

Social and environmental influences on egg size evolution in frogs

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Abstract

The reproductive strategies of frogs are highly diverse, but analysis of these strategies in a phylogenetic context has lagged behind other taxa. Here we investigate associations between aspects of parental care and egg size in a phylogenetic context. We obtained data on egg size and parental care strategies in various species of frogs from the scientific literature. We developed a phylogenetic supertree of frogs by combining the results of multiple phylogenetic analyses using matrix representation parsimony. We used phylogenetic pairwise comparisons to investigate the correlation between various forms of parental care and egg size across the order Anura. We also investigated correlations between tadpole carnivory and egg size, and phytotelm breeding and egg size. We also investigated the association of egg size with several environmental factors. Parental care, male parental care, direct development, stream breeding and montane breeding habitats were all associated with large egg size. Female care (in species with trophic egg feeding), carnivory, use of small pools (phytotelmata) and use of temporary pools were not associated with egg size.

Introduction

Theory and data indicate that parental care is likely to affect egg size and number in amphibians and other animals (e.g. Shine, 1978; Nussbaum & Schultz, 1989; Clutton-Brock, 1991). Ecological factors are also likely to affect egg and clutch size (Nussbaum, 1985; Stearns, 1992; Morrison & Hero, 2003). Frogs, which display an extraordinary diversity of reproductive strategies (Duellman & Trueb, 1986), are an excellent taxonomic assemblage in which to investigate the relationships between parental care, ecology and egg size in an evolutionary context.

Reproductive diversity in anurans is associated with a spectrum of parental behaviors, the primary aim of which is removing eggs or tadpoles from large bodies of water, such as ponds, to smaller pools or terrestrial sites (Magnusson & Hero, 1991). These behaviors include the production of foam nests, the construction of burrows and the use of phytotelmata (small pools that form in plant structures, such as bromeliad tanks). Many researchers have investigated these strategies (e.g. Crump, 1974; Resetarits & Wilbur, 1989; Caldwell & Araújo, 1998), and there have been several major reviews (e.g. Salthe & Duellman, 1973; Duellman & Trueb, 1986). Nevertheless, there is a dearth of investigations of the evolution of frog reproductive strategies using comparative methods that control for phylogenetic effects (e.g. Beck, 1998). In contrast, the use of phylogenetic trees to inform comparative analyses statistically

has become the rule rather than the exception in many other taxa (e.g. Garland, Huey & Bennett, 1991; Balshine *et al.*, 2001; Liker, Reynolds & Szekely, 2001; Bennett & Owens, 2002).

Until very recently there were few well-supported phylogenies available for frogs, making phylogenetically controlled comparative analyses difficult or impossible. The recent flood of molecular phylogenetic analyses has changed things dramatically over the last 10 years, yielding robust phylogenetic hypotheses for many clades of frogs. Here we utilize a phylogenetic supertree for several hundred species of frogs, which was developed by combining the results of a number of recent molecular phylogenetic analyses. We then use this tree to analyze the relationship between egg size and parental care, as well as other aspects of anuran ecology.

Previous studies have investigated various aspects of both egg and clutch size in the context of reproductive strategies in frogs (e.g. Salthe & Duellman, 1973; Crump, 1974; Kuramoto, 1978). There has also been considerable debate in the literature concerning the relationship between egg size and parental care (Shine, 1978; Nussbaum & Schultz, 1989). Some comparative analyses have indicated that egg size increases in the presence of parental care (e.g. Crump, 1995, 1996). However, none of the studies to date have taken phylogeny into account, calling the statistical validity of any conclusions into question (Harvey & Pagel, 1991).

In a previous study (Summers, McKeon & Heying, 2006) we carried out a comparative analysis of parental care and

egg size using the phylogenetic supertree mentioned above. That study dealt specifically with several general hypotheses for the evolution of egg size: particularly Shine's (1978) 'safe harbor hypothesis' and Nussbaum's (1985, 1987) hypothesis concerning the effect of ecological selection on juveniles. The focus of the paper was to use Pagel's maximum-likelihood method for discrete characters (Pagel, 1994, 1997, 1999*a,b*) to investigate the order of transitions between parental care and egg size (Shine and Nussbaum's hypotheses make opposite predictions in this regard). The results of the comparative analysis suggested that, in general, the evolution of large egg size tends to precede the evolution of parental care, rather than the reverse, although the evolution of parental care does appear to facilitate the evolution of large egg size.

Our previous study focused on these general hypotheses concerning the evolution of parental care and egg size, and did not subdivide parental care into different types. That study also did not deal with the effect of ecological factors that have been proposed to affect egg size in frogs. The importance of ecological factors in driving large egg size was stressed by Nussbaum (1985, 1987), and our previous study supporting a prediction of his hypothesis emphasizes the need for further investigation of the ecological factors influencing egg size. In this study, we use phylogenetic pairwise comparisons to investigate different forms of care separately (e.g. male vs. female care). We also investigate the effect of several general ecological factors that may affect egg size.

Our rationale for investigating subdivisions of parental care is related to previous work on egg size in dendrobatid frogs. In a recent comparative study of egg size in neotropical poison frogs (family Dendrobatidae), Summers & McKeon (2004) found that egg size in phytotelm breeders decreased in species with female care relative to species with male parental care. Hence, the relationship between parental care and egg size may be complex. In the case of the neotropical poison frogs, there was not enough variation among species to investigate the issues raised in a phylogenetically controlled statistical framework. Here we address some of these issues using data on frog species from across the order Anura.

Summers & McKeon (2004) focused specifically on the transition from terrestrial breeding to phytotelm breeding and its interaction with patterns of male and female care in the dendrobatids. They found that egg and tadpole size increased in phytotelm-breeding species with male parental care, but decreased in species with female care, relative to terrestrial breeders (all of which have male care).

These trends need to be seen in the context of the nature of parental care in the dendrobatids. First, species with female care have an extended period of care involving egg feeding: females return to pools where they have placed tadpoles and feed them infertile eggs over a period of weeks (Weygoldt, 1987). In contrast, species with uniparental male care do not feed their tadpoles. Summers & McKeon (2004) proposed that egg size may have decreased in species with female care (relative to an ancestral species with male care)

because continuous feeding mitigated the requirement to provide a large amount of nutrients to offspring at hatching. Offspring are repeatedly fed over the course of development, making this initial heavy investment unnecessary. In contrast, phytotelm-breeding species of dendrobatids with male care showed an increase in egg size and early-stage tadpole size relative to terrestrial-breeding ancestors with male care, although the increase in egg size was not quite significant using standard nonparametric tests (Summers & McKeon, 2004).

Summers & McKeon (2004) proposed two explanations for an increase in egg and tadpole size in phytotelm-breeding dendrobatids with male care. First, in species that breed in phytotelmata, the size of the pool in which the tadpole must develop is quite small, and hence it may be critical for the egg to receive extra nutrients in order to survive in a nutrient-poor environment. Second, in some species of phytotelm-breeding dendrobatids with male parental care, cannibalism is common (e.g. Summers, 1990; Caldwell & Araújo, 1998; Summers & Symula, 2001). In this situation, it may benefit parents to produce a large egg, and hence a large tadpole, to minimize the risk of depredation to the tadpole when it is first deposited in a pool.

With regard to other aspects of ecology, a variety of environmental factors have been proposed to affect egg size in frogs and other taxa (e.g. Gollmann & Gollmann, 1996; Liker *et al.*, 2001; Morrison & Hero, 2003). In this study, we gathered data on the type of waterbody used for tadpole deposition (particularly lentic vs. lotic systems), use of extremely small pools (phytotelmata) for tadpole deposition, breeding pool duration, longitude (temperate vs. tropical), altitude (montane vs. lowland), level of aridity (particularly desert breeding) and diet (particularly tadpole carnivory and cannibalism). Several of these factors have been proposed to have significant effects on egg size (e.g. Pettus & Angelton, 1967; Gollmann & Gollmann, 1996).

In this study, we first address the general question of whether egg size increases in species with parental care. A number of researchers have suggested that this is the case, but it is important to evaluate this question in a phylogenetic framework. We also investigate the effect of direct development and non-feeding tadpoles on egg size, as this has also been implicated in increased egg size. Next we evaluate how egg size changes in species with male care and in species with female care (with egg feeding), in species without direct development or non-feeding tadpoles. We then attempt to evaluate the effect of various environmental factors. We realize that our catalog of environmental factors is incomplete, but only a limited amount of information on environmental factors was available in most of the sources we consulted. Hence, we consider this to be a first step in evaluating the effects of ecological factors on egg size in frogs in a phylogenetically controlled comparative context.

Materials and methods

We address these hypotheses with phylogenetic pairwise comparisons. Pairwise comparisons are useful when

comparing species that differ in one categorical variable (the independent variable: e.g. presence or absence of parental care) and one continuous variable (the dependent variable: egg size). In our analysis, we used data on egg size directly. We did not control for either female body size or clutch size. Female body size was excluded from analysis because egg size and body size were not significantly associated in our data. A regression analysis of egg size on maximum female body size revealed no significant relationship ($n = 469$, $R^2 = 0.00016$, $F = 0.079$, $P = 0.78$). Similar results were found using average female body size. We did not control for clutch size because doing so might obscure the patterns we are hypothesizing. Egg and clutch size are typically correlated, but we think that the selective forces that we are attempting to evaluate should affect clutch size indirectly through their direct effects on egg size. Hence, it would be inappropriate to control for clutch size in the analysis, as this variable is free to evolve in response to the selection pressures we posit, given an inherent trade-off between egg and clutch size.

Comparative data

Data on egg size (diameter of the ovum in millimeters), presence or absence of parental care, parental care type (e.g. maternal vs. paternal), presence or absence of cannibalism/carnivory, latitude (tropical vs. temperate), elevation (montane vs. lowland), tadpole environment (lentic vs. lotic), pool duration (temporary vs. permanent) and type of pool used for tadpole deposition (phytotelmata vs. other) were taken from the scientific literature, from the references listed in Supplementary Material Appendix S1. We collected 790 records on 640 species. For species where we obtained more than a single record, we took the average value across records to represent the value for that species. Analyses were carried out only with data from those species that were incorporated into the phylogenetic supertree (see below). The final data set used for the analysis of egg size and parental care is provided in Supplementary Material Appendix S2 (other data are available on request from the first author).

Phylogenetic supertree

We constructed a supertree representing the phylogenetic relationships of as many species from our data set as possible using matrix representation parsimony (Sanderson, Purvis & Henze, 1998), as implemented in the program RADCON (Thorley & Page, 2000). This method represents the nodes from the topologies of component phylogenies as elements of a matrix. The matrix is analyzed via parsimony, yielding a consensus topology of the combined topologies. Trees from the literature were 'pruned' before analysis: species in the tree that were not in our data set were removed.

To construct our supertree we used a hierarchical approach. That is, we used a relatively small number of studies to address specific phylogenetic clades (e.g. the genus *Bufo*).

We started with studies addressing basal relationships in the order Anura, and then worked our way up to more recent clades. We preferentially used studies that employed DNA sequence data and maximum likelihood analysis of phylogenetic relationships. We preferred studies that used larger amounts of data, and we used the most recent studies preferentially when the same group of researchers published several different phylogenies on the same group of organisms using the same gene regions.

Our analysis utilized the following references for the following groups: order Anura (Hoegg *et al.*, 2004; Roelants, Jiang & Bossuyt, 2004); Neobatrachia (Darst & Cannatella, 2004; Hoegg *et al.*, 2004); African Asian Ranoids (Richards & Moore, 1996; Emerson, Inger & Iskandar, 2000a; Emerson *et al.*, 2000b; Marmayou *et al.*, 2000; Dawood, Channing & Bogart, 2002; Wilkinson, Drewes & Tatum, 2002; Van der Meijden, Vences & Meyer, 2004); African Ranoids (Madagascan taxa) (Richards, Nussbaum & Raxworthy, 2000; Glaw & Vences, 2002; Vences *et al.*, 2003b); Leptodactylids (Larson & O. de Sa, 1998); Hylids (DaSilva, 1997; Mendelson, Da Silva & Maglia, 2000; Chek *et al.*, 2001; Faivovich, 2002; Salducci *et al.*, 2002; Moriarty & Cannatella, 2003; Faivovich *et al.*, 2004); Bufonids (Graybeal & Cannatella, 1995; Graybeal, 1997); Dendrobataids (Clough & Summers, 2000; Santos, Coloma & Cannatella, 2003; Vences *et al.*, 2003a); Australian frogs (Schauble, Moritz & Slade, 2000; Read *et al.*, 2001); Microhylids (Zweifel, 1986). To carry out the pairwise comparisons, we used a fully resolved phylogenetic hypothesis. The final supertree was close to full resolution, but there were some polytomies remaining. These were arbitrarily resolved for the analysis. This did not appear to affect the results of the pairwise comparison analyses, as alternative resolutions of the polytomies did not qualitatively affect the results (data not shown). The phylogenetic tree used for the analysis is provided in nexus format in Supplementary Material Appendix S3.

Pairwise comparisons

We used phylogenetic pairwise comparisons (Healy & Guilford, 1990; Moller & Birkhead, 1992; Maddison, 2000) for the comparative analysis. This is a simple phylogenetically controlled comparative method that is very useful for comparing the evolution of a continuous character with that of a discrete character. In most published studies using phylogenetic pairwise comparisons, traits are compared between the focal taxon (a taxon characterized by a specific trait or environment, such as association with montane habitats) and its sister clade (e.g. Gotmark, 1994). We follow that tradition here. In order to analyze the results statistically, we used each pairwise comparison as an independent data point, and we compared the counts of each type of result using a χ^2 test with the Yates correction. We used Fisher's exact test when the expected cell count was below 5. Ancestral state reconstructions for parental care and egg size were made using maximum likelihood in the program MESQUITE (Maddison & Maddison, 2004).

Table 1 Pairwise comparisons

	Sample size	χ^2	<i>P</i> value	Effect
Parental care	26 larger, 9 smaller	7.79	<0.05	Positive
Paternal care	10 larger, 3 smaller	4.71	<0.05	Positive
Maternal care	3 larger, 4 smaller		>0.05	
Direct development	20 larger, 2 smaller	13.14	<0.01	Positive
Cannibalism/carnivory	7 larger, 6 smaller	0.15	>0.05	
Phytotelmata breeding	5 larger, 3 smaller	0.15	>0.05	
Lotic versus lentic	13 larger, 3 smaller	5.06	<0.05	Lotic larger
Montane versus lowland	10 larger, 1 smaller	5.82	<0.05	Montane larger
Pool duration	10 larger, 12 smaller	0.05	>0.05	

Results

Table 1 summarizes the results of our analyses. We identified 35 phylogenetically independent pairwise comparisons between lineages with parental care and sister lineages or clades without parental care. Using a χ^2 test with the Yates correction, we found a significant association between the evolution of parental care and the evolution of large egg size (larger 26, smaller 9, $\chi^2 = 7.79$, d.f. = 1, $P < 0.05$). This result confirms the results of our previous analyses using alternative comparative methods (Summers *et al.*, 2006). Hence parental care is strongly associated with increased egg size, even after controlling for phylogenetic effects.

A comparison of species with direct development or non-feeding tadpoles (with or without parental care) yielded a highly significant association (larger 20, smaller 2, $\chi^2 = 13.14$, d.f. = 1, $P < 0.01$). As direct development and non-feeding tadpoles may thus obscure the patterns investigated in this paper, we removed species with direct development or non-feeding tadpoles from the analysis (whether or not they had parental care) in the comparisons that follow.

Species with male parental care but without direct development or non-feeding tadpoles also had significantly larger eggs than their sister lineages or clades (larger 10, smaller 3, $\chi^2 = 4.71$, d.f. = 1, $P < 0.05$). These results demonstrate that there is a positive association between paternal care and egg size independent of direct development or non-feeding tadpoles.

To investigate the effect of female care in which eggs are fed to tadpoles by the mother (as in some dendrobatids), we compared egg size for those species known to display this behavior with their sister lineages or clades. The number of available comparisons in our data set was small (8). We found no significant relationship between female parental care with egg feeding and egg size (larger 3, smaller 5, Fisher's exact test, d.f. = 1, $P > 0.5$). The small sample size suggests that power could be an issue, but the trend was for species with female care to have smaller eggs than their sister taxa. Hence, it seems likely that these species have either smaller eggs or eggs of the same size as their close relatives without female care.

To investigate the influence of diet (particularly the presence or absence of cannibalism or carnivory), we compared the egg size of species known to exhibit either

cannibalism or carnivory with sister lineages. The sample size was small, but we found no trend in the data (larger 7, smaller 6, $\chi^2 = 0.15$, d.f. = 1, $P > 0.5$). Hence, species with cannibalistic or carnivorous tadpoles do not have particularly large or small eggs compared with closely related species.

To investigate the influence of phytotelmata use (excluding species with direct development or non-feeding tadpoles), we compared species known to breed in phytotelmata that had feeding tadpoles with other species. Sample size was again small, but the results were fairly evenly divided (larger 5, smaller 3, Fisher's exact test, d.f. = 1, $P > 0.5$). Hence we did not find any clear evidence in favor of the hypothesis that phytotelm breeding is consistently associated with the evolution of larger egg size.

Our comparison of species breeding in lentic versus lotic aquatic environments yielded 16 pairwise comparisons across the tree. In this case, there was a clear trend for species breeding in lotic systems to have larger eggs (larger 13, smaller 3, $\chi^2 = 5.06$, d.f. = 1, $P < 0.05$). Comparison between species associated with montane environments compared with lowland species also showed a significant trend toward larger eggs in montane species (larger 10, smaller 1, $\chi^2 = 5.82$, d.f. = 1, $P < 0.05$).

In contrast, our comparisons of species with short breeding seasons using temporary pools with their sister taxa did not reveal any significant differences in egg size (larger 10, smaller 12, $\chi^2 = 0.05$, d.f. = 1, $P > 0.5$). The number of pairwise comparisons for tropical versus temperate species was low, but no trends were suggested by the results (larger 3, smaller 4, Fisher's exact test, $P > 0.5$). There were not enough data to analyze the relationship between environmental aridity and egg size.

Discussion

Our results confirm previous claims that egg size increases with parental care. An association between presence of parental care and egg size has been noted frequently in the literature (e.g. Crump, 1995), but previous researchers did not control for phylogenetic effects. We also found a strong association between direct development or non-feeding tadpoles and large egg size. This association has also been noted frequently, but again without controlling for phylogeny.

The evolution of increased egg size in association with the evolution of direct development or the production of non-feeding tadpoles makes obvious sense, in that the embryos or tadpoles require all the nutrients they will need throughout development to metamorphosis.

This association does not completely explain the increase in egg size associated with parental care, because species with male parental care but without direct development or non-feeding tadpoles still show an increase in egg size. What accounts for this? Shine (1978) argued that the evolution of parental care would create a 'safe harbor' that would then favor the evolution of increased egg size. In contrast, Nussbaum (1985, 1987) argued that other aspects of the environment experienced by eggs or juveniles were likely to be the key determinants of egg size in most cases. In those cases where the environment selects for large egg size, selection should in turn be more likely to favor parental care. As noted above, the results from our comparative analysis of the order of parental care and egg size evolution (Summers *et al.*, 2006) provide some support for the prediction of Nussbaum's (1985, 1987) hypothesis: that the evolution of large egg size will typically precede the evolution of parental care. Given that, an important question becomes: What environmental factors might favor the evolution of large eggs or juveniles?

As noted above, Summers & McKeon (2004) argued that either diet (cannibalism/carnivory) or pool size (phytotelm breeding) might be critical factors. Our general comparative analysis did not support either of these factors as playing a pivotal role in the evolution of large egg size in anurans. First, cannibalism was not associated with an increase in egg size: species observed to be cannibalistic or carnivorous did not have larger eggs. Our sample size was small, but the data were quite evenly split with regard to the two categories. Second, species that used phytotelmata (but did not have direct development or non-feeding tadpoles) showed no significant increase in egg size. There was a slight trend in the predicted direction, and the sample size was small; therefore, this factor perhaps deserves further investigation with a larger sample of phytotelmata breeders. One could also argue that the exclusion of species with direct development or non-feeding tadpoles makes the test of this factor too restrictive. That is, one could argue that direct development or production of non-feeding tadpoles may have evolved in some cases as mechanisms that allow anurans to exploit very small, nutrient-limited pools such as phytotelmata. However, this did not seem to be the case: the phytotelm breeders in our data set generally did not have direct development or non-feeding tadpoles.

One environmental factor that has been proposed to have important effects on egg size by a variety of researchers is breeding in a lotic environment. In fact, Nussbaum's (1985, 1987) hypothesis focused on the effect of lotic environment in selecting for large juvenile size in salamanders. He argued that the large size of the available food in lotic (as opposed to lentic) environments favors large juvenile size and hence large egg size. Our comparison of lotic versus lentic systems supports the hypothesis that lotic environments favor large

egg size in frogs: there was a significant association between the use of lotic systems for breeding and the evolution of large egg size. However, the selective mechanism is not clear from our analysis, as we do not have data on food size. Other hypotheses, such as high flow rates allowing greater size to be supported by passive gas exchange, also provide possible explanations.

Another environmental factor that has been proposed to influence egg size is altitude. A number of researchers have found that frogs living in montane environments produce larger eggs within species (e.g. Pettus & Angelton, 1967; Berven, 1982; Beattie, 1987). Our results are consistent with the results of these studies: species associated with montane environments had significantly larger eggs than their sister lineages. The selective mechanism in this case is not known with certainty, but could involve reductions in developmental rate caused by low temperatures (e.g. Bradford, 1984). In turn, this could favor large size to allow the egg a longer development time (Downie, 2004). Alternatively, there could be an association between montane and lotic environments (because many montane species breed in streams). However, there were a substantial number of lotic species in the data set that were not montane.

Latitude has also been suggested as a factor affecting egg size, with egg size increasing with increasing latitude (e.g. Gollmann & Gollmann, 1996; Morrison & Hero, 2003). In this study, we did not find any significant differences between egg size in tropical relative to temperate species. Our sample size was small, but no trend was evident in the data. Our investigations of breeding duration (comparing frogs breeding for short times in temporary pools with frogs breeding over longer periods) did not reveal any significant association with egg size.

With respect to parental care type (male vs. female care), our results are consistent with the results from previous work on dendrobatids (Summers & McKeon, 2004). In dendrobatids, egg size in the lineage with female care (and egg feeding) is smaller than egg size in the sister lineage with male care. Across the order Anura, there was no significant association between egg size and female parental care (with egg feeding). Hence, species with egg feeding do not have larger egg size than species in sister lineages. This supports the argument that egg feeding mitigates the need for extra nutrients associated with parental care in general, as discussed above.

During the preparation of this paper, Frost *et al.* (2006) published a monograph presenting a comprehensive phylogeny for the entire class Amphibia. The anuran part of this phylogeny is obviously relevant to the results presented here, although it was published too late for us to use the tree in our formal analyses. It is also beyond the scope of this study to make a detailed comparison of all the factors we analyzed in this study between our tree and that of Frost *et al.* (2006). However, we have made a detailed comparison between the two trees as they relate to the evolution of parental care. This provides some insight into whether or not analyses based on the two different trees would be similar.

The short answer to this question is yes. There are several reasons for this. First, the evolution of parental care is widely and sparsely dispersed on the tree of anurans, and each 'parental care clade' (i.e. a monophyletic group in which all members have parental care) tends to be quite small. Hence, differences between the topologies of the two trees do not tend to either split up clades with parental care or clump groups with parental care (which are widely separated on both trees). Second, the two topologies are broadly similar. Although there are many topological differences, these do not generally impact the number of instances of the independent evolution of parental care. In those few cases where the number of instances of independent evolution is affected, the result tends to be a breakup of a clade that contained species with parental care (e.g. *Nectophryne* and *Nectophrynoidea*). The impact of this effect should be to increase the overall significance of the comparison between parental care and egg size, as it will increase the number of instances of phylogenetically independent association. In summary, we do not think that the use of the Frost *et al.* (2006) tree would have substantially changed the results of our analyses.

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Supplementary material

The following material is available for this article online:

Appendix S1 Parental care and egg size references

Appendix S2 Parental care categories

Appendix S3 Phylogenetic tree (nexus format)

This material is available as part of the online article from <http://www.blackwell-synergy.com>