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Morphological correlates of life-history variation: is lizard clutch size related to the number of germinal beds in the ovary?

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Clutch size varies widely in reptiles, both intraspecifically and interspecifically. The mechanisms that generate this variation have attracted detailed study, focusing primarily on ecological factors (e.g. food availability), trade-offs with other traits (e.g. offspring size), and physical constraints (e.g. maternal body shape). Does ovarian morphology, specifically the number of germinal beds from which ova are produced, also correlate with clutch size? Our review of published data on 58 lizard species reveals that clutch size is correlated with the number of germinal beds per ovary (more fecund species have more germinal beds), and that phylogenetic changes in germinal beds have been consistently associated with concurrent phylogenetic changes in fecundity. These correlations imply a causal connection: either clutch size is constrained by ovarian morphology, and/or ovarian morphology evolves to allow adaptive shifts in clutch size. The latter hypothesis is more consistent with available data. © 2008 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2008, **94**, 81–88.

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INTRODUCTION

Fecundity is a key life-history trait, both for individual fitness and population viability. Accordingly, identifying correlates (and hence possibly determinants or consequences) of variation in fecundity has been a central theme of evolutionary and ecological research (Slagsvold, 1982; Martin, Hannon & Rockwell, 1989; Zaviezo & Mills, 2000; Charnov & Ernest, 2006). The correlates of clutch size variation are of particular interest in a group such as squamate reptiles (lizards and snakes) in which fecundity can vary enormously both among and within species (Fitch, 1970; Ballinger, 1978; Jones *et al*., 1982; Köhler, 2005). Previous research on reptile fecundity has identified a series of correlates of clutch size variation, and experimental work has clarified the causal processes underlying some of that variation. For example, prey availability (and thus feeding rates) can modify clutch sizes, on a variety of temporal scales (James & Whitford, 1994; Madsen & Shine, 1996, 1999; Doughty & Shine, 1998), but only within a range set by physical constraints such as maternal body form and, thus, available abdominal space to hold the eggs (Vitt & Congdon, 1978; Shine, 1992; Du, Ji & Shine, 2005; Pizzatto, Almeida-Santos & Shine, 2007). Furthermore, allometric engineering studies convincingly demonstrate trade-offs between offspring size and number, so that selection on offspring size can modify clutch size as a correlated response (Sinervo *et al*., 1992) especially if egg size is heritable whereas clutch size is not (Brown & Shine, 2007).

Other potential correlates of squamate clutch size have attracted less attention. The number of eggs or offspring produced in a clutch ultimately depends upon the number of follicles produced, the proportion of those follicles that become vitellogenic, and the incidence of atresia of those vitellogenic follicles prior to ovulation, as determined by circulating hormonal factors such as follicle-stimulating hormone and peptides (Jones *et al*., 1982; Norris, 2007). The follicles, in turn, are derived from specialized germinal beds, small regions on the dorsal ovarian surface consisting of dividing oogonia, naked oocytes, and primordial

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*Corresponding author. E-mail: raju@mail.usyd.edu.au follicles (Jones *et al*., 1982; Klosterman, 1983; Gómez & Ramírez-Pinilla, 2004; Radder & Shine, 2007). The number of such germinal beds does not change over the lifetime of an individual reptile (Jones *et al*., 1982; Radder & Shine, 2007) but varies interspecifically from one to six (Jones *et al*., 1982; Jones & Summers, 1984; Shanbhag & Prasad, 1993; Uribe *et al*., 1995; Shanbhag, Subraya & Saidapur, 1998; Amey & Whittier, 2000; Stewart & Florian, 2000; Sica *et al*., 2001; Radder & Shine, 2007). Hence, we might expect species with more germinal beds per ovary to produce larger clutches (Jones *et al*., 1982). Such a correlation might arise if the number of germinal beds acted as a proximate constraint on the number of vitellogenic follicles produced, and also would be expected if selection on clutch size favoured the evolution of the appropriate number of germinal beds (i.e. as many as needed to produce the requisite number of eggs). Either process would be of interest, because previous studies have ignored potential quantitative mechanistic links between follicle production and fecundity. On a purely empirical level, a tight correlation between such variables might be valuable in predicting mean adult fecundity from ovarian morphology of nonreproductive females (Radder & Shine, 2007).

To clarify these issues, we reviewed published information on germinal bed condition in lizards. We have analysed these data in two ways. First, we looked for broad patterns among present-day species: for example, to determine whether germinal bed number shows phylogenetic conservatism, and to examine correlations between this trait and other phenotypic characteristics. If they exist, such correlations might help to predict fecundity from ovarian morphology (above). Second, we analysed the data in a phylogenetic framework, to determine whether evolutionary changes in the number of germinal beds per ovary correlate with changes in other traits such as fecundity, female body size, or age at maturation. This second (comparative) set of analyses tested for the predicted functional association between ovarian morphology and fecundity. We selected life-history traits such as body size, age at maturation, reproductive mode (i.e. oviparity versus viviparity), and clutch frequency (i.e. single versus multiple broods/season) identified by previous studies as correlates of clutch size variation within reptiles (Dunham & Miles, 1985; Fitch, 1985; Dunham, Miles & Reznick, 1988; Shine & Greer, 1991).

MATERIAL AND METHODS

We reviewed published literature on lizards for information on the number of germinal beds per ovary, age at maturation, reproductive mode, clutch frequency, and mean snout–vent length of adult females (see Supplementary material, Table S1). Because body size is a major correlate of clutch size variation in reptiles, any correlation between germinal bed number and clutch size (as predicted from mechanistic grounds), in either of our main analyses, might be an artefact of covariation with body size. To provide convincing evidence of a functional relationship between germinal bed number and clutch size, the relationship should be stronger between these two variables than between germinal bed number and body size; and should remain significant even when body size is incorporated in the analysis.

DATA ANALYSES

Statistical analyses of the data were performed using Statview, version 5.0 (SAS, 1998). Snout–vent length and clutch size values were ln-transformed to achieve variance homogeneity and normality. Our comparative analyses used Mesquite, version 1.12 (Maddison & Maddison, 2006). Hypotheses on phylogenetic relationships were obtained from the available literature (Hutchinson *et al*., 1990; Joger, 1991; Reeder & Wiens, 1996; Wiens & Reeder, 1997; Harris, Arnold & Thomas, 1998; Macey *et al*., 1999; Flores-Villela *et al*., 2000; Frost *et al*., 2001a, b; Wiens & Slingluff, 2001; Honda *et al*., 2002; Perry & Garland, 2002; Reeder, Cole & Dessauer, 2002; Honda *et al*., 2003; Reeder, 2003; Schulte, Valladares & Larson, 2003; Ham, Zhou & Bauer, 2004; Hugall & Lee, 2004; Poe, 2004; Schulte *et al*., 2004; Townsend *et al*., 2004; Lee, 2005; Vidal & Hedges, 2005; Carranza & Arnold, 2006; Wiens, Brandley & Reeder, 2006). In some cases, we had to assume that taxonomy accurately reflects phylogeny. Conspecific populations were treated as sister clades (or sometimes polytomies; the only species in our study for which we have data for two populations is *Heteronotia binoei* (Gray, 1845), a species complex that includes both parthenogenetic and sexual reproduction), and we used the supertree technique to solve incongruences among hypotheses (Bininda-Emonds, 2004). All of the comparative tests were run twice, based on two phylogenetic hypotheses about relationships among the major groups of lizards. One set of analyses used a cladogram based on molecular characters (Townsend *et al*., 2004; Vidal & Hedges, 2005) whereas the other was based on morphology (Lee, 2005). We used independent contrasts analyses to compare relationships between number of germinal beds and continuous life-history traits with the PDAP package for Mesquite (Felsenstein, 1985; Midford, Garland & Maddison, 2005). All branch lengths were set to 1 and relationships between variables were analysed by linear regressions forced through the origin (Garland, Harvey & Ives, 1992). Relationships between numbers of germinal beds and categorical life-history variables were analysed using Pagel's (1994) test for binary data (as modified by Maddison & Maddison, 2006; for details, see the Mesquite manual). Intensity of likelihood search was 10, and 1000 simulations were conducted to estimate *P*-values.

RESULTS

PATTERNS IN GERMINAL BED NUMBER

First, we describe patterns overall, treating each population as an independent data point (i.e. ignoring phylogeny). We located information on the number of germinal beds per ovary for 43 oviparous and 16 viviparous species of lizards representing nine families (see Supplementary material, Table S1). Among oviparous lizards, 18 species exhibit one germinal bed per ovary, 24 species exhibit two, and the remaining one taxon displays 4–6 germinal beds per ovary. In viviparous lizards, three species contain one germinal bed per ovary, 12 species have two, and one species has 4–6 (see Supplementary material, Table S1).

Overall, the number of germinal beds per ovary was higher in species with larger mean clutch sizes than in species with smaller mean clutch sizes (Spearman correlation $Z = 0.33$, $P < 0.012$, $N = 58$; Fig. 1). Also, the maximum number of vitellogenic follicles ovulated from each ovary was higher in species with a higher number of germinal beds per ovary (Spearman correlation $Z = 4.93$, $P < 0.0001$, $N = 58$). Mean adult female body size was linked to clutch size interspecifically ($r = 0.44$, $P < 0.0005$, $N = 58$) but was not significantly correlated with the number of germinal beds per ovary ($r = -0.01$, $P = 0.93$). Incorporating body size and ln clutch size in a multiple logistic regression, with number of germinal beds per ovary as the categorical dependent variable, revealed that clutch size remained statistically significant ($P = 0.0004$) but body size did not ($P = 0.17$). The number of germinal beds was not significantly associated with reproductive mode, frequency of reproduction, or any of the other morphological or life-history traits that we recorded (see Supplementary material, Table S1).

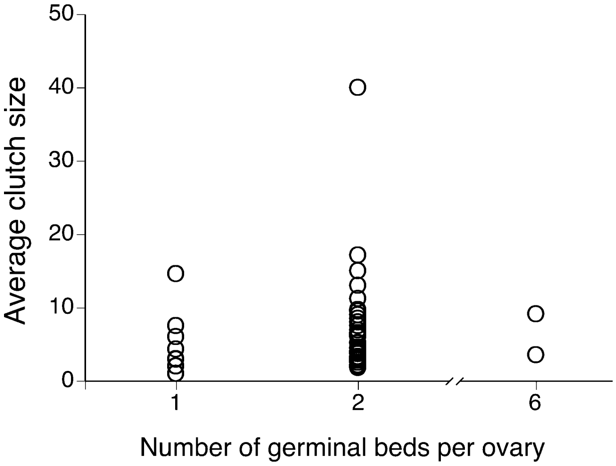

Figure 1. Relationship between number of germinal beds (GBs) per ovary and average clutch size in lizards ($N = 58$ species), including both oviparous and viviparous taxa. For species exhibiting four to six germinal beds per ovary, the maximum number (i.e. six GBs) is used to plot the distribution.

PHYLOGENETICALLY-BASED ANALYSES OF GERMINAL BED NUMBER

Many of the traits that we scored (including clutch sizes and germinal bed numbers per ovary) display significant phylogenetic conservatism (i.e. related species resemble each other more than would be expected by chance). For example, all nine of the gekkonid species listed in the Supplementary material (Table S1) have a single germinal bed per ovary, and a clutch size of two eggs. By contrast, other groups (such as the Scincidae) show more diversity in these respects, and traits such as female mean adult size also vary considerably among major lineages. Thus, any patterns documented from analyses treating species as independent units (above) might be due to phylogenetic conservatism rather than to causal (functional) relationships among variables. The phylogenetic (comparative) analysis avoids this problem by focusing on evolutionary changes in trait values rather than mean trait values *per se*. As predicted, evolutionary changes in the number of germinal beds per ovary were consistently associated with evolutionary changes in mean clutch size [Vidal & Hedges' (2005) tree: $N = 54$ contrasts, $r = 0.26$, one-tailed $P = 0.03$; appropriate because there is an a priori prediction of a positive correlation; Lee's (2005) tree, $N = 56$ contrasts, $r = 0.24$, one-tailed $P = 0.035$; Fig. 2]. Body size was not associated with germinal bed number (for contrasts analyses, all $P > 0.84$), and multiple regression with evolutionary changes in germinal bed number as the dependent variable revealed a significant effect of evolutionary shifts in clutch size (one-tailed $P < 0.025$ under both hypotheses) but not body size (one-tailed $P > 0.22$). Under both phylogenetic hypotheses, Pagel's (1994) analytic method detected no significant relationship between the number of germinal beds and the mode of reproduction ($P > 0.1$) but a positive relationship was found between the number of germinal beds and the mean number of follicles ovulated per ovary per clutch ($P < 0.05$) and number of broods (one-tailed $P < 0.04$).

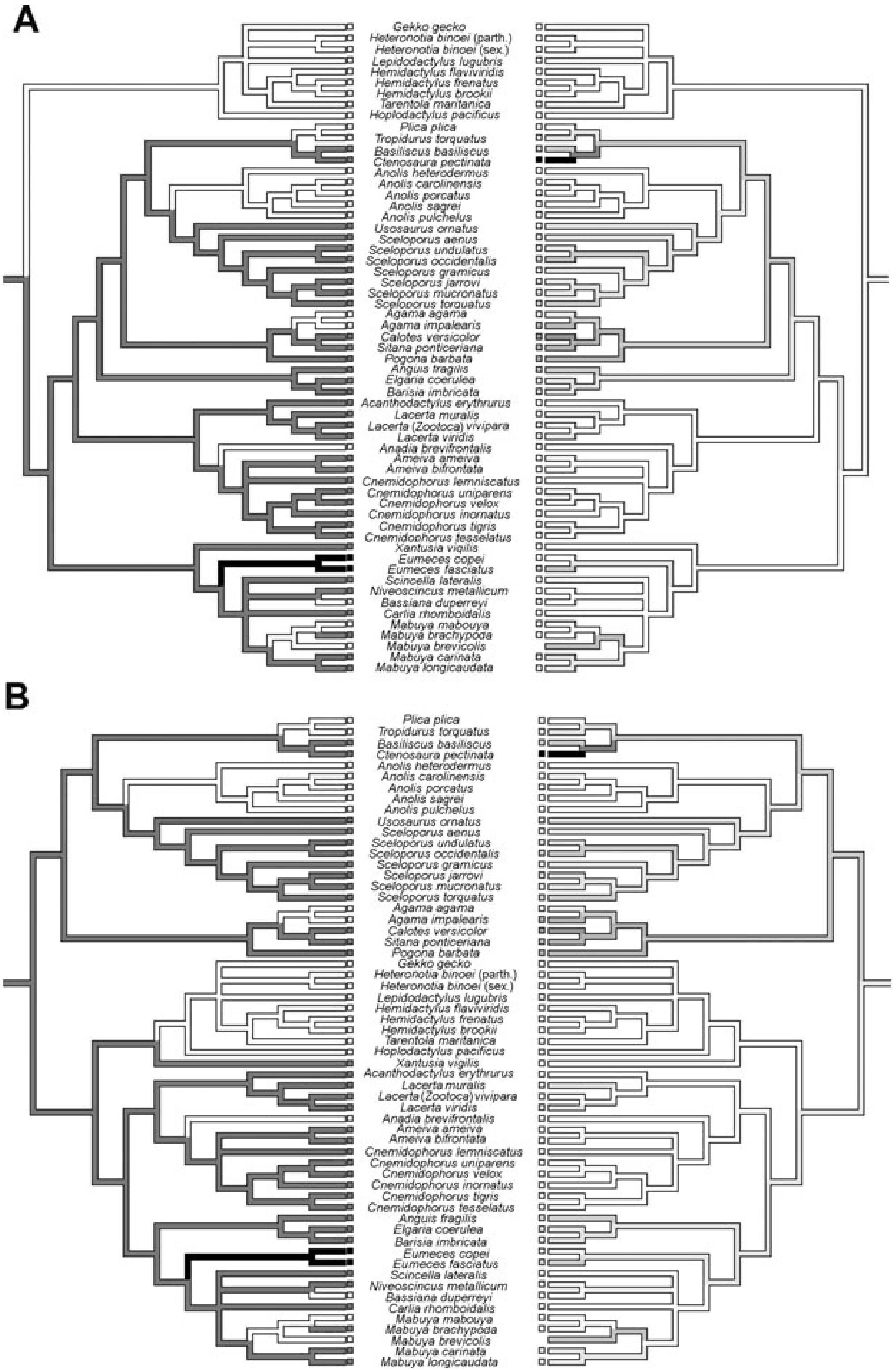

Figure 2. A, evolution of the number of germinal beds (left) and mean clutch sizes (right) among lizards according to a phylogenetic hypothesis based upon the work of Vidal & Hedges (2005) and Townsend *et al*. (2004). B, evolution of the number of germinal beds (left) and mean clutch sizes (right) among lizards according to a phylogenetic hypothesis proposed by Lee (2005). Reconstructions were made using squared parsimony in Mesquite, version 1.12 (Maddison & Maddison, 2006). Darker branches show higher variable values (i.e. unusually rapid rates of evolutionary change in the trait in question).

DISCUSSION

Among lizards, ovarian morphology is linked to fecundity. Lizard species that produce large clutches tend to produce vitellogenic follicles in multiple germinal beds per ovary. By contrast, most lizards that produce small clutches exhibit only a single germinal bed per ovary. This association is not surprising, because the morphological feature involved (number of germinal beds) is mechanistically linked to fecundity. Most of the other traits that we examined in the present study, encompassing a range of measures of body size and reproductive biology, were not significantly linked to the number of germinal beds per ovary (but see number of broods, above).

Although our data confirm a relationship between clutch size and ovarian morphology, they do not reveal the causal basis for that relationship. We can envisage two possibilities: constraint or adaptation. Under the ‘constraint’ hypothesis, the number of germinal beds per ovary sets an upper limit to clutch size and thus generates a linkage between the two traits. Under the ‘adaptation’ hypothesis, the causal link is reversed, with selection on clutch size favouring the evolution of the number of germinal beds needed to produce the requisite number of follicles. The ‘constraint’ hypothesis appears to be implausible because: (1) clutch size determines individual fitness and hence is likely to be under intense selection; we doubt that a mechanistic constraint could prevent adaptively favoured increases in clutch size and (2) clutch size varies intraspecifically over a wide range in most lizard populations (e.g. reflecting factors such as maternal body size and food availability; Shine & Greer, 1991) and thus, any constraint imposed by the number of germinal beds must encompass the population’s maximum value for clutch size. That value will generally be much higher than the mean, so that a constraint on maximum fecundity would translate into a highly-skewed distribution of clutch sizes with most clutches comprised of a similar, high number of eggs. In practice, distributions of clutch size within lizard populations are approximately normal (R. Shine, unpubl. data). We doubt that germinal bed number constrains clutch size, except perhaps in rare instances where a rapid phylogenetic increase in maternal body size generates selection for massively increased fecundity.

An alternative hypothesis to explain the link between clutch size and number of germinal beds is one of adaptation. As selection favours increases in clutch size, ovarian morphology evolves to increase the number of germinal beds and thus allow greater follicle production. Presumably, the reverse also occurs, with selection for clutch size reduction favouring concentration of follicle production into a single germinal bed per ovary. Why do high-fecundity species tend to have more germinal beds instead of producing additional follicles from a single germinal bed? The link between clutch size and ovarian morphology is not obligate, as indicated by the substantial variation evident in the relationship between these two variables. For example, some high-fecundity species have only a single germinal bed per ovary [e.g. *Agama impalearis* (Boettger, 1874) has a mean clutch size of 14.6 eggs, but only a single germinal bed per ovary], and some low-fecundity taxa have multiple beds [e.g. *Eumeces copei* (Taylor, 1933) averages only 3.5 offspring per litter, but has four to six germinal beds per ovary; see Supplementary material, Table S1]. That diversity also shows that a low number of germinal beds does not necessarily constrain clutch size.

Instead, the number of germinal beds may reflect selective pressures related to the spatial distribution of vitellogenic follicles within the female’s body. In species with multiple germinal beds per ovary, these beds tend to be well-separated spatially within the ovary (Shanbhag, 2002: fig. 7C; R. Radder, pers. observ.) Additional germinal beds thus may allow follicles to develop at multiple locations instead of in clumps. This spacing out may confer a fitness benefit by spreading the burden spatially and, hence, reducing the degree of abdominal distension at any one point in the maternal body. Vitellogenic follicles are very large, approximating the size of eggs by the time of ovulation and, hence, may severely constrain locomotor performance of females (Shine, 1980, 2003; Bauwens & Thoen, 1981; Garland, 1985; Sinervo, 1990; Sinervo, Hedges & Adolph, 1991; Miles, Sinervo & Frankino, 2000). Females of many multiple-clutching species simultaneously contain both vitellogenic follicles and oviductal eggs (Radder & Shanbhag, 2003), and such eggs sometimes may be retained in the oviduct for prolonged periods (Radder, Shanbhag & Saidapur, 1998). Especially in slender-bodied and arboreal species, disadvantages to such distension may favour a redistribution of developing follicles along the ovaries such that the burden is distributed more widely than if a single germinal bed produced all of the follicles in each ovary (for a similar argument concerning overlap between left and right ovaries in arboreal snakes, see Pizzatto *et al*., 2007). This hypothesis predicts an increase in the number of germinal beds per ovary with the evolution of arboreality or slender body form. The best opportunities to test such adaptive hypotheses may come from lineages with labile ovarian morphology; for example, *Mabuya mabouya* (Bonnaterre, 1789) reportedly displays both one and two germinal beds per ovary within the same population (Gómez & Ramírez-Pinilla, 2004).

Other adaptive hypotheses also are plausible. For example, more germinal beds may allow variability at the upper end of the clutch size range. Low initial resource availability may not trigger the functioning of all germinal beds (through hormonal mechanisms). Later income resources may modulate and trigger each functioning bed. As in humans, alternation in the functioning of the right and left ovary has been reported in reptiles (Norris, 2007). Some turtles produce most of their eggs in one ovary during one season and most from other ovary the next season (Norris, 2007). The regulatory mechanisms are not yet understood, and current data are inadequate to address some of the above predictions robustly because information on germinal beds is available for only < 2% of lizard species (see Supplementary material, Table S1) out of a total of > 4470 species listed to date (Uetz, 2000). These areas of research warrant further study.

In summary, our analyses of the available published data support the hypothesis of a functional link between a critical life-history attribute (i.e. clutch size) and an easily measured aspect of ovarian morphology (i.e. the number of germinal beds). The gap between fields such as reproductive anatomy and life-history variation has rarely been bridged, but our study is encouraging for the potential feasibility of integration across these areas of research.

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SUPPLEMENTARY MATERIAL

The following material is available for this article online:

Table S1. Number of germinal beds per ovary in 58 species of lizards.

This material is available as part of the online article from:
http://www.blackwell-synergy.com/doi/abs/10.1111/j.1095-8312.2008.00959.x
(This link will take you to the article abstract).