Testing the persistence of phenotypic plasticity after incubation in the western fence lizard, *Sceloporus occidentalis*

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ABSTRACT

Hypothesis: Phenotypic variation in traits induced by different incubation temperatures does not persist into the lifetime of young lizards, and therefore contributes little to variation in long-term fitness.

Organism: Western fence lizard (*Sceloporus occidentalis*).

Methods: Split-clutch laboratory incubation experiment including eggs from two different populations under two different incubation regimes, measurement of morphological traits at hatching, and tracking of morphology and temperature preference behaviour for 7 weeks after hatching.

Results: Several morphological traits, including body mass, hindlimb length, inter-limb length, and tail length, initially differed between the two incubation treatments, but only the difference in tail length persisted to age 7 weeks. Thermal preference was relatively conserved, with juveniles showing no difference in mean selected body temperatures across treatments; however, warm-incubated lizards thermoregulated more precisely than their cool-incubated counterparts.

Conclusion: Studies of incubation effects can reveal changes in animal phenotypes post-hatching, but if these effects do not persist, they may not be subject to natural selection and consequently be of little ecological relevance.

Keywords: incubation, performance, phenotypic plasticity, *Sceloporus occidentalis*, thermoregulation.

INTRODUCTION

In nature, animals often exhibit substantial intraspecific geographic variation in their average phenotypes (Endler, 1977; Forsman and Shine, 1995). Historically, researchers have recognized

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genetic differentiation as the main source of geographic variation. However, we now recognize that many cases of geographic variation result from phenotypic plasticity in addition to genetic divergence. The geographic variation that results from phenotypic plasticity is not necessarily adaptive; it may simply be the result of physical or chemical effects of environmental factors on developing phenotypes (Smith-Gill, 1983; Stearns, 1989). Therefore, it is important to examine whether environmentally induced phenotypic variation is consistent with optimized organismal phenotypes in particular environments. Recent common-garden studies have attempted to distinguish the relative contributions of genetic variation and phenotypic plasticity to phenotypic variation among populations that are distributed along latitudinal or altitudinal gradients (Smith-Gill, 1983; Ford and Seigel, 1989; Qualls and Shine, 1996; Travis *et al.*, 1999; Olsson and Uller, 2002; Sears and Angilletta, 2003). These studies are an important first step to understanding the possible adaptive significance of phenotypic plasticity.

In recent years, the recognition of phenotypic plasticity as an important evolutionary phenomenon has led to an explosion of studies examining its mechanisms and adaptive significance (Smith-Gill, 1983; Gotthard and Nylin, 1995; Schlichting and Pigliucci, 1998; Pigliucci, 2001; West-Eberhard, 2003). Many studies on animals, especially ectotherms, have focused on developmental plasticity during incubation as a source of phenotypic variation in neonates. For example, variables such as temperature and moisture of the incubation environment can result in changes in the phenotypes of hatchling lizards (Elphick and Shine, 1998; Qualls and Shine, 1998; Qualls and Andrews, 1999), snakes (Burger, 1990, 1991a), turtles (Du and Ji, 2003; Glen *et al.*, 2003), and fishes (Hansen and Petersen, 2001; Einum and Fleming, 2002). A wealth of empirical data reveals substantial physiological effects of the incubation environment on offspring olfaction (Burger, 1991b), morphology (Shine *et al.*, 1997; Elphick and Shine, 1998; Qualls and Shine, 2000), performance and behaviour (Marsh and Bennett, 1986; Qualls and Shine, 1998; Du and Ji, 2003), and growth rates (Sinervo and Adolph, 1994; Du and Ji, 2003). In particular, lizards have proven to be an ideal system for studying plasticity (Sinervo and Adolph, 1994; Sears and Angilletta, 2003).

Many empirical studies assume that the phenotypic results of developmental plasticity are adaptive (Ferguson and Brockman, 1980; Packard *et al.*, 1987; Qualls and Shine, 1998). Incubation effects may play a significant role in hatchling survival because they occur during the important first stages of hatchling growth and development (Hansen and Petersen, 2001). However, several factors complicate this interpretation. First, environmental factors such as temperature almost inevitably affect the phenotypes of animals, especially ectotherms, in ways that may not be adaptive, but simply developmentally unavoidable (Smith-Gill, 1983; Stearns, 1989; Sinervo and Adolph, 1994). Second, the phenotypic effects of incubation environments may be transitory (Qualls and Shine, 2000; Watkins and Vraspir, 2006). This is especially important, because if induced morphological changes are 'visible' to natural selection only very briefly, the adaptive 'value' of such temporary induced morphologies is accordingly reduced. However, few studies have determined how long these effects persist through ontogeny (Elphick and Shine, 1998; Qualls and Shine, 2000). Other factors, including the environment experienced after birth, may prove more important to a young organism than its incubation temperature. Indeed, several recent studies have hypothesized that whereas developmental plasticity may occur across a wide variety of taxa, induced phenotypes may not contribute to long-term fitness unless they persist into the lifetime of the animal (Elphick and Shine, 1998; Qualls and Shine, 2000). Experiments exploring the persistence of phenotypes beyond hatching are thus important to understand the ecological relevance of these induced phenotypes.

In this study, we examined the effects of incubation temperature on offspring traits, and specifically addressed whether any effects observed in hatchlings persisted through the first

several months. We also examined whether lizards from different populations differed in the manner by which incubation temperature influenced hatchling traits, given that these populations experience different temperatures. Adult fence lizards from the desert site tend to be smaller [snout-to-vent length and mass (C. Buckley, unpublished data)] and more arboreal (Adolph, 1990a, 1990b; Sinervo and Losos, 1991) than lizards from the mountain site; however, adults from the two populations differ only slightly in their field body temperatures (Adolph, 1990a). As mentioned above, incubation temperature is known to induce substantial phenotypic changes in hatchling lizards, affecting traits ranging from morphology to olfaction (Van Damme *et al.*, 1992; Shine *et al.*, 1997; Qualls and Shine, 1998; Downes and Shine, 1999; Qualls and Andrews, 1999; Andrews *et al.*, 2000). To remain consistent with previous studies on other species, we altered the incubation environment to observe its relative effect on hatchling phenotypes.

Finally, we examined a third factor that has been rarely considered in studies of developmental plasticity – age. Rearing hatchling lizards in the laboratory and observing changes in their phenotypes as they mature enables researchers to discern the persistence of phenotypic effects induced by population and incubation factors. Our experimental design allows us to examine the interactions among these three factors (population, incubation, and age) in shaping the phenotypes of western fence lizards. We designed a laboratory experiment meant to approximate nest temperatures experienced by populations at two different altitudes. We chose two populations of the western fence lizard, *Sceloporus occidentalis*, which occur in two different habitats in the San Gabriel Mountains of southern California. The desert population (Largo Vista), located at the southern edge of the Mojave Desert, occurs at 1370 m in elevation and experiences an average daytime summer temperature that is 8.8°C warmer than the mountain population (Table Mountain), which occurs at 2230 m elevation on the eastern edge of the San Gabriel mountains (Adolph, 1990a). We measured juvenile body, limb and tail morphology, and thermal preference in the laboratory as indicators of lizard phenotypes at hatching, and repeated these measurements at regular intervals (every 2 weeks after the first week) until 7 weeks of age.

We tested the null hypothesis that the population of origin and incubation temperature (cool = 24°C and warm = 30°C) will have no effect on hatchling phenotypes in these lizards. The alternative hypothesis was that source population and incubation environment will influence hatchlings' resultant phenotypes. The temperature–size rule predicts that within most animal species, body size increases with decreasing temperature (reviewed by Atkinson and Sibly, 1997). Phylogenetic evidence shows that squamates (lizards and snakes) usually reverse this trend, tending to reduce in size at higher latitudes (Ashton and Feldman, 2003), but some studies have revealed larger lizards at higher temperatures or higher latitudes (Van Damme *et al.*, 1992; Qualls and Shine, 1998). Based on our own observations of adult *S. occidentalis* across populations in nature, we predict that cooler temperatures will produce larger hatchlings with shorter limbs in this species. In this case, there are two likely outcomes of rearing hatchlings in the laboratory. First, the combination of source population and incubation environment could affect juvenile lizards' phenotypes in a consistent and parallel fashion, or even accentuate their phenotypes, irrespective of the lizards' age. Second, these two factors may interact with the age of the lizard to create a relationship between phenotypic traits that does not persist through time, but rather is altered by the environment the juvenile experiences after hatching. We predict that the phenotypic variation produced by different incubation environments will not persist into the lifetime of the juvenile lizards, but rather that lizards from different experimental treatments and from different

populations converge on an intermediate phenotype over time. Here we discuss our results in the context of the ecological relevance of incubation studies in lizards.

MATERIALS AND METHODS

Study organism

The western fence lizard, *Sceloporus occidentalis*, is a medium-sized (56–87 mm snout-to-vent length) semi-arboreal lizard that occurs along the western coastal states of the United States and south into Mexico. This lizard ranges from northern Baja California into Washington and occupies a wide variety of open and dry habitats from sea level to about 2700 m in elevation (Behler, 2000). Females in most populations lay one clutch of eggs per reproductive season, regardless of elevation or altitude (Goldberg, 1974; Davis and Ford, 1983). The large ranges of latitude and elevation in these lizards makes them an ideal study organism for studies of geographic variation.

Collection and maintenance

During the months of May and June, we collected gravid female *Sceloporus occidentalis* from two elevations in the San Gabriel Mountains in southern California: (1) Largo Vista, 8 km east of Valyermo, Los Angeles County, on the edge of the Mojave Desert (elevation 1370 m; hereafter, Largo Vista), and (2) Table Mountain, 2 km northwest of Wrightwood, San Bernardino County (elevation 2230 m; hereafter, Table Mountain). The two sites are 11.6 km apart and the area between them is continuously inhabited by *S. occidentalis*, so the two sites are not completely isolated from one another. We collected eggs from 30 females (15 from Largo Vista, 15 from Table Mountain). Females collected at each site were transported within 24 h to the laboratory in Claremont, CA, and housed individually in 37-litre glass terraria in an animal room kept at room temperature. The lizards' substrate consisted of mixed peat moss and sand at a depth of 4 cm, and several pieces of bark were supplied for cover. Each lizard was given a heat lamp cycling at 10 h light/14 h dark, creating a thermal gradient of approximately 40°–20°C in which the lizards could thermoregulate. Lizards were fed crickets dusted with vitamin powder and sprayed with water *ad libitum*.

We checked each cage at least once daily for the presence of eggs. Females readily buried eggs in the peat moss/sand substrate, and became visibly gaunt after laying eggs, so the date of oviposition was determined despite eggs being buried. Eggs were removed from cages, weighed, cleaned off, and placed individually in 200-ml plastic cups filled with moistened vermiculite [10:1 ratio of vermiculite to water by mass to produce a water potential of approximately −200 kPa (Tracy *et al.*, 1978)], and covered with plastic wrap to prevent water loss. Vermiculite was replaced every 7 days and eggs were weighed to the nearest 0.01 g using an analytical balance (A&D model #FX-400). We split each clutch (clutches ranged from 4 to 12 eggs) and assigned the eggs randomly to one of two incubation treatments: warm (30°C) or cool (24°C). We chose these temperatures as extremes that produce viable, healthy individuals in *Sceloporus* lizards. Females laying eggs in the laboratory also tend to choose mean temperatures between these two extremes (C. Buckley, unpublished data). Incubators were checked daily for the presence of neonates.

Juveniles were housed individually in plastic terraria with a damp sand substrate, fed 2-week-old crickets, and supplied with water in excess. After one week, all hatchlings were

weighed, measured and tested for thermal preference (see below). Hatchlings were then kept in a constant-temperature incubator with a cycling temperature of 32°C during the day and 20°C at night, with a lighting cycle of 10 h light/14 h dark.

Morphology, growth, and thermoregulation of juveniles

Juvenile lizards were subjected to a number of tests at hatching, and at 1, 3, 5, 7, and 9 weeks of age. Body morphology measurements were recorded to the nearest 0.01 cm using digital calipers (Mitutoyo, series 500), including snout-to-vent length (SVL), hindlimb length, forelimb length, inter-limb distance (the distance between the posterior insertion of the forelimb and the anterior insertion of the hindlimb), and tail length. Mass was also recorded to the nearest 0.01 g (A&D model FX-100 analytical balance).

Juvenile-preferred body temperatures (T_b) were recorded beginning at one week of age. We placed each juvenile in a thermal gradient box to determine thermal preference. The temperature in these boxes was controlled by placing a heat bulb (Philips 250 W) above one end of the 2-m gradient box; temperature ranged from approximately 50°C at one end to 25°C at the other end of the box. We covered the bottom of the gradient boxes with approximately 3 cm of sand to mimic the animals' natural habitats. We measured substrate operative temperature as a proxy for lizard internal body temperatures by using a thermo-couple wire within a sealed copper tube that was approximately the size of the juvenile and was painted grey to approximate the reflectivity of western fence lizards. Taking cloacal temperatures of small juveniles is stressful to small lizards, and the body temperature of a small hatchling can change dramatically after only a few seconds of handling (Andrews and Rose, 1994). Substrate operative temperatures correlate closely with body temperatures in hatchlings of the eastern fence lizard, *Sceloporus undulatus*, a sister species to *S. occidentalis* (Warner and Andrews, 2002); thus, substrate operative temperature was considered a viable predictor of hatchling body temperature. The juvenile's position in the gradient was marked at both one and two-hour intervals in the gradient box. Upon entering the thermal gradient, juveniles sometimes fled to the far side of the thermal box, away from the researcher. If lizards had not settled into a thermoregulation position (i.e. body in basking position near the heat lamp) after one hour, they were prompted to return to the heat lamp by nudging with a small stick. If they still had not assumed a thermoregulatory position after a further 10 min, they were excluded from the analysis. After removal of the hatchling from the thermal gradient box, we immediately recorded the substrate temperatures for the lizard's two chosen positions.

Statistical analysis

We used analysis of covariance (ANCOVA) to analyse increase in egg mass (growth) as a function of egg age between the two incubation treatments. We used Student's *t*-tests to evaluate differences between incubation-by-population treatment groups at hatching for morphological and thermal preference variables. We also used two-way repeated-measures analysis of variance (ANOVA), with source population and incubation environment as factors and hatchling age as the repeated measure, to determine the relative effect each has on the various morphological and behavioural characters.

RESULTS

Incubation and egg characteristics

Clutch size averaged 8.4 ± 1.85 (standard deviation) eggs, and did not differ between sites ($t_{1,14} = 1.554$, $P = 0.142$); therefore, since we split clutches, each incubation treatment contained, on average, four eggs from each clutch. There was no difference in egg mass at laying between eggs assigned to each incubation treatment ($t_{1,28} = 1.367$, $P = 0.183$) or between eggs from the different source populations ($t_{1,28} = 0.295$, $P = 0.770$). During incubation, warm-incubated eggs gained mass more quickly (by taking up water) than did cool-incubated eggs (ANCOVA, slopes test: $F_{1,427} = 7.19$, $P = 0.008$; Fig. 1), and between 33 and 40 days (one week before the average hatching time for warm-incubated eggs) the warm-incubated eggs were therefore heavier than the cool-incubated eggs ($t_{1,28} = 3.674$, $P = 0.001$). The survival rate to hatching was lowest for Table Mountain individuals in the warm environment, and eggs incubated in the cool environment took almost twice as long to hatch as did eggs incubated in the warm environment, irrespective of source population (Table 1).

Hatchling morphology

We analysed data on a total of 170 hatchlings. Incubation regime had variable effects on hatchling characteristics for animals from both source populations. Cool-incubated hatchlings were significantly heavier than warm-incubated hatchlings immediately after hatching ($t_{1,167} = 7.37$, $P < 0.001$), had shorter hindlimbs ($t_{1,87} = 2.36$, $P = 0.021$), shorter
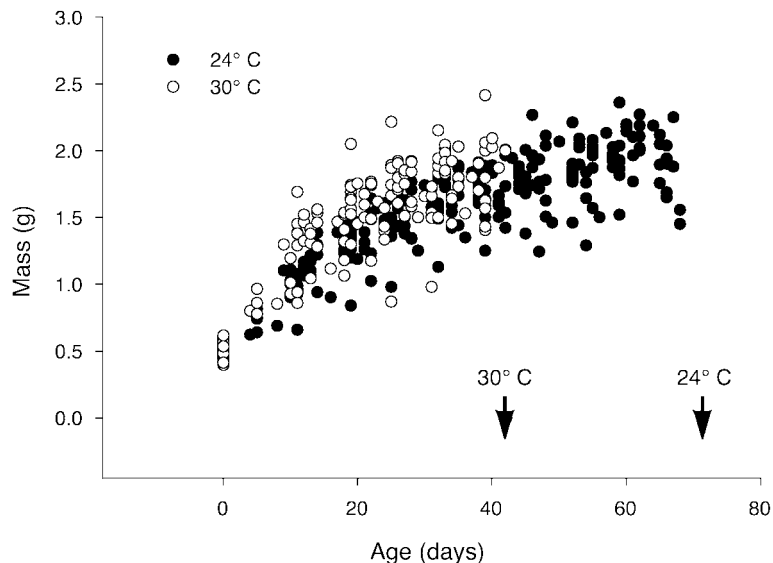

Fig. 1. Mass of eggs incubated at two temperatures, 24°C and 30°C, plotted over time. Eggs from the same clutch were averaged to create one data point. Eggs were weighed on a weekly basis until hatching. The solid arrows indicate the average hatching time for warm-incubated and cool-incubated neonates.

Table 1. The effects of incubation temperature regimes on incubation period, egg survival, and hatchling characteristics in the western fence lizard from two populations (mean ± standard error)

Trait	Largo Vista		Table Mountain	
	Cool ($n = 59$)	Warm ($n = 50$)	Cool ($n = 63$)	Warm ($n = 79$)
Incubation length (days)	71.5 ± 0.28	42.1 ± 0.20	71.2 ± 0.29	42.2 ± 0.24
Egg survival to hatching (%)	76	68	73	56
Hatchling mass (g)	0.75 ± 0.01	0.67 ± 0.01	0.75 ± 0.01	0.67 ± 0.01
Snout-to-vent length (mm)	27.4 ± 0.15	27.5 ± 0.18	27.45 ± 0.19	27.12 ± 0.17
Forelimb length (mm)	8.42 ± 0.08	8.63 ± 0.13	8.61 ± 0.10	8.57 ± 0.09
Hindlimb length (mm)	11.07 ± 0.14	11.16 ± 0.11	11.3 ± 0.12	11.4 ± 0.11
Inter-limb length (mm)	12.8 ± 0.12	13.66 ± 0.19	13.02 ± 0.13	13.49 ± 0.14
Tail length (mm)	34.5 ± 0.64	37.36 ± 0.61	34.7 ± 0.51	37.2 ± 0.50

Table 2. Summary of *F*-statistics for repeated-measures ANOVAs conducted to determine the effect of population of origin and incubation temperature on hatchling morphology

Dependent variable	Between subjects				Within subjects			
	Pop	Inc	Pop × Inc	d.f.	Age	Age × Pop	Age × Inc	Age × Pop × Inc
Mass	1.008	0.95	0.042	1, 73	127.587***	0.032	4.305**	0.965
SVL	1.071	0.729	1.147	1, 73	195.031***	0.726	1.085	0.844
Forelimb	0.029	0.794	6.88*	1, 73	42.338***	0.497	1.571	0.378
Hindlimb	3.169	5.119*	1.426	1, 73	79.237***	0.977	1.572	0.35
Inter-limb	1.354	6.97*	0.474	1, 73	72.114***	0.372	8.145***	1.272
Tail	1.867	0.859	0.242	1, 73	19.294***	0.478	3.256*	1.113
Mean body temperature (T_b)	0.038	0.093	0.407	1, 52	0.04	0.763	1.284	2.911*
T_b precision	1.256	4.301*	0.31	1, 43	1.004	1.844	0.986	0.7

Note: Offspring age (1, 3, 5, and 7 weeks of age) was the repeated factor. Pop = population, Inc = incubation temperature.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

inter-limb lengths ($t_{1,167} = 4.76$, $P < 0.001$), and shorter tails ($t_{1,167} = 4.65$, $P < 0.0001$) than warm-incubated hatchlings (Table 1). On the other hand, incubation environment did not have a significant effect on hatchling SVL or forelimb length. There were no effects of source population on any hatchling morphological variables (Table 1).

Persistence of incubation effects

Lizard age had a significant effect on all morphological variables measured (within-subject age effect; Table 2). Mass showed a significant interaction between age and incubation temperature (Table 2), as hatchlings decreased in mass between their first and third weeks before gaining mass again throughout the rest of the study (Fig. 2). Snout-to-vent length
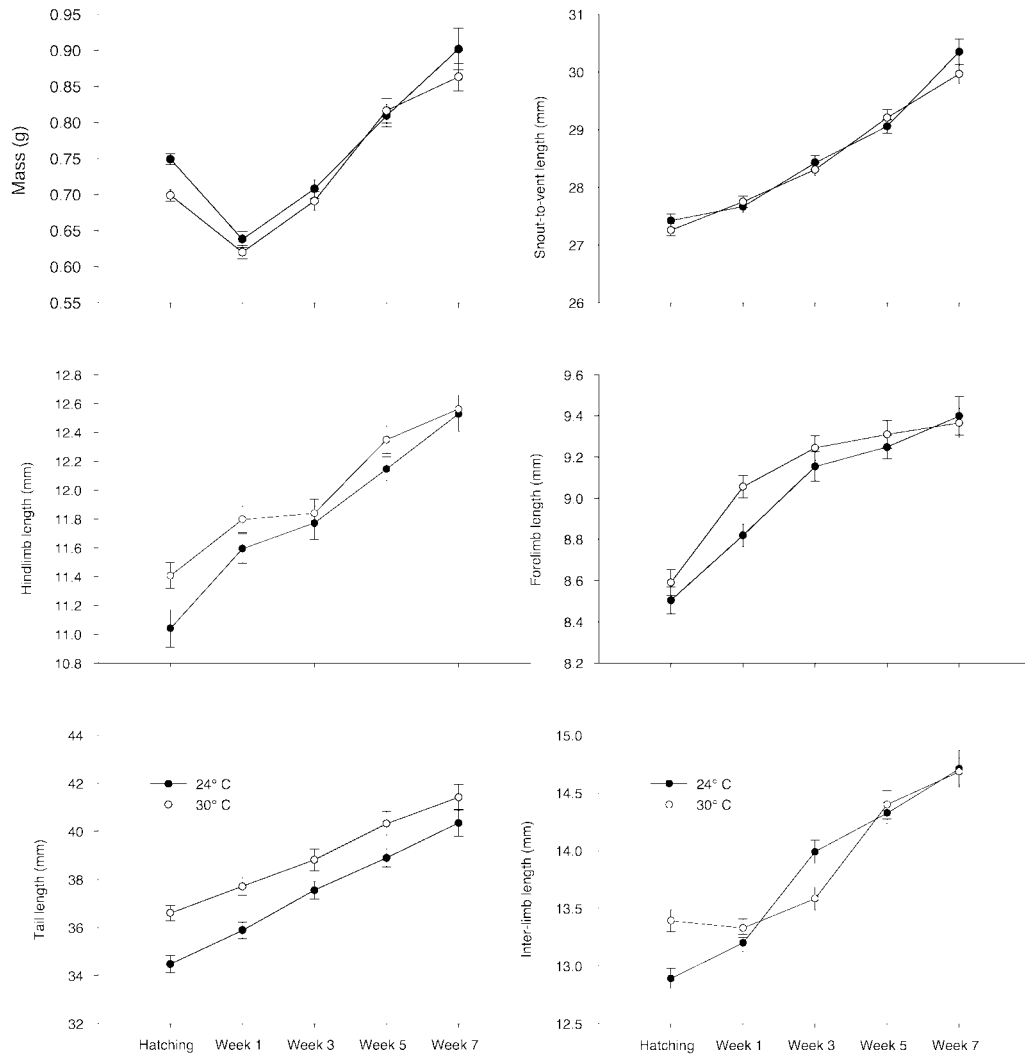
Fig. 2. Morphology of juvenile *Sceloporus occidentalis* at biweekly intervals, with the exception of the first tick mark, which occurred at hatching. There were no detectable morphological differences between animals from the Largo Vista and Table Mountain sites, so animals from the two populations were pooled for analysis. Error bars indicate 1 standard error on each side of the mean. Solid circles = cool-incubated lizards; open circles = warm-incubated lizards.

did not differ among hatchlings from either source population or incubation environment throughout the study, and growth in length between the incubation environments was virtually identical (Fig. 2). There was a significant incubation temperature × population interaction affecting forelimb length, with animals from the warmer incubation environment showing a trend towards longer forelimbs (Table 2, Fig. 2). Incubation environment exhibited a significant direct effect on offspring hindlimb length, with warm-incubated animals showing consistently longer hindlimbs than those incubated at the cool

temperature (Table 2, Fig. 2). Inter-limb length was affected both directly and indirectly by incubation temperature, but the direction of this trend was difficult to discern (Table 2, Fig. 2). Tail length was significantly affected by incubation environment, and this trend persisted until the seventh week of the experiment (age × incubation interaction; Table 2, Fig. 2).

Temperature preference

We used average selected body temperature as a measure of the lizards' thermal preference and the difference between the two temperatures selected as a measure of precision of thermoregulation. Lizards from both source populations and incubation environments selected similar average temperatures, independent of age, throughout all weeks of the experiment (two-factor repeated-measures ANOVA; Table 2, Fig. 3). Precision of
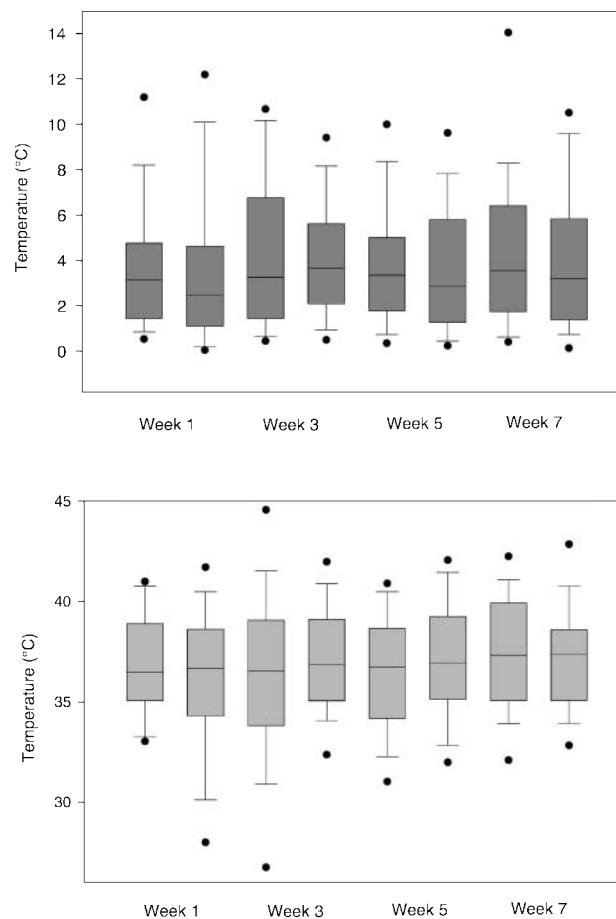

Fig. 3. Temperature preference and precision of thermoregulation recorded for hatchling *Sceloporus occidentalis*. Temperature precision is measured as the absolute value of the difference in two temperatures selected within 2 h of basking. Whiskers and boxes represent the 90th, 75th, 25th and 10th percentiles. Filled circles represent the 95th and 5th outlier percentiles.

thermoregulation was significantly affected by incubation regime, such that warm-incubated lizards thermoregulated more precisely (i.e. displayed slightly smaller overall differences between temperatures chosen) than did cool-incubated animals (two-factor repeated-measures ANOVA; Table 2, Fig. 2).

DISCUSSION

It is well-documented that the incubation environment can alter reptiles' body shape (Beuchat, 1988; Qualls and Shine, 1998; Du and Ji, 2003). Interspecific phylogenetic comparisons suggest that adult lizards living at high altitudes and latitudes often have smaller, shorter bodies than do their warm-climate counterparts (Ashton and Feldman, 2003); however, some lizards show the reverse trend (Van Damme *et al.*, 1992; Qualls and Shine, 1998), and distinguishing between genetic and environmentally induced geographic variation has been problematic. On the whole, we found that the phenotypic contributions of population, incubation environment, and age of individuals post-hatching were complex. Furthermore, we observed that most morphological differences did not persist up to 7 weeks into the lifetime of these juveniles.

We found that cool-incubated eggs gained mass more slowly than did warm-incubated eggs, but that cool-incubated eggs took nearly twice as long to hatch and were heavier at hatching than their warm-incubated counterparts. This size advantage likely trades off with the extended incubation time experienced by offspring in the cool incubation environment, leaving them less time to feed and gain mass before the onset of the winter months. In addition, we found that although hatchling lizards did not differ in SVL between the two incubation temperatures, cool-incubated lizards had shorter hindlimbs, inter-limb lengths, and tail lengths than their warm-incubated counterparts. The stout body shape that results from relatively short extremities and heavy body mass produced by cold incubation environments (Van Damme *et al.*, 1992; Qualls and Shine, 1998) has been associated with a syndrome of traits that presumably reflect lower offspring quality, including smaller body sizes, slower growth rates, and greater fluctuating asymmetry. However, the functional consequences of this stout body shape remain unclear, since cool-incubated hatchlings may in fact exhibit faster running speeds than warm-incubated hatchlings (Qualls and Andrews, 1999). Further studies should examine the potential effects of altered body shape on hatchling locomotor performance.

To understand the ecological relevance of induced phenotypes, it is necessary to observe the changes in offspring traits during subsequent life-history stages. In our case, we found little evidence that phenotypic variation induced during incubation persisted into the lives of young lizards. We also detected statistical interactions among population of origin, incubation temperature, and age of the hatchlings. The time period immediately after hatching is crucial to the survival of young organisms, since juveniles are often poorer performers on an absolute scale than their adult counterparts, and make easy prey for many predators (Arnold, 1983). It is possible, although largely unproven, that even traits that do not persist past the hatchling stage may be important for survival over short time periods [i.e. immediately post-hatching (Benoit and Pepin, 1999)], although the long-term consequences for survival are less obvious. Environmentally induced variation in phenotypic traits might not persist into the lifetime of an animal because the relative magnitude of the phenotype differences could be 'swamped' by either its genetics or the environment in which the young animal lives. Therefore, the short-term adaptive nature of an induced trait may have little bearing on the future survival of an animal, and may not prove ecologically relevant to

subsequent life-history stages. The most robust way to test this idea would be to induce different phenotypes in the laboratory that are known to persist for only short periods, and then release these animals into the field to determine long-term effects on fitness.

We observed some trends that showed clear changes in morphology within the first several weeks. For example, cool-incubated hatchlings were more massive at the outset, but this trend did not persist past the first 3 weeks of life. Similarly, inter-limb length did not show persistent effects of incubation treatments, despite displaying a marked difference immediately post-hatching. It is generally assumed that small body size results in lower survival rates in the wild (Ferguson and Fox, 1984); therefore, a faster growth rate would be advantageous to the smaller, warm-incubated hatchlings, and might also explain the short-lived increase in growth rate of the forelimbs observed in warm-incubated hatchlings. The shorter hindlimbs displayed by cool-incubated lizards upon hatching also did not persist past several weeks of life; however, the fact that cool-incubated lizards had consistently, if not significantly, shorter hindlimbs suggests that further study is needed. Hindlimb morphology plays a major role in performance capacities in these and other animals that rely on locomotion to escape predators (Marsh and Bennett, 1986; Sinervo and Losos, 1991). The role of locomotion may be especially important for young animals that are often under greater threat than adults, and are also poorer performers (Carrier, 1996). Future studies that document the locomotor capacities of developmentally plastic lizard species could discern the relationship between age and relative performance capacities.

Another issue concerns the applicability of our laboratory study to the field ecology and life-history of *Sceloporus* lizards and other ectotherms. Laboratory studies can more carefully control unwanted variation in variables that are not under study (e.g. humidity, hours of sunlight), but provide only one view of the role of the environment in the lives of animals, whereas field studies offer less control over environmental variables, but also provide a greater degree of ecological realism. We argue that in the absence of information on persistence of temperature effects on morphology, our controlled laboratory experiment provides a first step, but we encourage other researchers to pursue field studies, such as using enclosures at different elevations (and hence exposing lizards to more natural ranges of temperature), to better understand the persistence of morphological traits in a more ecologically realistic setting.

Hatchlings incubated at a warmer temperature had longer tails than did cool-incubated hatchlings, and this environmentally induced difference persisted for 7 weeks. A simple explanation for this trend could be that warm-incubated embryos produced more or larger vertebrae relative to their body size than did cool-incubated embryos. Lizards are known to exhibit inter- and intra-specific variation in growth rate of caudal vertebrae (Bergmann *et al.*, 2004), but the developmental mechanisms responsible for these effects are currently unknown. Effects of developmental temperature on vertebral number have been observed in other vertebrates (Fowler, 1970; Jockusch, 1997). Another explanation might involve differential spacing, not size or number, of caudal vertebrae induced by the incubation environment. Studies involving X-ray analysis could help to answer these questions.

Unlike morphology, average thermal preference showed no marked differences between treatments, including animals incubated at different temperatures and animals from different populations. We observed that warm-incubated juveniles showed slightly greater precision (indicated by smaller numbers; Fig. 3) in their thermoregulatory behaviour than cool-incubated juveniles. As mentioned above, cool incubation temperatures are sometimes

known to produce offspring of lower quality, and thus a cool-incubated juvenile may not thermoregulate as precisely as a warm-incubated juvenile. It is also possible that the observed difference in temperature preference was not a product of active temperature selection, but a by-product of different activity levels across lizards from different incubation treatments; however, lizards of this species tend toward inactivity (C.R. Buckley and S.C. Adolph, personal observation). Between age groups, juvenile-selected body temperatures in this study averaged from 4 to 6°C in variation in the laboratory. This apparent lack of consistency could have arisen from the juveniles' low thermal inertia in comparison to adults, making narrower ranges in variation in body temperature difficult for juveniles to maintain. The preferred body temperatures measured in the present study are slightly higher than those in previous laboratory studies of hatchling fence lizard temperature preference [31–33°C (Sinervo, 1990; Sinervo and Adolph, 1994)]. This could reflect a methodological difference, as those studies measured cloacal body temperature rather than operative temperature at the locations chosen by lizards.

Adult *S. occidentalis* have been shown to select temperatures from 33 to 36°C in the field and in the laboratory (Wilhoft and Anderson, 1960; Brattstrom, 1965; McGinnis, 1966; Adolph, 1990a; Wang and Adolph, 1995), and adults of *S. occidentalis* from both of our study populations select temperatures of approximately 36°C at both of our study sites (S.C. Adolph, unpublished data). Despite differences in environmental temperature at the two sites, the hatchlings from these source populations preferred similar temperatures in the laboratory. When observed in full sun, adults of this species show little difference in field body temperatures across the two sites (Adolph, 1990a). The absence of change in thermal preference, despite our reciprocal incubation experiment, suggests that temperature preference is resilient to incubation conditions. This finding further corroborates the finding that, because optimal body temperature is crucial to performance in nature, lizards maintain relatively narrow and conserved ranges of thermal preference within species (Marsh and Bennett, 1986; Adolph, 1987; Bennett and Huey, 1990; Mathies and Andrews, 1995; Angilletta *et al.*, 2002). Previous studies have, however, observed significant differences in selected body temperatures in the laboratory of adults from the Largo Vista site and from another population in Oregon (Sinervo, 1990). The Table Mountain and Largo Vista populations are very similar genetically [$F_{ST} = 0.017$ for 7 allozyme loci (C.S. McFadden and S.C. Adolph, unpublished data)], indicating high levels of contemporary gene flow between these two populations. High gene flow would impede differentiation in temperature preference and other traits. At the same time, high gene flow can increase the likelihood that adaptive phenotypic plasticity will evolve (Sultan and Spencer, 2002).

Studies of developmental plasticity in ectotherms have generally focused on the effects of the incubation environment, particularly temperature, on the phenotypes of juvenile animals. Here, we observed that while some post-hatching incubation effects may initially be large, they may also be short-lived. Placed in a larger context of life-history theory, phenotypic variation that is induced during the egg stage may not be ecologically relevant to future life-history stages and should be interpreted with caution. Future studies of the persistence of incubation effects should include a longer time period for evaluating persistence of these altered traits, in addition to expansion of the experiment to include other behaviours (e.g. sprint performance) to determine if the morphological changes we observed here translate into an equivalent change in organismal function.

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REFERENCES

Adolph, S.C. 1987. Physiological and behavioral ecology of the lizards *Sceloporus occidentalis* and *Sceloporus graciosus*. Dissertation, Department of Zoology, University of Washington, Seattle, WA.

Adolph, S.C. 1990a. Influence of behavioral thermoregulation on microhabitat use by two *Sceloporus lizards*. *Ecology*, **71**: 315–327.

Adolph, S.C. 1990b. Perch height selection by juvenile *Sceloporus* lizards – interspecific differences and relationship to habitat use. *J. Herpetol.*, **24**: 69–75.

Andrews, R.M. and Rose, B.R. 1994. Evolution of viviparity: constraints on egg retention. *Physiol. Zool.*, **67**: 1006–1024.

Andrews, R.M., Mathies, T. and Warner, D.A. 2000. Effect of incubation temperature on morphology, growth, and survival of juvenile *Sceloporus undulatus*. *Herpetol. Monogr.*, **14**: 420–431.

Angilletta, M.J., Hill, T. and Robson, M.A. 2002. Is physiological performance optimized by thermoregulatory behavior? A case study of the eastern fence lizard, *Sceloporus undulatus*. *J. Therm. Biol.*, **27**: 199–204.

Arnold, S.J. 1983. Morphology, performance, and fitness. *Am. Zool.*, **23**: 347–361.

Ashton, K.G. and Feldman, C.R. 2003. Bergmann's Rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution*, **57**: 1151–1163.

Atkinson, D. and Sibly, R. 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. *Trends Ecol. Evol.*, **12**: 235–239.

Behler, J.L. 2000. *National Audubon Society Field Guide to North American Amphibians and Reptiles*. New York: Alfred A. Knopf.

Bennett, A.F. and Huey, R.B. 1990. Studying the evolution of physiological performance. In *Oxford Surveys in Evolutionary Biology* (D.J. Futuyma and J. Antonovics, eds.), pp. 251–284. Oxford: Oxford University Press.

Benoit, H.P. and Pepin, P. 1999. Interaction of rearing temperature and maternal influence on egg development rates and larval size at hatch in yellowtail flounder (*Pleuronectes ferrugineus*). *Can. J. Fish. Aquat. Sci.*, **56**: 785–794.

Bergmann, P.J., Hobbs, A.M., Kavalench, M.L. and Russell, A.P. 2004. Modulated but conserved segmental growth of the original tail in *Callisaurus draconoides* (Phrynosomatidae) and *Calotes versicolor* (Agamidae). *Herpetologica*, **60**: 62–74.

Beuchat, C.A. 1988. Temperature effects during gestation in a viviparous lizard. *J. Therm. Biol.*, **13**: 135–142.

Brattstrom, B. 1965. Body temperatures of reptiles. *Am. Midl. Nat.*, **73**: 376–422.

Burger, J. 1990. Effects of incubation temperature on behavior of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *J. Herpetol.*, **24**: 158–163.

Burger, J. 1991a. Effects of incubation temperature on behavior of hatchling pine snakes: implications for reptilian distribution. *Behav. Ecol. Sociobiol.*, **28**: 297–303.

Burger, J. 1991b. Response to prey chemical cues by hatchling pine snakes (*Pituophis melanoleucus*): effects of incubation temperature and experience. *J. Chem. Ecol.*, **17**: 1069–1078.

Carrier, D.R. 1996. Ontogenetic limits on locomotor performance. *Physiol. Zool.*, **69**: 467–488.

Davis, J. and Ford, R.G. 1983. Home range in the western fence lizard (*Sceloporus occidentalis occidentalis*). *Copeia*, **1983**: 933–940.

Downes, S.J. and Shine, R. 1999. Do incubation-induced changes in a lizard's phenotype influence its vulnerability to predators? *Oecologia*, **120**: 9–18.

Du, W.-G. and Ji, X. 2003. The effects of incubation thermal environments on size, locomotor performance, and early growth of hatchling soft-shelled turtles, *Pelodiscus sinensis*. *J. Therm. Biol.*, **28**: 279–286.

Einum, S. and Fleming, I.A. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? *Am. Nat.*, **160**: 756–765.

Elphick, M. and Shine, R. 1998. Long term effects of incubation temperatures on the morphology and performance of hatchling lizards (*Bassiana duperreyi*, Scincidae). *Biol. J. Linn. Soc.*, **63**: 429–447.

Endler, J.A. 1977. *Geographic Variation, Speciation, and Clines*. Princeton, NJ: Princeton University Press.

Ferguson, G.W. and Brockman, T. 1980. Geographic differences of growth rate of *Sceloporus* lizards (Sauria: Iguanidae). *Copeia*, **1980**: 259–264.

Ferguson, G.W. and Fox, S.F. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards, *Uta stansburiana*: its causes and evolutionary significance. *Evolution*, **38**: 342–349.

Ford, N.B. and Seigel, R.A. 1989. Phenotypic plasticity in reproductive traits: evidence from a viviparous snake. *Ecology*, **70**: 1768–1774.

Forsman, A. and Shine, R. 1995. Parallel geographic variation in body shape and reproductive life history within the Australian scincid lizard *Lampropholis delicata*. *Funct. Ecol.*, **9**: 818–828.

Fowler, J.A. 1970. Control of vertebral number in teleosts – an embryological problem. *Q. Rev. Biol.*, **45**: 148–167.

Glen, F., Broderick, A.C., Godley, B.J. and Hays, G.C. 2003. Incubation environment affects phenotype of naturally incubated green turtle hatchlings. *J. Mar. Biol. Assoc. UK*, **83**: 1183–1186.

Goldberg, S.R. 1974. Reproduction in mountain and lowland populations of lizard *Sceloporus occidentalis*. *Copeia*, **1**: 176–182.

Gotthard, K. and Nylin, S. 1995. Adaptive plasticity and plasticity as an adaptation – a selective review of plasticity in animal morphology and life-history. *Oikos*, **74**: 3–17.

Hansen, T.K. and Petersen, I.B. 2001. The influence of rearing temperature on early development and growth of spotted wolffish *Anarhichas minor* (Olafsen). *Aquaculture Res.*, **32**: 369–378.

Jockusch, E.L. 1997. Geographic variation and phenotypic plasticity of number of trunk vertebrae in slender salamanders, *Batrachoseps* (Caudata: Plethodontidae). *Evolution*, **51**: 1966–1982.

Marsh, R.L. and Bennett, A.F. 1986. Thermal dependence of sprint performance of the lizard *Sceloporus occidentalis*. *J. Exp. Biol.*, **126**: 79–87.

Mathies, T. and Andrews, R.M. 1995. Thermal and reproductive biology of high and low elevation populations of the lizard *Sceloporus scalaris* – implications for the evolution of viviparity. *Oecologia*, **104**: 101–111.

McGinnis, S.M. 1966. *Sceloporus occidentalis* – preferred body temperature of western fence lizard. *Science*, **152**: 1090–1091.

Olsson, M. and Uller, T. 2002. Developmental stability and genetic architecture: a comparison within and across thermal regimes in tadpoles. *J. Evol. Biol.*, **15**: 625–633.

Packard, G.C., Packard, M.J., Miller, K. and Boardman, T. 1987. Influence of moisture, temperature and substrate on hatchling snapping turtle embryos. *Ecology*, **68**: 983–993.

Pigliucci, M. 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. Baltimore, MD: Johns Hopkins University Press.

Qualls, C.P. and Andrews, R.M. 1999. Cold climates and the evolution of viviparity in reptiles:

cold incubation temperatures produce poor-quality offspring in the lizard, *Sceloporus virgatus*. *Biol. J. Linn. Soc.*, **67**: 353–376.

Qualls, C.P. and Shine, R. 1996. Reconstructing ancestral reaction norms: an example using the evolution of reptilian viviparity. *Funct. Ecol.*, **10**: 688–697.

Qualls, F.J. and Shine, R. 1998. Geographic variation in lizard phenotypes: importance of the incubation environment. *Biol. J. Linn. Soc.*, **64**: 477–491.

Qualls, F.J. and Shine, R. 2000. Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, *Lampropholis guichenoti*. *Biol. J. Linn. Soc.*, **71**: 315–341.

Schlichting, C. and Pigliucci, M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sunderland, MA: Sinauer Associates.

Sears, M.W. and Angilletta, M.J. 2003. Life-history variation in the sagebrush lizard: phenotypic plasticity or local adaptation? *Ecology*, **84**: 1624–1634.

Shine, R., Elphick, M. and Harlow, P. 1997. The influence of natural incubation environments on the phenotypic traits of hatchling lizards. *Ecology*, **78**: 2559–2568.

Sinervo, B. 1990. Evolution of thermal physiology and growth-rate between populations of the western fence lizard (*Sceloporus occidentalis*). *Oecologia*, **83**: 228–237.

Sinervo, B. and Adolph, S.C. 1994. Growth plasticity and thermal opportunity in *Sceloporus* lizards. *Ecology*, **75**: 776–790.

Sinervo, B. and Losos, J. 1991. Walking the tight rope: arboreal sprint performance among *Sceloporus occidentalis* lizard populations. *Ecology*, **72**: 1225–1233.

Smith-Gill, S.J. 1983. Developmental plasticity: developmental conversion versus phenotypic modulation. *Am. Zool.*, **23**: 47–55.

Stearns, S.C. 1989. The evolutionary significance of phenotypic plasticity. *BioScience*, **39**: 436–445.

Sultan, S.E. and Spencer, H.G. 2002. Metapopulation structure favors plasticity over local adaptation. *Am. Nat.*, **160**: 271–283.

Tracy, C.R., Packard, G.C. and Packard, M.J. 1978. Water relations of chelonian eggs. *Physiol. Zool.*, **51**: 378–387.

Travis, J., McManus, M.G. and Baer, C.F. 1999. Sources of variation in physiological phenotypes and their evolutionary significance. *Am. Zool.*, **39**: 422–433.

Van Damme, R., Bauwens, D., Brana, F. and Verheyen, R.F. 1992. Incubation temperature differentially affects hatching time, egg survival, and hatchling performance in the lizard *Podarcis muralis*. *Herpetologica*, **48**: 220–228.

Wang, J.P. and Adolph, S.C. 1995. Thermoregulatory consequences of transmitter implant surgery in the lizard *Sceloporus occidentalis*. *J. Herpetol.*, **29**: 489–493.

Warner, D.A. and Andrews, R.M. 2002. Laboratory and field experiments identify sources of variation in phenotypes and survival of hatchling lizards. *Biol. J. Linn. Soc.*, **76**: 105–124.

Watkins, T.B. and Vraspir, J. 2006. Both incubation and posthatching temperature affect swimming performance and morphology of wood frog tadpoles (*Rana sylvatica*) *Physiol. Biochem. Zool.*, **79**: 140–149.

West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford: Oxford University Press.

Wilhoft, D.C. and Anderson, J.D. 1960. Effect of acclimation on the preferred body temperature of the lizard, *Sceloporus occidentalis*. *Science*, **131**: 610–611.