Adaptation to elevation but limited local adaptation in an amphibian

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We performed a reciprocal transplant experiment to estimate "parallel" adaptation to elevation and "unique" adaptation to local sites at the same elevation, using the frog *Rana temporaria* in the Swiss Alps. It is important to distinguish these two processes because they have different implications for population structure and ecological specialization. Larvae were reared from hatching to metamorphosis within enclosures installed in their pond of origin, in three foreign ponds at the same elevation, and in four ponds at different elevation (1500–2000 m higher or lower). There were two source populations from each elevation, and adults were held in a common environment for 1 year before they were crossed to produce offspring for the experiment. Fitness was a measure that integrated larval survival, development rate, and body size. Parallel adaptation to elevation was indicated by an advantage at the home elevation (11.5% fitness difference at low elevation and 47% at high elevation). This effect was stronger than that observed in most other studies, according to a survey of previous transplant experiments across elevation (*N* = 8 animal species and 71 plants). Unique local adaptation within elevational zones was only 0.3–0.7 times as strong as parallel adaptation, probably because gene flow is comparatively high among nearby wetlands at the same elevation. The home-elevation advantage may reduce gene flow across the elevational gradient and enable the evolution of habitat races specialized on elevation.

KEY WORDS: Altitude, amphibian, spatial scale, temperature.

Local adaptation is recognized when genotypes that originate at a particular site perform better when tested there than genotypes that are foreign to the site. The classic study design for detecting local adaptation—a reciprocal transplant experiment in which genotypes are tested in "home" and "away" sites—has been applied in a wide variety of organisms (Kawecki and Ebert 2004). These experiments demonstrate that local adaptation is common in nature. Meta-analyses by Leimu and Fischer (2008) and Hereford (2009) concluded that 70% of studies detected a fitness advantage of local genotypes over foreigners when tested at the site of origin of the locals. However, there are two distinct kinds of local adaptation, termed "unique" and "parallel" local adaptation by Kawecki and Ebert (2004). Most reciprocal transplant experiments are not designed to differentiate between these kinds, although they have different consequences for evolutionary dynamics. Hence, although the meta-analyses demonstrate that local adaptation is widespread, we cannot yet evaluate the relative importance of unique and parallel local adaptation.

Unique local adaptation occurs when genotypes are adapted to the specific site at which they occur. The agents of selection that promote adaptation can be diverse, including combinations of environmental features and other organisms occurring at the site. Gene flow among sites usually opposes the evolution of unique local adaptation because it introduces genotypes that are not adapted to local conditions (Slatkin 1987; Lenormand 2002). The outcome of unique local adaptation is a mosaic of demes each adapted to locally relevant ecological conditions. Parallel local adaptation exists when genotypes are adapted to habitat types rather than specific sites. Gene flow does not oppose parallel adaptation if it primarily connects sites with similar habitat, as can occur when dispersing individuals exhibit habitat choice or immigrants from other habitats have reduced fitness (Nosil et al. 2005; Wang and Bradburd 2014). The outcome of parallel adaptation is a set of habitat-races or host-races, each adapted to its characteristic environment no matter where it occurs. Differentiating parallel from unique local adaptation is important because

they create distinct patterns of population structure (Bradburd et al. 2013; Sexton et al. 2014; Van Buskirk and Jansen van Rensburg 2020). Moreover, parallel adaptation, if it unfolds without interruption, may lead to ecological speciation (Via 1999; Dres and Mallet 2002; Nosil 2012). An experiment can differentiate unique and parallel local adaptation if it rears genotypes in their home site, in away sites of the same habitat type, and in away sites of different habitat type. The outcome would support unique local adaptation if fitness were higher at home than in away-same-habitat and away-different-habitat sites, whereas parallel adaptation would be indicated if fitness were higher in home and away-same-habitat sites than in away-different-habitat sites.

We implemented the experimental design described above to estimate the extent of unique local adaptation and parallel adaptation to elevation in populations of a frog (*Rana temporaria*) in an alpine landscape. The design included reciprocal transplants across a 2000-m elevational gradient and among local sites at similar elevation. Hence, the experiment aimed to detect parallel adaptation to elevation (or to environmental conditions associated with elevation) and unique local adaptation to any conditions that vary among local sites. In our study area, frog populations at 500 m elevation and above 2000 m are separated by a steep gradient that can be as short as 5 km. Variation in elevation also shows a mosaic geographic structure because high-elevation sites occur on different mountains separated by low valleys. Habitat variation among populations at the same elevation – involving features such as canopy cover and predator density – occurs over scales of a few tens or hundreds of meters (Van Buskirk 2005), well within the dispersal capability of juvenile and adult frogs (Sinsch 2014).

Many reciprocal transplant experiments have been performed across elevational gradients, but they have not clearly resolved the prevalence of parallel adaptation to elevation. A recent meta-analysis of studies on plants concluded that survival was usually better in the home site than in sites at a different elevation ($N = 6$ studies; Halbritter et al. 2018). But a wider sample of many similar experiments not included in Halbritter et al.'s review indicates that the home-elevation advantage is often weak (e.g., Mark 1965; Byars and Hoffmann 2009; Grassein et al. 2014; Latreille and Pichot 2017; Hamala et al. 2018). The results for animals are even less convincing: we are aware of no transplant experiment demonstrating adaptation to elevation in an animal (Bernardo 1994; Morrison and Hero 2003; Iraeta et al. 2006, Iraeta et al. 2008; Caruso et al. 2019; Berven 1982a, b). In section Discussion, we present a thorough overview of reciprocal transplants testing for adaptation to elevation in plants and animals.

The mixed results of earlier experiments are surprising for two reasons. First, environmental gradients associated with elevation can be dramatic, and therefore, selection favoring adaptation to elevation is expected to be strong. Temperatures are lower and the growing season is shorter at high elevation, and there can be important differences associated with moisture and solar radiation (Billings and Mooney 1968; Körner 2003; Barry 2008). These environmental factors can impose divergent selection across elevational gradients for phenology, development rate, or thermal tolerance (Kooyers et al. 2019; Peterson et al. 2020). A second reason is that the transplant results are contradicted by common garden experiments showing quantitative genetic divergence along elevational gradients in diverse taxa. In plants, genotypes from high-elevation localities tend to be smaller when assessed in a common environment (Halbritter et al. 2018). In animals, many experiments reveal that genotypes originating from cold, high-elevation sites exhibit rapid development rate, a phenomenon known as counter-gradient variation (Conover et al. 2009; Keller et al. 2013; Bachmann et al. 2020). Elevational divergence is frequently noted in other characters that may be functionally relevant with respect to temperature or seasonality (Ellers and Boggs 2002; Caro et al. 2013; Levy and Nufio 2015; Noguerales et al. 2016). These studies indicate that phenotypic variation with elevation is consistent with functional expectations of adaptation, particularly in animals. However, they cannot provide strong evidence on the question of whether phenotypic divergence is adaptive (Kawecki and Ebert 2004). This is partly because common garden studies can never be certain that they have measured the characters responsible for adaptation. Also, such experiments do not estimate the fitness consequences of phenotypic variation in a natural context. This can be done only with a reciprocal transplant experiment, in which the performance of individuals from each source population is measured both in the home environment and in environments occupied by other populations (Kawecki and Ebert 2004; Keller et al. 2013; Halbritter et al. 2018).

These issues motivated our study of parallel adaptation to elevation and unique local adaptation in frogs. Given the scarcity of evidence in the literature for adaptation to elevation, we first asked whether populations enjoy a fitness advantage when reared at their home elevation. Secondly, we estimated the relative magnitudes of parallel adaptation to elevation and local adaptation to unique environmental variation. This distinction is important because the balance between these two processes influences the pattern of population structure and reflects the geographic scale of selection and gene flow (Via 1999; Kawecki and Ebert 2004; Bradburd et al. 2013; Van Buskirk and Jansen van Rensburg 2020).

Methods

RECIPROCAL TRANSPLANT EXPERIMENT

The experiment focused on the aquatic tadpole stage of *R. temporaria*. One reason for this was practical: the entire larval stage

can be studied over a period of about 2 months in this species, whereas the terrestrial stage lasts many years (Miaud et al. 1999). In addition, local adaptation may be especially pronounced in larval anurans because they are confined to discrete wetlands where they cannot avoid large-scale variation in predation risk, water chemistry, or climate. Juvenile and adult frogs in the terrestrial phase of the life cycle may be better able to select microhabitats with moderate environmental conditions, and models suggest that this can reduce the likelihood of local adaptation (Edelaar et al. 2017; Schmid and Guillaume 2017).

We reared tadpoles originating from four source populations (two low-elevation and two high-elevation) at their home and away elevations, in a reciprocal transplant field experiment during spring and summer of 2015. Animals from each source were reared in eight test ponds (four at each elevation). We included extra replication of test ponds because transplant experiments often discover wide variation in performance among test sites (Bowman et al. 2008; Kaufmann et al. 2017). This design is suitable for estimating the two kinds of local adaptation discussed above: unique local adaptation (to unknown features of local sites) and parallel local adaptation (to a specific feature of the environment, in this case elevation) (Kawecki and Ebert 2004).

The locations of the source and test sites are given in Figure 1 and Table S1. We produced eggs for the experiment from adult frogs that were collected a year in advance, which was necessary because low- and high-elevation populations breed at different times of year. This means that adult frogs were held in a common environment for a year before the experiment began, which probably reduced maternal environmental variation among populations and between elevations. We caught 45–65 adult frogs in each source population when they gathered to breed in March or June 2014, and held them over the summer in six outdoor enclosures, each 10 m^2, at the University of Zurich. The enclosures were grassy, partly shaded, and contained a small pond in a buried plastic wading pool (0.7 m^2). We added crickets to each enclosure once a week (*Acheta domesticus* and *Gryllus assimilis*). In late October, the frogs were moved to an indoor room at 20°C, placed in groups of three into 0.28 m^2 plastic boxes with a leaf litter substrate, and fed regularly with crickets. On December 16, 2014, we transferred the frogs to a 4°C climate chamber, where they were kept in same-sex pairs in 1-L plastic boxes with 0.3 L water and numerous small pieces of foam rubber.

Tadpoles for the experiment came from full-sib families produced by performing within-population crosses as described by Browne et al. (2006). Half the frogs from each source population, chosen at random, were transferred on March 21, 2015 from the climate chamber to a laboratory room at 22°C, and crossed 3 days later. The remaining individuals were moved to the same room on June 16, 2015 and crossed 4 days later. We injected females 1 day before crossing with a solution made from 2 mg
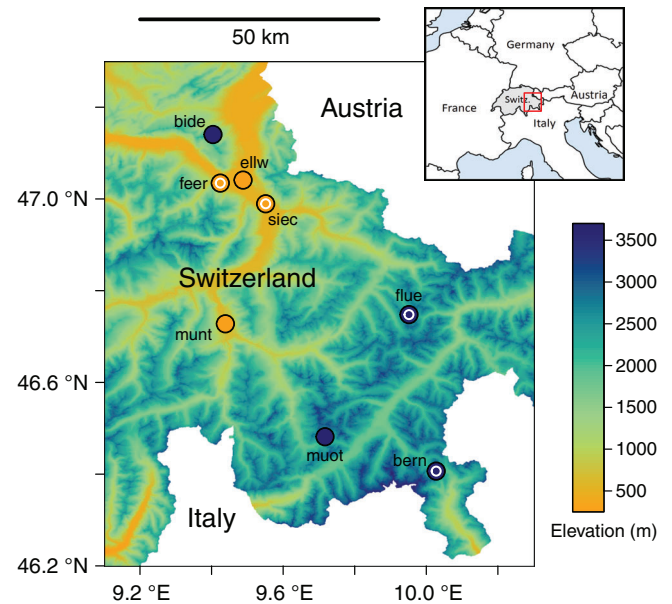

Figure 1. Map of eastern Switzerland illustrating the *Rana temporaria* populations included in the experiment. Yellow symbols indicate the four low-elevation test sites (<650 m); dark blue symbols are the four high-elevation test sites (>2000 m). The four source populations are marked with a white bulls-eye inside the symbol. The background elevation raster is from the Swiss Bundesamt fuer Landestopographie (Wabern, Switzerland; https://shop.swisstopo.admin.ch/en/ products/height_models/alti3D). Names, geographic coordinates, and other information about the sites are available in Table S1.

LHRH fish hormone (Bachem H-7525, Bubendorf, Switzerland) dissolved in 100 mL Ringer's solution. On the day of crossing, males were injected with the same solution and, 2 h later, were induced to release spermic urine by gently massaging the pelvic girdle. Eggs were stripped from the females and directly added to the sperm suspension. These procedures created 9/6 (in March/June) healthy full-sib families for source population *siec*, 9/7 families for population *feer*, 7/7 families for *flue*, and 8/8 families for *bern*. Fertilized eggs and hatchlings were kept at 14°C until the experiment began.

There were eight replicates of the four source populations at each of the eight test sites (Table S1). We installed 32 enclosures in each site, arranged into eight spatial blocks of four enclosures each. Enclosures within a block were placed about 10–20 cm apart. Each enclosure contained 12 tadpoles from a single source population. Enclosures were cylindrical open-top cages (40 cm diameter; 0.13 m^2), constructed of fiberglass window screen with a hardware cloth bottom. They were placed in shallow water near the edges of the pond, where most tadpoles naturally occur, and each received about 1 L of pond sediment scooped from the substrate adjacent to the enclosure and inspected to remove predators. In some cases, enclosures were moved during the

experiment to follow the receding water of a drying pond. Figure S1 includes photographs of test ponds and enclosures.

Tadpoles were introduced to the experiment when they were at Gosner (1960) stage 25 (4-7 days old; 16.4 mg ± 0.61 SD). As far as possible, every full-sib family contributed equally to the 12 individuals in each enclosure. The experiment began on April 8 at low elevation and on June 25 or 26 at high elevation. Enclosures were checked at least once per week thereafter, and each enclosure was removed when its first tadpole reached metamorphosis (Gosner stage 42; forelimb emergence). We counted and weighed survivors when the enclosure was removed, and individually reared those that had not yet metamorphosed in 200 mL plastic cups at 18°C in the laboratory until they reached stage 42. The 1786 surviving tadpoles spent an average of 87% (median 89%) of their larval period in the field enclosures (details in Fig. S2). Results for survival and body mass at the point when the enclosure was removed were similar to those at metamorphosis (stage 42), so hereafter we report data on performance measured at the completion of metamorphosis. For every animal in the experiment, we recorded whether it survived and, if it survived, its development rate (stages/day between stage 25 and 42) and mass at metamorphosis (mg).

ENVIRONMENTAL CONDITIONS

We measured environmental variables at the eight test sites during the time when tadpoles were in the water. Temperature came from HOBO data loggers (Onset Corp., Bourne, MA) installed 5 cm below the water surface at the center of each block of four enclosures, and programmed to record temperature at 30 min intervals for 35 days. Dissolved oxygen, pH, and salinity were measured with a portable meter (Hach Lang GmbH, Düsseldorf, Germany) about 30 days after the experiment began and before the onset of metamorphosis (May 13 at low elevation and July 25 at high elevation). Readings were made at three locations at each test site (center of the pond, 2 m from the northern edge, and 2 m from the southern edge).

STATISTICAL ANALYSES

Estimating individual fitness in organisms with complex life cycles is not straightforward when data are available for only one life stage. However, in amphibians, field studies have shown that measures of adult performance such as survival and body size at reproduction are positively correlated with size and age at metamorphosis (Smith 1987; Semlitsch et al. 1988; Berven 1990, 2009; Scott 1994; Altwegg and Reyer 2003). Large size at emergence may enhance foraging opportunities for juvenile amphibians, facilitate survival in the terrestrial habitat when predators are present, or improve desiccation resistance. Early metamorphosis allows juveniles to accomplish some terrestrial growth before conditions deteriorate late in the season. In *Pelo-*

phylax water frogs, Altwegg and Reyer (2003) estimated that terrestrial survival depends on metamorphic size and age as follows: logit[survival to age 1] = -0.693 + 0.870·(mass) - 0.021 × (age), where mass and age at metamorphosis are standardized to mean = 0 and SD = 1. We applied this relationship to animals in our field experiment to calculate a quantity that we call "expected fitness"; individuals that died before metamorphosis were assigned a value of 0. The main advantage of this quantity is that it implements an empirically measured relationship between larval performance and success (survival) through the first year of life. An alternative would be to integrate the three measures of larval performance using multivariate analysis of variance, but this is inferior because it implicitly weights them equally or in proportion to their variances, neither of which accurately represents their contributions to terrestrial survival (Smith 1987; Altwegg and Reyer 2003). We will also present separate results for larval survival, development, and metamorphic mass, but our intention is mostly to understand sources of variation in expected fitness. Analysis of separate fitness components can produce unreliable results (McGraw and Caswell 1996; Shaw et al. 2008).

The significance of parallel and unique local adaptation were evaluated in separate analyses. For parallel adaptation to elevation, we fitted a mixed effects linear model with fixed effects of the elevation of the test site (low/high), the elevation of the source population (low/high), and their interaction. The test elevation-by-source elevation interaction evaluated whether tadpoles performed better when tested at their home elevation. For unique local adaptation to source ponds independent of elevation, we fitted two models corresponding to the two criteria for testing local adaptation outlined by Kawecki and Ebert (2004). One criterion compares local and foreign individuals within test sites. This analysis used data from the four source populations, comparing animals that were of local origin with those of the other source population from the same elevation. The model included the fixed effects of test elevation (low/high), immigrant status (local/foreign), and their interaction. The other criterion compares performance at the home site with that in away sites. Here, we used data from all eight test sites, comparing tadpoles when reared at home versus the three away sites at the same elevation. This model included the fixed effects of test elevation (low/high), immigrant status (home/away), and their interaction. All models included random effects of the enclosure nested within block and test site.

These models were each fitted to data on expected fitness, larval survival, development, and metamorphic mass using the MCMCglmm package in R 3.5.2 (Hadfield 2010). For expected fitness, we assumed a zero-inflated Poisson distribution because 35.5% of the 2760 individuals perished before metamorphosis and therefore had a value of 0 (Fig. S3). To create Poisson counts, we divided expected fitness by its mean, multiplied this value by

10, and then rounded it to the nearest integer to produce a quantity that averaged 10.0 (SD = 9.77). The rounding process introduced a small error, but the correlation between relative fitness before and after rescaling was 0.9975. A binomial distribution was assumed for survival and Gaussian distributions for development rate and mass. Parameter estimates and their credible intervals came from 2000 essentially independent samples from the posterior distribution. We ran three parallel chains for every model and used Gelman and Rubin's (1992) method to confirm that convergence was achieved.

Finally, we evaluated the relative magnitudes of unique local adaptation and parallel adaptation to elevation by comparing animals of three origin types within each test site: local (*loc*), foreign from the same elevation (*same.elev*), and the two populations of foreign from the other elevation (*away.elev*). This comparison used data from the four test sites that served as source populations. The magnitudes of different kinds of adaptation were expressed in terms of specific differences between the origin types. The difference [*loc* – *sam.ele*] represents the increase in fitness due to unique local adaptation; the difference [(*loc* + *same.elev*)/2 – *away.elev*] represents parallel adaptation to elevation; and the difference [*loc* – *away.elev*] represents the total increase in fitness due to both kinds of adaptation. We calculated these differences from predicted values for the three origin types as defined above, computed for each of 2000 samples from the posterior distribution of a linear mixed effects model fitted in MCMCglmm. Fixed effects were test elevation (low/high), origin type, and their interaction. Random effects were block and cage nested within block.

LABORATORY EXPERIMENT

Frogs that produced eggs for the high-elevation portion of the experiment were held at 4°C in the climate chamber for 87 days longer than frogs used at low elevation. We conducted a laboratory experiment to check whether this influenced the performance of their offspring. The experiment had a 2 × 2 factorial design, with the elevation of the source population (low/high) crossed with the duration of the overwintering period (short/long). Two source populations were nested within each combination of elevation and duration, the 61 full-sib families were nested within populations, and there were two replicate individuals of each family. This gave a total of 122 tadpoles. Each tadpole was reared in a cup containing 200 ml water, assigned at random to a position in one of 32 baths maintained at 18°C by a flow-through water system. The light:dark regime was 13:11 h. Hatchlings were added to the experiment when they were 7–8 days old and at stage 25. Water was changed three times per week, and food was a 4:1 mixture of ground rabbit pellets and fish flakes provided ad libitum. We weighed metamorphs (mg) and recorded their development rate (stages/day) when they reached stage 42.

The analysis used mixed-effects linear models to test whether the overwinter duration (short/long corresponding to low/high elevation transplants), the elevation of the source population (low/high), and their interaction affected tadpole survival, development rate, or mass at metamorphosis. Clutch nested within source population were included as random effects. Models were fitted using the lme4 package in R (Bates et al. 2015).

Results
LOCAL ADAPTATION
The field experiment revealed strong parallel local adaptation to elevation in *Rana temporaria*. In tests conducted at both elevations, tadpoles with the highest expected fitness were those that originated from the test elevation (Fig. 2A). This was particularly true at high elevation, where animals from high-elevation populations had expected fitness about twice as high as those from low elevation. The significance of adaptation to elevation was indicated by interactions between test elevation and source elevation for both logistic and Poisson processes of the zero-inflated Poisson model (Table 1A).

The home-elevation fitness advantage arose from variation in survival and size at metamorphosis, but not in development rate (Fig. 2). At high elevation test sites, survival of high-source tadpoles was much higher than survival of low-source tadpoles, and survival was unrelated to source elevation at low elevation test sites. For mass at metamorphosis, animals from both high and low source populations were comparatively large when tested at their home elevation. Interactions between test elevation and source elevation indicated that these patterns were significant (Table 1). Data from the four test sites at each elevation illustrate that adaptation to elevation was consistent across sites, especially for mass and overall expected fitness (Fig. S4).

There was limited evidence for unique local adaptation to specific ponds within elevational zones. The comparison of local with foreign animals from the same elevation suggested that locals experienced a decided fitness advantage, but only in tests at low elevation (Fig. 3A). This was reflected in the significant interaction between test elevation and local/foreign status in the logistic part of the zero-inflated Poisson model (Table 2, left side). However, there were no effects of local/foreign status or interactions between status and test elevation in separate analyses of survival, development rate, and mass at metamorphosis (Table S2). Inspection of the separate performance responses for each test pond confirms that locals performed better only in terms of survival, and mostly at low elevation (upper row in Fig. S5).

The comparison between animals tested home and away at the same elevation gave no indication of local adaptation

Table 1. Linear mixed-effects models testing for parallel adaptation to elevation, fitted using Markov chain Monte Carlo techniques in MCMCglmm. The four responses are (A) expected fitness (zero-inflated Poisson distribution), (B) proportion surviving to metamorphosis (binomial distribution), (C) development rate in stages/day (Gaussian distribution), and (D) mass at metamorphosis in mg (Gaussian distribution). Expected fitness is the predicted survival to age 1, multiplied by 10 and rounded to the nearest integer (see Fig. S3). In all cases, the full model with random effects of enclosure, block, and test pond received the highest support, and all three random effects were highly significant according to the deviance statistic.

Source of variation	Level	Estimate (95% HPD interval)	*P*-value
A. Expected fitness (N = 2760)			
Logistic process[a]			
Test elevation	low	-0.734 (-1.621, 0.286)	0.1370
Source elevation	low	1.066 (0.776, 1.337)	0.0005
Test × Source elevation	low, low	-0.774 (-1.211, -0.380)	0.0005
Poisson process			
Test elevation	low	-0.095 (-1.276, 1.131)	0.8570
Source elevation	low	-0.365 (-0.755, 0.048)	0.0810
Test × Source elevation	low, low	0.668 (0.133, 1.208)	0.0140
B. Survival to metamorphosis (N = 2760)			
Test elevation	low	0.627 (-0.704, 2.076)	0.3110
Source elevation	low	-1.312 (-1.802, -0.883)	0.0005
Test × Source elevation	low, low	1.108 (0.462, 1.721)	0.0005
C. Development rate (N = 1786)			
Test elevation	low	-0.043 (-0.144, 0.058)	0.3400
Source elevation	low	-0.004 (-0.012, 0.003)	0.3110
Test × Source elevation	low, low	0.002 (-0.007, 0.012)	0.7570
D. Mass at metamorphosis (N = 1779)			
Test elevation	low	26.062 (-139.91, 209.36)	0.7140
Source elevation	low	-54.183 (-73.20, -33.77)	0.0005
Test × Source elevation	low, low	107.062 (83.08, 134.00)	0.0005

[a] The logistic process estimates the probability that a zero value of fitness is caused by the zero-inflation process. Thus, negative coefficients indicate that the covariate was associated with higher survival.

independent of elevation (Fig. 3B; Table 2, right side). This was partly because performance varied greatly among test ponds. For example, all tadpoles originating from low elevation survived well and developed rapidly in the low-elevation test site of *munt*, regardless of their population of origin (Fig. S5, bottom row). At high elevation, both survival and development were high for all animals assessed at the test site of *bide*.

We estimated that parallel adaptation to elevation was 1.4–3.2 times greater in magnitude than unique local adaptation. This came from comparison of expected fitness among tadpoles of three origin types: local animals (*loc*), foreign from the same elevation (*same.elev*), and foreign from the other elevation (*away.elev*). Treatment means for the three origin types are in Figure 4; the MCMC distributions of differences between model-predicted values of the three origin types are in Figure S6. *Loc* animals performed much better than *away.elev* animals at all four sites, but expected fitness of *same.elev* was usually not much below that of *loc*. Hence, the proportional change in expected fitness caused by unique local adaptation (0.224 at low elevation [80%

HPDI: -0.222 to 0.519]; 0.156 at high elevation [-0.389 to 0.510]) was weaker than the change attributed to parallel local adaptation (0.311 at low elevation [0.107 to 0.488]; 0.495 at high elevation [0.290 to 0.730]).

ENVIRONMENTAL CONDITIONS

The four high-elevation test sites had similar pH and marginally higher dissolved oxygen in comparison with low-elevation sites, and significantly lower salinity (Table S1; $P = 0.005$, ANOVA). Water temperature when larvae were in the ponds did not vary with elevation in cloudy weather, but under clear skies the daily maximum temperature at high elevation was 2°C higher and the minimum nighttime temperature averaged 2°C lower (Fig. S7).

LABORATORY EXPERIMENT

There was no effect of the duration of the adult overwintering period in the climate chamber on performance of their offspring in the laboratory experiment (Fig. S8; Table S3). Tadpoles from high-elevation populations developed faster and reached

Table 2. Mixed-effects models testing for unique local adaptation unrelated to elevation, fitted using Markov chain Monte Carlo techniques in MCMCglmm (Hadfield 2010). The dependent variable is expected fitness, which exhibited a zero-inflated distribution (Fig. S3). Coefficients were estimated for a logistic process related to mortality[a] and a Poisson process related to growth and development. In both analyses, the random effect structure with enclosure, block, and test pond received the highest support. The weak effects of immigrant status in both tests, and marginally significant interactions with test elevation, suggest that unique local adaptation was inconsistent. The corresponding analyses for separate components of performance are summarized in Appendix Table S2.

Source of variation	Level	Local-vs-foreign criterion ($N = 684$) Estimate (95% HPD interval)	MCMC P-value	Home-vs-away criterion ($N = 1404$) Estimate (95% HPD interval)	MCMC P-value
A. *Logistic process*[a]					
Test elevation	low	-0.099 (-0.572, 0.430)	0.693	-0.267 (-1.442, 0.896)	0.626
Immigrant status	local/home	-0.123 (-0.685, 0.402)	0.659	-0.030 (-0.568, 0.516)	0.925
Status × Test elevation	local, low	-0.729 (-1.449, -0.023)	0.046	-0.711 (-1.456, 0.044)	0.072
B. *Poisson process*					
Test elevation	low	0.155 (-0.935, 1.582)	0.880	0.253 (-0.963, 1.577)	0.679
Immigrant status	local/home	0.080 (-0.691, 0.846)	0.827	0.083 (-0.706, 0.820)	0.836
Status × Test elevation	local, low	-0.301 (-1.338, 0.708)	0.585	-0.287 (-1.3, 0.731)	0.594

[a] The logistic process estimates the probability that a zero value of fitness was caused by the zero-inflation process. Thus, a negative coefficient means that the covariate was associated with higher survival.

a smaller size at metamorphosis, but this was true for both short-winter (March) and long-winter (June) animals. There were no significant effects of duration or its interaction with source elevation.

Discussion

This experiment demonstrated that larvae of the common frog, *Rana temporaria*, are strongly adapted to environmental conditions that vary along an elevational gradient in the Swiss Alps. When we reared tadpoles in low-elevation ponds, those that originated at low elevation performed slightly better due to their larger body size at metamorphosis. But when tadpoles were reared at high elevation, those that originated at high elevation survived much better and grew larger than tadpoles from low elevation. Comparison of animals transplanted among ponds at the same elevation and between very different elevations showed that adaptation to elevation was two to three times stronger than adaptation to local ponds at the same elevation.

The relative importance of parallel adaptation to elevation probably arose from the geographic scale and spatial structure of habitat variation in this system. Evolutionary theory highlights a balance between the homogenizing effects of gene flow and diversifying effects of local selection (Felsenstein 1976; Slatkin 1987; Garcia-Ramos and Kirkpatrick 1997; Blanquart et al. 2012; Polechova and Barton 2015). Gene flow in these models counteracts the change in allele frequency favored by selection and therefore usually causes some degree of maladaptation. However, the extent of dispersal connecting demes depends on their arrangement in space, and therefore the maladaptive influence of gene flow is sensitive to the spatial scale of variation in selection coefficients (Slatkin 1973; May et al. 1975; Endler 1977; Lenormand 2002). This prediction explains our finding that adaptation to elevation was stronger than unique local adaptation within elevational zones. Selection varies strongly among environments at both scales. The relevant difference between high and low elevation environments is probably related to temperature, duration of the growing season, and possibly solar radiation (Blumthaler et al. 1997; Körner 2003; Halbritter et al. 2013; Bachmann et al. 2020). Within elevational zones, and particularly at low elevation, wetlands vary considerably in predation risk, canopy cover, and density of competitors (Van Buskirk 2005; Werner et al. 2007). Habitat variation at both scales can impose strong natural selection for divergent characters (Van Buskirk 2017; Kooyers et al.
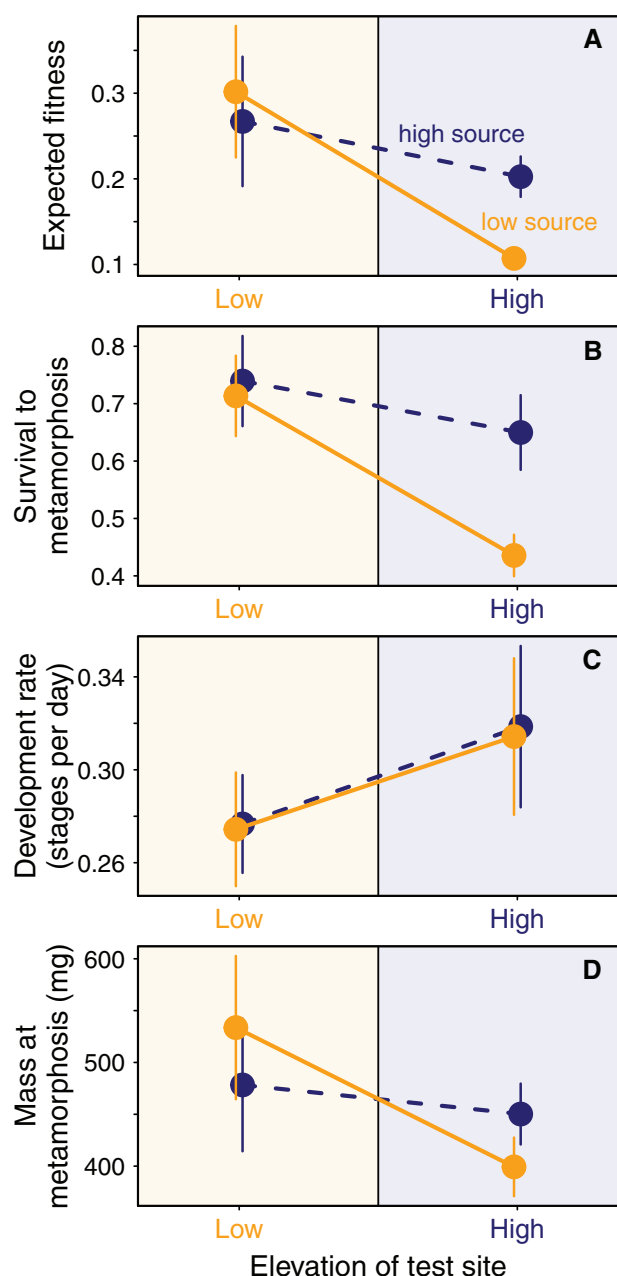
Figure 2. Adaptation to elevation in *Rana temporaria*. Tadpoles originating at both low and high elevation enjoyed a home-elevation advantage arising mostly from survival and body size. Expected fitness in panel A is the estimated probability of survival from the hatchling stage to age 1 year, integrating larval survival, development rate, and mass at metamorphosis. Error bars, which are sometimes obscured by symbols, represent ± 1 SE of the mean (*N* = 4 test ponds).

2019; Bachmann et al. 2020). But gene flow connecting different environments is potentially much greater at the spatial scale of ponds within elevational zones (tens or hundreds of meters apart) than between elevational zones (4–10 km apart). Moreover, the spatial structure of environmental variation differs between the

two scales. Within elevational zones, habitat variation shows a mosaic spatial arrangement and dispersing individuals may encounter a wide array of environments (Van Buskirk 2005, 2014). In contrast, habitat variation related to elevation is arrayed along a gradual spatial gradient, so that dispersing individuals are likely to settle in an environment that is not too different from their natal environment. Hence, the scale and structure of environmental variation ensures that gene flow occurs more frequently between different habitats at the same elevation than between different elevations, and this enables adaptation to elevation to evolve.

The three components of performance that contribute to fitness—survival, body size, and development rate—showed distinct patterns of response across elevation (Fig. 2). Results for development rate were particularly unexpected. Development rate was faster at high elevation, presumably because mid-afternoon water temperatures were warmer. However, development did not vary with elevation of origin, although much evidence indicates that amphibian larvae originating from populations in cold climates exhibit rapid development (countergradient variation; Bachmann et al. 2020). Our laboratory experiment confirmed this pattern for the four source populations in this experiment. These findings probably reflect differential survival with respect to development rate in the field experiment, shifting the distribution of phenotypes within the set of surviving animals. This would produce a pattern of response different from what is observed in laboratory studies because survival is usually very high in the laboratory and therefore strong selection is less important (Laugen et al. 2003; Orizaola and Laurila 2009; Luquet et al. 2019; Bachmann et al. 2020). In the case of development rate, this explanation requires that differential mortality caused convergence of high- and low-elevation populations at both elevations. This is exactly what natural selection is expected to do. For example, if selection at high elevation acts against slow development, as predicted by the counter-gradient model (Conover et al. 2009; Berven 1982b), it would disproportionately target low-elevation genotypes and lead to increasingly similar development rates measured at high elevation. The corresponding process at low elevation would also cause convergent development rates in the low-elevation test sites, just as we observed.

One implication of the home-elevation advantage is that gene flow connecting different elevations will be reduced. The performance trade-off visible in Figure 2 indicates that natural selection acts against the offspring of immigrants that move between elevational zones. This mechanism could be especially effective because it acts early in the life cycle, during the larval stage, and hence leaves a reduced number of individuals exposed to isolating mechanisms that may act during later life stages (Nosil et al. 2005). Reduced gene flow then tips the migration-selection balance toward selection, promoting further adaptation to elevation (Slatkin 1987; Lenormand 2002). Hence, parallel
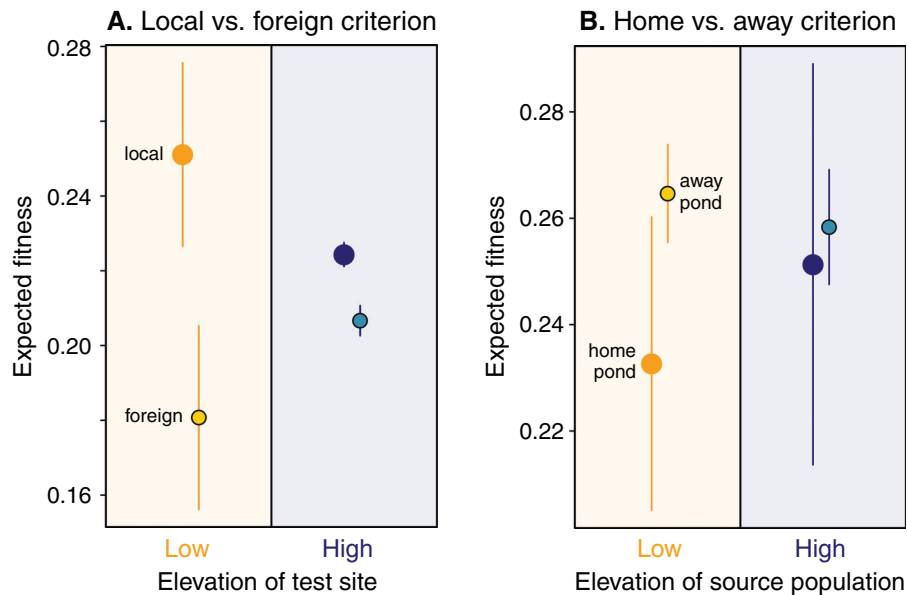
Figure 3. Tadpoles of *Rana temporaria* showed little adaptation to their local site, independent of elevation. Expected fitness is the estimated probability of survival to age 1, integrating larval survival, development rate, and mass at metamorphosis. The local-versus-foreign criterion (A) compares individuals of local origin with those from a different pond at a similar elevation. The home-versus-away criterion (B) compares expected fitness in the pond of origin with that in the other three ponds at a similar elevation. Data illustrated here were centered by test site (A) and source population (B). The significance tests in Table 2 analyzed relative fitness rescaled as described in *Methods*. Symbols are ± 1 SE (*N* = 2 source populations).
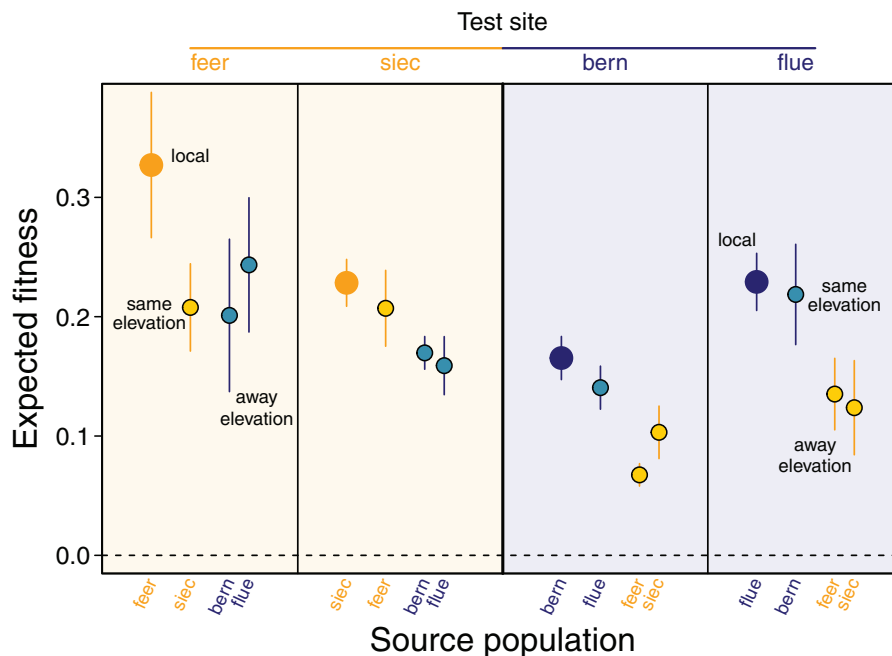

Figure 4. Unique and parallel local adaptation of *Rana temporaria* tadpoles in the field experiment. Yellow and blue text and shading refer to low- and high-elevation test sites. Unique local adaptation is the difference between the first two symbols in each panel: that is, between local tadpoles in their home pond and those in a foreign pond at their home elevation. Parallel local adaptation to elevation is the difference in expected fitness between the first two symbols and the third and fourth symbols in each panel: that is, between the tadpoles at their home elevation and those at the away elevation. These comparisons indicate that unique local adaptation was relatively weak except in test site *feer*.

Table 3. Summary of evidence for adaption to elevation in reciprocal transplant field experiments. There are two criteria for assessing adaptation: local/foreign is the proportion of gardens in the experiment within which populations originating from the local elevation performed better than populations from different elevation; home/away is the proportion of source populations that performed better at home elevation than in gardens at different elevation. Direction indicates whether the response was consistent with adaptation to elevation or maladaptation. We recorded four components of performance: lambda from a matrix population model or another integrated measure of fitness, survival, size (*e.g.*, body mass or plant height), and reproduction (*e.g.*, seed set or flower number). For each species, performance components that were available were averaged, and the table reports the mean ± SD over species. The distribution of separate responses recorded for all species is in Fig. S9.

		Taxon	
Criterion	Direction	Animals ($N = 8$)	Plants ($N = 71$)
Local/foreign	Adaptation	0.230 ± 0.292	0.276 ± 0.281
	Maladaptation	0.104 ± 0.145	0.143 ± 0.224
Home/away	Adaptation	0.208 ± 0.209	0.355 ± 0.244
	Maladaptation	0.239 ± 0.191	0.289 ± 0.213

adaptation to elevation could create a positive feedback mechanism that intensifies adaptation to the gradient (Hendry 2004; Kawecki and Ebert 2004).

It may be necessary to temper this conclusion with the observation that selection against immigrants was asymmetric, with animals from low elevation performing particularly poorly when tested at high elevation. Many previous studies have also detected asymmetric barriers to gene flow in sister species or habitat races (Tiffin et al. 2001; Coyne and Orr 2004, p. 226). Kawecki and Holt (2002) highlighted a potential consequence of asymmetric gene flow in which isolated sink populations receive limited genetic variation and suffer reduced capacity for local adaptation. This process appears not to have occurred in our system. Although there is strong selection at high elevation against migrants from lower elevation, demographic or genetic isolation does not appear to have prevented adaptation to high elevation.

Previous transplant experiments across elevational gradients have generated mixed evidence for adaptation to elevation (Table S4 lists studies of eight animal species and 71 plants). To be sure, some studies observe a strong home-elevation fitness advantage (Galen et al. 1991; Byars et al. 2007). But most do not. Averaging over several measures of performance and using both the local/foreign and home/away criteria, about 20–35% of comparisons have detected adaptation to elevation, 10–30% have detected maladaptation, and the majority of comparisons detected neither (Table 3). In fact, the evidence may be weaker than these values suggest, for three reasons. First, many estimates confound unique local adaptation with parallel adaptation to elevation because they include only one population or one test site each at low and high elevation. Our experiment could also be criticized for low replication, especially with the two low elevation source ponds only 11 km apart, but the eight test sites and four source populations nevertheless permitted us to distinguish unique from parallel local adaptation (Fig. 4). Second, the false impression of local adaptation can be created whenever all populations perform best in certain gardens, usually at low elevation (Grassein et al. 2014; Vitasse et al. 2014; Figures 2 and S4 in this study). When this situation is subjected to a home-away comparison, low populations appear to be adapted to elevation and high populations appear maladapted. Similarly, some studies find that certain populations perform best at all elevations (Hall et al. 1990; Gonzalo-Turpin and Hazard 2009; Grassein et al. 2014). When subjected to a local/foreign comparison, this situation suggests adaptation to elevation in gardens at the home elevation and maladaptation at other elevations. And third, some studies supporting adaptation to elevation are complicated by the fact that populations from different elevation have different ploidies or belong to different subspecies (Flegrova and Krahulec 1999; Geber and Eckhart 2005; Martin and Husband 2013). Taken together, these considerations suggest that adaptation to elevation is detected in no more than 30% of relevant comparisons.

The importance of parallel local adaptation in our study may be related to the geography of the elevational gradient occupied by *R. temporaria* in the Swiss Alps. This gradient is longer and steeper than most others that have been subjected to reciprocal transplant experiments. Source populations in this study covered an elevational range of 1871 m, and the range in growing season among ponds exceeded that in 92% of previous studies (Fig. 5A). This favors adaptation to elevation by creating strongly divergent selection regimes between low and high sites. Indeed, our literature survey found that longer gradients were associated with more positive evidence for adaptation to elevation (Fig. S10). At the same time, though, the Swiss elevational gradient is relatively steep in terms of environmental change per distance (Fig. 5B), and this is known to counteract local adaptation because gene flow can more readily connect distinct environments (Bridle et al. 2009; Bachmann et al. 2020). However, in *R. temporaria* there is evidence that gene flow between ponds at different elevations
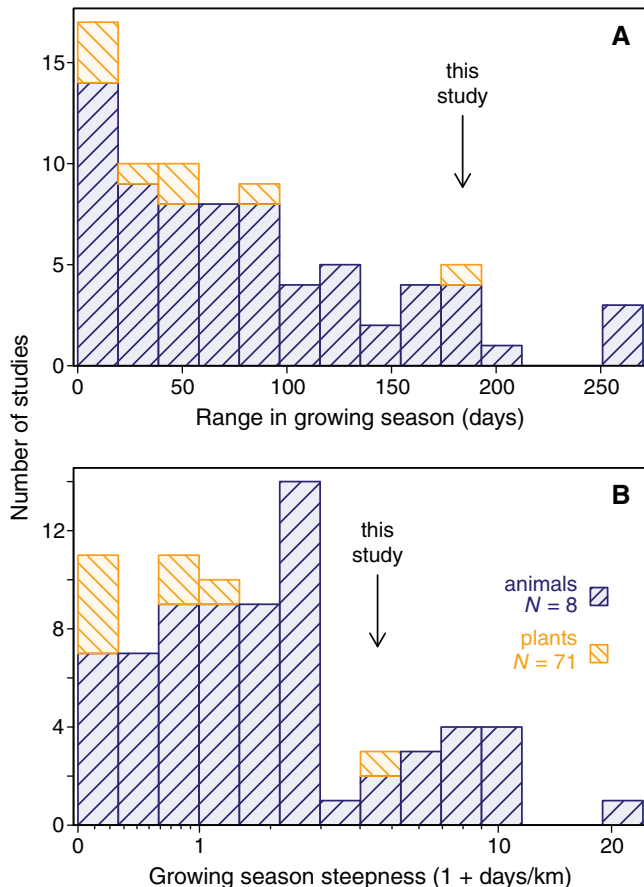
Figure 5. Reciprocal transplant experiments across elevation have been performed on gradients covering a wide range of environments. Growing season is the number of days per year with mean temperature >5°C estimated using a spline interpolation fit to monthly average temperatures. Steepness is the range in growing season length along the gradient divided by the geographic distance between sites with the most extreme growing seasons. Temperature data at 30-s resolution are from WorldClim 1970–2000 (Fick and Hijmans 2017). Details of the original studies are given in Table S4.

is lower than would be expected based on geographic distance and other factors that influence dispersal (Van Buskirk and Jansen van Rensburg 2020). Reduced gene flow may be caused by selection against immigrants or their offspring from other elevations, as discussed above, or by adaptive habitat choice of dispersing frogs (Nosil et al. 2005; Wang and Bradburd 2014). In summary, strong parallel adaptation to elevation in this system is probably enabled by modest gene flow across a gradient of highly divergent environments.

Nearly 60 years ago, Mayr (1963, p. 524) observed that a stable distribution boundary implies that the power of natural selection is limited. Otherwise, Mayr imagined that dispersal into adjacent areas would result in adaptation and gradual expansion of the range "like the rings of a tree." In contrast, our results demonstrate good potential for adaptation in populations at the edge of the distribution. *Rana temporaria* tadpoles originating from high-elevation sites, which approach the upper margin of the species' distribution in the Swiss Alps, possess a clear performance advantage at high elevation in comparison with low-elevation animals. Thus, evolutionary change at the range boundary has not been overwhelmed by gene flow from the center of the distribution or prevented by genetic drift due to small population size and fragmentation, both of which can occur in theoretical models (Garcia-Ramos and Kirkpatrick 1997; Kirkpatrick and Barton 1997; Peischl et al. 2015; Polechova and Barton 2015; reviewed in Willi and Van Buskirk 2019). This may argue against Mayr's proposal that the boundary forms where adaptation fails, but it leaves unanswered the mystery of how the upper margin of the elevational distribution is enforced.

AUTHOR CONTRIBUTIONS
J.C.B. designed the study, conducted the experiment, analyzed data, and wrote the first draft. J.V.B. designed the study, obtained funding and permits, analyzed data, and wrote the manuscript.

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DATA ARCHIVING
Data and analysis scripts are available on Dryad: https://doi.org/10.5061/dryad.4j0zpc891.

CONFLICT OF INTEREST
The authors declared no conflict of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix Table S1. Sites used in the transplant experiment. Locations are given in decimal degrees North and East. The number of *Rana temporaria* clutches was counted just after oviposition in two years (2013 and 2014).

Appendix Table S2. Tests of unique local adaptation independent of elevation. Tadpole performance is represented by survival (A), development (B), and size at metamorphosis (C).

Appendix Table S3. Linear mixed-effects models testing whether tadpole performance varied with the duration of the overwintering period experienced by their parents.

Appendix Table S4. Summary of studies reporting reciprocal transplant experiments across elevation in plants. These studies support two kinds of comparison.

Appendix Figure S1. Photographs of a low-elevation test site (A; *ellw* on 7 May 2015), a highelevation test site (B; *bern* on 26 June), and a block of four enclosures (C; *muot* on 26 June).

Appendix Fig. S2. Time course of the experiment for the 1786 tadpoles that survived to metamorphosis in low-elevation (yellow) and high-elevation (blue) test sites.

Appendix Figure S3. Distribution of fitness across all individuals in the field experiment. We calculated expected fitness as explained in the main text, integrating tadpole survival, development rate, and mass at metamorphosis.

Appendix Figure S4. Adaptation to elevation in *Rana temporaria* tadpoles. The figure depicts mean $\pm$ 1 SE ($N = 8$ enclosures) of the results for each source population evaluated in the eight test sites.

Appendix Figure S5. Weak evidence for unique local adaptation in the field experiment. The upper panels are the comparisons of local and foreign tadpoles within-test sites; performance was standardized by test site (left side of Table 2 and Table S3).

Appendix Figure S6. Estimated relative magnitudes of unique local adaptation and parallel adaptation to elevation in the field experiment. The three kinds of differences were calculated within each of the four test ponds.

Appendix Figure S7. Water temperature measured by data loggers positioned at a depth of 5 cm below the water surface at the center of each experimental block and programmed to record temperature at 30-minute intervals.

Appendix Figure S8. Results of the laboratory experiment testing for an effect of the duration of the overwintering period on tadpole performance.

Appendix Figure S9. Evidence for adaptation and maladaptation to elevation in studies that performed reciprocal transplants across elevational gradients in animals and plants, including this study.

Figure S10. Two measures of adaptation to elevation from reciprocal transplant experiments across elevational gradients.