

## PATERNAL GENOTYPE INFLUENCES INCUBATION PERIOD, OFFSPRING SIZE, AND OFFSPRING SHAPE IN AN OVIPAROUS REPTILE

MATS OLSSON,<sup>1,2</sup> ANNICA GULLBERG,<sup>3</sup> RICHARD SHINE,<sup>2</sup> THOMAS MADSEN,<sup>2</sup> AND HÅKAN TEGELSTRÖM<sup>3</sup>

<sup>1</sup>University of Göteborg, Department of Zoology, Section of Animal Ecology, Medicinaregatan 18, S-413 90 Göteborg, Sweden

<sup>2</sup>The University of Sydney, School of Biological Sciences, Zoology Building A08, N.S.W. 2006, Australia

<sup>3</sup>Uppsala University, Department of Genetics, Box 7003, S-750 07 Uppsala, Sweden

**Abstract.**—Theoretical models for the evolution of life-history traits assume a genetic basis for a significant proportion of the phenotypic variance observed in characteristics such as hatching date and offspring size. However, recent experimental work has shown that much of the phenotypic variance in hatchling reptiles is induced by nongenetic factors, such as maternal nutrition and thermoregulation, and the physical conditions experienced during embryogenesis. Thus, there is no unambiguous evidence for strictly genetic (intraspecific) influences on the phenotypes of hatchling reptiles. We report results from a technique that uses a genetic marker trait and DNA fingerprinting to determine paternity of offspring from multiply sired clutches of European sand lizards, *Lacerta agilis*. By focusing on paternal rather than maternal effects, we show that hatchling genotypes exert a direct influence on the duration of incubation, the size (mass, snout-vent length) and shape (relative tail length) of the hatchling, and subsequent growth rates of the lizard during the first 3 mo of life. Embryos with genes that code for a few days' delay in hatching are thereby larger when they hatch, having undergone further differentiation (and hence, have changed in bodily proportions), and are able to grow faster after hatching. Our data thus provide empirical support for a crucial but rarely tested assumption of life-history theory, and illuminate some of the proximate mechanisms that produce intraspecific variation in offspring phenotypes.

**Key words.**—*Lacerta agilis*, incubation period, multiple paternity, offspring size and shape, paternal genes.

Received December 1, 1994. Accepted August 4, 1995.

Hatching (or birth) is an important stage in the life history of any organism, and factors such as developmental rate, the time of emergence from the egg or mother, and the size and shape of the offspring, are liable to have a profound effect on that hatchling's probability of survival and later reproduction (e.g., Ford and Huxley 1927; Gould 1977). Studies on many different types of organisms have demonstrated significant influences of the hatchling's phenotype, and its plasticity, on organismal fitness (e.g., Ferguson and Fox 1984; Sinervo 1990; Newman 1994; Via et al. 1995). Thus, the determinants of offspring phenotype are of central interest in life-history theory, and there have been numerous attempts to model the evolutionary processes at work in adaptation of this life-history stage (e.g., Smith and Fretwell 1974; Brockelman 1975; Shine 1978; Nussbaum 1981; Lloyd 1987; Parker and Begon 1986; Nussbaum and Schultz 1989; Winkler and Wallin 1987). These studies deal primarily with adaptive evolution of mean hatchling size, using optimality theory. Considerably less attention has been paid to adaptive variation in hatchling size (Gillespie 1987). Nevertheless, all these models rely upon the assumption that there is a genetic underpinning to the observed phenotypic variance among hatchlings within a clutch or population. Without such a link between genotype and phenotype, even intense selection on offspring phenotypes will have no deterministic effect on allelic frequencies, and thus the system will not evolve (i.e., will not show cumulative changes in gene frequencies in response to selection).

Although the assumption is thus a crucial one, we have little evidence to support it in many types of animals. There are quantitative genetic studies of vertebrates such as birds (e.g., Väisänen et al. 1972; van Noordwijk et al. 1981; Price and Grant 1985) and mammals (e.g., Austin and Short 1984; Cheverud et al. 1983), which provide unequivocal support of

genetic influence on hatchling traits when maternal effects are removed. However, recent experimental studies on a variety of reptilian taxa have shown that important characteristics of the offspring (e.g., its time of hatching, as well as its size, shape, behavior, thermoregulatory preferences, and performance abilities) are highly dependent upon the physical conditions that the embryo experiences prior to hatching (e.g., Lang 1985; Burger 1989, 1990; Packard 1991; Shine and Harlow 1993; Shine 1995, and papers cited therein). Thus, the typically low within-clutch variance in offspring characteristics, compared with the typically high among-clutch variance in these characteristics (Vitt and Congdon 1978; Sinervo and Adolph 1989), may be due to proximate environmental factors influencing phenotypes within an adaptive reaction norm. That is, particularly in reptiles, eggs within a clutch may produce similar hatchlings not because of high genetic relatedness among the offspring, but simply because the eggs have been exposed to similar hydric and thermal conditions throughout embryogenesis. The adaptive phenotypic response of the embryo (and hence hatchling) to the biotic and abiotic factors that it experiences during pre- and postoviposition life-history stages, provides exciting problems in studies of evolutionary biology. In this work, we restrict ourselves to investigating if significant variation in hatchling morphology can be referred to paternal genes. If this link can be established, we may have a tool for partitioning genetic variance from environmental variance for hatchling traits. We could then go on to determine how variance components of such traits during early ontogeny affect lifetime fitness. Quite possibly these relationships will be different in reptiles to those in other groups such as birds and mammals.

It might seem that the problem of demonstrating strictly genetic effects on hatchling size and shape can be overcome

in oviparous reptiles, by dividing the clutch at oviposition and incubating siblings under a variety of different conditions (Shine 1995). This technique can factor out any apparently "maternal" (= genetic) effects that are in fact due to incubation conditions but does not offer a complete solution because most oviparous reptiles undergo a significant proportion of embryonic development prior to oviposition (Shine 1995). In addition, the thermal regimes selected by gravid females immediately prior to laying can significantly influence the phenotypes of the hatchlings (Shine 1995). Similarly, food availability (and thus, maternal conditions) has been shown to affect several components of the hatchling phenotype (King 1993). Thus, a low within-clutch variance in hatchling phenotypes, compared with a high among-clutch variance in the same characteristics, cannot be used as evidence of genetic influence on offspring traits.

The confounding influence of proximate environmental factors such as maternal nutrition and thermoregulation can be reduced but not eliminated by examining the consistency of offspring phenotypes produced by individual females through time. For example, if differences among females in traits such as offspring size are consistent through several breeding seasons, it seems more likely that such traits are under genetic control (e.g., Madsen and Shine 1992). Again, however, other confounding factors may play a role. Females may differ in characteristics such as parasite loads, or there may be long-term effects of feeding opportunities early in life (the "silver-spoon effect," Grafen 1988). Ultimately, then, differences among females in offspring traits are consistent with the hypothesis that these traits are under genetic control but do not provide unequivocal support for this idea. How can we obtain a more rigorous assessment of strictly genetic influences on offspring characteristics? Quantitative genetics techniques (cross-breeding, etc.) can provide data to answer this question, but these techniques require large numbers of animals and long periods of time (e.g., Bulmer 1985; Falconer 1989). In this paper, we use an alternative, less complex technique to assess the influence of genetics on offspring traits in an oviparous lizard. By establishing paternity of eggs in multiply sired clutches, we can examine paternal (and thus, strictly genetic) influences without the host of confounding nongenetic factors associated with any attempt to interpret maternal influences on offspring characteristics. This is possible because reptiles yolk their follicles simultaneously from circulating yolk precursors (vitellogenin) and ovulate all the eggs at the same time. Thus, energetic and nutritional within-clutch variance is typically low in lizards compared with (for example) birds; birds lay eggs sequentially, and foraging success prior to oviposition may vary between eggs.

#### MATERIALS AND METHODS

We used females from a sand lizard (*Lacerta agilis agilis*) population with low genetic diversity on the Swedish west coast (Asketunnan, 50 km South of Gothenburg). The females were mated in the laboratory, eight of them by two different males each. Four of the females were mated with two Swedish males, whereas the other four females were mated with one Swedish male and one male from a Romanian population of

sand lizards (*Lacerta agilis chersonensis*). After matings, the females were kept separately. As soon as the eggs were laid, they were transferred to boxes with vermiculite into which water had been mixed in the ratio 10:1 (milliliters, vermiculite: water). The eggs were incubated at 25°C ( $\pm 1^\circ\text{C}$ ) until hatching. Because the eggs were adherent, we did not separate them for weighing, as this separation may damage the surface of the soft eggshell. We used offspring body size and body shape to look for paternal effects, because these traits can be estimated with accuracy and are correlated with offspring survival in sand lizards (Olsson 1992). Hatchlings were measured to the nearest 0.5 mm snout to vent and total length, and weighed to the nearest 0.001 g.

Offspring paternity was determined by a genetic marker trait (dorsal stripes, Olsson et al. 1994a) and by DNA fingerprinting (Olsson et al. 1994a,b). To investigate the consistency in the genetic marker trait (dorsal stripes), we performed the following crossing experiment prior to commencing the present study. Striped (dominant) males and non-striped (recessive) females were mated, and so were their offspring. These brother-sister matings resulted in 56 young from 10 clutches. The empirical result was 15 nonstriped and 41 striped young, which were tested against the predicted frequencies for single-locus heterozygous crossings (i.e., 3:1 ratio). A  $\chi^2$  test for goodness-of-fit did not reject the null hypothesis of heterozygote crossings ( $\chi^2 = 0.10$ ,  $df = 1$ ,  $P > 0.8$ ). Only males and females that were homozygous dominant or homozygous recessive for stripes were used in the multiple matings reported in the present study, and paternity of offspring could therefore be safely assigned using stripes (Olsson et al. 1994a). To investigate paternity with DNA fingerprinting, bands greater than 3 to 4 kb were used. An offspring was considered sired by a certain male if the offspring had at least one male-specific band (a band not shared by the female or the other male). The probability of occurrence of a mutant band is negligible. No offspring showed bands specific for both males (Olsson et al. 1994a).

Because the volume and the nutrient content of the yolk may vary among clutches, the statistical analyses of clutches sired by two males were performed as pairwise tests of trait means of the half sibs within each clutch. We used  $[(X_1 - X_2)/s^2(1/n_1 - 1/n_2)]$  as the test statistic. By using theory for multiple tests (Hochberg and Tanhame 1987), we calculated a simultaneous probability value for the offspring in the four clutches in each group.

We also considered the possibility that differences between males in the size of their offspring (half sibs) were due to the fact that one male's sperm by mere chance fertilized the largest eggs, or the eggs containing the most nutrients, in the clutch. Conservatively, we assume that there is enough variation in egg energy or nutrient content within a clutch to yield the observed differences in hatchling size (although we doubt this assumption). From simple combinatorial theory, we can calculate the probability that a male sires  $k$  of the largest of the  $n$  eggs in a clutch; when a male sires for example three out of eight eggs in a clutch this probability equals  $(3 \cdot 2 \cdot 1)/(8 \cdot 7 \cdot 6)$ . In the general case, this can be expressed  $n(n-1) \dots (n-k+1)/1 \cdot 2 \dots k$ , which is derived from the definition of the binomial coefficient ( $k$  = number of offspring sired by a given male in a clutch of  $n$  eggs;  $n$  = number of eggs

TABLE 1. Maternal and paternal influences on offspring phenotypes in the sand lizard, *Lacerta agilis*. Table shows values from two-factor analyses of variance, with male and female identification numbers as the factors and offspring traits as the dependent variables. "Relative tail length," residual score from the general regression of total length on snout-vent length; "body shape," residual score from the general regression of mass on snout-vent length.

Dependent variable	Maternal effect			Paternal effect			Interaction between maternal and paternal effects		
	df	F	P	df	F	P	df	F	P
Snout-vent length (mm)	11,117	6.02	0.0001	9,117	0.66	0.75	2,117	7.75	0.0007
Mass (g)	11,116	7.69	0.0001	9,116	0.81	0.61	2,116	10.40	0.0001
Incubation period (d)	7,70	217.0	0.0001	8,70	319.0	0.0001	1,70	36.4	0.0001
Relative tail length (residual score)	11,115	2.99	0.002	9,115	0.72	0.69	2,115	0.58	0.56
Body shape	11,115	7.67	0.0001	9,115	1.47	0.17	2,115	2.86	0.06

in a clutch, ranked in order of "quality," e.g., energy or nutrient content, Olle Häggström, Department of Mathematics, Chalmers University of Technology, pers. comm., 1993).

### RESULTS

As in most previous analyses of reptilian reproduction, we found strong maternal effects on the phenotypes of the offspring. Single-factor analysis of variance (ANOVA), with female identification number as the factor, revealed strong differences among females in all of the traits we measured on their offspring: snout-vent length ( $F_{14,128} = 12.76$ ,  $P < 0.0001$ ), tail length ( $F_{14,126} = 24.79$ ,  $P < 0.0001$ ), mass ( $F_{14,127} = 10.07$ ,  $P < 0.0001$ ), relative tail length (residual scores from the regression of total length to snout-vent length:  $F_{14,126} = 5.62$ ,  $P < 0.0001$ ), and body shape (residual from the regression of mass to snout-vent length:  $F_{14,127} = 6.87$ ,  $P < 0.0001$ ). Offspring from different females also differed significantly in their subsequent growth rates, in terms of both snout-vent length ( $F_{10,82} = 10.53$ ,  $P < 0.0001$ ) and mass ( $F_{10,81} = 6.24$ ,  $P < 0.0001$ ). Because we knew the paternity as well as the maternity of each offspring, we were able to analyze these data further, using a two-factor ANOVA with female identification number and male identification number as the two factors, and offspring traits as the dependent variables. This analysis confirmed the importance of

among-female effects, but was ambiguous with respect to paternal effects (Table 1). Offspring sired by different males differed significantly in incubation periods but not in any of the other traits we examined. Nonetheless, significant interaction terms between maternal and paternal effects were common, suggesting that the phenotype of the offspring depends not only on maternal factors (genotype, condition, etc.) but also on some interaction between maternal factors and paternal genotypes.

The nature of this paternal effect was clarified when we compared offspring sired by the Romanian male with their half sibs sired by Swedish males. In clutches with mixed paternity, sperm from the Romanian male consistently gave rise to larger young than did sperm from the Swedish males (SVL -  $P < 0.002$ ; total length -  $P < 0.003$ ; mass -  $P = 0.044$ ; see Table 2). The offspring sired by the Romanian male also differed significantly from their Swedish half sibs in terms of incubation periods (means = 39.09 versus 37.04 d, unpaired  $t = 4.09$ ,  $df = 78$ ,  $P < 0.0001$ ) and relative tail lengths (residual scores from the general regression of total length on SVL: means = 1.06 versus -0.04, unpaired  $t = 2.49$ ,  $df = 78$ ,  $P < 0.014$ ).

The number of young sired by the Romanian male (out of each entire clutch) were, three out of eight, two out of four, two out of five, and one out of nine hatchlings. We used the generalized expression derived from combinatorial theory for calculating the probability that the Romanian male by pure chance fertilized the "largest eggs" (p. 8). Because females are independent observations, we multiplied the probabilities for each clutch to obtain the corresponding probability for the entire experiment. This probability could then be described by the expression  $p = (2 \cdot 1/4 \cdot 3) \cdot (2 \cdot 1/5 \cdot 4) \cdot 1/9 \cdot (3 \cdot 2 \cdot 1/8 \cdot 7 \cdot 6) = 1:30240$  ( $P = 0.00003$ ).

These analyses indicate that the genes of the Romanian male resulted in a longer incubation period, and the production of heavy offspring with longer bodies and shorter tails than their half sibs sired by Swedish males. Are these independent effects, or are these traits part of a correlated suite of characteristics related to different developmental pathways? To answer this question, we investigated correlations among these traits within the offspring sired by Swedish males only (i.e., omitting all offspring from the Romanian males). Our correlational analyses showed that more prolonged incubation periods resulted in offspring that were longer (incubation period versus SVL:  $r = 0.26$ ,  $N = 69$ ,  $P < 0.04$ ) and heavier (incubation period versus mass:  $r = 0.24$ ,

TABLE 2. Mean snout-vent length (SVL, mm), total length (TOT, mm), and mass (g) of offspring sired by Swedish female sand lizards mated with a Swedish male (S), and a Romanian male (R).

Fe- male num- ber	Male number	Offspring N =	SVL $\bar{x} \pm SD$	TOT $\bar{x} \pm SD$	Mass $\bar{x} \pm SD$
1	1 (R)	2	30.5 $\pm$ 0.71	75.0 $\pm$ 1.41	0.720 $\pm$ 0.0
1	2 (S)	2	30.0 $\pm$ 0.0	72.0 $\pm$ 1.41	0.620 $\pm$ 0.03
2	1 (R)	2	31.0 $\pm$ 1.41	69.0 $\pm$ 4.24	0.539 $\pm$ 0.01
2	3 (S)	3	29.0 $\pm$ 1.0	67.0 $\pm$ 1.41	0.573 $\pm$ 0.06
3	1 (R)	1	30.0 $\pm$ 0.0	70.0 $\pm$ 0.0	0.500 $\pm$ 0.0
3	4 (S)	8	27.6 $\pm$ 0.52	64.5 $\pm$ 1.51	0.490 $\pm$ 0.06
4	1 (R)	3	30.0 $\pm$ 1.0	70.0 $\pm$ 7.07	0.751 $\pm$ 0.06
4	5 (S)	5	28.6 $\pm$ 1.14	68.8 $\pm$ 3.90	0.636 $\pm$ 0.08
Mean values					
Romanian					
father	(N = 8)		30.4 $\pm$ 0.92	71.8 $\pm$ 3.49	0.659 $\pm$ 0.12
Swedish					
fathers	(N = 18)		28.4 $\pm$ 1.09	65.9 $\pm$ 3.00	0.558 $\pm$ 0.09

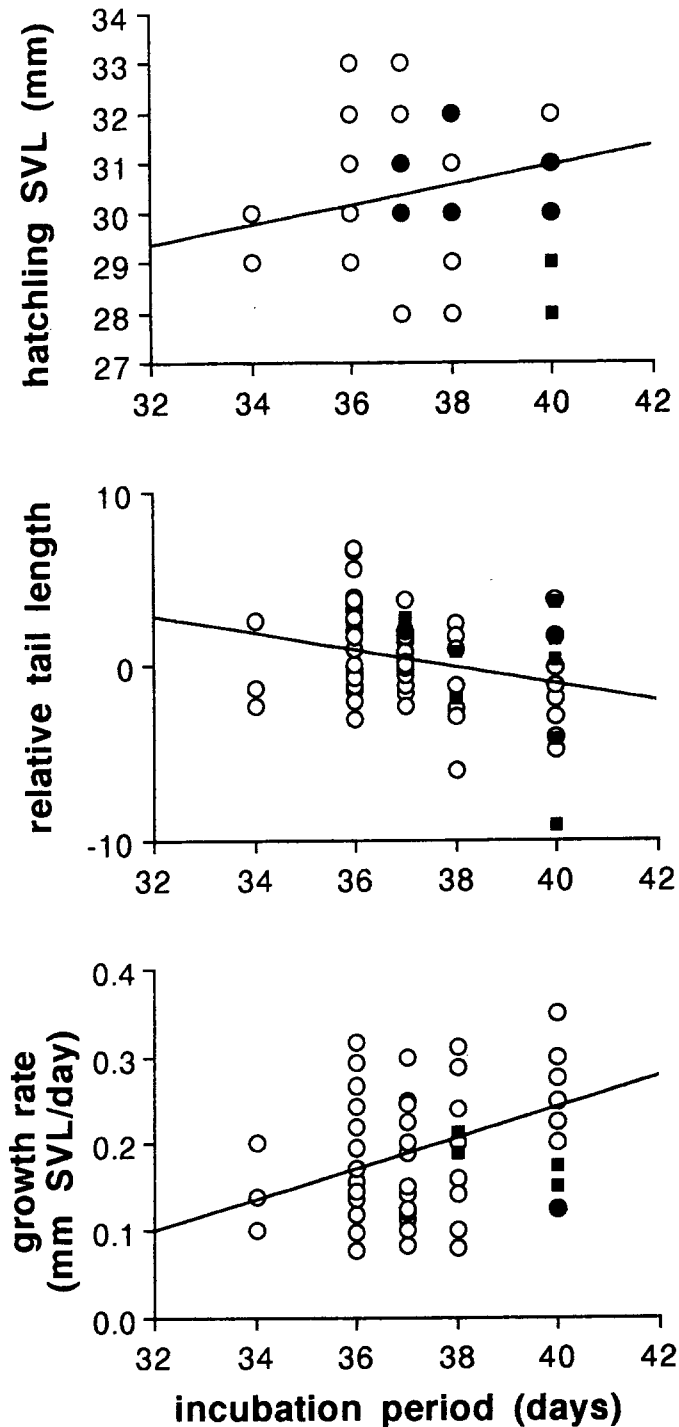


FIG. 1. The figure illustrates how a suite of morphological traits in the sand lizard hatchling changes with increasing incubation period of the egg. Prolonged incubation results in a hatchling with a longer body (top graph), shorter tail (middle graph), and which grows at a faster rate during its first 40 d of life (bottom graph).

$N = 68$ ,  $P < 0.05$ ), and with shorter tails relative to SVL (incubation period versus residual scores of total length on SVL:  $r = 0.31$ ,  $N = 67$ ,  $P < 0.013$ ). Figure 1 shows two of these relationships and also shows that hatchlings that emerged after longer incubation periods grew significantly

faster in the first few months of life than did hatchlings from briefer incubation (incubation period versus subsequent growth rate in SVL:  $r = 0.39$ ,  $N = 67$ ,  $P < 0.001$ ; versus growth in mass:  $r = 0.29$ ,  $N = 66$ ,  $P < 0.02$ ).

These data suggest that the four "atypical" characteristics of hatchlings sired by the Romanian male (mass, length, relative tail length, and subsequent growth rate) are somehow correlated in occurrence, rather than being independent effects of the Romanian genotype. Given longer incubation period of the Romanian hatchlings, the correlates of prolonged incubation in the Swedish offspring are such that we would predict that the Romanian offspring would be longer and heavier, have shorter tails, and have more rapid growth as juveniles. In each case, the magnitude of these differences between Swedish and half Romanian offspring is close to that predicted by the difference in incubation periods (Fig. 1). For example, the predicted offspring sizes for Swedish lizards with a 39.1-d incubation period (the mean for the Romanian-sired lizards) are 0.67 g (vs. 0.65 g for Romanians) and 30.8 mm SVL (vs. 30.7 mm for Romanians). These results suggest that the underlying relationships among offspring traits are similar in the lizards from Sweden and Romania: the genotype of the Romanian male is simply at one end of a continuum also seen in Swedish populations.

#### DISCUSSION

Sand lizards provide an excellent model system in which to investigate genetic influences on offspring phenotypes. Because this species is oviparous, there is no significant placental transfer of nutrients to the oviductal embryo (eggshell deposition in oviparous lizards commences soon after ovulation: Guilette 1992; Guilette and Jones 1985; Heulin 1990). Thus, the total allocation of energy and nutrients to each offspring is determined at ovulation, and cannot be influenced by the phenotype of the embryo or subsequent maternal allocation "decisions" based on that embryonic phenotype. The prevalence of multiple mating and consequent multiple paternity in sand lizards allows us to control for maternal (genetic and nongenetic) effects by comparing half sibs rather than more distantly related individuals. In sand lizards, number of matings does not correlate with offspring mass and, hence, females do not accumulate more resources for their young by obtaining more ejaculates (Olsson et al. 1994b,c). Therefore, the sperm provides only genes to the embryo, and any effect of paternal identity on offspring phenotypes must be a genetic effect.

Our calculations of probabilities (above) enable us to reject the possibility that offspring sired by the Romanian male are larger than their half sibs because of a chance effect whereby the Romanian sperm happened to fertilize larger eggs. Thus, any difference in offspring phenotypes between the offspring of Romanian and Swedish males must result from some difference in the way in which the embryonic genotype controls the processes of differentiation, growth, and developmental timing. Our data suggest that developmental timing is crucial in this respect, and that the mechanistic (proximate) basis for the modifications of hatchling phenotype (size, shape, and subsequent growth rate) lie in the embryo's control of its

duration of incubation (essentially, the embryo's "decision" as to when it should hatch).

Why should a prolongation of incubation influence offspring size and shape? The likely answer is that embryogenesis proceeds during this time, such that (1) body length increases as the embryo grows; (2) although dry mass decreases during embryogenesis (due to metabolic energy expenditure), the embryo's wet mass actually increases over this period because of an influx of water as yolk is converted into body tissue (Shine 1977); and (3) the relative proportions of the body and tail change in accordance with normal growth allometry. The enhancement of subsequent growth is more difficult to understand but may relate to competitive advantages of larger hatchling sizes in social encounters between juvenile lizards (Stamps and Krishnan 1994a,b). Given that prolongation of incubation is advantageous, why do not all genotypes code for delayed hatching? Perhaps because in the brief cool Swedish summers, early hatching (prior to the onset of lethally low soil temperatures in autumn) may confer such advantages as to outweigh the minor disadvantages of smaller hatchling size. In keeping with this interpretation, *L. agilis* are among the northernmost oviparous reptiles, and our study area is relatively close to the northern (cold-climate) limit of their geographic distribution (Bischoff 1984). Advantages to early hatching may be less important in warmer areas, such as Romania.

Could our results be effects (such as heterosis or outcrossing deficiency) that are due to the combination of genes from different geographic origins and thus not representative of the genetic effects that normally occur within natural populations? We doubt this interpretation. If the larger size of the Romanian-sired offspring was due to heterosis rather than to genes coding for prolonged incubation, we would not expect to find the same relationship between incubation period and hatchling morphology within the offspring sired by the Swedish males alone. Moreover, the difference in incubation periods between the Romanian and Swedish sand lizards is a relatively modest one, and part of a general clinal variation in this trait with latitude within the range of *L. agilis* (Rykena 1988). Thus, the mean values for developmental period, as well as for the morphological traits we measured, lie well within the range of the Swedish offspring.

The explanation we have proposed above identifies incubation period as the controlling variable for all zygotes, independent of parental geographic origin, with other phenotypic effects as consequences of prolonged embryogenesis. We note, however, that our data are correlational and not experimental: thus, it remains possible that the longer incubation period is a result of the larger offspring size (etc.) rather than the other way around. We doubt this interpretation because (1) our calculations show that the probability of Romanian and Swedish offspring originating from different-sized eggs is so low as to be negligible; and (2) we would not expect body-shape differences (e.g., in relative tail length) if different-sized offspring were all at the same developmental stage at hatching.

In summary, our data on sand lizards demonstrate a significant link between offspring genotype and phenotype. By focusing on paternal rather than maternal influences, we have been able to avoid a host of confounding influences that

would otherwise make it very difficult to identify any such genetic effect. The finding that particular genotypes produce particular phenotypes is not surprising: it is a basic tenet of evolutionary biology. Nonetheless, there is remarkably little empirical support for this crucial assumption for most types of animals. Our data are also of interest in clarifying some of the proximate mechanisms underlying the ways in which genotypes function to produce specific phenotypes. In particular, the role of developmental timing, and the genotype's manipulation of the relative amount of time that the organism spends in different life-history stages, provides an interesting perspective on the proximate determinants of offspring phenotypes in reptiles. With the increasing availability of molecular techniques for identifying paternity, it should soon be possible to evaluate the relative magnitudes of genetic and nongenetic influences on hatchling phenotypes both in the laboratory and in the field. Such information could greatly enhance our understanding of the ways in which natural selection on offspring phenotypes can feed back into macro-evolutionary changes in relevant traits.

#### ACKNOWLEDGMENTS

We thank the following institutions for financial support: M. Olsson: Collianders Stiftelse, Långmanska Kulturfonden, the Swedish National Environmental Protection Board, and Lamm's Stiftelse. A. Gullberg and H. Tegelström: the Swedish Natural Science Research Council. R. Shine and T. Madson: the Australian Research Council. M. Olsson also thanks T. Helin for excellent field assistance and the University of Sydney for logistical support. We are all grateful for the insightful comments offered by D. Winkler, and an anonymous reviewer.

#### LITERATURE CITED

- AUSTIN, C. R., AND R. V. SHORT. 1984. Reproduction in mammals, Vol. 4. Reproductive fitness. Cambridge University Press, London.
- BISCHOFF, W. 1984. *Lacerta agilis* 1758—Zauneidechse. Pp. 23–68 in W. Böhme, ed. Handbuch der Reptilien und Amphibien Europas, Band 2/I:23–68. Echsen (Sauria) II. AVLA-Verlag, Wiesbaden.
- BROCKELMAN, W. Y. 1975. Competition, the fitness of offspring, and optimal clutch size. *Am. Nat.* 109:677–699.
- BULMER, M. 1985. The mathematical theory of quantitative genetics. Clarendon Press, Oxford, UK.
- BURGER, J. 1989. Incubation temperature has long-term effects on behavior of young pine snakes (*Pituophis melanoleucus*). *Behav. Ecol. Sociobiol.* 24:201–207.
- . 1990. Effects of incubation temperature on behavior of young black racers (*Coluber constrictor*) and kingsnakes (*Lampropeltis getulus*). *J. Herpetol.* 24:158–163.
- CHEVERUD, J. M., L. J. LEAMY, W. R. ACHLEY, AND J. J. RUTLEDGE. 1983. Quantitative genetics and the evolution of ontogeny 1. Ontogenetic changes in quantitative genetic variance components in randombred mice. *Genet. Res.* 42:62–75.
- FALCONER, D. S. 1989. Introduction to quantitative genetics, 3rd ed. Longman Scientific and Technical, Burnt Mill, England.
- FERGUSON, G. W., AND S. F. FOX. 1984. Annual variation of survival advantage of large juvenile side-blotched lizards *Uta stansburiana*; its causes and evolutionary significance. *Evolution* 38:342–349.
- FORD, E. B., AND J. S. HUXLEY. 1927. Mendelian genes and rates of development in *Gammarus chevreuxi*. *Brit. J. Exp. Biol.* 5:112–134.

- GILLESPIE, J. H. 1977. Natural selection for offspring numbers: A new evolutionary principle. *Am. Nat.* 111:1010-1014.
- GOULD, S. J. 1977. *Ontogeny and phylogeny*. The Belknap Press of Harvard University Press, Cambridge, MA.
- GRAFEN, A. 1988. On the uses of data on lifetime reproductive success. Pp. 454-471 in T. H. Clutton-Brock, ed. *Reproductive success. Studies of individual variation in contrasting breeding systems*. Chicago University Press, Chicago.
- GUILLETTE, L. J., JR. 1992. Morphology of the reproductive tract in a lizard exhibiting incipient viviparity (*Sphenomorphus fragilis*) and its implication for the evolution of the reptilian placenta. *J. Morphol.* 212:1-11.
- GUILLETTE, L. J., JR., AND R. E. JONES. 1985. Ovarian, oviductal, and placental morphology of the reproductively bimodal lizard species, *Sceloporus aeneus*. *J. Morphol.* 184:85-98.
- HEULIN, B. 1990. Étude comparative de la membrane coquillière chez les souches ovipare et vivipare du lézard *Lacerta vivipara*. *Can. J. Zool.* 68:1015-1019.
- HOCHBERG, Y., AND A. TANHAME. 1987. *Multiple comparison procedures*. John Wiley, New York.
- KING, R. B. 1993. Determinants of offspring number and size in the brown snake, *Storeria dekayi*. *J. Herpetol.* 27(2):175-185.
- LANG, J. W. 1985. Incubation temperature affects thermal selection of hatchling crocodiles. *Am. Zool.* 25:18A.
- LLOYD, D. C. 1987. Selection of offspring size at independence and other size-versus-number strategies. *Am. Nat.* 129:800-817.
- MADSEN, T., AND R. SHINE. 1992. Determinants of reproductive success in female adders, *Vipera berus*. *Oecologia* 92:40-47.
- NEWMAN, R. A. 1994. Genetic variation for phenotypic plasticity in the larval life history of spadefoot toads (*Scaphiopus couchii*). *Evolution*, 48(6):1773-1785.
- VAN NOORDWIJK, A. J., L. C. P. KEIZER, J. H. VAN BALEN, AND W. SCHARLOO. 1981. Genetic variation in egg dimensions in natural populations of the great tit. *Genetica* 55:221-232.
- NUSSBAUM, R. A. 1981. Seasonal shifts in clutch-size and egg-size in the side-blotched lizard, *Uta stansburiana*. *Oecologia* 49:8-13.
- NUSSBAUM, R. A., AND D. L. SCHULTZ. 1989. Coevolution of parental care and egg size. *Am. Nat.* 133:591-603.
- OLSSON, M. 1992. Sexual selection and reproductive strategies in the sand lizard, *Lacerta agilis*. Ph.D. diss. University of Göteborg, Sweden.
- . 1994. Nuptial coloration in the sand lizard (*Lacerta agilis*): An intrasexually selected cue to fighting ability. *Anim. Behav.* 48:607-613.
- OLSSON, M., GULLBERG, AND H. TEGELSTRÖM. 1994a. Sperm competition in the sand lizard (*Lacerta agilis*). *Anim. Behav.* 48:193-200.
- OLSSON, M., A. GULLBERG, H. TEGELSTRÖM, T. MADSEN, AND R. SHINE. 1994b. "Promiscuous" females have more viable offspring. Published under "Can adders multiply," scientific correspondence. *Nature* 369:528.
- OLSSON, M., T. MADSEN, R. SHINE, A. GULLBERG, H. TEGELSTRÖM. 1994c. Rewards of promiscuity. *Nature* 372:230.
- PACKARD, G. C. 1991. Physiological and ecological importance of water to embryos of oviparous reptiles. Pp. 213-228 in D. C. Deeming, and M. W. J. Ferguson, eds. *Egg incubation: Its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, UK.
- PALMER, B. D., V. DEMARCO, AND L. J. GUILLETTE, JR. 1994. Oviductal morphology and eggshell formation in the lizard *Sceloporus woodi*. *J. Morphol.* *In press*.
- PARKER, G. A., AND M. BEGON. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. *Am. Nat.* 128:573-592.
- PRICE, T. D., AND P. R. GRANT. 1985. The evolution of ontogeny in Darwin's finches: A quantitative genetic approach. *Am. Nat.* 125(2):169-188.
- RYKENA, S. 1988. Innerartliche differenzen bei der Eizeitungsdauer von *Lacerta agilis*. *Mertensiella* 1:41-53.
- SHINE, R. 1977. Reproduction in Australian Elapid snakes II. Female reproductive cycles. *Aust. J. Zool.* 25:655-666.
- . 1978. Propagule size and parental care: the "safe harbor" hypothesis. *J. Theor. Biol.* 75:417-424.
- . 1995. A new hypothesis for the evolution of viviparity in reptiles. *Am. Nat.* 145:809-823.
- SHINE, R., AND P. HARLOW. 1993. Maternal thermoregulation influences offspring viability in a viviparous lizard. *Oecologia* 96:122-127.
- SINERVO, B. 1990. The evolution of maternal investment in lizards: An experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 42:885-899.
- SINERVO, B., AND S. C. ADOLPH. 1989. Thermal sensitivity of growth rate between populations in *Sceloporus* lizards: Environmental, behavioral, and genetic aspects. *Oecologia* 83:228-237.
- SMITH, C. C., AND S. D. FRETWELL. 1974. The optimal balance between size and number of offspring. *Am. Nat.* 108:499-506.
- STAMPS, J., AND V. V. KRISHNAN. 1994a. Territory acquisition in lizards: I. First encounters. *Anim. Behav.* 47:1375-1385.
- . 1994b. Territory acquisition in lizards: II. Establishing social and spatial relationships. *Anim. Behav.* 47:1387-1400.
- VIA, S., R. GOMULKIEWICZ, G. DE JONG, S. M. SCHEINER, C. D. SCHLICHTING, AND P. H. VAN TIENDEREN. 1995. Adaptive phenotypic plasticity. *Trends Ecol. Evol.* 10(5):212-217.
- VITT, L., AND J. D. CONGDON. 1978. Body shape, reproductive effort, and relative clutch mass in lizards: resolution of a paradox. *Am. Nat.* 112:595-608.
- VÄISÄNEN, R., O. HILDÉN, M. SOIKKELI, AND S. VOULANTO. 1972. Egg dimension variation in five wader species: the role of heredity. *Ornis. Fennica.* 49:25-44.
- WINKLER, D. W., AND K. WALLIN. 1987. Offspring size and number: A life-history model linking effort per offspring and total effort. *Am. Nat.* 129:708-720.

Corresponding Editor: D. Howard