

Life in the land of the midnight sun: are northern lizards adapted to longer days?

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The relative importance of genetic and environmental factors for explaining differences in trait distributions between populations is one of the major issues in evolutionary biology. In ectotherms, temperature can have a major impact on morphology, physiology, and life history strategies, and has often been inferred to explain differences between populations. In species with active thermoregulation, however, the ambient temperature may not be as important as the opportunity for thermoregulation. We studied growth in juvenile common lizards (*Lacerta vivipara*) originating from two environments differing in such thermal opportunity (i.e. day length). The populations differed significantly in their norms of reaction, with lizards experiencing long days in the wild showing a steeper increase in growth rate with increasing thermal opportunity. The environment with longer days also has a lower mean temperature in the wild, and in accordance with evolutionary predictions, lizards from this population had higher endurance at low temperatures. Both populations showed genetic variation in degree of phenotypic plasticity in growth rate as evident from the extensive crossing in norms of reaction.

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The extent to which differences between populations reflect genetic divergence (due to local adaptation or genetic drift) or the same genotype responding differently to local environmental conditions (phenotypic plasticity) is important for understanding the evolutionary process and its outcome. Furthermore, phenotypic plasticity may itself be under selection, with divergence in norms of reaction for populations inhabiting different environments. For example, many species of reptiles show variation in a broad range of morphological, physiological, and life history traits between populations (Dunham et al. 1988, Tsuji 1988a, b, Niewiarowski and Roosenburg 1993), and are therefore well suited for studies of the relative importance of genetic and environmentally plastic factors underlying differences in phenotypes between populations. To assess whether populations have diverged genetically or only differ as a result of pheno-

typic plasticity, the conventional approach is to perform a common garden experiment, where organisms are raised under controlled conditions differing in the relevant aspect under study (e.g. temperature). Alternatively, reciprocal transplants can be compared under free-ranging conditions. If the genotypes express the same phenotype under the same conditions, phenotypic plasticity is the cause of the observed differences between populations. If differences between populations are maintained, however, some degree of genetic divergence has occurred (reviewed by Pigliucci 2001).

Undisputably, one of the most conspicuous differences between habitats is their thermal regime. For example, mean temperature differs between high and low altitudes, which has been suggested to explain general patterns of inter- and intraspecific variation in, e.g. body size and life history traits (Mayr 1963,

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Adolph and Porter 1993, Atkinson 1994). In ectotherms, such variation in temperature can have a major impact on the distribution of morphological, physiological, and life history traits (Avery 1984, Huey and Kingsolver 1989, Adolph and Porter 1993) and has been shown to cause geographical trait variation in, e.g. amphibian and reptilian populations (Berven 1982a, b, Niewiarowski and Roosenburg 1993, Bronikowski 2000, Ståhlberg et al. 2001). Studies of correlations between preferred body temperatures (T_p) and optimal performance in vertebrates experiencing different temperature regimes have, however, produced ambiguous results, with little or no evidence for evolutionary response to the thermal environment in many taxa (Van Damme et al. 1990, Castilla et al. 1999, Gvozdik and Castilla, 2001, Jonsson et al. 2001, Angilletta et al. 2002), and with differences between studies largely being a result of different experimental protocols (Bauwens et al. 1986, Gvozdik and Castilla 2001). One reason for this inconsistency could be that for species with active thermoregulation (such as lizards and snakes), the ambient temperature is not as important as thermal opportunity, i.e. the number of hours per day that an individual can achieve its preferred body temperature by basking in the sun. Indeed, lizards and snakes can increase their body temperature to levels permitting activity by active basking even in early spring when the ambient temperature is still low. Thermal opportunity have a major impact on ingestion and growth rate in lizards (Avery 1984), but despite the potential for evolutionary divergence between populations differing in thermal opportunity, relatively few studies have addressed this question compared to studies of differences in performance related to differences in mean temperatures (Sinervo 1990, Sinervo and Adolph 1994).

Northern Scandinavia is characterized by relatively short summers, but long days (the midnight sun). Thus, although the overall activity season for animals that are dependent on high temperatures for activity (such as lizards and snakes) is shorter in the north, animals can be active for longer each day with favourable weather. Therefore, we might expect northern ectotherms to have evolved a better ability to cope with long activity periods per 24 h (and lack of sleep) than populations living under more "normal" light:dark conditions. In lizards, growth rate relates to fitness by affecting age and size at maturity and is likely to be under selection for increased performance (Adolph and Porter 1993). To test whether or not populations experiencing different opportunities for thermoregulation have diverged genetically, we therefore studied growth rate in juvenile common lizards, *Lacerta vivipara*, from northern and southern Sweden in a common garden experiment mimicking long versus short day length.

Materials and methods

The common lizard, *Lacerta vivipara*, is a small, 50–70 mm snout-vent length (SVL), 3–5 g, ground-dwelling lizard. It has one of the largest distribution ranges of all reptiles, occurring in most parts of Europe and northern Asia, and inhabiting a variety of habitats throughout its distribution. Thus, *L. vivipara* is an ideal species for studies of geographic differences in thermal determinants of growth (Sorci et al. 1996, Lorenzon et al. 1999, 2001, Sorci and Clobert 1999). In Sweden, the common lizard is live-bearing with a mean clutch size of 5 young. Mating begins a couple of weeks subsequent to emergence from hibernation, which occurs in March–April in southern Sweden and May–June in the northern part.

Because lizard activity is governed by thermal opportunity, data on sunrise and sunset was collected from the Swedish Almanack (Esselte Almanacksförlag). Mean day lengths during the respective activity seasons are 21 h 20 min for the northern population (late May–August) and 15 h 40 min for the southern population (April–early September). This difference in day length reflects patterns of activity, with lizards from the southern part being active approximately from 07.00 until 20.00 at favourable weather, whereas in northernmost Sweden, lizards can achieve high body temperatures from 03.00 until 24.00, a difference in 8 hours (the sun actually never sets during part of the summer in northern Sweden, but in most habitats it comes too close to the horizon during the midnight hours to permit activity, T. Uller, pers. obs.). Mean air temperatures collected from the Swedish Meteorological Institute show that the mean ambient temperature during the same period are higher for the southern population (13.24 versus 10.47°C, south and north respectively).

38 gravid females from four localities in the southwest of Sweden (Öjersjö: N 57°42' E 12°8'; Sandsjöbacka: N 57°32' E 12°2'; Asketunnan: N 57°22' E 11°58'; Hållsundsudde: N 57°21' E 12°0'), and 18 from two localities in the north (Markitta: N 67°10' E 21°30'; Mettä-Markitta N 67°12' E 21°28') were caught during April–June 2001 and transported to the University of Göteborg. At capture, females were weighed to the nearest 0.01 g, measured snout-vent length (SVL) and total length to the nearest mm and head width and head length to the nearest 0.01 mm. They were kept in 500 × 400 × 350 mm cages, 4–5 lizards in each cage, with peat and bark as bottom substrate, and rocks and piles as shelter. The females were fed mealworms (*Tenebrio* larvae) and crickets (*Gryllus* sp.) once a day with water given ad libitum. A 40 W light bulb allowed the females to thermoregulate for 10 h per day, with the ambient temperature being 22°C during daytime and 18°C during night. Potential differences in preferred body temperature of females from the different regions, and thus differences in temperature experienced by the

embryos, were analysed by monitoring 10 lizards from the south and 13 lizards from the north every 30 minutes with respect to cloacal temperature with a thermometer (Schultheiss, Germany) during 2.5 h at midday on two subsequent days (thermal gradient in cages being 22–38°C).

Immediately before parturition, the females were assigned to separate cages to ensure accurate scoring of maternity. Cages were checked at least twice daily for hatchlings. At parturition, hatchlings were toeclipped for individual identification, weighed to the nearest mg, and measured (SVL and total length) to the nearest 0.5 mm with a ruler, and head width and head length to the nearest 0.01 mm with a pair of callipers. The neonates were kept in cages as described for adults, with 15–20 juveniles per cage. Water and *Drosophila* flies were provided ad libitum.

On day 1–3 after parturition, the lizards were tested for endurance in a physiological performance trial. Physiological performance was estimated by letting the neonates swim in a 700 × 400 × 350 mm thermally insulated aquarium filled with water. Each juvenile lizard was subjected to three swimming trials, one on each consecutive day, at three different temperatures (24, 30 or 36°C, in random order). Juveniles were transferred directly from their cages into the water, with swimming immediately commencing, predominantly by undulating movements of the tail. When the lizard stopped swimming, it was encouraged to continue by a light tap on its body side with a plastic ruler. When it did not resume swimming after three consecutive taps, the trial was interrupted. Experience from earlier studies show that this method gives a repeatable estimate of endurance in neonate common lizards with more repeatable scores than, e.g. sprint speed and tread mill endurance (Olsson et al. 2002, M. Olsson et al., pers. obs.)

Subsequent to the swimming trials, the lizards were transferred to small separate circular-bottomed cages (diameter × height: 100 × 140 mm), with paper as substrate and rocks as shelter. The cages were placed in thermal incubators at two different light regimes, 18:6 L:D, and 6:18 L:D. In the L phase the neonates were kept at 30°C, within the preferred body temperature for common lizards (Van Damme et al. 1986, Uller unpubl., and Results), and in the D phase they were kept at 15°C. This design forced the lizards to experience long or short days of their preferred body temperature and thus excluded confounding thermoregulatory behaviour (Sinervo 1990). Families were split, with half the young in each treatment. The cages were sprayed with water once a day and the juveniles were fed *Drosophila* and crickets ad libitum. After 15 days, the growth experiment was interrupted and the lizards again weighed and measured as described above. Growth rate was calculated as (mass subsequent to treatment) – (mass before treatment). The same quali-

tative results were obtained using ratios. Only families represented by at least two juveniles in each treatment were used in analyses of growth rates.

Results

Female lizards from northern Sweden were significantly larger than those from the south (mean ± SE mm; north: SVL = 63.6 ± 1.56, tot = 150.5 ± 15.86, N = 18; south: SVL = 57.0 ± 0.97, tot = 125.0 ± 3.76, N = 38; t-test; SVL: $t = 3.69$, $p = 0.0005$, tot: $t = 2.12$, $p = 0.039$, $df = 55$). There was no difference in female preferred body temperature between populations (north: 30.12 ± 0.61°C; south: 30.04 ± 0.83°C; t-test; $t = 0.26$, $p = 0.79$, $df = 22$). Of the 56 females, 41 gave birth to live young, with difference in mean clutch size between populations showing borderline significance (north: 5.21 ± 0.53; south: 4.04 ± 0.35; Wilcoxon two-sample test, $Z = 1.93$, $p = 0.053$). To avoid the problems inherent in an unbalanced nested analysis, we used mean scores per female in analyses of differences between populations. An ANOVA with latitude as factor, and clutch size as covariate (to control for differences in size due to differences in clutch size), revealed that northern hatchlings were both longer and heavier than their southern conspecifics (birth weight: $F_{[1, 39]} = 5.05$, $p = 0.030$; SVL: $F_{[1, 39]} = 23.80$, $p < 0.001$; total length: $F_{[1, 39]} = 12.80$, $p < 0.001$). Body mass at parturition was significantly correlated with endurance at all temperatures, and we therefore incorporated birth mass as a covariate in an ANOVA with endurance as dependent variable and latitude as factor. Northern juvenile lizards had significantly higher endurance at 24°C, whereas the corresponding differences at 30 and 36°C fell short of significance (24°C: $F_{[1, 29]} = 16.01$, $p < 0.001$; 30°C: $F_{[1, 29]} = 0.56$, $p = 0.46$; 36°C: $F_{[1, 29]} = 0.15$, $p = 0.70$).

To look for differences in growth rate, we first ran an ANOVA with family, and the interaction between latitude and day length (short/long), as factors, and with birth mass as a covariate. The model revealed significant effects of family and the latitude × treatment interaction on growth rate, with northern lizards having a higher increase in growth rate with increasing thermal opportunity than southern lizards (family: $F_{[28, 117]} = 3.51$, $p < 0.001$; latitude × day length: $F_{[2, 117]} = 167.45$, $p < 0.001$, Fig. 1). Separate t-tests (from mean scores per female) for each day length, i.e. ignoring the latitude × day length interaction, did not show significant differences between populations (short day: $t = 0.55$, $p = 0.58$, $df = 38$; long day: $t = 1.25$, $p = 0.22$, $df = 36$). To look for differences in genetic and maternal effects between populations and treatments, we also performed separate ANOVAs for each latitude and day length, with family as factor and body mass as covariate.

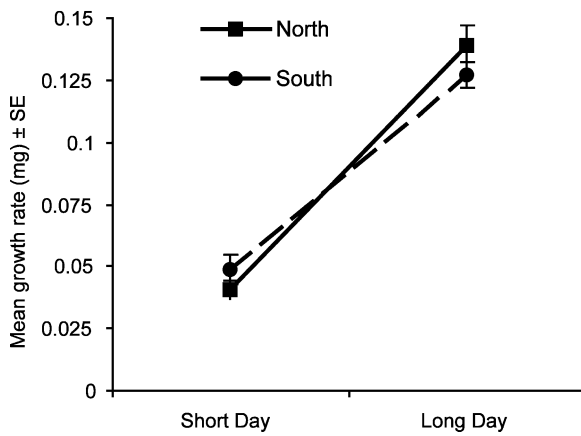


Fig. 1. Mean growth rates \pm SE for north (solid line) and south (broken line) populations in short and long day length treatments. See text for test statistics.

Family effects were significant or showed borderline significance in all these analyses, whereas birth mass only influenced growth rate for the northern population in the long day treatment (Table 1). Southern lizards showed no significant correlation between growth rates in short versus long day treatment, whereas in northern lizards it bordered on significance (south: $r = 0.11$, $p = 0.59$; north: $r = 0.55$, $p = 0.0508$). Extensive crossing of reaction norms for growth rate between treatments suggests that there was significant genetic variability for phenotypic plasticity in this trait in both populations (Fig. 2).

Discussion

Animals inhabiting northern Scandinavia experience relatively short summers compared to their conspecifics in more southern parts. Days are longer in the north, however, which allow animals dependent on thermoregulation to be active for longer periods per 24 h when weather permits. This scenario should select for genotypes that can maintain physiological processes for prolonged periods, withstand lack of sleep, uphold vigilance against predators, and endure long foraging bouts. This is supported by our comparison of growth rates for juvenile lizards originating from southern and

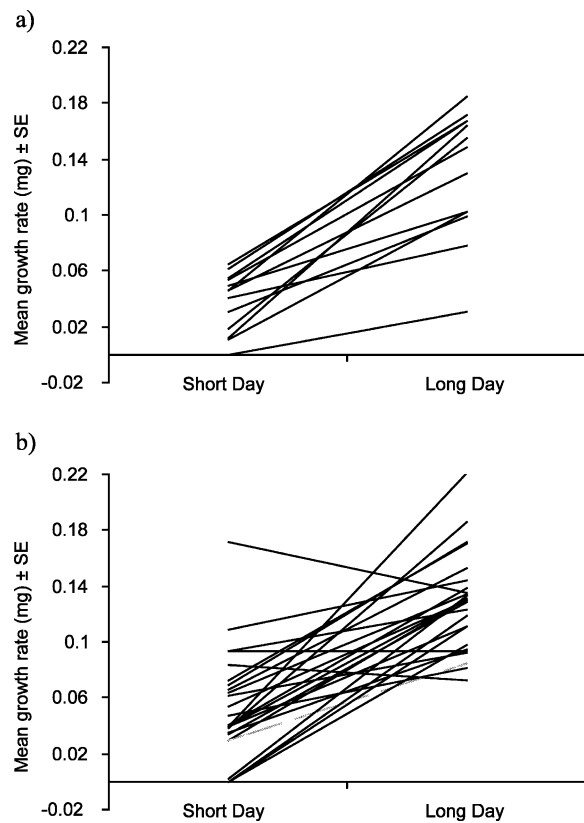


Fig. 2. Family reaction norms for growth rate for north (a) and south (b) populations in short and long day length treatments.

northern Sweden which shows that lizards from northern Sweden have a higher increase in growth rate with increasing thermal opportunity than lizards from the south (Fig. 1, 2). Thus, northern and southern populations seem to have diverged with respect to response to thermal opportunity, which parallels the thermal conditions experienced in natural populations. Both populations showed variation in phenotypic plasticity for growth rate, as evident from the extensive crossing of reaction norms (Fig. 2).

That thermal opportunity can select for population divergence with respect to growth rate has also been shown in intra- and interspecific comparisons of growth rates in the lizards *Sceloporus graciosus* and *S. occiden-*

Table 1. Results from one-way ANOVAs with growth rate (in mass) as dependent variable, family as factor and birth mass as a covariate.

Latitude	Day length	Family			Birth mass		
		F	p	df	F	p	df
North	Short	2.62	0.030	11, 20	0.28	0.60	1, 20
	Long	3.62	0.005	11, 23	8.85	0.007	1, 23
South	Short	3.17	0.006	14, 25	0.19	0.66	1, 25
	Long	1.99	0.070	16, 21	0.02	0.89	1, 21

talis (Sinervo and Adolph 1994). In these species, norms of reaction paralleled differences in opportunity for activity in natural populations, both when contrasting the different species and within *S. occidentalis* inhabiting different thermal environments (Sinervo and Adolph 1994).

In the present study, juveniles from the northern range of the distribution had higher endurance at 24°C, whereas the corresponding difference at 30 and 36°C were non-significant. This is in accordance with the overall ambient temperature data from the populations, which on average is lower in the north through the activity season in terrestrial habitats. Furthermore, being active for longer times each day is also likely to involve activity at lower temperatures (e.g. having to move during the colder period of the day, when thermoregulation is still possible), further selecting for increased endurance at relatively low temperatures in northern populations. Additional support for selection for endurance may also stem from the significant difference in birth mass between populations (controlling for differences in clutch size). In all our thermal treatments, endurance was positively correlated with body size, which may give the larger size in northern hatchlings greater possibility to actively forage over long periods of time. Indeed, body size at parturition had a significant influence on growth rate in the long day treatment for northern, but not southern lizards. At short day lengths, however, birth mass did not influence growth rate for any of the populations. Another potential influence on positive directional selection for birth mass in the north could be to withstand the longer hibernation period, as fat reserves is likely to influence probability of survival in juvenile common lizards (Avery 1970, Bauwens 1981).

Given the overall short activity season in the north, one would perhaps have expected northern lizards evolving towards thermal opportunists, i.e., having a higher growth rate in general and even more so at relatively long days. In some fish species, populations inhabiting relatively colder water have a lower growth rate in the wild than populations living under warmer conditions, but when reared in common garden experiments, the populations experiencing the harsher environment grow faster than their conspecifics even at relatively warm conditions (Conover and Present 1990). Thus, there is a negative covariation between genotype and environmental conditions which reduces the phenotypic variation in nature (countergradient variation, Conover and Schultz 1995). Our study does not support this scenario, however, as northern lizards did not show an overall increased growth rate compared to southern lizards. The reason for the lack of support for the counter-gradient hypothesis in this study may be that the short activity season is balanced by the longer days in the north, and therefore that the overall time for growth (i.e. days that allow foraging and high

metabolism) is similar for both populations. Unfortunately, data on number of days that permit activity over the whole season are lacking for both populations. Even if the overall time permitting activity was similar, however, we would still expect northern lizards to be relatively better able to cope with long days, which is supported by the significant differences in norms of reaction between the populations.

In conclusion, our study suggests that common lizards from populations differing in day length have diverged with respect to norms of reaction of growth rate, with northern lizards having a steeper increase in growth rate with increasing thermal opportunity, and with both populations showing genetic variation in response to thermal opportunity as evident from crossing norms of reaction.

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