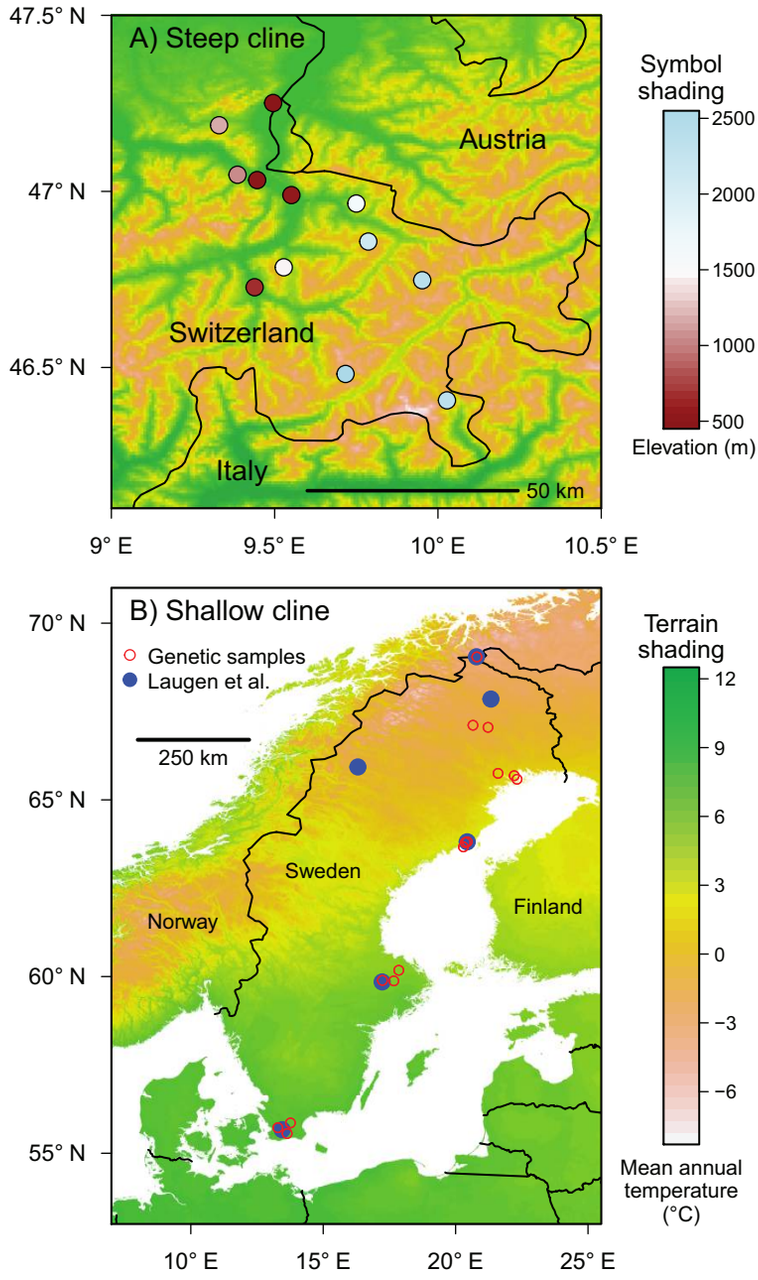

Figure 2: Locations of populations of *Rana temporaria* sampled along elevational (*A*) and latitudinal (*B*) gradients, superimposed on a color-contoured map representing mean annual temperature. The symbols in *A* are color coded to reflect the elevation of the site (ranging from 445 to 2,542 m). *B* shows the six sites included in the laboratory experiment of Laugen et al. (2003, 2005) and the 15 populations that contributed data on single-nucleotide polymorphism variation.

on cloudy days during the larval period between 500 and 2,500 m; on sunny days water temperature was warm even at high elevation (fig. A1*H*). We anticipated that predation risk would decline with elevation, as has been reported in many previous studies (de Mendoza and Catalan 2010; Roslin et al. 2017; Moreira et al. 2018; Hargreaves et al. 2019). But survey data collected after the experiment was underway suggested that predator density was not strongly associated with elevation in our study area (fig. A1*B*). Hence, the predictions discussed in the introduction are mainly relevant for adaptation to temperature; results from the predator treatment are included here for the sake of completeness and to improve estimation of statistical error.

Tadpoles were reared individually in 200-mL cups that floated in large plastic tubs in a windowless room. There were 32 tubs, each containing 80 L of water and a maximum of 39 tadpoles in a 5 × 8 grid (one position was occupied by a device to regulate water level). The photoperiod was 13L : 11D.

Nominal temperatures were 15° and 21°C. These temperatures realistically reflect midafternoon water temperature in shallow areas where tadpoles spend much of their time (fig. A1*H*), and the duration of the larval stage in our experiment was similar to that observed in nature (Loman 2002; Laugen et al. 2003). We controlled water temperature with a flow-through system in which four reservoirs were maintained at constant temperature using aquarium heaters and water coolers. There were two reservoirs per temperature treatment; from each reservoir, water was pumped into eight of the 80-L plastic tubs arranged in random positions around the room. Five data loggers recorded temperature at 20-min intervals within each treatment, and this confirmed that actual temperatures were 15.0°C (SD, 0.16) and 21.4°C (SD, 0.46). Eighty percent of all readings were between 14.94° and 15.18°C in the cold treatment and between 21.09° and 21.68°C in the warm treatment.

Apparent predation risk was manipulated by adding water with or without chemical cues of predation to every cup three times per week. Water containing predator cues was prepared by feeding a 100-mg *R. temporaria* tadpole to each of three late-instar dragonfly larvae (*Aeshna cyanea*), kept individually in 5-L containers. Immediately after the *Aeshna* fed, we combined water from the three containers and added 20 mL to each cup containing a tadpole assigned to the predator treatment. This created a concentration of 6 mg tadpole L^{-1} consumed per week. Cups in the predator-free treatment received 20 mL of aged tap water. To minimize the chance of spilling the wrong water into a cup, we assigned predator treatments at random to entire rows of five cups within tubs.

We collected 10 freshly laid clutches of *R. temporaria* in each of the 12 source populations. Collection dates ranged from March 21 to June 18, 2014, following the progression of breeding phenology with increased elevation (fig. A1*D*). Eggs were held at 14°C in the laboratory for about 10 days until they hatched. Tadpoles were added to the experiment when they reached Gosner (1960) developmental stage 25, at an average of 5.5 days after hatching. The experiment contained 960 individuals: 2 temperatures × 2 predator treatments × 12 populations × 10 sibships × 2 replicates.

We fed the tadpoles 15% of their mass per day on a finely ground 4 : 1 mixture of rabbit food and TetraMin fish flakes, and we changed the water three times per week. Tadpoles were weighed and returned to their cups when they were at about Gosner stage 35, which occurred at an average age of 32 days (SD, 9.7; range, 20–51 days [depending on temperature]). We calculated proportional growth rate until stage 35 as $\exp[\log(m_2/m_1)/t]$, where m_1 is the mass at the start, m_2 is the mass at stage 35, and t is the number of days from the start to weighing. When an individual reached metamorphosis—defined as emergence of the first forelimb (stage 42)—it was removed from the cup and weighed. We calculated development rate (stages day^{-1}) as $(42 - 25)/t$, where t is the number of days between stages 25 and 42. These measurements assume that early growth rate is exponential and development rate is linear, both of which are approximately correct (Alford and Jackson 1993; Van Buskirk 2002; Muenst 2015).

Results were analyzed with mixed effects models using restricted maximum likelihood to test the effects of experimental treatments and elevation of origin on growth rate, development rate, survival to metamorphosis, and log-transformed mass at metamorphosis. Elevation was centered and treated as a continuous fixed effect at the level of the population. Categorical fixed effects were temperature at the level of the tub and predator treatment at the level of the individual tadpole. All interactions were included. Random intercepts were included for tub, population, and sibship nested within population. We judged the significance of random effects using likelihood ratio tests after refitting models using maximum likelihood. Because the random effect of tub was never important, fixed effects were evaluated using models without tub. We determined the significance of fixed effects by inspecting 95% profile likelihood confidence intervals (Venzon and Moolgavkar 1988). Models were fitted using the lme4 package in R version 3.3.2 (Bates et al. 2015).

Comparison with the Latitudinal Gradient

We compared results from the Swiss elevational gradient with those of Laugen et al. (2003, 2005), who performed a laboratory experiment on five to six populations of *R. temporaria* collected from 55.7°N to 69°N along a latitudinal gradient in Fennoscandia (fig. 2*B*). Laugen et al. used methods similar to ours: tadpoles were held in individual cups under three temperatures (14°, 18°, 22°C), food was a 3 : 1 mixture of rabbit food and fish flakes, and the light regime was 16L : 8D. Laugen et al. did not include a predator treatment and we did not have an 18°C treatment, so these two treatments were not used here.

The comparison between the elevational and latitudinal gradients and among other climate gradients elsewhere in this study was accomplished by expressing each gradient on a common scale corresponding to the duration of the growing season at the source populations. On the Swiss and Fennoscandian gradients, growing season varies linearly with elevation and latitude, yielding a lapse

rate of 92 m of elevation per degree of latitude (fig. A1*A*). Other comparative measures of climate are available, but we used the duration of the growing season for several reasons. First, in various organisms the amount of time available is known to impose selection favoring rapid development (Rowe and Ludwig 1991; Conover et al. 2009; Sniegula et al. 2012). Second, *R. temporaria* behave as if they are sensitive to the duration of the growing season. For example, on both gradients the date of oviposition shows a very similar linear and negative relationship with growing season (fig. A1*D*). This is presumably caused by the sensitivity of breeding phenology to the date of snowmelt, which is related to local temperature and season length (Beebee 1995; Phillimore et al. 2010). And third, the upper distribution limits of plants on latitudinal and elevation gradients are strongly correlated with each other and with season- and temperature-related measures, with some indication that distributions extend into slightly colder environments on elevation than on latitude (Halbritter et al. 2013; Randin et al. 2013). This information suggests that adaptation may respond specifically to season length, although other environmental variables may be associated with season length. We defined the duration of the growing season as the number of days with a mean air temperature exceeding the developmental zero temperature, T_{DZ} (the temperature below which development ceases). Below, we review several studies suggesting that T_{DZ} averages 6.4°C in larval *R. temporaria*. Spatial temperature data for the elevational gradient came from Gugerli et al. (2008) at 200-m resolution, and those for the latitudinal gradient came from WorldClim version 2 (Fick and Hijmans 2017) at 1-km resolution. Growing season length was highly correlated with both elevation ($r = -0.988$) and latitude ($r = -0.987$; fig. A1*E*).

We used linear models to compare mass at metamorphosis and development rate between the two gradients. The unit of observation was the treatment mean; for Laugen et al. (2003, 2005), these values came from figures and tables because the original data were not available. Independent variables were the length of the growing season of the source population (centered), the identity of the gradient (latitude or elevation), the temperature treatment (14/15° or 21/22°C), and their interactions. We did not compare growth rates between gradients because the only available measure of growth along the latitudinal gradient comparable to our measure was made at 18°C (Lindgren et al. 2018).

Estimating Gene Flow

We indirectly inferred rates of gene flow across both gradients by measuring population divergence using genomic single-nucleotide polymorphisms (SNPs). Our sample of the elevational gradient was the 12 populations from the common-garden experiment, collected in 2013 (fig. 2*A*; table A1): these were part of a larger set of 82 populations sampled across all of Switzerland (Jansen van Rensburg 2018). The latitudinal gradient was sampled in 2014 and included 15 sites between 55.6°N and 69°N; these broadly represented the same gradient studied by Laugen et al. (2003), and four sites were coincident or nearly so with populations used by Laugen et al. (fig. 2*B*; table A2). One egg from each of 20 clutches was collected at each site; tadpoles were reared in the laboratory and preserved in ethanol when they reached stage 36. We implemented a modified version of the double-digest restriction site–associated DNA protocol (Peterson et al. 2012), described in the appendix (available online), to discover genome-wide SNP markers. The variant file was filtered to maximize the number of individuals and loci in the data set while minimizing spurious SNPs and loci that may be under selection or that deviate from expectations such as Hardy-Weinberg equilibrium. We obtained 1,827 SNPs genotyped in 148 individuals in the 12 Swiss populations (7–20 individuals per population; median, 10) and 2,081 SNPs in 132 individuals in the 15 Fennoscandian populations (2–17 per population; median, 9).

We used the slope of the relationship between genetic divergence and distance to compare the extent of gene flow in Switzerland and Fennoscandia with respect to geographic space (measured in kilometers) and the environmental gradient (measured as difference in the duration of the growing season, defined above). Population divergence with respect to pairwise geographic or environmental distance was measured using linearized F_{ST}: $F_{ST}/(1 - F_{ST})$. Rousset (1997) demonstrated that b, the slope of the regression of linearized F_{ST} against log distance, is proportional to $1/N_e\sigma^2$, where N_e is the average effective population size and σ is the average natal dispersal distance (i.e., the distance between the positions at which parents and their offspring reproduce). Using F_{ST} to reflect population divergence is reasonable in this instance because the mutation rate of SNPs is low ($<10^{-8}$ in various taxa; Schrider et al. 2013; Krasovec et al. 2018) and the demographic history of *R. temporaria* within Switzerland and Fennoscandia is relatively homogeneous (Teacher et al. 2009; Jansen van Rensburg 2018). Our SNP data revealed mostly weak geographic trends within regions: genetic diversity declined weakly with elevation and latitude, and N_e showed no trend (table A3; fig. A1*C*). These are conditions under which F_{ST} provides a relatively interpretable measure of population structure (Whitlock 2011). However, we caution that violation of assumptions related to mutation, dispersal, and demography can bias the extent of gene flow implied by F_{ST} (Whitlock and McCauley 1999). In spite of this,

Rousset's (1997) isolation-by-distance method for estimating effective migration distance has been shown to be robust to violations of many of these assumptions (Rousset 2001; Sumner et al. 2001; Leblois et al. 2003, 2004; Robledo-Arnuncio and Rousset 2010).

Weir and Cockerham's (1984) F_{ST} was estimated with the hierfstat package in R (Goudet 2005). We used Mantel correlograms with 9,999 permutations (function mantel.correlog in the vegan package; Legendre and Legendre 2012, ch. 13) to evaluate the significance of relationships between linearized F_{ST}, calculated for each pair of populations, and both the log of the geographic distance between them and the difference in growing season length. We estimated N_e in Fennoscandia and Switzerland using the single-sample linkage disequilibrium method LDNe, implemented in NeEstimator version 2.1 (Do et al. 2014). Details are given in figure A1. We then compared average dispersal distance between gradients by combining information on N_e and the slope of the isolation-by-distance relationship, b: $\sigma_{fenn}/\sigma_{switz} = \text{sqrt}[(N_{e(switz)} \cdot b_{switz})/(N_{e(fenn)} \cdot b_{fenn})]$. The analogous comparison of dispersal with respect to growing season was made using information on N_e and the slope of linearized F_{ST} regressed against the difference in growing season duration.

Meta-analysis

We used meta-analysis to compare our results with the broader literature on amphibian population divergence along temperature gradients. The hypothesis was that countergradient variation in development rate will be less apparent when the environmental gradient is steep. We located relevant literature by searching the Web of Science for "temperature" and "population*" and "amphibian* or tadpole*"; studies were included if they (1) sampled eggs from at least two populations occupying different thermal environments, (2) reared larvae under at least two constant-temperature treatments, (3) reported either of two measures of larval performance (size at metamorphosis [mass or body length] or development rate to metamorphosis [stages day^{-1}]), and (4) reported variation in larval performance among replicates within temperature treatments. We did not analyze larval growth rate because it was available for only four studies (Olsson and Uller 2002; Meier 2007; Lindgren and Laurila 2009; this study). The inclusion criteria yielded 19 studies for which we could evaluate evidence of counter- or cogradient variation: phenotypic plasticity came from comparison of temperature treatments, and population genetic divergence came from comparison of multiple populations of known origin reared under common environmental conditions.

We characterized the environmental gradient sampled by each study from the duration of the growing season at the source localities of the populations, calculated as described above using the species-specific developmental zero temperature (T_{DZ}) and WorldClim temperature data. T_{DZ} was calculated as $-a/b$ averaged across all studies of the same species, where a and b are the intercept and slope from a regression of development rate against experimental temperature. The effective steepness of the environmental gradient in each study was the maximum difference in growing season divided by the geographic distance between the pair of populations with the greatest difference in growing season. The appendix describes how we estimated the environmental gradient for studies of adaptation to microgeographic variation in water temperature (Orizaola and Laurila 2009; Richter-Boix et al. 2010, 2015; Edge et al. 2013).

The two measures of larval performance were analyzed within a mixed effects meta-analytic framework, in which observations i in study j were modeled as

$$y_{j,i} = \mu + \text{covariates} + s_j + e_{j,i} + m_{j,i}, \qquad (1)$$

where y is larval performance, μ is the intercept, s are study-level deviations, e are observation-level residuals, and m are measurement errors. The random effect of study modeled potential covariance among multiple observations from the same study. Measurement error was assumed to be proportional to a diagonal matrix of the standard deviation of the performance measure among replicates. In this case, measurement error includes any source of deviation at the level of the replicate, including variation due to genotype, spatial position within the experiment, and measurement itself (Ives et al. 2007). The fixed covariates were temperature, growing season length in the source locality, the effective steepness of the geographic temperature gradient, the temperature-by-season interaction, and the growing season–by–steepness interaction. The latter interaction tests our central hypothesis that the extent of population divergence depends on steepness of the gradient. The steepness of the gradient was log transformed because it showed a right-skewed distribution and because a unit change in the geographic length of a gradient creates a greater change in steepness for the steepest gradients. We scaled performance measures to comparable units by dividing by the standard deviation among replicates pooled at the level of the study. Experimental temperature, growing season, and steepness were centered. The models were fitted with Markov chain Monte Carlo techniques implemented in the R package MCMCglmm (Hadfield 2010); equation (1) is model 5 from the online appendix of Hadfield and Nakagawa (2010). Parameter estimates and their credible intervals came from 2,000 essentially independent samples from the posterior distribution.

A preliminary analysis compared four possible structures of the study-level random effect: no study, study

intercepts only, intercepts along with heterogeneity among studies in slopes on season length, and the latter model including the covariance between slopes and intercepts (i.e., unstructured variance-covariance matrix). For both performance measures, the deviance information criterion (Spiegelhalter et al. 2002) indicated that the best-supported model was that with only study intercepts and parallel slopes. Hence, we evaluated the importance of fixed effects using equation (1).

All data and analysis scripts used in this study are available in the Dryad Digital Repository (https://doi.org/10.5061/dryad.41ns1rn96; Bachmann et al. 2020).

Results

Common-Garden Experiment

Growth rate of *Rana temporaria* tadpoles early in the larval period was higher in the warm treatment (fig. 3*A*; table 1). Populations originating from high elevation tended to grow more slowly, although the confidence interval for the effect of elevation included zero (−3.296 to 0.0001). The direction of this trend indicated cogradient variation (i.e., the opposite of countergradient variation; Conover et al. 2009). A negative interaction between elevation and temperature indicated that high-elevation populations enjoyed less benefit from warm conditions than did low-elevation populations, although figure 3*A* suggests that this effect was small. Early growth rate was not influenced by the predator treatment.

Survival to metamorphosis averaged 89% (fig. 3*B*). Survival was affected by a temperature-by-predator interaction: warm conditions caused a reduction in survival of about 7% in the predator treatment but a slight increase in the predator-free treatment. Survival was not significantly related to the elevation of origin or interactions involving elevation.

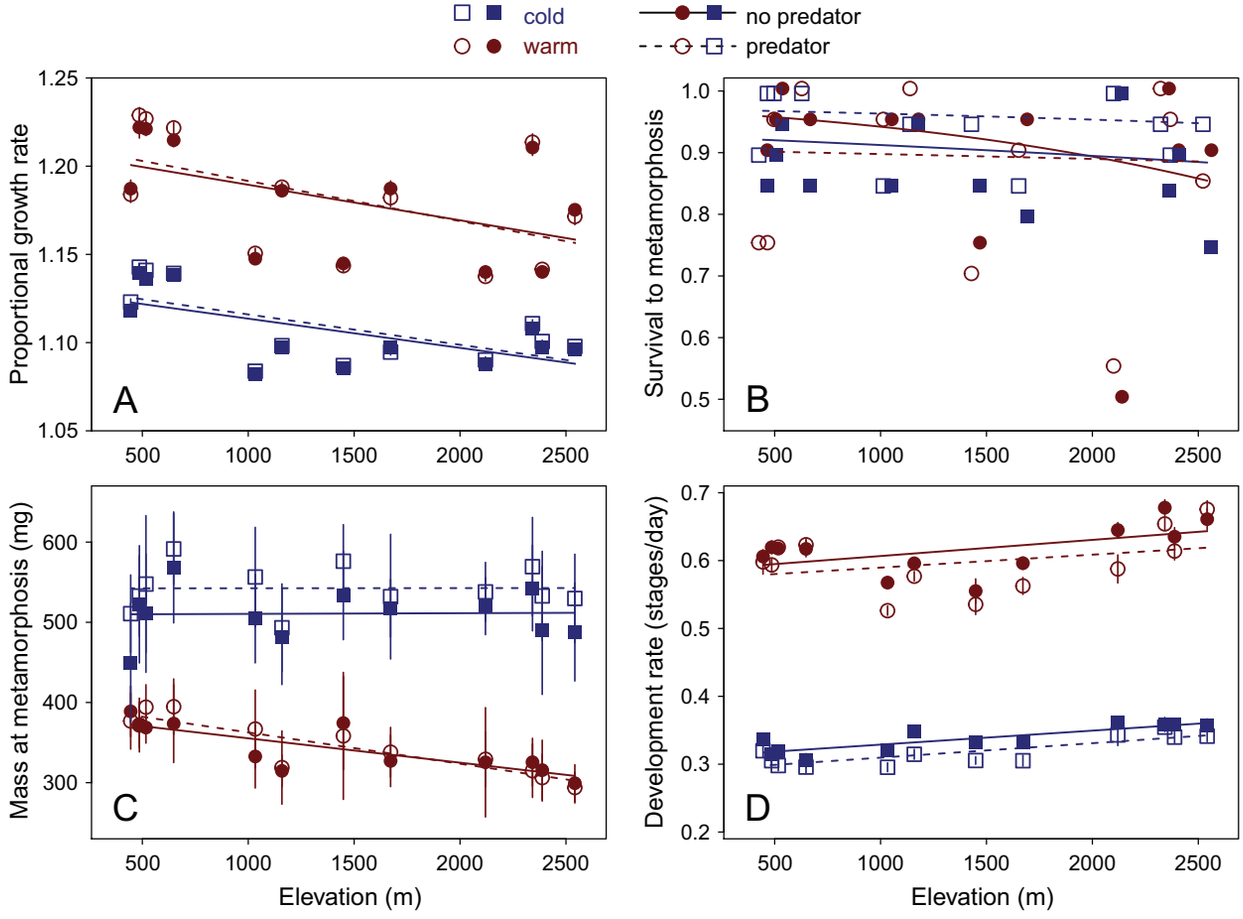

Figure 3: Early tadpole growth rate (*A*), survival to metamorphosis (*B*), mass at metamorphosis (*C*), and development rate (*D*) in the laboratory experiment of *Rana temporaria* originating from populations at different elevation. Blue symbols are population means for the cool treatment (15°C), and red is the warm treatment (21°C). Open symbols and dashed lines represent the predator treatment. In *B*, symbols are offset to improve visibility, and lines show fitted curves from the model in table 2. Error bars (±1 SE) are sometimes obscured by the symbols.

Table 1: Summary of mixed effects models on four measures of tadpole performance in the laboratory experiment

		Dependent variable			
	Level	Early growth rate (proportional)	Survival (proportion)	Mass at metamorphosis (mg)	Development rate (stages day^{-1})
Fixed effects (SE):					
Elevation	…	−1.648 (.849)	−.203 (.341)	.345 (2.020)	**2.230 (.852)**
Temperature	Warm	**7.527 (.112)**	.263 (.310)	**−40.069 (1.282)**	**27.943 (.391)**
Predation	Present	.199 (.111)	**.909 (.353)**	**6.030 (1.262)**	**−1.961 (.385)**
Elevation × temperature	Warm	**−.304 (.147)**	−.458 (.406)	**−9.448 (1.671)**	.067 (.509)
Elevation × predation	Present	−.076 (.145)	−.044 (.466)	−.470 (1.648)	−.117 (.502)
Temperature × predation	Warm, present	−.111 (.159)	**−1.295 (.467)**	**−5.022 (1.800)**	.113 (.548)
Elevation × temperature × predation	Warm, present	−.244 (.208)	.621 (.614)	−1.794 (2.345)	−.316 (.715)
Random effects (LR statistic):					
Population	…	**4.98e−4 (1060)**	**2.67e−2 (10.0)**	**3.45e2 (45.8)**	**3.93e−4 (114)**
Sibship (population)	…	**3.37e−5 (64.7)**	**6.57e−1 (8.57)**	**3.64e2 (18.1)**	**2.39e−4 (31.0)**

Note: Entries in the table are the coefficient with standard error in parentheses (for fixed effects) and the variance component with the likelihood ratio (LR) statistic on 1 df in parentheses (for random effects). Coefficients for early growth rate, mass, and development rate were multiplied by 100. Mass at metamorphosis was log transformed before analysis. Elevation was measured in kilometers and centered. Boldface highlights effects for which the 95% profile confidence interval did not overlap zero (fixed effects) or the LR test was significant at $\alpha = .05$ (random effects).

Mass at metamorphosis was on average 53% smaller in the warm treatment than in the cold treatment (fig. 3*C*; table 1). Exposure to predator chemicals caused mass to increase by about 5%, and the temperature-by-predator interaction reflected a larger predator-induced increase in body size at cold temperature. An interaction between elevation and temperature arose because body size declined with elevation at 21°C but not at 15°C. This pattern, too, suggested cogradient variation, although the main effect of source elevation was not significant.

Tadpole development rate increased by 85% in the warm treatment relative to cold, and predator cues caused a decrease of about 4% (fig. 3*D*). Tadpoles from high-elevation populations developed significantly faster than those from low-elevation populations. This is countergradient variation. For development rate and all other traits, there was significant variation among populations and full-sib families within populations (table 1).

Comparison with the Latitudinal Gradient

The range in duration of the growing season was greater on the elevational gradient (40–226 days) than on the latitudinal gradient (90–195 days; figs. 4, A1*A*). For mass at metamorphosis, there was no overall trend with, nor interactions involving, growing season length (table 2). For development rate, a strong three-way interaction among growing season duration, gradient, and temperature was caused by variation in slopes against growing season (fig. 4*B*). Population divergence in development rate was steeper with latitude in the warm treatment. The overall effect of growing season was negative: tadpoles from colder climates developed more rapidly along both gradients. This indicates countergradient variation in both studies. The results in figure 4*B* were therefore similar to the prediction illustrated in figure 1. Slope estimates for the latitudinal gradient were 2.8 times more negative than those for the elevational gradient at cold temperature and 9.5 times more negative at warm temperature (latitude vs. elevation [$\times 10^{-4}$]: −6.51 vs. −2.36 for cold, −26.92 vs. −2.83 for warm). Confidence intervals did not include zero for any of the four estimates evaluated separately. The strong difference between gradients in table 2 is not meaningful because experimental conditions, including containers, food, and temperature treatments, were not identical in the two studies.

Estimates of Gene Flow

The matrices of pairwise F_{ST} values for the Swiss and Fennoscandian populations are in tables A4 and A5. Isolation by distance was significant in both Fennoscandia and Switzerland: Mantel correlograms revealed significant positive spatial autocorrelation in $F_{ST}/(1 - F_{ST})$ over shorter distances (fig. 5*B*). The slopes of the regressions of $F_{ST}/(1 - F_{ST})$ against log distance were similar in Fennoscandia ($b = 0.0398 \pm 0.0224$ jackknifed SD) and Switzerland ($b = 0.0513 \pm 0.0574$ SD; fig. 5*A*). Effective population size estimated by LDNe, $\hat{N}_e$, did not significantly differ between Fennoscandia ($\hat{N}_e = 291$; table A2) and Switzerland ($\hat{N}_e = 231$ for all 82 populations; $\hat{N}_e = 388$ for the populations used in the experiment; table A1). $\hat{N}_e$ did not vary significantly with elevation or latitude (details in

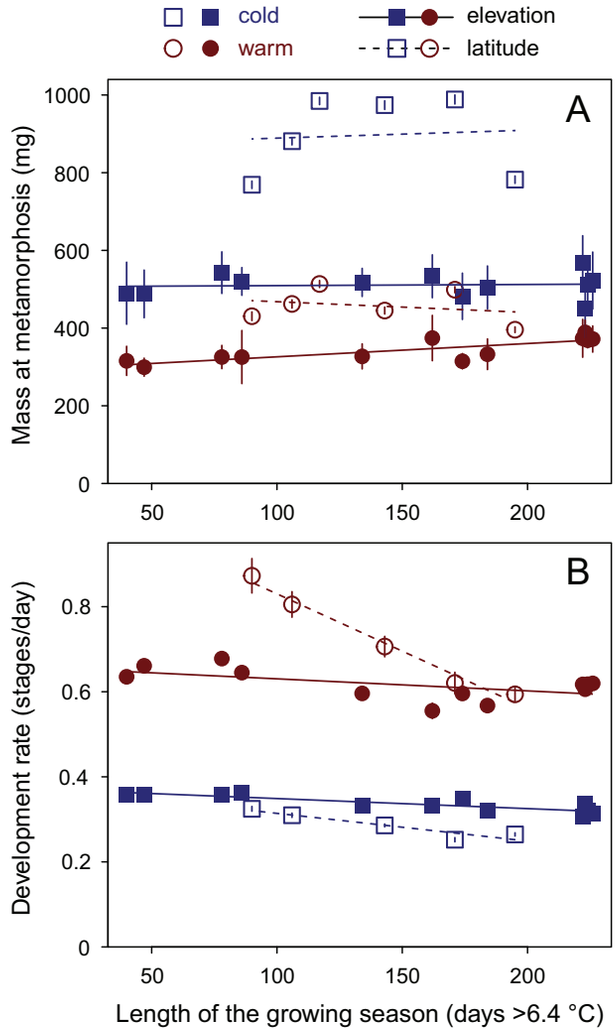

Figure 4: Comparison of population divergence of *Rana temporaria* in tadpole mass at metamorphosis and development rate, from across gradients in elevation (filled symbols; this study) and latitude (open symbols; Laugen et al. 2003, 2005). Both gradients are expressed in units of growing season duration, defined as the number of days per year with an average temperature >6.4°C. A long growing season occurs at low latitude or elevation. Blue symbols and lines are the cool treatment (14°C), and red is the warm treatment (21° or 22°C). Error bars (±1 SE), which are sometimes obscured by the symbols, were calculated over sample sizes of ~400 individuals (*A*) or eight sibships (*B*) for latitude and of 10 sibships for elevation.

fig. A1*C*). These values of $\hat{N}_e$ are somewhat higher than published estimates for most anurans (Schmeller and Merilä 2007; Phillipsen et al. 2011), and they imply that natal dispersal distance averages 63.2 m in Switzerland and 82.8 m in Fennoscandia.

Divergence along the axis of growing season duration was highly significant on the latitudinal gradient but not on the elevational gradient (fig. 5*C*, 5*D*). This difference was due to the geography of the two gradients: in Fennoscandia, growing season length is highly spatially autocorrelated up to about 500 km, whereas positive spatial autocorrelation is weaker and extends ≤30 km in Switzerland (fig. A2). As a result, the slope of $F_{ST}/(1 - F_{ST})$ regressed against the difference in growing season length was 20 times steeper on the latitudinal gradient ($b = 1.82 \times 10^{-3}$) than on the elevational gradient ($b = 9.12 \times 10^{-5}$). After accounting for the values of $\hat{N}_e$ in Fennoscandia and Switzerland, gene flow along the environmental gradient of season length was estimated to be 3.9 times higher in Switzerland than in Fennoscandia.

Meta-analysis

Meta-analysis supported the hypothesis that adaptive population divergence is weaker on steep climate gradients. Across all studies listed in table 3, animals from populations in warmer climates exhibited strongly reduced development rate and smaller body size at metamorphosis (fig. 6; table 4). This represented countergradient variation in the case of development because temperature-induced plasticity was positive. In other words, evolved divergence in development compensated for phenotypic plasticity induced by environmental temperature. But in the case of size (fig. 6*A*), the decreasing trend along the gradient in growing season length represented cogradient variation because the induced response to temperature was in the same direction. Both measures of performance had significant interactions between season length and the steepness of the gradient: that is, the slopes in figure 6 were less negative for studies that sampled steeper environmental gradients. For development rate, this finding indicates that countergradient variation, while significant overall, was weaker when measured along steep climate gradients (compare fig. 6*B* with fig. 1). The temperature–by–season length interaction, also significant for both size and development rate, reflected higher detectability of population divergence at warm experimental temperatures (fig. 6; table 4). Variance components due to measurement error and study were always important.

Discussion

Our results support the prediction that quantitative genetic divergence along environmental gradients should be most pronounced on shallow gradients. This prediction comes from classic theory describing the balance between the spatial change in selection and the spatial extent of gene flow (Haldane 1948; Antonovics 1968; Slatkin 1973; Endler 1977; Kirkpatrick and Barton 1997; Lenormand 2002). Many empirical studies illustrate that gene flow erodes the influence of local selection (e.g., Storfer and Sih 1998; Hendry

Table 2: Comparison of body size and development rate measured at metamorphosis in *Rana temporaria* tadpoles sampled along an elevational gradient in Switzerland (this study) and a latitudinal gradient in Fennoscandia

		Mass at metamorphosis			Development rate		
Source of variation	Level	Coefficient	SE	*P*	Coefficient	SE	*P*
Growing season length (days)		.0293	.2181	.894	**−2.36e−4**	**.91e−4**	**.016**
Gradient	Latitude	**387.55**	**27.18**	**.001**	**−5.38e−2**	**1.16e−2**	**.001**
Temperature	Warm	**−170.41**	**21.25**	**.001**	**2.79e−1**	**8.84e−3**	**.001**
Growing season × gradient	Latitude	.1759	.6122	.776	−4.15e−4	2.63e−4	.127
Growing season × temperature	Warm	.3133	.3085	.319	−4.70e−5	1.29e−4	.719
Gradient × temperature	Latitude, warm	**−273.92**	**38.44**	**.001**	**1.44e−1**	**1.64e−2**	**.001**
Growing season × gradient × temperature	Latitude, warm	−.7946	.8658	.367	**−1.99e−3**	**3.73e−4**	**.001**

Note: Both gradients were expressed on a scale of growing season length, measured in days and centered before analysis. Temperatures in the cold/warm treatments were 15°/21.4°C in Switzerland and 14°/22°C in Fennoscandia. This analysis was done on mean values at the level of population and temperature treatment.

and Taylor 2004; Bridle et al. 2009; Tack and Roslin 2010; Kalske et al. 2016), but earlier evidence that gene flow can moderate the slope of a cline along a spatial environmental gradient is more limited (Bridle et al. 2009; Levy and Nufio 2015). Our study supports this prediction in two ways. First, we observed stronger countergradient variation in larval development rate among frog populations occupying a long, shallow latitudinal gradient in Fennoscandia than along a short, steep elevational gradient in Switzerland (fig. 4). Second, a meta-analysis of 19 studies found that this pattern is widespread in anurans: countergradient variation in larval development rate was more pronounced on shallow climate gradients (fig. 6). These findings suggest that presumably adaptive divergence on steep gradients is eroded by gene swamping due to frequent dispersal among distinct environments.

The intuitive explanation of our results is that dispersing individuals along shallow geographic gradients settle in an environment that is not very different from that in which they originated. On steeper gradients, dispersing individuals will frequently move into new environments, and this counteracts local adaptation by importing genotypes adapted to other conditions (Keller et al. 2013). This explanation is closely related to Levins's (1968) concept of environmental grain, which also emphasizes the scale of environmental variation relative to the mobility of the organism. On a continuous spatial environmental gradient, two length scales are relevant (Slatkin 1973). One is called the "characteristic length" of the cline ($l_c = \sigma/s^{1/2}$), which weighs the average gene flow distance (σ) relative to the total difference in selection between opposite ends of the gradient (s). A population cannot adapt precisely to changes in the local phenotypic optimum that occur on a spatial scale less than l_c. Our comparison between the Fennoscandian and Swiss gradients suggests that l_c may be twice as large on the long latitudinal gradient as on the short elevational gradient, assuming that the difference in selection across a gradient is related to its amplitude of environmental change. The latitudinal gradient had somewhat greater estimated gene flow distance, and the elevational gradient had somewhat greater environmental amplitude. The second length scale is the spatial distance over which the change in selection occurs (k). This value is much larger for a long latitudinal gradient than for a short elevational gradient. For large values of k, adaptation closely follows the optimum and gene flow is not important, especially toward the center of the gradient (Fisher 1950; Slatkin 1973). But for smaller values of k, gene flow prevents local demes from tracking their local selection regime, and they instead adapt to environmental conditions averaged over a broader range of the spatial gradient. Hence, populations adapting to a steep gradient (small k) should exhibit shallow phenotypic clines and suffer maladaptation over much of the gradient (Slatkin 1973). A similar prediction emerges from models of quantitative traits, in which a critical parameter B balances gene flow against the steepness of the environmental gradient and hence combines information from Slatkin's two length scales (Kirkpatrick and Barton 1997; Polechová and Barton 2015; Polechová 2018). In our study, the much smaller value of k in Switzerland suggests that gene flow should keep *R. temporaria* populations on the elevational gradient away from their local optima, and indeed the slope of the cline in development rate was shallower on the elevational gradient in Switzerland (fig. 4). Assuming that populations in Fennoscandia are closely tracking the phenotypic optimum, those in Switzerland show maladaptive values of development rate over at least part of the gradient. Measured against the common scale of growing season length, population divergence on the elevational gradient was reduced by 64% (cold treatment) or 89% (warm treatment).

The preceding argument about length scales of the two gradients is not greatly affected by our finding that estimated effective population size was slightly higher in

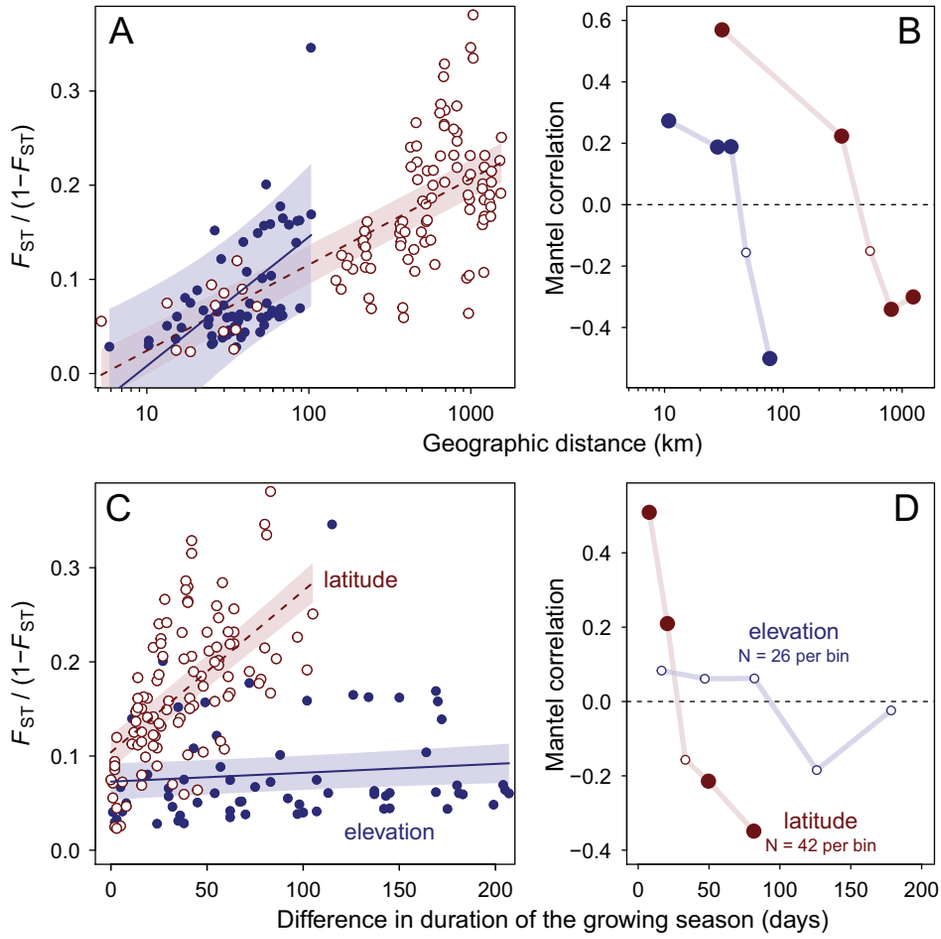

Figure 5: Patterns of population divergence at single-nucleotide polymorphism markers with respect to geographic distance and the difference in growing season length. *A* depicts isolation by distance among the 12 experimental populations of *Rana temporaria* on the elevational gradient in Switzerland (blue symbols) and 15 populations on the latitudinal gradient from southern Sweden to northern Finland (red symbols). Each point represents a pair of populations. Lines are major axis regressions, and shading represents 95% confidence intervals estimated by jackknifing over populations. *B* is the spatial correlogram of genetic divergence relative to distance, with pairs of populations sorted into five bins of equal sample size. The Mantel correlation is a Pearson correlation coefficient between the above-diagonal elements of two matrices representing genetic distance and geographic distance ($F_{ST}/[1 - F_{ST}]$ and the log of great circle distance). Significant correlations, indicated with larger symbols, were judged by permuting rows and columns of the genetic divergence matrix 9,999 times and controlling the false discovery rate for five tests (Benjamini and Hochberg 1995). *C* and *D* illustrate population divergence with respect to the difference in the length of the growing season between sites (measured in days >6.4°C). Gene flow among environmentally divergent sites was greater across elevation than across latitude.

Switzerland than in Fennoscandia. The 30% difference in $\hat{N}_e$ lowers the estimate of dispersal distance in Switzerland, and therefore l_c is somewhat shorter. But the great length of the Fennoscandian gradient nevertheless ensures that k, the distance over which selection changes, is at least 100 times greater for latitude than for elevation. This suggests that estimated gene flow among distinct selective environments would be much higher in Switzerland even if the difference between gradients in $\hat{N}_e$ were substantially greater. This accounts for the shallower cline observed on the elevational gradient.

The meta-analysis produced a quantitatively similar picture of the consequences of gene flow on steep climate gradients. Based on the statistical model in table 4, the predicted slope of development rate against length of the growing season at the source locality is −0.0345 SD units °C^{-1} (highest posterior density interval: −0.0457 to −0.0236) for the shallowest environmental gradients in the data set. These were primarily long latitudinal or longitudinal gradients (Laurila et al. 2008; Orizaola et al. 2010; Edge et al. 2013; Liess et al. 2013). The model predicts that the corresponding slope for a gradient as steep as the

Table 3: Common-garden experiments that manipulated temperature and compared populations sampled across gradients in environmental temperature

Species	T_{DZ} (°C)	No. populations	Maximum geographic extent (km)	Maximum season difference (days)	Steepness (days/km)	Temperature treatments (°C)	Traits	Source
Bufo calamita	15.5	6	56.1	6.0	.147	20/27	Development rate, size	Rogell et al. 2010
Rana arvalis	4.9	7	35.6	32.8[a]	1.058	15/20	Development rate, size	Richter-Boix et al. 2015
		13	1,638.2	94.0	.058	16/19	Development rate, size	Luquet et al. 2019
Rana clamitans	15.55	4	217.5	45.0	.207	18/23/28	Development rate, size	Berven et al. 1979
Rana lessonae	10.25	3	5.2	14.2[a]	3.186	20.3/25.4	Development rate, size	Orizaola and Laurila 2009
		8	901.1	31.0	.035	19/22/26	Development rate, size	Orizaola et al. 2010
Rana sylvatica	10.0	9	218.2	52.0	.239	13–25[b]	Development rate, size	Berven 1982
	10.0	8	1,408.6	55.0[a]	.039	20/22/24	Development rate, size	Edge et al. 2013
Rana temporaria	6.4	2	1,412.6	90.0	.064	14/22	Development rate, size	Merilä et al. 2000
		2	1,531.2	106.0	.069	16/20	Development rate, size	Laurila et al. 2001
		2	123.8	68.0	.549	14/19.7	Development rate, size	Sommer and Pearman 2003
		6	1,534.7	105.0	.068	14/18/22	Development rate, size	Laugen et al. 2003, 2005
		7	1,513.0	106.0	.071	15/18	Development rate, size	Laurila et al. 2008
		8	1,471.6	95.0	.067	15/18	Size	Lindgren and Laurila 2009
		6	224.7	31.6[a]	1.711	15.2/18.3	Development rate, size	Richter-Boix et al. 2010
		8	503.0	39.0	.078	18/23	Development rate, size	Liess et al. 2013
		10	49.6	103.0	2.083	10/15/20	Size	Muir et al. 2014
		2	2.7	19.0	6.970	16/20	Development rate, size	Oromi et al. 2015
		12	102.4	186.0	3.734	15/21.4	Development rate, size	This study

Note: T_{DZ} is the temperature below which development rate is zero. T_{DZ} was averaged at the level of the species from the studies listed in this table. Maximum geographic extent is the great circle distance between the two most distant populations. Maximum season difference is the difference in length of the growing season (number of days with mean temperature above T_{DZ}) between the two most environmentally distinct populations. Steepness is the maximum season difference divided by the geographic distance between the two most environmentally distinct populations.

[a] Variation in length of the growing season was estimated from local average water temperatures reported in the article (see the appendix).

[b] Six temperature treatments: 13°, 15°, 18°, 20°, 22°, and 25°C.

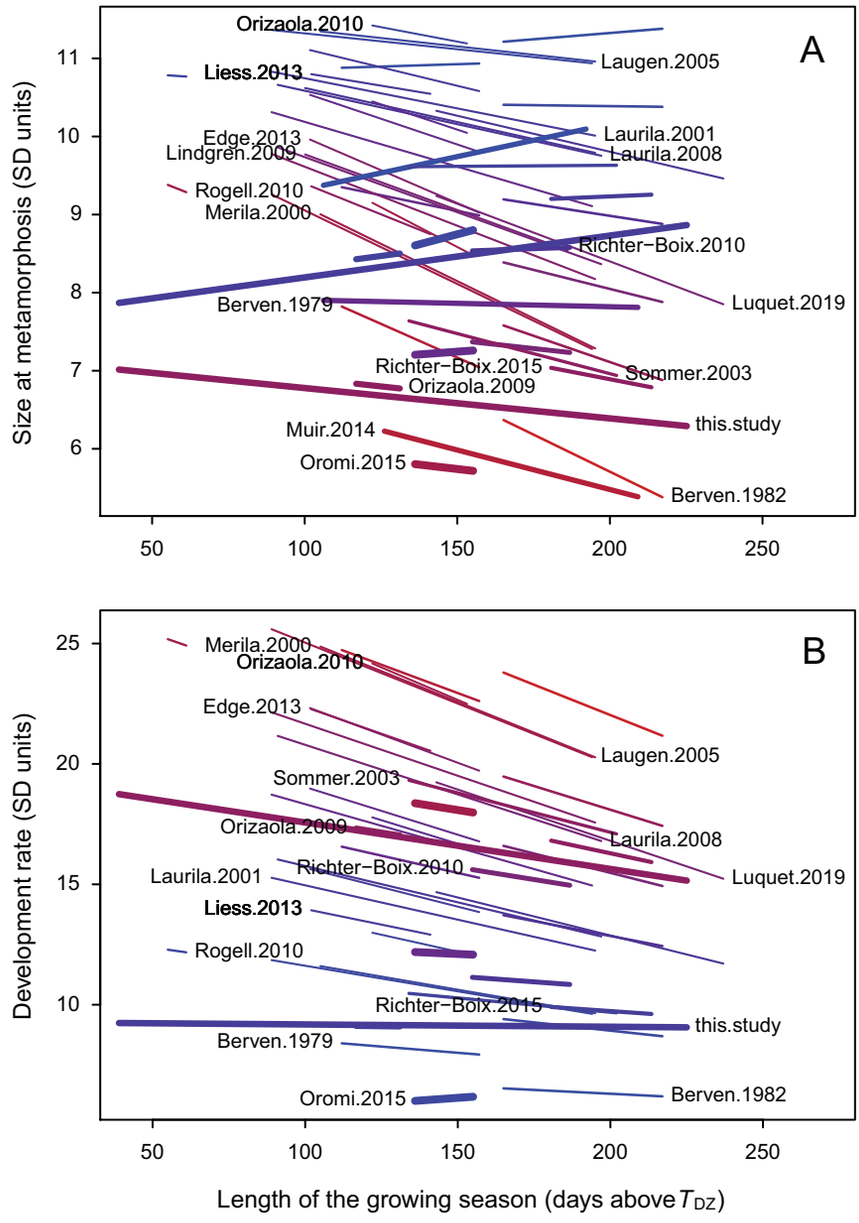

Figure 6: Size at metamorphosis (*A*) and development rate (*B*) in relation to growing season duration (days per year at which average local temperature exceeds the developmental zero temperature of the species) from studies of countergradient variation in amphibians. The vertical axes are in units of pooled standard deviation among replicates within treatments. The lines are fitted curves from the models in table 4. The color ramp from blue to red corresponds to temperature treatments from cool to warm, and line thickness is proportional to the steepness of the environmental gradient. For each study, the first author and publication year are labeled adjacent to the fitted curves for one temperature (details of all studies are in table 3).

Swiss elevational gradient is −0.0102 (−0.0224 to 0.0023). Hence, divergence in development rate on the steepest gradients was only 29.7% as strong as that on a shallow gradient. This 70% difference, we argue, is a measure of maladaptation due to higher gene flow on steep gradients.

Clinal divergence in development rate is predicted by models on adaptation under time constraints, which find that larval development should accelerate as the time window available for development shortens (Ludwig and Rowe 1990; Rowe and Ludwig 1991; Abrams et al. 1996). As expected, diverse taxa show a pattern of countergradient variation in development with along elevation or latitude (Hodkinson 2005; Keller et al. 2013; fig. 6*B* in this study). Therefore, our interpretation assumes that countergradient

Table 4: Summary of Bayesian mixed effects models on measures of tadpole performance reported in studies of countergradient variation in amphibians

	Dependent variable	
	Size at metamorphosis	Development rate
Fixed effects (*P* value):		
Temperature treatment	**−.3752 (.001)**	**1.4975 (.001)**
Season length	**−.0077 (.005)**	**−.0288 (.001)**
Steepness of gradient	−.5136 (.443)	−.9630 (.259)
Temperature × season length	**−.0018 (.001)**	**−.0037 (.002)**
Season length × steepness	**.0031 (.002)**	**.0066 (.001)**
Random effects (LR statistic, *P* value):		
Study	**24.67 (1,214, .001)**	**35.75 (2,176, .001)**
Measurement error	**1.052 (336, .001)**	**10.52 (1,966, .001)**
Sample sizes:		
Observations	367	327
Studies	19	17
Species	6	6

Note: Entries in the table are the coefficient with the Markov chain Monte Carlo *P* value in parentheses (for fixed effects) and the variance component with the likelihood ratio (LR) statistic and *P* value on 1 df in parentheses (for random effects). Fixed covariates are temperature treatment (°C; centered by study), season length (days above T_{DZ}; centered), and steepness of the gradient (log-transformed days km^{-1}; centered). Boldface highlights significant effects. Studies contributing to this analysis are listed in table 4, and the results are illustrated in figure 6.

variation in development rate is an adaptation to the abbreviated growing season in cold climates.

However, the results for body size and growth rate were different: population divergence was either not associated with the gradient in season length or showed a trend toward cogradient variation (figs. 3*A*, 4*A*). For body size, other empirical studies of ectotherms along climate gradients show all possible patterns (Blanckenhorn and Demont 2004; Hodkinson 2005; Keller et al. 2013; Rollinson and Rowe 2018; fig. 6*A* in this study). Blanckenhorn and Demont (2004) argued that geographic trends in body size are unpredictable in ectotherms because a short season favors small size whereas cold temperature induces large size, and the causal mechanisms are completely independent. In Abrams et al.'s (1996) model of life-history evolution under time constraints, the change in optimal body size at metamorphosis with the length of the growing season depends on two relationships: how adult fitness scales with size at emergence and how larval survival scales with larval growth rate. Body size is predicted to be insensitive to season length when log fitness increases linearly with size and larval mortality increases linearly with growth. Both relationships are probably positive in anurans (Gibbons and McCarthy 1986; Arendt 2003; Lardner and Loman 2003; Dmitriew 2011), but little is known of whether they may be linear, accelerating, or decelerating.

For growth rate, the weak cogradient pattern measured in our experiment may contradict the outcome predicted by theory, which is that optimal growth rate will decrease as the growing season becomes longer; this is because the costs of high growth should be avoided when time constraints relax (Abrams et al. 1996). Exceptions occur, however, if the log-fitness advantages of large body size increase linearly with size (or greater than linearly), in which case optimal growth rate will then be unrelated to or may even increase with season length (Abrams et al. 1996).

Empirical evidence for predictions about growth rate are difficult to evaluate. Dmitriew (2011) cited numerous studies that report countergradient variation in growth on latitudinal gradients and hence appear to support the prediction that optimal growth declines with season length. But all of these studies calculated growth as the ratio of final size to development time, which creates several problems. First, growth rate is not independent of development rate, and this means that growth may appear to increase with latitude or elevation simply because the duration of the larval phase declines so strongly (Merilä et al. 2000; Armbruster and Conn 2006; Laurila et al. 2008; Muir et al. 2014). A second issue is that this growth metric assumes a linear growth model originating at the origin, which is inappropriate for volumetric measures of body size such as mass. Amphibians and many invertebrates grow nearly exponentially during the first part of the larval phase (Wilbur 1984; Alford and Jackson 1993; Peacor and Pfister 2006; Mansano et al. 2014; Muenst 2015; Meister et al. 2017). Nonlinearity is especially obvious in amphibians, which lose substantial weight late in the larval stage, so that the entire growth trajectory can be approximated as an exponential process that damps exponentially (Wilbur and Collins 1973). Incorrectly assuming a linear growth model can bias

comparison of "growth rate" among populations for which development time also differs. And a third issue is that empirical data on linear growth are difficult to compare with theoretical predictions because the models refer to proportional (or relative) growth rate (Rowe and Ludwig 1991; Abrams et al. 1996). Of the four studies in our literature survey for which proportional growth rate could be estimated, just one showed any indication of countergradient variation (in this case, with latitude; Lindgren and Laurila 2009). Available amphibian data therefore do not strongly demonstrate an increase in intrinsic growth rate in cold climates. In the end, this may be unsurprising given that growth rate is subject to trade-offs and environmental influences other than temperature and duration of the growth season (Blanckenhorn 2000; Dmitriew 2011; Meister et al. 2017).

We have focused on the swamping effect of gene flow on adaptation, but what about positive effects? Theory suggests two ways that gene flow can increase the rate of adaptation. First, dispersal into marginal populations can increase population size, augment genetic variation, and consequently enhance the rate of response to local natural selection (Holt and Gomulkiewicz 1997; Alleaume-Benharira et al. 2006; Yeaman and Guillaume 2009; Polechová 2018). This is predicted to occur at low to intermediate levels of immigration into populations that are demographically inviable and subject to genetic drift. Second, gene flow can enhance the rate of adaptation if individuals move nonrandomly, adaptively selecting habitats for which they are well suited (Armsworth and Roughgarden 2008; Bolnick and Otto 2013; Edelaar et al. 2017). This process is most effective when dispersal is high relative to the change in selection, such as on a steep environmental gradient (Bolnick and Otto 2013). Both mechanisms therefore predict that adaptation tracks the phenotypic optimum more closely on steep gradients, which is opposite to what we observed. Nevertheless, our results do not exclude either possibility: clinal variation in development rate may be steeper in some instances than it would have been in the complete absence of gene flow, and this would be difficult to detect in a comparative study such as ours. At minimum, however, our study suggests that the swamping impact of gene flow greatly outweighs any positive impacts and therefore supports the conclusion that the dominant impact of gene flow along climate gradients is to erode local adaptation.

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Statement of authorship: J.C.B. designed the study, conducted the experiment, analyzed the data, and wrote the first draft. A.J.v.R. collected the Swiss genetic samples, did the molecular analyses, and commented on the manuscript. M.C.-C. collected the Fennoscandian samples. A.L. obtained funding and permits and commented on the manuscript. J.V.B. designed the study, obtained funding and permits, collected the Swiss genetic samples, analyzed data, did the meta-analysis, and wrote the manuscript.

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Left, pair of common frogs (*Rana temporaria*), already in amplexus, approaching a wetland. *Right*, male common frogs gather to await females at a communal oviposition site. Photo credit: Josh Van Buskirk.