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Geographic variation in lizard phenotypes: importance of the incubation environment

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Geographic variation in phenotypes can result from proximate environmental effects as well as from underlying genetic factors. Reciprocal transplant experiments, in which organisms are moved from one area to another, offer a powerful technique to partition the effects of these two factors. However, many studies that have utilized this technique have focused on the post-hatching organism only and ignored potential effects of environmental influences acting during embryonic development. We examined the phenotypic responses of hatchling scincid lizards (*Lampropholis guichenoti*) incubated in the laboratory under thermal regimes characteristic of natural nests in two study areas in southeastern Australia. Although the sites were less than 120 km apart, lizards from these two areas differed in thermal regimes of natural nests, and in hatchling phenotypes (morphology, locomotor performance). We incubated eggs from each area under the thermal regimes typical of both sites. Some of the traits we measured (e.g. hatchling mass and snout-vent length) showed little or no phenotypic plasticity in response to differences in incubation conditions, whereas other traits (e.g. incubation period, tail length, inter-limb length, body shape, locomotor performance) were strongly influenced by the thermal regime experienced by the embryo. Thus, a significant proportion of the geographic variation in morphology and locomotor performance of hatchling lizards may be directly induced by differences in nest temperatures rather than by genetic divergence. We suggest that future studies using the reciprocal transplant design should consider environmental influences on all stages of the life-history, including embryonic development as well as post-hatching life.

ADDITIONAL KEY WORDS:—reciprocal incubation – reciprocal transplant – *Lampropholis guichenoti* – phenotypic plasticity – nest – egg.

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INTRODUCTION

Recent years have seen considerable progress in the methods used to identify underlying causes of phenotypic variation. One major advance has been the increasing use of experimental (manipulative) studies to elucidate the relative importance of genetic influences and phenotypic plasticity as contributors to geographic variation (e.g. McCauley, 1978; Stearns & Sage, 1980; Berven & Gill, 1983; James, 1983; Sinervo & Adolph, 1989; Sinervo, 1990a, b; Adolph, 1991; Sinervo *et al.*, 1992; Ferguson & Talent, 1993; Niewiarowski & Roosenburg, 1993). Among the most promising of these techniques is the reciprocal transplant experiment, in which organisms from one environment are transplanted to an area occupied by conspecifics with different phenotypes (and vice versa). Comparisons of the subsequent phenotypes of transplanted versus control subjects can reveal the relative importance of population-specific (including genetic) factors compared to proximate environmentally-induced sources of variation. A related technique, the reciprocal incubation experiment, investigates the effects of population specific and environmental influences on embryos (in our case the eggs of an oviparous lizard species). Reciprocal incubations are most often conducted in the laboratory where the effects of specific environmental parameters on hatchling phenotypes (e.g. temperature, water potential) can more easily be assessed.

Geographic variation in phenotypes is widespread in lizards, and clearly reflects both genetic and non-genetic factors (see Ballinger, 1983; Dunham, Miles & Reznick, 1988, for reviews). However, previous work in this field has not specifically examined the possible role of environmentally-induced effects acting prior to hatching; instead, authors have focused on the responses of the animals during post-hatching life. Could plastic responses acting during embryonic development cause a biologically significant component of intraspecific geographic variation in lizard phenotypes? We know that incubation conditions in lizard nests can vary among geographic areas, and that variation in incubation conditions can induce phenotypic variation in hatchlings (e.g. morphology: Fox, Gordon & Fox, 1961; Osgood, 1978; Morris *et al.*, 1983; Packard & Packard, 1987; Phillips *et al.*, 1990; Harlow, 1996; behaviour: Ferguson & Fox, 1984; Van Damme *et al.*, 1992; Shine & Harlow, 1996; thermal preference: Qualls, 1996a; gender: Charnier, 1966; Bull, 1980; Ferguson & Joanen, 1982; Schwarzkopf & Brooks, 1985; Cree, Thompson & Daugherty, 1995) Hence, the issue is not whether or not such effects occur, but their magnitude relative to the observed degree of geographic variation. If nest-site characteristics vary only slightly, or if hatchling phenotypes show little sensitivity to incubation conditions, then such plasticity may contribute little to geographic variation in these phenotypic traits. Alternatively, if hatchling phenotypes are very sensitive to incubation conditions, and nest-site characteristics show marked geographic variation, then we would expect phenotypic plasticity alone to generate biologically significant geographic variation in hatchling phenotypes. Moreover, such environmental influences

on phenotypes may extend well beyond hatching. For example, incubation temperature has been shown to affect size-corrected growth rates (Van Damme *et al.*, 1992), thermoregulatory behaviour (Qualls, 1996a; Shine & Harlow, 1996), and activity levels (Shine & Harlow, 1996) of post-hatching lizards. Thus, lizards that are physically indistinguishable at hatching may grow at different rates, have different thermal preferences, and exhibit different levels of activity, entirely as a result of differences in the thermal regimes they experienced during incubation. Experiments that transplant wild-caught hatchlings from populations with different nest environments, and then monitor the subsequent phenotypes of these lizards (e.g. Niewiarowski & Roosenburg, 1993), would not be able to separate such environmentally-induced differences in growth rates from inter-populational genetic differences.

Theoretical discussions generally partition contributions to geographic variation into genetic and environmental components. However, in manipulations of complex biological systems such as that under study here, we can only distinguish a 'source population' effect from specific, experimentally-manipulated sources of environmental variation. Thus, 'population-based' differences among our hatchlings may be entirely genetic, entirely environmentally-induced, or (most likely) they may contain both environmental components (e.g. dam-specific and population-wide non-genetic maternal influences) and genetic contributions to phenotype. In this paper, we investigate the relative importance of one proximate environmental factor (incubation temperature) and population-specific influences as determinants of geographic variation in the phenotype of hatchling Australian garden skinks (*Lampropholis guichenoti*). We do this by comparing the phenotypes of hatchlings from two geographically separate populations, incubated in a reciprocal incubation experiment under thermal regimes similar to those in natural nests used by each population. The use of a 2 population $\times 2$ incubation-regime factorial design allows us to separate phenotypic variance into two components: that which is environmentally induced by differences in thermal environments during incubation, and that which is inherent in lizards from each source population.

In this study we have manipulated only one aspect of the nest environment: incubation temperature. There are many other environmental factors that potentially could influence the phenotypes of hatchling lizards (see above). However, the aim of our study was not to fully elucidate the role of all factors affecting lizard phenotype. The first step is to see whether any such effects are significant. If even one aspect of the embryonic environment is shown to have a substantial influence on hatchling phenotype relative to population-specific effects, then caution should be exercised when designing future experiments that aim to partition sources of geographic variation in phenotype.

MATERIAL AND METHODS

Study species and study areas

Lampropholis guichenoti is a small (to 51 mm snout-vent length, 2.1 g body mass) heliothermic scincid lizard that is widely distributed along the eastern coast of Australia (Cogger, 1992). In our study populations clutch size ranges from one to

four eggs, although more typically, two or three eggs are laid (Qualls, 1996b). Like most wide-ranging lizard species that have been studied in detail, *L. guichenoti* shows significant geographic variation in traits such as body size, reproductive output, growth rate, and age at sexual maturity (e.g. Pengilley, 1972; Joss & Minard, 1985; Simbotwe, 1985; Forsman & Shine, 1995; Qualls, 1996b). Our unpublished analyses show that adult female *L. guichenoti* from our high-elevation study site (Blue Mountains) are generally larger (in snout-vent length [SVL], inter-limb length [ILL], total length [TL], pre-oviposition and post-oviposition mass) than lizards from our lowland (Sydney) study site, they produce heavier clutches containing heavier eggs, and when gravid they run significantly faster in laboratory trials (Qualls, 1996b; Qualls & Shine, 1997). Additionally, male *L. guichenoti* from our Blue Mountains site are larger (SVL, ILL, TL) and heavier than Sydney conspecifics (Qualls, 1996b).

We collected lizards from two sites in New South Wales, Australia: central Sydney (Newtown, 33°53′S 151°10′E, 6 m asl) and the Blue Mountains (Blackheath, 33°41′S 150°19′E, 1010 m asl). Although the two areas are separated by <120 km, they experience very different climates. The low-elevation coastal site (Sydney) has warm summers (mean midsummer thermal minimum 18.6°C, maximum 26.1°C) and mild winters (mean midwinter thermal minimum 6.5°C, maximum 16.8°C) whereas the Blue Mountains region experiences colder temperatures (mean midsummer minimum 12.7°C, maximum 23.0°C, mean midwinter minimum 2.4°C, maximum 9.1°C, Australian Bureau of Meteorology, 1988).

Thermal regimes of natural nests and laboratory incubation

We used miniature data loggers ('Hobo-temp', Onset Computer Corp., Pocasset, Mass.) to record the thermal regimes of natural nests at both Sydney and Blue Mountains sites. A thermal probe was placed among freshly laid eggs within each nest, such that eggs lay above as well as below the tip of the probe. Temperatures were monitored (± 0.2°C) in eight Sydney nests, and five Blue Mountains nests, during the summers of 1993/94 and 1994/95 (Fig. 1). In the laboratory, we programmed two Clayson 10-step cycling incubators to approximate thermal conditions in the nests at the two study sites. The temperature profiles of the laboratory incubation treatments were identical in the shape and magnitude of their daily thermal fluctuations, but differed substantially in their temperatures: the 'Blue Mountains' incubator was 7°C lower than the 'Sydney' incubator at all times (Fig. 2).

All of the Sydney eggs ($n = 188$) and approximately 40% of the Blue Mountains eggs used in this study were laid in captivity (captive-laid $n = 66$ of 154 eggs), from females captured and brought into the laboratory prior to oviposition (mean $\pm$ SD = 13 ± 7 days in captivity before laying). We supplemented our Blue Mountains sample with eggs from natural nests, and included data from the resulting hatchlings in our analyses if most of embryonic development (>80%, based on laboratory incubation periods) occurred in the controlled laboratory environment. Data for hatchlings from wild-laid eggs were pooled with those from laboratory-laid eggs after we tested for (but did not detect) differences in phenotype between the groups.

We divided each clutch between the two incubation treatments, such that each female contributed no more than one egg to each treatment. Equal numbers were assigned to each of the hot and cold incubation treatments. In many cases we could distinguish discrete clutches of wild-laid eggs, as groups of two or three eggs of

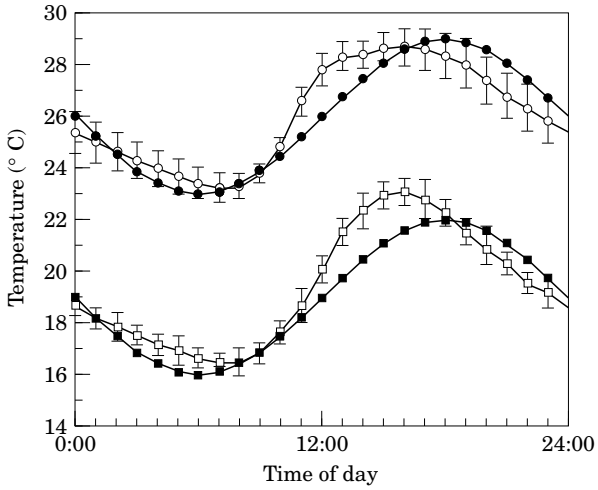

Figure 1. Mean daily temperature regimes in natural Sydney ($n=8$) and Blue Mountains ($n=5$) nests of *Lampropholis guichenoti* and the thermal regimes used to incubate eggs in the laboratory. Circles represent 'Sydney profiles', squares represent 'Blue Mountains profiles', open symbols represent field nest temperatures (error bars indicate 1 SE each side of the mean), filled symbols represent laboratory incubation regimes.

similar size were often stuck together. In cases when we could not distinguish clutches we used a 'best guess' strategy, erring on the conservative side, by not using eggs we suspected to be part of a clutch of more than two eggs. There were likely some inaccuracies in this method but, as clutch sizes were small ($n \leq 3$ eggs for all laboratory-laid clutches, with $n=2$ for 70% of laboratory-laid clutches, Qualls, 1996b) and overall sample sizes large, it is extremely unlikely that the incubation treatments were significantly biased by any one female.

All eggs were incubated individually in 64 ml glass jars containing moist vermiculite (water potential $= -200$ kPa, as calculated from a calibration curve of the ratio water mass:dry vermiculite mass for this batch of vermiculite, P. Harlow unpubl. data) and covered with plastic food wrap. Reweighing of the vermiculite in a sample of jars at the end of incubation showed that water potential did not differ between incubation treatments (one-factor ANOVA, $F_{1,18}=0.02$, $P=0.88$; cold incubator mean $= -287$ kPa, $n=10$; hot incubator mean $= -283$ kPa, $n=10$).

We retained a subsample of healthy eggs from each population, in addition to some damaged eggs, for determination of the developmental stages of embryos at oviposition (Sydney: $n=15$, Blue Mountains: $n=18$). Prior to dissection, we froze these eggs to kill the embryos. We then examined each embryo under a dissecting microscope and assigned it an approximate developmental stage using the *Lacerta vivipara* series of Dufaure and Hubert (1961) as a guide.

Hatchling morphology and locomotor performance

We weighed hatchlings (± 0.001 g) and measured their snout–vent (SVL), total (TL) and inter-limb (ILL: straight line distance between the posterior insertion of

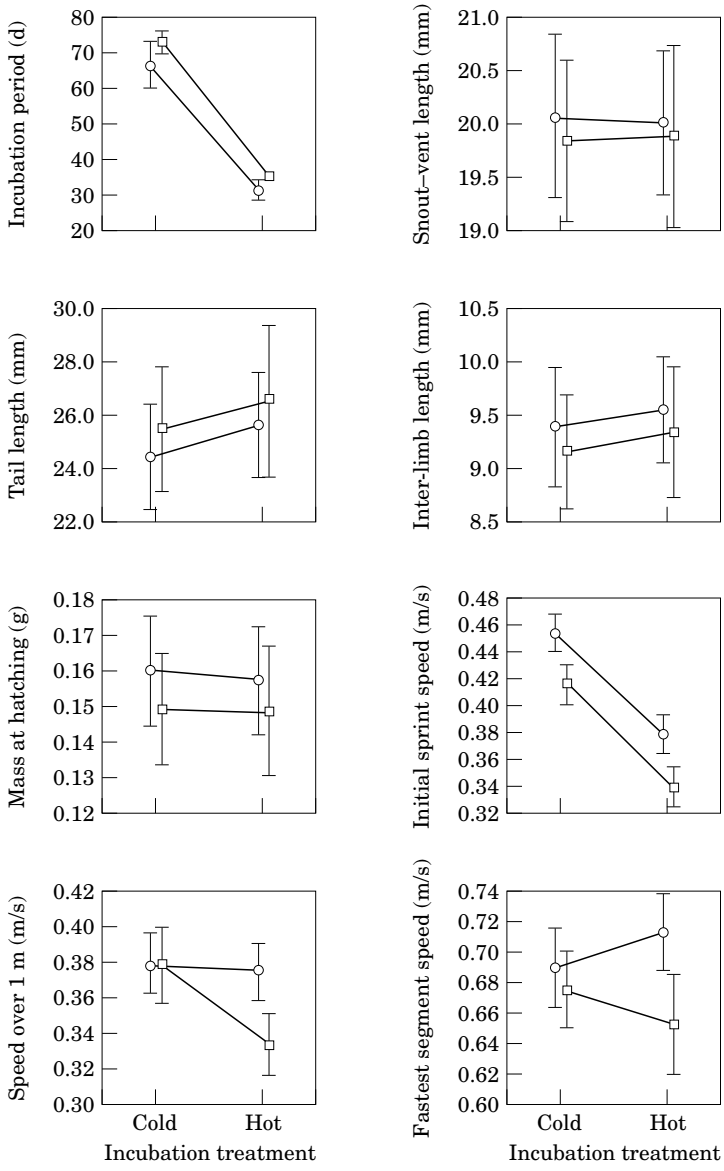

Figure 2. The influences of source population and incubation treatment on phenotypic traits of hatchling *Lampropholis guichenoti*. (○) Blue Mountains hatchlings, (□) Sydney hatchlings. Error bars indicates 1 SD each side of the mean. See Table 2 for statistical tests.

the fore-limb and the anterior insertion of the hind limb) lengths (all ± 0.5 mm), and calculated tail length (TL–SVL) from these measurements.

We quantified the locomotor performance of lizards within 4 days of hatching, by chasing each hatchling along an electronically-timed 'racetrack'. The racetrack was 1 m long and 5 cm wide, and was made of wood with sand glued to the surface to increase traction. Five pairs of infra-red photocells were positioned at 25 cm intervals along the track and these were connected to an electronic stopwatch. As

a lizard passed the first set of photocells the stopwatch was activated. The photocells were then turned off serially as the lizard passed through the beam linking each pair. Running speeds were measured in a temperature-controlled room at 25°C (± 1°C). Hatchlings were given at least 2 h to equilibrate to room temperature before the first trial. After this time, a hatchling was tipped directly from its holding cage into the racetrack and chased down the track with a paintbrush. We ran each lizard twice per trial with at least 30 minutes of rest between successive runs.

We recorded the time taken and whether the lizard stopped or turned during each run. From these measurements we calculated: the mean speed (average of two runs) over 1 m; mean initial sprint speed (measured over the first 25 cm of each run); the fastest speed over any 25 cm segment, and whether the lizard stopped or turned during each run. Because some hatchlings stopped part-way along the racetrack and could not be chased further, our analyses excluded values that fell further than two standard deviations from the mean.

Analyses

We analysed our data using analysis of variance (ANOVA), analysis of covariance (ANCOVA), correlation analysis and χ^2 analysis. All data conformed to normality (Shapiro–Wilk test) and homogeneity of variance (Bartlett's test). We log-transformed data on incubation period before analysis. In ANCOVAs, unless specifically discussed, slopes are homogeneous ($P>0.05$). When ANCOVA interaction terms were not significant at $P \leq 0.05$ they were removed and the model recalculated to increase the power of the tests.

We did not apply group-wide simultaneous inference techniques to compensate for the increased likelihood of spuriously significant results that occurs as a by-product of performing large numbers of statistical tests (Holm, 1979). There are major difficulties in the application of such techniques, particularly an increased probability of type II errors, and a strong reliance on subjectivity in judgment of what constitutes a 'single group' within which such corrections should be made. In practice, none of our major conclusions would be changed by the application of such techniques.

RESULTS

Our initial tests (two-factor ANOVAs: wild/laboratory-laid and incubation treatment as factors) showed that the source of Blue Mountains eggs (i.e. whether they were laid in the laboratory or collected from the wild) did not significantly affect any of the morphological traits that we measured at hatching ($P>0.40$ in all tests, wild-laid $n=88$, laboratory-laid $n=43$). Thus, we pooled the data for these two groups for subsequent tests. Similarly, differential mortality of eggs among groups was minimal, and hence did not confound our comparisons: mortality rates during incubation of laboratory-laid eggs were not influenced by either source population ($\chi^2=0.54$, 1 df, $P=0.46$) or incubation regime ($\chi^2=0.09$, 1 df, $P=0.76$). Additionally, mortality did not differ between eggs incubated under their 'natural' thermal conditions, and eggs exposed to conditions they would not experience in the wild ($\chi^2=0.06$, 1 df, $P=0.80$).

TABLE 1. Mean trait values and one-factor ANOVA comparisons of the phenotypes of hatchling *Lampropholis guichenoti* from Sydney and the Blue Mountains incubated under temperature regimes typical of their source populations ('natural' hatchlings). Analyses of locomotor performance are restricted to hatchlings that did not stop running during their trials. SH = hot-incubated Sydney eggs; BMC = cold-incubated Blue Mountains eggs

	SH mean	BMC mean	df	*F*	*P*	residual df
Incubation period (d)	34.24	66.37	1	1625.26	<0.0001	114
Hatchling snout-vent length (mm)	19.90	20.08	1	1.40	0.239	114
Hatchling inter-limb length (mm)	9.34	9.37	1	0.08	0.780	114
Hatchling tail length (mm)	26.66	24.50	1	22.59	<0.0001	113
Mass at hatching (g)	0.15	0.16	1	11.81	0.0008	113
Mean initial sprint speed (m/s)	0.34	0.45	1	10.36	0.002	42
Mean speed over 1 m (m/s)	0.40	0.50	1	11.78	0.001	42

We begin our investigation of the sources of phenotypic variation between our study populations with a comparison of the phenotypes of hatchlings incubated under 'natural' thermal regimes (i.e. Sydney lizards incubated at temperatures that simulated Sydney nests, and Blue Mountains lizards incubated at cooler Blue Mountains temperatures). Our analyses reveal significant differences in hatchling phenotypes between these two groups. Sydney hatchlings had shorter incubation periods, they were lighter, their tails were longer, and they ran slower than Blue Mountains lizards over both distances measured (one-factor ANOVAs, Table 1). Thus, wild *Lampropholis guichenoti* also likely show significant geographic variation in hatchling phenotypes. We did not directly compare the phenotypes of our experimentally incubated neonates with field-caught hatchlings, as we do not expect our laboratory-incubated hatchlings necessarily to be identical in phenotype to their wild counterparts. Our incubation regimes mimicked only interpopulational variation in nest temperatures: it is likely that other physical factors acting during embryonic development (e.g. hydric conditions, Gutzke & Packard, 1987; Miller *et al.*, 1987) also influence the phenotypes of hatchling *L. guichenoti*. Our following analyses include traits that did not differ between the two populations in the above comparisons between 'natural' Blue Mountains and Sydney neonates, because of the possibility that an overall similarity in mean values between lizards from the two areas (Table 1) might mask incubation and source population effects acting in opposing directions.

Incubation period

Eggs in the cold incubator took more than twice as long to hatch as those kept under warmer conditions (Table 2, Fig. 2). More surprisingly, eggs from Blue Mountains lizards completed incubation sooner than those from Sydney when both were kept under the same thermal regime (Table 2, Fig. 2). As Blue Mountains eggs are heavier at laying (Qualls, 1996b), we performed an analysis of covariance (source population and incubation treatment as factors, egg wet mass as covariate, incubation

TABLE 2. Two-factor analyses of variance showing the effects of source population and incubation treatment on the phenotypes of hatchling *Lampropholis guichenoti* incubated in a reciprocal incubation experiment

	population (popn)			incubation treatment (inc)			popn × inc			residual
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df
ln incubation period	1	12.95	0.0004	1	15956.19	<0.0001	1	2.59	0.110	135
Mass at hatching	1	20.10	<0.0001	1	0.37	0.545	1	0.22	0.642	228
Snout-vent length	1	3.09	0.080	1	0.005	0.983	1	0.20	0.651	229
Inter-limb length	1	8.21	0.005	1	5.98	0.015	1	0.04	0.837	229
Tail length	1	11.35	0.0009	1	14.60	0.0002	1	0.01	0.926	228
Mean initial sprint speed	1	6.63	0.011	1	24.51	<0.0001	1	0.01	0.924	194
Mean speed over 1 m	1	1.18	0.278	1	1.74	0.189	1	1.31	0.254	187
Fastest segment speed	1	1.82	0.179	1	0.001	0.974	1	0.71	0.399	196

period as dependent variable) to see if differences in egg mass could account for the additional incubation time. However, the covariate was not significant in the analysis (egg wet mass: $F_{1,132}=2.19$, $P=0.15$). Thus egg wet mass did not explain the population-specific differences in incubation periods. An alternate explanation is that the Blue Mountains eggs were laid at a more advanced developmental stage. However, our staging of *L. guichenoti* embryos lacked the precision necessary to determine whether differences in developmental stage at oviposition could potentially explain the small but statistically very significant differences in incubation period between the populations (all eggs were at stage 30 or 31 at oviposition).

Hatchling morphology

Hatchling morphology was influenced by both the population that a lizard came from and the incubation regime to which it was subjected. Only one morphological trait (hatchling SVL) was unaffected by either intrinsic or environmental influences (Table 2, Fig. 2), while some others were very labile (Table 2, Fig. 2). For example, hatchling tail lengths and ILLs were affected by both factors: Sydney hatchlings had longer tails than those from the Blue Mountains, and hot-incubated lizards were longer-tailed than their cold-incubated siblings (Table 2, Fig. 2). This divergence reflects differences between treatments in absolute tail length, as well as differences in body shape (tail length relative to SVL: two-factor ANCOVA, with source population and incubation treatment as factors, SVL as covariate and tail length as the dependent variable: population: $F_{1,236}=15.48$, $P<0.0001$; incubation: $F_{1,236}=13.52$, $P=0.0003$, SVL: $F_{1,236}=31.26$, $P<0.0001$). While the influence of incubation treatment on ILL was similar to its effect on tail length (in both cases, hot-incubated lizards were longer than those incubated under the colder regime), Sydney hatchlings had shorter ILLs than their Blue Mountains counterparts. Analysis of covariance (source population and incubation treatment as factors, SVL as covariate and ILL as the dependent variable) revealed that, in addition to differences between treatments in the absolute values of ILL, there were also significant differences in ILL relative to SVL (SVL: $F_{1,236}=323.96$, $P<0.0001$, population: $F_{1,236}=6.20$, $P=0.01$, Blue Mountains > Sydney; incubation: $F_{1,236}=16.68$, $P<0.0001$, hot-incubated > cold-incubated).

Blue Mountains neonates were heavier than Sydney hatchlings but their masses were not influenced by incubation conditions (Table 2, Fig. 2). 'Stockiness' (mass relative to SVL) of neonates also differed between populations but not between incubation treatments. Analysis of covariance revealed that the offspring of Blue Mountains females were heavier-bodied at hatching than their Sydney counterparts (incubation treatment and source population as factors, SVL as covariate and hatchling mass as dependent variable; population: $F_{1,235}=22.82$, $P<0.0001$; incubation: $F_{1,235}=0.87$, $P=0.35$, SVL: $F_{1,235}=255.61$, $P<0.0001$).

Hatchling locomotor performance

Unless otherwise stated, locomotor performance was analysed using two-factor ANOVAs with source population and incubation treatment as factors. When differences between treatments in locomotor performance were detected, two-factor ANCOVAs were performed (source population and incubation treatment as factors,

SVL as covariate) to see if variation in hatchling size could account for the observed differences in performance.

Hatchling locomotor performance, as measured by running speeds and the proportion of lizards that stopped when being chased, was significantly influenced by both incubation treatment and population of origin. Mean initial sprint speeds of hatchlings differed between source populations and between incubation treatments (Table 2). Blue Mountains hatchlings ran faster over the first 25 cm of the racetrack than did those from Sydney (Fig. 2), and lizards incubated in cold conditions were faster than hot-incubated hatchlings over this distance (Fig. 2). Allometry does not explain this pattern, as the effects of incubation treatment and source population remained significant when body-size differences were factored out of the analysis (two-factor ANCOVA with hatchling SVL as covariate: population: $F_{1,194}=5.43$, $P=0.02$, incubation treatment: $F_{1,194}=25.10$, $P<0.0001$, SVL: $F_{1,194}=4.72$, $P=0.03$). Similar patterns were not apparent when the lizards' running speeds were measured over 1 m (Table 2) until a third factor—whether or not a lizard stopped during either run—was added to the analysis. With inclusion of this factor, differences between the populations and incubation treatments in speeds over 1 m became significant (stopped or not: $F_{1,183}=131.84$, $P<0.0001$, population: $F_{1,183}=8.64$, $P=0.004$, incubation treatment: $F_{1,183}=7.04$, $P=0.009$). Thus, the higher speeds of Blue Mountains and cold-incubated hatchlings were masked by differences between the groups in inclination to stop running. Variation in hatchling SVL did not explain a significant amount of the differences in speed over 1 m (three-factor ANCOVA: SVL: $F_{1,186}=0.40$, $P=0.53$, population: $F_{1,186}=7.93$, $P=0.005$, incubation treatment: $F_{1,186}=6.62$, $P=0.01$, stopped or not: $F_{1,186}=137.00$, $P<0.0001$).

Although tail length was highly labile, variation in this trait did not explain the observed differences in locomotor ability between populations or incubation treatments over either of the distances measured (two-factor ANCOVAs: population and incubation treatment as factors, tail length as covariate, mean initial sprint speed and mean speed over 1 m as dependent variables: covariate $P>0.05$ in both cases). Additionally, lizards with tails that were longer relative to their SVLs did not run at speeds different to their shorter-tailed conspecifics (correlation analysis of residuals from a plot of tail length versus SVL against running speed; mean initial sprint speed: $n=109$, correlation $=-0.05$, $P=0.62$, mean speed over 1 m: $n=109$, correlation $=-0.07$, $P=0.48$).

The maximum speeds attained by the lizards over any segment in either trial were not influenced by either their incubation regime or their population of origin (Table 2, Fig. 2).

The propensity of a lizard to stop or turn during a trial did not differ between incubation treatments ($\chi^2=0.81$, 1 df, $P=0.37$), however offspring from Blue Mountains eggs were more likely to stop during a trial ($\chi^2=3.92$, 1 df, $P=0.048$).

DISCUSSION

Our data show that differences in nest temperatures alone are sufficient to induce significant geographic variation in hatchling phenotypes. Thus, geographic differences in hatchling phenotypes (morphology, performance, incubation period) within a wide-ranging lizard species are likely to reflect direct influences of incubation

TABLE 3. Division of the phenotypic variation in 'labile' traits between 'naturally-incubated' hatchling *Lampropholis guichenoti* from Sydney (hot incubation treatment) and the Blue Mountains (cold incubation treatment) into that which is attributable to source population and that resulting from differences in incubation regimes. See text for explanation of calculations

Phenotypic trait	Percentage of difference attributable to incubation treatment (%)	Percentage of difference attributable to source population (%)
Incubation period	96	4
Mean initial sprint speed	66	34
Mean speed over 1 m	46	54
Inter-limb length	46	54
Tail length	53	47

conditions as well as source population (including genetic) effects. Although our two study areas were separated by <120 km, the thermal regimes in natural nests differed significantly (mean of 7°C difference) between the two areas. Hatchling phenotypes were very sensitive to incubation conditions, hence the thermal difference between the incubation treatments caused a high proportion of the interpopulational difference that we documented in the morphology and behaviour of the hatchlings. Our experimental design held other factors that may affect hatchling phenotypes (e.g. hydric conditions during incubation) constant, and we may thus have underestimated (or overestimated) the contribution of incubation effects to interpopulational divergence. In the field, the two areas may well differ in other nest-site characteristics that influence hatchling phenotypes.

Our main result—that phenotypic variation is due to direct environmental effects as well as population-specific influences—mirrors results from transplant experiments that have dealt with effects on post-hatching phenotypes (e.g. Berven, 1982; Brown, 1985; Hinch & Bailey, 1986; Hinch & Green, 1989; Niewiarowski & Roosenburg, 1993; Bernado, 1994). Our findings show, however, that ignoring effects induced by the local environment (i.e. nest conditions) on the embryo could lead to significant error in the interpretation of experimental findings. Because the embryo precedes the free-living animal in the life cycle, even small-scale variation in environmental influences exerted during embryogenesis may have dramatic effects on the resultant adult phenotype (Gould, 1977). Thus experiments that only consider the phenotypic responses of organisms to their environment during the post-hatching phase may not accurately reflect the responses observed if the organisms had been exposed to the 'new' environment for their entire life-cycle.

The phenotypic traits that we measured show a considerable range in the magnitude of variation, and in the relative importance of population-specific *versus* incubation-induced contributions to this variation (Tables 2 and 3). For example, hatchling SVL did not differ between lizards from the two populations or the incubation treatments. The same was true of the fastest sprint speed over any 25 cm segment. Other traits, such as hatchling mass and stockiness (mass:SVL), differed between the populations but were not influenced by incubation conditions. Additionally, many traits (such as incubation periods, ILLs, tail lengths, body shapes [tail length:SVL, ILL:SVL] and two measures of running speed) were influenced by incubation treatment *and* the population of origin. In such cases, the relative influence of incubation treatment and population varied over a considerable range.

We quantified the relative magnitude of population and incubation effects on traits influenced by *both* of these factors (we call these 'labile traits') as follows. First, we calculated the magnitude of the incubation effect on each trait by holding source population constant and determining the magnitude of the difference between the mean trait value for hot-incubated Sydney hatchlings and that of cold-incubated Sydney hatchlings, and the magnitude of the difference between the mean trait value for hot-incubated Blue Mountains hatchlings and that of cold-incubated Blue Mountains hatchlings. The mean of these two values calculated for each trait gave an overall average change in magnitude of each trait as a result of differences in incubation conditions. We performed a similar series of calculations to quantify the average magnitude of the source population effect on the hatchings' phenotypes (i.e. hot-incubated Sydney hatchlings *versus* hot-incubated Blue Mountains hatchlings, and cold-incubated Sydney hatchlings *versus* cold-incubated Blue Mountains hatchlings). These calculations produced two values for each trait: the magnitude of the effect of the incubation treatments on hatchling phenotype (inc) and the magnitude of the source population effect (popn). We then converted each value to a percentage of the total variation in phenotypes resulting from differences in our two experimental factors (Table 3).

Table 3 shows that both population-specific and incubation temperature-induced factors played significant roles in determining the values of some phenotypic traits, with the relative importance of these two factors differing substantially among the traits we measured. For example, incubation periods were highly responsive to incubation temperatures, as has been documented often in other taxa (e.g. Hagstrum & Milliken, 1991; Packard & Packard, 1988, and references therein), and presumably reflects a simple slowing of chemical reactions at lower temperatures. The cause of the population-based difference in incubation periods is less obvious, but may reflect selection for earlier hatching in the cooler climate (and shorter summer) of the Blue Mountains area (e.g. Olsson *et al.*, 1996; Rykena, 1988). Similarly, initial sprint speeds were influenced more by incubation treatment than by population (Table 3). However, hatchling tail length and the mean running speed of lizards over 1 m were affected to a similar degree by both population of origin and incubation regime (Table 3). Inter-limb length and stockiness (mass:SVL) were influenced more by population of origin than by incubation conditions (Table 3).

In summary, our work indicates that developmental plasticity manifested during embryogenesis may contribute significantly to observed patterns of geographic variation in phenotypic traits. Because events early in development (especially during differentiation) potentially can have more impact on the final (adult) phenotype than later events, such embryonic plasticity may play an important role. This result has at least three implications. First, we need to look more closely at the nest environment, and the responses of embryogenesis to that environment, if we are to fully understand the causal processes responsible for geographic variation within wide-ranging species. Second, there are methodological implications: reciprocal transplant experiments that do not consider the embryo's environment may be confounded, because they rely on hatchling phenotypes that may be unrepresentative of those that would emerge from eggs laid under natural conditions in the environments of interest. Third, lizards may be ideal model systems with which to further investigate these issues: apart from numerous other advantages (e.g. see Vitt & Pianka, 1994), they show extreme phenotypic plasticity during embryogenesis, in response to an environmental trait (nest temperature) that is easily measured in the field and easily

controlled in the laboratory (e.g. Packard *et al.*, 1987). Thus, we see enormous potential for further studies, in particular for the incorporation of embryo biology into reciprocal transplant experiments.

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