

Eggshell morphology and gekkotan life-history evolution

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Abstract Eggshell structure is related to fundamental aspects of embryonic development (via water and gas exchange), adult ecology and behavior (via nest site selection), and demography (via effects on survival). We compared life-history characteristics between gekkotans that lay rigid- versus parchment- shelled eggs to determine if evolutionary shifts in eggshell structure are associated with life-history evolution. Ancestral gekkotans laid parchment-shelled eggs, with rigid-shelled eggs evolving later. Clutch size in oviparous gekkotans is fixed at one or two eggs, and this characteristic eliminates an egg size versus clutch size tradeoff as a life-history strategy. We found that species laying rigid-shelled eggs exhibit (1) smaller eggs relative to adult body size, (2) smaller hatchlings relative to the size of the egg, (3) earlier embryonic stage at oviposition, (4) longer incubation periods, and (5) smaller adult body sizes than species laying parchment-shelled eggs. These patterns hold when accounting for phylogenetic relatedness, and are not explained by geographic distributions of climate and habitat. In general, our data support the hypothesis that the spherical shapes of rigid-shelled eggs limit their size (volume), which in turn has restricted hatchling size and adult body size. In contrast, while parchment-shelled eggs are similarly constrained in width, elongate shapes allow egg sizes, and hence hatchling sizes, to increase relative to adult body sizes. Finally, the evolution of rigid-shelled eggs may have allowed gekkotans to become so successful; over 1,000 species lay rigid-shelled eggs, as compared to about 200 species that lay eggs exhibiting the ancestral parchment-shelled condition.

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Introduction

Extant amniotes exhibit one of two types of eggshell: a parchment (leathery) shell that is relatively permeable to water vapor and metabolic gases or a highly mineralized rigid shell that is relatively impermeable (Packard and Seymour 1997). A parchment shell appears to be the ancestral state for amniotes, given that this is the ancestral state for modern mammals (i.e., monotremes) and lepidosaurs (tuatara, snakes, and lizards; Stewart 1997; Oftedal 2002). A derived rigid shell characterizes all archosaurs (crocodilians and birds). The ancestral shell type for turtles is problematic because of variation in shell type both within and among major clades (Iverson and Ewert 1991). The lack of resolution for turtles does not affect the assessment of the ancestral shell type for modern amniotes. It simply means that, given the likely placement of turtles as the sister-group of archosaurs (Shedlock and Edwards 2009), the common ancestor of these taxa either had a: (1) parchment shell; turtles and archosaurs would thus have evolved rigid shells independently (with possible reversals to parchment shells in turtles); or (2) rigid shell; turtles thus would have evolved parchment shells multiple times (with possible reversals to rigid shells). The multiple transitions between shell types beg the question of the adaptive value of these radically different barriers that separate embryos from their external environment.

The physical differences between rigid and parchment eggshells are profound (Schleich and Kästle 1988; Packard and DeMarco 1991); rigid-shelled eggs have a thick mineral layer that overlies a thin fibrous shell membrane, while parchment-shelled eggs have a thin mineral layer that overlies a thicker fibrous shell membrane. This arrangement renders rigid-shelled eggs hard and inflexible (i.e., with dimensions fixed shortly after oviposition), whereas parchment-shelled eggs are pliable and stretchy (and can thus expand after oviposition). These physical differences influence the water relations and gas exchange capabilities of eggs (Deeming and Thompson 1991; Packard 1991; Thompson and Speake 2004), and hence the development of the embryos that lie within. For example, water vapor permeability of rigid-shelled eggs is low and eggs tend to decrease in mass throughout incubation (Deeming and Unwin 2004). In contrast, water vapor permeability of parchment-shelled eggs is high and eggs tend to increase in mass during incubation as a result of water uptake from the environment (Ackerman et al. 1985; Packard and Packard 1988; Deeming and Unwin 2004). The pattern of oxygen permeability of eggshells parallels that of water vapor permeability (Deeming and Thompson 1991). This suggests that oxygen diffusion could limit development in rigid-shelled eggs under certain conditions, such as at high relative humidity and high incubation temperature, especially towards the end of development when the metabolic demands of embryos are highest (Thompson and Russell 1999).

Whereas the vast majority of squamates produce parchment-shelled eggs, gekkotan lizards provide a notable exception (Pough et al. 2004). Only about 200 species, members of the families Diplodactylidae, Carphodactylidae, Pygopodidae, and Eublepharidae (family-level taxonomy of Gamble et al. 2008) lay parchment-shelled eggs. These families are basal to the monophyletic lineage comprised of members of the families Gekkonidae, Phyllodactylidae, and Sphaerodactylidae. The more than 1,000 species in these taxa lay rigid-shelled eggs. The numerical dominance of gekkotans that lay rigid-shelled eggs

suggests that the adaptive radiation of this clade is associated with either the origin of the rigid-shelled egg itself or with the origin of other adaptations in combination with the rigid-shelled egg. Our main objective was thus to assess why gekkotans that lay rigid shelled eggs have been so successful.

We addressed this question in two ways. First, we determined whether the origin of the rigid-shelled egg affected life history evolution of gekkotans by testing a series of hypotheses based on morphological and physiological attributes of rigid- and parchment-shelled eggs. Gekkotan lizards have an invariant clutch size, with a given species either laying a one or a two-egg clutch, and gekkotans typically lay fewer clutches per season than do other lizards of comparable body sizes (Doughty 1997; Kratochvíl and Kubicka 2007; Xu and Ji 2007; Zhang et al. 2009). This trait makes life-history comparisons powerful because gekkotans have little opportunity to alter reproductive effort by way of egg size vs clutch size, or clutch size vs clutch frequency tradeoffs (i.e., common life-history tradeoffs). Consequently, we anticipated that the life histories of gekkotan lizards that lay rigid- vs parchment-shelled eggs would differ, and would do so in predictable ways. For example, rigid-shelled eggs tend to be spherical, whereas parchment-shelled eggs are elongate (Kratochvíl and Frynta 2006). Egg width is correlated with female body size for both types of eggshells, but rigid-shelled eggs have smaller volumes due to their more spherical shape (Kratochvíl and Frynta 2006). These observations imply that (1) egg size is smaller for species that produce rigid- versus parchment-shelled eggs. At the time of oviposition, rigid-shelled eggs contain all of the water necessary for embryonic development; in contrast, parchment-shelled eggs must absorb water from the environment for normal development (Packard and Packard 1988; Deeming and Unwin 2004). As a consequence, eggs of rigid-shelled species contain relatively more water and less yolk at oviposition than do parchment-shelled eggs (Belinsky et al. 2004). We therefore predicted that (2) when controlling for initial egg size, hatchlings from rigid-shelled eggs would be smaller than hatchlings from parchment-shelled eggs. Rigid-shelled eggs have even lower oxygen permeability after oviposition than before (Deeming and Thompson 1991) because shell membranes would be filled with fluid rather than air, and oxygen diffusion is far lower in fluid than in air (Cronin and Seymour 2000). We therefore predicted that embryos within rigid-shelled eggs would (3) be at earlier stages of development at the time of oviposition because oviposition would increase oxygen availability to embryos, and (4) would develop more slowly after oviposition because of low oxygen availability and therefore have longer incubation periods than embryos developing in parchment-shelled eggs.

We also predicted that a reduction in reproductive output per reproductive episode, and reduced developmental rates associated with the evolution of the rigid-shelled egg, should influence other attributes of the life histories of gekkotan lizards. Because of strong correlations between adult body size, clutch size, egg size, and offspring size (Andrews 1982; Bauwens and Díaz-Uriarte 1997), shifts in egg size of gekkotans also should influence adult body size. A priori, the direction of such a shift is not obvious. Under an egg size compensation scenario, an increase in body size would allow gekkotans with rigid-shelled eggs to lay larger eggs, thus compensating for reduced reproductive output through the production of larger hatchlings. Under a demographic compensation scenario, a decrease in body size would bring a clutch of two small eggs into line with a reduction in the energetic cost of reproduction (Warne and Charnov 2008). This means that females would be able to reproduce at smaller body sizes (i.e., younger), with a resultant increase in population growth rate. We therefore contrasted adult body sizes of gekkotans that produce rigid- and parchment-shelled eggs with the general prediction that (5) the overall distribution of body

sizes of these two groups would differ. Each of these questions was examined both with and without accounting for phylogenetic relatedness.

The second way we assessed why gekkotans that lay rigid shelled eggs are so successful was to examine their geographic distributions. We hypothesized that the success of gekkotans that lay rigid-shelled eggs was due to their invasion of environments not utilized effectively by gekkotans that lay parchment-shelled eggs. For example, the rigid-shelled egg could be a pre-adaptation for arid environments because the eggs are fully supplied with water at the time of oviposition. We therefore contrasted the geographic distributions of gekkotans that lay rigid- versus parchment-shelled eggs with respect to latitude (tropical versus temperate) and habitat (mesic versus arid). We chose these variables because they are applicable to all gekkotans. We did not partition distributions more finely because specific habitat types do not occur on all continents (e.g., fynbos, seasonally flooded forest, sand dunes, etc.), although all continents have gekkotans. Moreover, while current distributions of gekkotans with rigid- and parchment-shelled eggs are broadly overlapping, the continents have different subsets of the seven gekkotan families. For example, broad sympatry of the two shell types occurs in Australia (e.g., gekkonids and diplodactylids), in North America (e.g., sphaerodactylids and eublepharids), and in Central Asia (e.g., gekkonids and eublepharids). We also hypothesized that adult body size may be related to geographic distribution rather than, or in addition to, any association between shell type and adult body size. To test this hypothesis, we contrasted the geographic distributions of gekkotans that lay rigid- versus parchment-shelled eggs with respect to latitude (tropical versus temperate) and habitat (mesic versus arid).

Materials and methods

Data collection

We collected data on gekkotan life-history attributes from literature reviews (Simbotwe 1983; Greer 1989; Henkel and Schmidt 1995; Branch 1998; Zhao et al. 1999; Annable 2004; Köhler 2005), and filled in gaps using other primary literature sources focused on individual species and unpublished data provided by other researchers. Data compiled were: maximum adult body size (snout-vent length in mm), egg size at oviposition (length and width in mm), stage of embryonic development at oviposition (after Dufaure and Hubert 1961), incubation duration (days), hatchling body size (snout-vent length and total length in mm), and clutch size. When more than one source was available for any individual taxon, we used the mean value of all available data.

Linear dimensions of eggs were more commonly reported in the literature than was mass, although volume is more meaningful biologically because it is directly related to the amount of nutrient provisioning. We therefore converted linear dimensions into egg volume using the formula: $V = (\pi LW^2/6)[1 + (2c_2)/5 + (3c_2^2)/35]$ where V (mm^3) is the volume of the egg, L is the length of the egg in mm, W is the width of the egg in mm, and c_2 is calculated for each species using the formula $c_2 = 0.196(L/W) - 0.216$ (Iverson and Ewert 1991).

Because temperature influences incubation duration (Andrews 2004), we limited our dataset to constant incubation temperatures ranging from 27 to 29°C (28°C was the most commonly reported incubation temperature for gekkotans; Henkel and Schmidt 1995; Köhler 2005). When incubation temperature and incubation duration were reported as ranges, we used the midpoint for analysis. When hatchling total length was reported rather

than snout-vent length (SVL), we estimated SVL from total length using regressions of hatchling total length on hatchling SVL for each family.

Distributional information was obtained from regional field guides (e.g., Branch 1998), The Reptile Database (<http://www.reptile-database.reptarium.cz/>), Global Species (<http://www.globalspecies.org/>), and the primary literature. Species ranges were characterized as largely or entirely at temperate or at tropical latitudes, and as largely or entirely in mesic or arid habitats. Tropical latitudes were between the Tropics of Capricorn and Cancer and temperate latitudes were north and south of the Tropics. Mesic habitats included forests, woodlands, savannahs, and moist grasslands. Arid habitats included desert and arid scrublands and arid grasslands. Species that have extensive distributions at both temperate and tropical latitudes and/or in mesic and arid habitats (e.g., *Heteronotia binoei*) were excluded from analyses.

Statistical analyses

Prior to analysis, we restricted our dataset to species laying two-egg clutches and excluded the pygopodids (gekkotans with snake-like body forms) from analyses involving adult and hatchling SVL and egg volume, because their body shape is highly deviant from that of “conventional” gekkotans. Pygopodids were, however, included in comparisons of embryonic stage at oviposition and incubation duration.

Conventional linear regressions were used to analyze relationships between egg volume and adult body size, between hatchling size and egg volume, and between incubation duration and egg volume. We used ANCOVAs, with adult body size as the covariate, for comparisons of egg volumes between shell types, and with egg volume as the covariate for comparisons of hatchling size and incubation duration between shell types. We also re-ran these ANCOVAs using family as a random effect to account for autocorrelation within families that have similar eggshell types (e.g., Bomford et al. 2009). Because these random-effects ANCOVAs had substantially lower degrees of freedom, we regarded differences as being significant when $P < 0.10$ (for all other analyses we assumed statistical significance at $P < 0.05$).

To determine if results of conventional statistical analyses were biased by phylogenetic relationships among gekkotans, we used the PDAP:PDTREE module in Mesquite (Maddison and Maddison 2006; Midford et al. 2005) for phylogenetically controlled (PC) analyses. Phylogenetic relationships of gekkotan lizards were based on Gamble et al. (2008). To avoid bias, we selected one species to represent each gekkotan genus. If more than one species in a genus had both phylogenetic and life history information, we selected the species for which we had the greatest amount of life history data. The resultant data set contained 68 species with data for both adult body size and egg size. This phylogeny was pruned to 31 species for analyses that incorporated hatchling snout-vent length. For PC analyses, branch lengths were set to 1.0 if diagnostic criteria were met ($P > 0.05$); otherwise, Grafen branch lengths with $Rho = 0.5$ were used ($P > 0.05$). If two variables were significantly correlated in both the conventional (PU) and phylogenetically corrected (PC) analyses, we judged their association to be independent of phylogenetic relationships (Midford et al. 2005).

When conventional statistical analyses and PC analyses were in agreement, we used conventional ANOVA's and ANCOVA's to contrast the two groups. Whereas it might appear most desirable to contrast gekkotans that lay rigid-shelled and parchment-shelled eggs using PC ANOVA and ANCOVA'S (e.g., Shattuck and Williams 2010), the problem is that the singular origin of the rigid shell in gekkotans means that the sample size in such

a PC analysis is two, a group of related species with rigid shells and its sister-group made up of related species with parchment shells. Following Felsenstein's (1997) admonitions about this issue, we judged this approach inappropriate for our data. Instead, we relied on the consistency between a range of diverse statistical techniques to determine whether life history patterns evolved in parallel in the gekkotan lineages with rigid and parchment shells.

Geographic distributions of gekkotans were analyzed using conventional statistical analyses. We used likelihood ratio tests to determine associations among shell type, latitude, and habitat and a three-factor ANOVA to determine if Log_{10} adult body size was related to shell type, latitude, and habitat.

Conventional statistical analyses were conducted using JMP Software (Copyright 2007, SAS Institute, Cary, NC, USA). We tested raw data for normality (Kolmogorov–Smirnov tests) and homogeneity of variances (Bartlett tests), and when necessary, we used Log_{10} transformations to meet assumptions of parametric statistical tests. Prior to carrying out ANCOVAs, the assumption of homogeneity of slopes was satisfied by testing for significant interactions of the covariate with class variables. Interactions with P 's >0.10 were dropped from final models. We also compared the distribution of adult body sizes between eggshell types using a Kruskal–Wallace non-parametric test. Means are followed by $\pm 1\text{SE}$. Sample sizes reflect all available data for particular sets of variables unless otherwise noted.

Results

We obtained life-history data for 289 species that produce rigid-shelled eggs and 92 species that produce parchment-shelled eggs. Sample sizes varied among life history traits and families, reflecting the availability of data (Table 1).

Overall, our analyses confirmed previous observations and supported our independent predictions. As reported by Kratochvíl and Frynta (2006), species that lay rigid-shelled eggs produce smaller eggs than species that lay parchment-shelled eggs. Egg volume (Log_{10}EV) increased as a function of adult body size ($\text{Log}_{10}\text{SVL}$; $R^2 = 0.81$, $F_{1,317} = 1,327.94$, $P < 0.0001$; PC: $R^2 = 0.66$, $P < 0.001$). The relationship for rigid-shelled species was $\text{Log}_{10}\text{EV} = 2.07 * \text{Log}_{10}\text{SVL} - 1.0$ ($R^2 = 0.78$, $F_{1,251} = 869.44$, $P < 0.0001$), and for parchment-shelled species was $\text{Log}_{10}\text{EV} = 2.57 * \text{Log}_{10}\text{SVL} - 1.83$ ($R^2 = 0.80$; $F_{1,64} = 260.43$, $P < 0.001$). Egg volume was significantly smaller relative to adult body size for rigid-shelled species than for parchment-shelled species (ANCOVA, $F_{1,316} = 17.78$, $P < 0.0001$; random effects ANCOVA, $F_{1,312} = 2.86$, $P = 0.09$; Fig. 1).

Hatchling body size was strongly and positively associated with egg volume (Log_{10}EV ; PU: $R^2 = 0.72$, $F_{1,68} = 175.23$, $P < 0.0001$; PC: $R^2 = 0.70$, $P < 0.0001$). The relationship for rigid-shelled species was $\text{HSVL} = 15.61 * \text{Log}_{10}\text{EV} - 17.14$ ($R^2 = 0.73$, $F_{1,46} = 123.57$, $P < 0.0001$), and for parchment-shelled species was $\text{HSVL} = 20.39 * \text{Log}_{10}\text{EV} - 26.95$ ($R^2 = 0.63$, $F_{1,20} = 33.89$, $P < 0.0001$). Hatchlings of rigid-shelled species were smaller relative to egg volume than those of parchment-shelled species (ANCOVA, $F_{1,67} = 10.91$, $P = 0.002$; random effects ANCOVA, $F_{1,63} = 7.33$, $P = 0.009$; Fig. 2).

Rigid-shelled species laid their eggs earlier in development (average stage = 25.0, range: stages 23–28, $n = 12$) than did parchment-shelled species (average stage = 27.7, range: stages 20–30, $n = 7$; ANCOVA, $F_{1,18} = 23.89$, $P < 0.0001$; random effects ANCOVA, $F_{1,11} = 15.07$, $P = 0.003$).

Table 1 The number of gekkotan taxa (species or subspecies) for which we collected data on reproductive traits, shown by family

	Rigid-shelled species			Parchment-shelled species			
	Gekkonidae	Phyllodactylidae	Sphaerodactylidae	Diplodactylidae	Eublepharidae	Carpodactylidae	Pygopodidae
Adult snout-vent length	202	22	37	35	14	20	n/a
Egg volume	216	26	38	35	14	18	n/a
Hatchling snout-vent length	39	9	4	11	5	7	n/a
Developmental stage at oviposition	9	1	1	3	2	1	2
Incubation duration	67	9	4	22	9	4	5

These data omit species laying clutches with only one egg, and data on body size and egg volume for Pygopodidae because their snake-like body shape is vastly different from that of all other gekkotans

Fig. 1 The relationship between adult body size and egg volume in gekkotan species. *Regression lines represent parchment-shelled (closed circles and solid line, $N = 66$ species) and rigid-shelled species (open circles and dashed line, $N = 253$ species)*

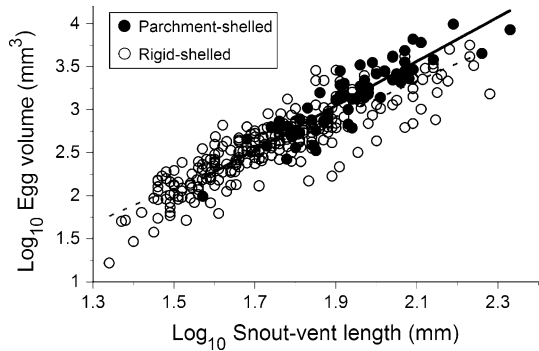
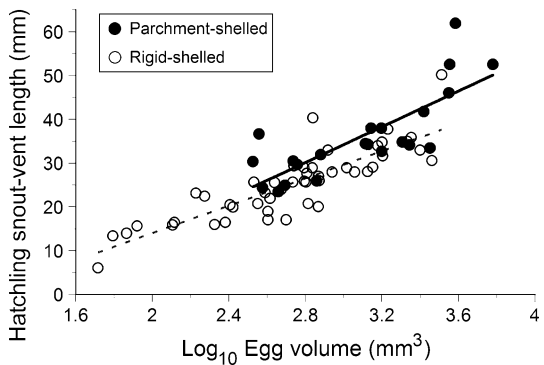


Fig. 2 The relationship between egg volume and hatchling body size for parchment-shelled (closed circles and solid line, $N = 22$) and rigid-shelled (open circles and dashed line, $N = 48$) gekkotan species



Incubation duration (Log_{10}ID) was positively correlated with egg volume (Log_{10}EV) in both PU and PC analyses ($R^2 = 0.21$, $F_{1,113} = 29.51$, $P < 0.0001$; PC: $R^2 = 0.14$, $P = 0.011$) and was unrelated to temperature ($R^2 = 0.01$, $F_{1,117} = 1.02$, $P = 0.31$). The relationship between incubation duration and egg volume for rigid-shelled species was $\text{Log}_{10}\text{ID} = 0.16 * \text{Log}_{10}\text{EV} + 1.37$ ($R^2 = 0.29$, $F_{1,78} = 32.61$, $P < 0.0001$) and for parchment-shelled species was $\text{Log}_{10}\text{ID} = 0.10 * \text{Log}_{10}\text{EV} + 1.54$ ($R^2 = 0.26$, $F_{1,33} = 11.65$, $P = 0.002$). Incubation duration of rigid-shelled eggs was significantly longer than that for parchment-shelled eggs (ANCOVA, $F_{1,112} = 8.92$, $P = 0.003$; random effects ANCOVA, $F_{1,108} = 3.12$, $P = 0.08$; Fig. 3) with adjusted least squared means of 68.4 and 59.1 days, respectively.

The distribution of adult body size also differed by eggshell type (Mann–Whitney $U = 14,280.5$, $P < 0.0001$); species producing rigid-shelled eggs were smaller than species producing parchment-shelled eggs. Mean maximum SVL's were 63.3 ± 2.01 mm ($n = 246$, median = 55.7 mm) and 81.2 ± 3.62 mm ($n = 85$, median = 80.4 mm), respectively (ANCOVA, $F_{1,329} = 54.73$, $P < 0.0001$; random effects ANCOVA, $F_{1,324} = 5.04$, $P = 0.025$; Fig. 4). Gekkotans with body sizes within the smallest ten percentile of the overall distribution of body sizes in our dataset belong to the families Gekkonidae, Phyllodactylidae, Sphaerodactylidae (all species with rigid eggshells), and Diplodactylidae (species with parchment eggshells).

The proportion of gekkotans with rigid and with parchment shells did not differ between tropical and temperate latitudes ($\chi^2 = 0.00$, $P = 1.00$, $n = 343$), with about twice as many species at tropical than temperate latitudes in both cases ($n = 237$ and 106,

Fig. 3 The relationship between egg volume and incubation duration of parchment-shelled (*closed circles and solid line, N = 35*) and rigid-shelled (*open circles and dashed line, N = 80*) gekkotan species

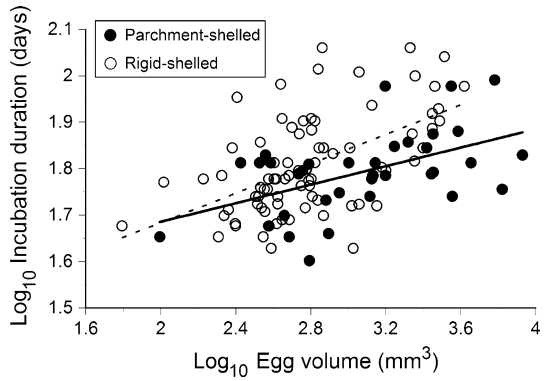
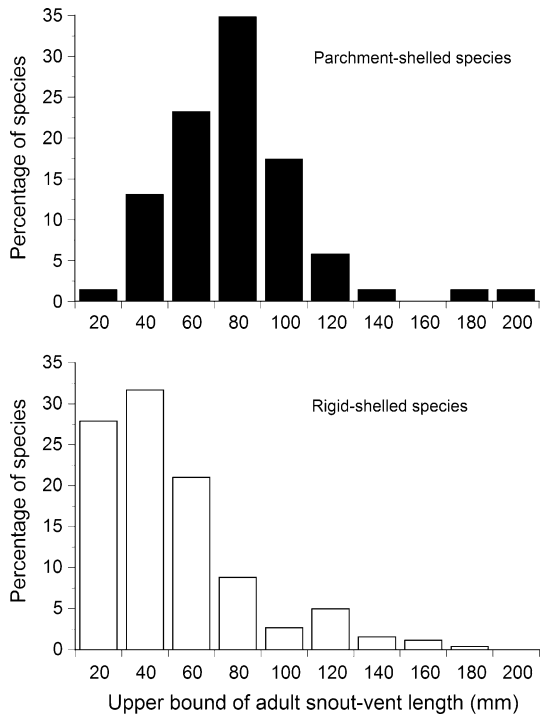


Fig. 4 Distribution of adult body sizes in parchment-shelled (*top, N = 69*) and rigid-shelled (*bottom, N = 262*) gekkotan species



respectively). Habitats occupied by gekkotans differed between latitudes ($\chi^2 = 69.5$, $P < 0.001$, $n = 343$); 80% of tropical habitats were mesic and 67% of temperate habitats were arid. Overall, the proportion of gekkotans laying rigid- and parchment-shelled eggs did not differ between habitats ($\chi^2 = 0.03$, $P = 0.87$, $n = 343$) with 65 and 66% of species, respectively, associated with mesic habitats. The same result was obtained in contrasts of habitat for tropical and temperate latitudes independently (Tropical: $\chi^2 = 0.35$, $P = 0.55$, $n = 237$; Temperate: $\chi^2 = 1.11$, $P = 0.29$, $n = 106$).

Body sizes of gekkotans were related to eggshell type, but not to either latitude or to habitat (three-factor ANOVA, Overall Model: $F_{3,310} = 17.7$, $P < 0.001$; Eggshell type:

$F_{1,310} = 51.5$, $P < 0.001$; Latitude: $F_{1,310} = 0.98$, $P = 0.32$; Habitat: $F_{1,310} = 0.01$, $P = 0.94$).

Discussion

Eggshell type, geographic distribution, and life-history evolution in gekkotans

We considered that the rigid-shelled egg by itself, or in conjunction with the invasion of novel environments, could be related to the adaptive radiation of sphaerodactylid, phyllodactylid, and gekkonid gekkotans. For example, exploitation of arid habitats could have been facilitated by the evolution of the rigid-shelled egg. Assuming that modern geographic distributions reflect patterns of distribution in the past, this hypothesis is rejected; modern distributions of gekkotans that lay rigid- and that lay parchment-shelled eggs are unrelated to latitude or habitat. Therefore, we will discuss insights into why gekkotans that lay rigid-shelled eggs are so successful in terms of life history patterns.

Life history patterns of gekkotans that lay rigid-shelled eggs differ from those of gekkotans that lay parchment-shelled eggs in several important respects that are independent of phylogenetic history. First, their reproductive output per clutch is relatively low. Gekkotans that lay rigid-shelled eggs have smaller eggs relative to adult body size, and smaller hatchlings relative to egg size, than gekkotans that lay parchment-shelled eggs. The inference that these shifts are causally related to egg type is especially strong because clutch size of gekkotans is fixed at two eggs (the few species that lay single-egg clutches were excluded from our analyses), thereby eliminating the possibility of a tradeoff between egg or hatchling size and the number of eggs produced per clutch. Second, their incubation periods are relatively long. Below we discuss possible functional constraints that explain or contribute to these life history differences.

Gekkotans with rigid-shelled eggs produce small eggs relative to female body size because of apparent constraints on egg shape. Rigid-shelled eggs have spherical shapes, while parchment-shelled eggs are elongate (Kratochvíl and Frynta 2006). Kratochvíl and Frynta (2006) argue that a spherical shape minimizes surface area relative to volume, which in turn reduces the amount of calcium that must be mobilized, assuming that shell thickness is not altered and that calcium is a limiting resource to egg production. In contrast, parchment-shelled eggs are weakly calcified (Schleich and Kästle 1988; Deeming 1988) and shape is thus less affected by the need to minimize the surface area of the egg. An alternative explanation is that a spherical egg is more resistant to mechanical stress than an elongate one (Bain 1991). Whatever the limiting factor, spherical eggs are characteristic of gekkotans that lay rigid-shelled eggs and elongate eggs are characteristic of gekkotans that lay parchment-shelled eggs.

Egg shape limits the size (volume) of rigid-shelled eggs relative to female body size. For gekkotans, the width of eggs is related to female body size, irrespective of shell type (Kratochvíl and Frynta 2006), and presumably to the pelvic aperture through which eggs must pass (Congdon and Gibbons 1987; Michoud and Echernacht 1995). While eggs of gekkotans are soft at the time of oviposition, their shape at oviposition is roughly the same as when they have hardened. For example, the length to width ratio of eggs of *Phelsuma dubia* at oviposition is 1.3 (from photographs of eggs emerging from the cloaca in Osadnik 1984), and the length to width ratio for eggs of this species in our data set is 1.2. The diameter of rigid-shelled eggs thus limits their size relative to the body size of the female (Sinervo and Licht 1991). While the width of parchment-shelled eggs is similarly

constrained, length is not; for the same adult body size and egg width, elongate eggs have greater sizes than spherical eggs (Kratochvíl and Frynta 2006).

Gekkotans with rigid-shelled eggs produce small hatchlings relative to egg size. The explanation is unusually straightforward because it reflects a direct functional constraint imposed by the rigid shell. Rigid-shelled eggs contain more water and less yolk than do parchment-shelled eggs (Belinsky et al. 2004). The rigid-shell precludes water uptake after oviposition, and hence eggs must contain all the water required for development at oviposition. In contrast, parchment-shelled eggs take up water from the environment after oviposition; per unit volume, such eggs have a higher initial yolk provisioning and less water than rigid-shelled eggs.

Gekkotans with rigid-shelled eggs have longer incubation durations relative to egg size, and, by extension, relative to hatchling size, than gekkotans that lay parchment-shelled eggs. The inference that developmental rates are accordingly slower is confounded by differences in the stage of the embryo at oviposition. As we predicted, gekkotans that lay rigid-shelled eggs oviposit when embryos are at an earlier developmental stage (stage 25 on average) than gekkotans that lay parchment-shelled eggs (stage 28 on average). Does a three-stage difference in development at the time of oviposition explain an average 9-days difference in incubation duration of rigid- and parchment-shelled gekkotan eggs? At 28°C, the most common incubation temperature in our dataset, lizard embryos at stages 25–28 advance by about one stage per day (Andrews 2004; Wise et al. 2009). At 22°C, embryos advance about 0.5 stages per day (Andrews 2004). Because incubation temperatures of 22–28°C likely bracket nest temperatures for many gekkotans (Henle 1990; Pike et al. 2010), these rates of development indicate that a three-stage difference in development at oviposition accounts for at least some (3–6 days) of the 9-day difference in incubation length. Incubation lengths of gekkotans that lay rigid-shelled eggs thus may be longer than those that lay parchment-shelled eggs, but our analyses are suggestive and not conclusive. The appropriate direct comparison of development rates (the reciprocal of incubation length), would have to account for stage at oviposition, hatchling mass, incubation temperature; such a comprehensive data set is currently available for too few species for a meaningful statistical analysis.

Benefits of the rigid-shell and the radiation of gekkotans

Gekkotans that lay rigid-shelled eggs have a reduced reproductive output per clutch, and, perhaps, reduced developmental rates. Nonetheless, almost 1,000 species of gekkotans lay such eggs—clearly a successful adaptive radiation. The numerical dominance of species that lay rigid-shelled eggs suggests that the origin of the rigid-shelled egg may have been the key innovation (Bond and Opell 1998) that facilitated the adaptive radiation of this gekkotan clade. Did the rigid shell allow gekkotans to utilize resources or exploit niches that were previously unavailable?

Defining features of gekkotan lizards are nocturnality and a fixed clutch size fixed at one or two eggs. These features are characteristic of all gekkotans, at least ancestrally, so they presumably do not account for the extensive radiation of the families characterized by rigid-shelled eggs. We suggest that the water economy of rigid-shelled eggs may account for the success of the derived gekkotans. Rigid-shelled eggs contain all of the water necessary for embryonic development at oviposition, thus eliminating the need for females to locate nest sites that will maintain high moisture levels throughout the incubation period. The highly calcified shell per se limits water loss from the egg (Deeming and Thompson 1991). Egg mortality from desiccation thus should be virtually non-existent for

rigid-shelled eggs and the rigid-shell may provide protection from pathogens such as fungi and bacteria and predators such as small invertebrates (e.g., Moreira and Barata 2005).

Nest sites and modes of deposition of gekkotans that lay rigid-shelled eggs are highly variable compared to those of gekkotans that lay parchment-shelled eggs. Eggs of the latter are typically placed in nests in the ground, under logs or rocks, or in debris; the common denominator of nest sites is that substrates are sufficiently moist for water uptake by eggs during most if not all of incubation. In contrast, nests of species that lay rigid-shelled eggs are often aboveground, and eggs may be placed loose in cavities or glued to exposed surfaces of trees or rocks or attached to overhanging rock faces (Dunson 1982; Henkel and Schmidt 1995; Deeming and Unwin 2004; Ineich 2010). Gluing eggs to the substrate, in particular, may reduce mortality of eggs as they would not be accidentally dislodged and given enhanced protection from predation by some predators. While rigid-shelled eggs also are placed in nests excavated in the ground or under debris, these sites are relatively dry during all or part of the incubation period. The features of rigid-shelled eggs that facilitate successful incubation in dry microhabitats thus would have promoted the invasion of arboreal habitats by adults, and perhaps extended breeding seasons in seasonally-dry habitats. Because rigid-shelled eggs are desiccation resistant, a number of these species are associated with marine beaches where eggs are exposed to both saltwater spray and inundation (Brown and Alcalá 1957; Brown and Duffy 1992; Gardner 1985). Using beaches would have facilitated dispersal to islands via eggs attached to floating debris (Brown and Alcalá 1957; Brown and Duffy 1992; Gardner 1985; Carranza et al. 2000), situations in which members of rigid-shelled lineages have been particularly successful as colonizers.

Taxa, such as turtles (Iverson and Ewert 1991), that exhibit multiple origins of rigid and parchment shell types within and among lineages could offer a powerful means of testing some of the more general predictions of the life-history consequences of eggshell evolution in vertebrates. The outcome of such analyses, however, will likely differ substantially from those found for gekkotans because of potential trade-offs between egg size and clutch size.

Body size evolution in gekkotans

Gekkotans laying rigid-shelled eggs have small body sizes relative to those of species that lay parchment-shelled eggs. In fact, 28% of gekkotans that lay rigid-shelled eggs have adult SVLs <40 mm, while only one of the species that lay parchment-shelled eggs has a body size this small (Fig. 4). While some species of gekkotans that lay rigid-shelled eggs are as large as the largest species that lay parchment-shelled eggs (100–200 mm SVL), they are not represented proportionately to their abundance (80% of gekkotans lay rigid-shelled eggs but only 60% of gekkotans 100 mm SVL or more lay rigid-shelled eggs; Chi-squared one-sample test, $\chi^2 = 12.5$, $P < 0.001$, $df = 1$, $n = 50$). Moreover, large gekkotans that lay rigid-shelled eggs are more likely to have insular than continental distributions than large gekkotans that lay parchment-shelled eggs (Likelihood Ratio test, $\chi^2 = 5.45$, $P = 0.020$, $df = 1$, $n = 50$). Adult body size of gekkotans that lay rigid-shelled eggs thus shifted to smaller sizes across the size range attained by gekkotans in general. One explanation for this phenomenon is that small body sizes may be adaptive in particular climates or habitats. Because shell type is not associated with either tropical or temperate latitudes or with mesic or arid habitats, this explanation can be rejected.

We initially proposed two alternative life history scenarios for the outcome of reduced reproductive output on adult body size. One was that species producing rigid-shelled eggs

would compensate by evolving *larger* body sizes than species producing parchment-shelled eggs. Doing so could potentially allow females to produce eggs that are at least equivalent in size to those of parchment-shelled eggs. Whereas most reptiles that produce small eggs relative to the body size of the female compensate by increasing clutch size (e.g., Bauwens and Díaz-Uriarte 1997; Warne and Charnov 2008), gekkotans cannot do this because of an invariant clutch size of either one or two eggs. The body-size compensation scenario is thus clearly rejected; gekkotans that lay rigid-shelled eggs are smaller than gekkotans that lay parchment-shelled eggs.

An alternative scenario was that body size would decrease because of demographic compensation. A decrease in average body size would: (1) reduce maintenance costs of adults, hence bringing the energy available for reproduction in line with low reproductive output (Warne and Charnov 2008), and (2) reduce the age at first reproduction (assuming equivalent growth rates) that would in turn increase the intrinsic rate of increase of gekkotan populations. If this scenario is correct, then small body size is a consequence of the rigid shell itself.

This idea that the evolution of the rigid-shelled egg drove the evolution of small body size is speculative. Nonetheless, body size distributions are not associated with current climate or habitat. This indicates that such physical factors do not currently favor one shell type over another, and perhaps this has been the case in the past. In fact, the association between gekkotans that lay rigid-shelled eggs and that have large body sizes with islands suggests that biotic factors such as competition or predation are important determinates of their distribution. Moreover, because of constraints on both egg size and clutch size, a decrease in adult body size as a direct consequence of the evolution of the rigid-shelled egg is a hypothesis that begs further consideration.

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