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Author(s): Jason L. Brown, Victor Morales, and Kyle Summers
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A Key Ecological Trait Drove the Evolution of Biparental Care and Monogamy in an Amphibian

Jason L. Brown,[1,*] **Victor Morales,**[2] **and Kyle Summers**[1,†]

1. Department of Biology, East Carolina University, Greenville, North Carolina 27858; 2. La Facultad de Ciencias Biológicas, Universidad Ricardo Palma, Lima, Peru

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abstract: Linking specific ecological factors to the evolution of parental care pattern and mating system is a difficult task of key importance. We provide evidence from comparative analyses that an ecological factor (breeding pool size) is associated with the evolution of parental care across all frogs. We further show that the most intensive form of parental care (trophic egg feeding) evolved in concert with the use of small pools for tadpole deposition and that egg feeding was associated with the evolution of biparental care. Previous research on two Peruvian poison frogs (*Ranitomeya imitator* and *Ranitomeya variabilis*) revealed similar life histories, with the exception of breeding pool size. This key ecological difference led to divergence in parental care patterns and mating systems. We present ecological field experiments that demonstrate that biparental care is essential to tadpole survival in small (but not large) pools. Field observations demonstrate social monogamy in *R. imitator*, the species that uses small pools. Molecular analyses demonstrate genetic monogamy in *R. imitator*, the first example of genetic monogamy in an amphibian. In total, this evidence constitutes the most complete documentation to date that a single ecological factor drove the evolution of biparental care and genetic and social monogamy in an animal.

Keywords: parental care, mating systems, sexual selection, frogs, Dendrobatidae, *Ranitomeya*.

Introduction

The relationship between the form of parental care and the mating system within a species is an issue of fundamental importance to the study of animal breeding systems (Trivers 1972; Clutton-Brock 1991; Arnold and Duvall 1994). Despite decades of research, the specific ecological factors that drive the evolution of parental care remain a subject of debate, particularly in the case of vertebrates (e.g., Burley and Johnson 2002; Wesolowski 2004).

In turn, the form of parental care is thought to have an important influence on the evolution of mating systems in general (Trivers 1972; Kokko and Jennions 2008) and monogamy in particular, but the details of these relationships are still debated (Reichard and Boesch 2003). Furthermore, the recent explosion in genetic analyses of mating systems has revealed that monogamy (with both social and genetic mate fidelity) is a rare phenomenon (Jennions and Petrie 2000; Westneat and Stuart 2003).

One long-standing hypothesis posits that when biparental care becomes crucial to offspring survival, this can favor the evolution of monogamy, since both members of a pair may receive higher reproductive returns by investing in mutual offspring rather than seeking extrapair reproductive opportunities (Kleiman 1977; Wittenberger and Tilson 1980). Research on a variety of monogamous species has produced evidence consistent with this hypothesis (e.g., Gubernick and Teferi 2000; Reichard and Boesch 2003). Nevertheless, it has proven difficult to identify specific ecological factors that drove the evolution of biparental care and monogamy in concert in particular species compared with closely related species that are promiscuous.

In this study, we use comparative, experimental, and genetic approaches to investigate the evolution of parental care and mating strategies in an ecological context in anurans. Frogs, with a diversity of reproductive modes and breeding habitats, offer unique opportunities to investigate the influence of specific ecological and social factors on reproductive strategies (Summers et al. 2006, 2007; Wells 2007). Many frogs reproduce away from large bodies of water, a trend likely driven by predation on eggs and larvae (Crump 1974; Duellman and Trueb 1986; Magnusson and Hero 1991). Moving away from these large water bodies entailed a trade-off: predation was reduced, but environmental "harshness" (Wilson 1971) increased for eggs and larvae, especially in terms of increased desiccation risk

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[*] Present address: Biology Department, Duke University, Durham, North Carolina 27708.

[†] Corresponding author; e-mail: summersk@ecu.edu.

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(Touchon and Warkentin 2008). This trend of avoiding oviposition in large bodies of water likely selected for parental care in order to ameliorate the increased harshness of the terrestrial environment (McDiarmid 1978; Bickford 2004). Some species of tropical frogs evolved the habit of placing their offspring in small pools, such as phytotelmata (e.g., water-filled leaf axils), and the use of these small pools may have driven the evolution of more intensive parental care, such as trophic egg feeding (Weygoldt 1987). In turn, consistent feeding of offspring may have favored the evolution of biparental care (Summers and Earn 1999). This hypothetical sequence of events is logical, and elements of the sequence have appeared repeatedly in the scientific literature. Nevertheless, these elements have not been tested in any rigorous way. Here we present tests of the major links in this chain of logic, using phylogenetic comparative analyses.

Traditionally, research on biparental care has focused on birds and mammals, and the general assumption is that maternal care is primitive and, hence, that the evolution of male parental care plays a critical role in the evolution of biparental care and monogamy (Kleiman 1977; Reichard and Boesch 2003). In frogs, male parental care is typically primitive, so it is the evolution of maternal care that is critical to the evolution of biparental care (Summers and Earn 1999; Summers et al. 2006).

To investigate the effect of breeding pool size on patterns of parental care and mating strategies in more detail, we carried out research on two species of Peruvian poison frogs, *Ranitomeya imitator* (the mimic poison frog) and *Ranitomeya variabilis* (the variable poison frog). Both of these species were formerly in the genus *Dendrobates* (for details, see Grant et al. 2006). By comparing two closely related species of frogs whose breeding systems differ with respect to a single major ecological variable, we were able to focus on the effect of breeding pool size on parental care and mating system evolution.

Previous research on these species demonstrated that *R. variabilis* deposited their tadpoles in pools that averaged 112 mL in volume, compared with 24 mL for *R. imitator* (Brown et al. 2008*b*). Field experiments demonstrated that *R. imitator* preferred to use extremely small pools, whereas *R. variabilis* preferred large pools (Brown et al. 2008*a*). These species also evolved differences in space use, territorial defense, parental care, mating strategies, and larval ecology in association with the use of different pool sizes (Brown et al. 2008*a*, 2008*b*, 2009*b*, 2009*c*). *Ranitomeya imitator* showed biparental care, in which the male and the female cooperated over long periods (months) to care for offspring, regularly feeding tadpoles with trophic eggs (Brown et al. 2008*b*; fig. 1). In contrast, *R. variabilis* showed uniparental male care: females did not participate in care, and tadpoles were not fed (Brown et al. 2008*b*, 2009*c*).

In this article, we investigate the hypothesis that a transition to the use of very small breeding pools (i.e., small phytotelmata) drove the evolution of biparental care. A key prediction of this hypothesis is that small phytotelmata contain insufficient nutrients for tadpole growth and survival, producing the need for trophic eggs. In order to test this prediction, we carried out a series of reciprocal transplant experiments in the field.

In frogs, as in other taxa, the evolution of biparental care may favor the evolution of monogamy (Caldwell 1997; Bourne et al. 2001). Previous research on *R. imitator* showed pair bonding and social monogamy: members of pairs exhibited regular affiliative interactions (e.g., tactile courtship) over long periods (months), pair member home ranges were tightly overlapping, and both members aggressively defended territory boundaries against conspecifics and heterospecifics (Brown et al. 2008*b*, 2009*b*). In contrast, *R. variabilis* had a highly promiscuous mating system, with frequent partner switching (Brown et al. 2008*b*). Home ranges were not exclusive, and neither territorial boundaries nor pools were defended (Brown et al. 2008*b*, 2009*b*).

Here we investigate the hypothesis that the evolution of biparental care in association with small breeding pool size led to the evolution of genetic monogamy, in addition to social monogamy. To test for genetic monogamy, we made long-term observations on families of *R. imitator* and carried out molecular genetic analyses of relatedness on all socially pair-bonded adults and their putative offspring.

Our broad comparative analyses are carried out on a much larger scale than our experimental and genetic analyses both in terms of phylogenetic scope (i.e., across the order Anura vs. two closely related species) and in terms of the range of reproductive resources (comparing across lakes, ponds, streams, small pools, and phytotelmata vs. two types of small phytotelmata). Figure 2 presents these different scales and their connections in a graphical format.

Methods

Comparative Analyses

We used data from 404 frog species distributed across the entire order Anura and maximum likelihood reconstruction of the evolution of these binary characters to test for a correlation between evolutionary transitions to phytotelm breeding and to parental care. We summarized data on parental care, breeding site, and egg feeding from the scientific literature (Dryad data [http://hdl.handle.net/10255/dryad.1093]; Breeding Site Refs, Supertree [Nexus], PhytoEvol, and PCEvol are available in a zip file in the

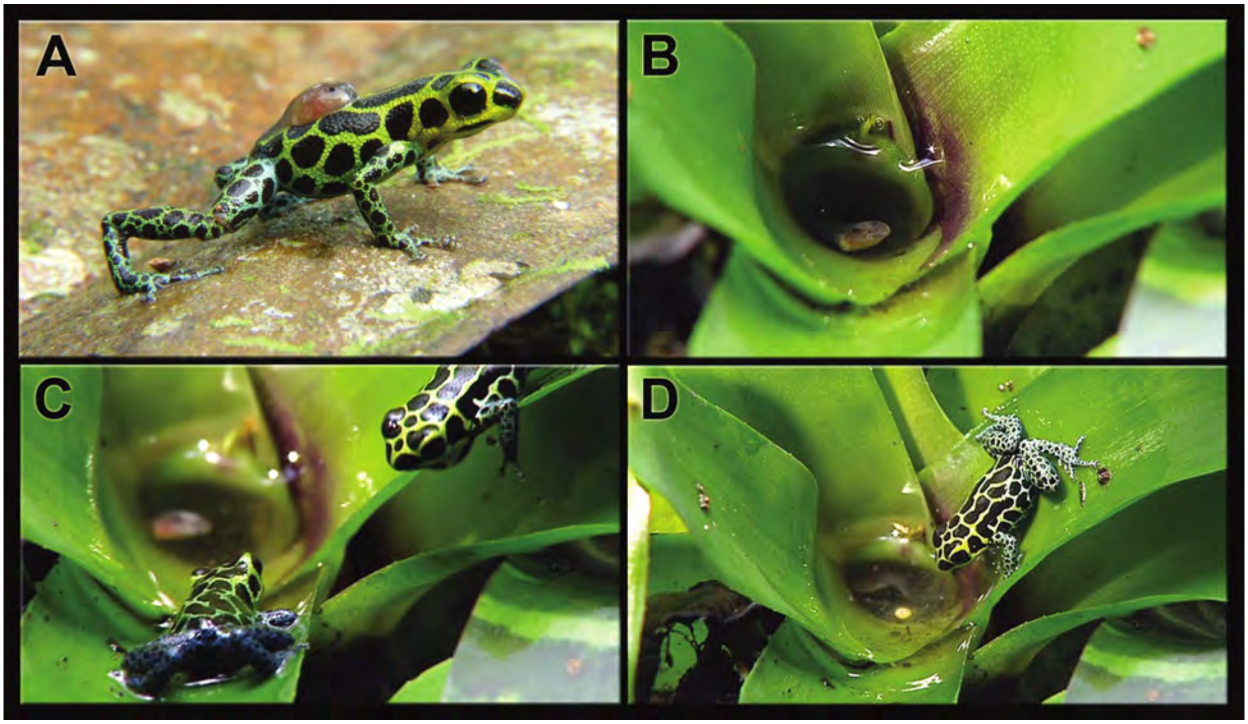

Figure 1: Biparental care in *Ranitomeya imitator*. The male leads a female to a deposition site within their territory. The female will then oviposit one to four eggs on the surface of a leaf, away from water. *A*, A week after oviposition, the male returns and retrieves its tadpoles, breaking the tadpole free from the egg sack and allowing the tadpole to wriggle onto its back. *B*, The male then deposits the tadpole in an unoccupied pool of water within the pair's territory. The male will then survey the pool every couple of days and determine when the tadpole is fed trophic egg(s). *C*, To initiate egg feeding, the male begins to call from the surface of the pool, and after a short period, the tadpole's mother arrives. *D*, After an extended bout of calling, eventually the female will dive into the pool and deposit a trophic food egg (depicted in white within the center of the plant; female is within the pool). This behavior is repeated on average every 7.3 days throughout the duration of development of the tadpole. Images *B–D* are the property of the BBC Natural History Unit.

online edition of the *American Naturalist*). We focused on species for which we could obtain reliable information on patterns of parental care and breeding site and that we could also place in a phylogenetic context with reasonable confidence. The topology of the phylogenetic tree used for our comparative analyses was a supertree constructed in a hierarchical manner (Summers et al. 2006). Basal relationships in the order Anura were based on a recently published phylogeny of the Amphibia (fig. S1 in Santos et al. 2009). Lower-level relationships were based on a less recent but more comprehensive phylogeny of the Amphibia (Frost et al. 2006). Lower-level relationships that were not resolved in these trees were resolved on the basis of a previously published phylogenetic supertree, constructed from numerous published phylogenetic analyses and developed for comparative analyses of the evolution of parental care and egg size in frogs (Summers et al. 2006, 2007). The final tree is provided in Nexus format and in graphical format (Supertree [Nexus], PhytoEvol, PCEvol, available in a zip file in the online edition of the *American Naturalist*). For alpha taxonomy, we used the online resource Amphibian Species of the World (Frost 2009).

We tested associations between characters by using maximum likelihood models (continuous-time Markov models) for the analysis of correlations between discrete characters across an evolutionary tree (Pagel 1994), implemented in the Discrete module in the BayesTraits suite of programs developed by M. Pagel and A. Meade (http://www.evolution.rdg.ac.uk). Each trait used in a comparative analysis (phytotelm breeding, parental care, egg feeding, biparental care) was coded as a discrete character: yes = 1, no = 0. In the Discrete module of BayesTraits, we used the maximum likelihood algorithm to estimate character state changes on our evolutionary tree and compared the likelihood of a model that assumed the independent evolution of the two binary characters being compared (e.g., breeding site and presence/absence of parental care) with that of a model that allowed for correlated evolution between these characters.

Statistical comparisons were implemented with likeli-

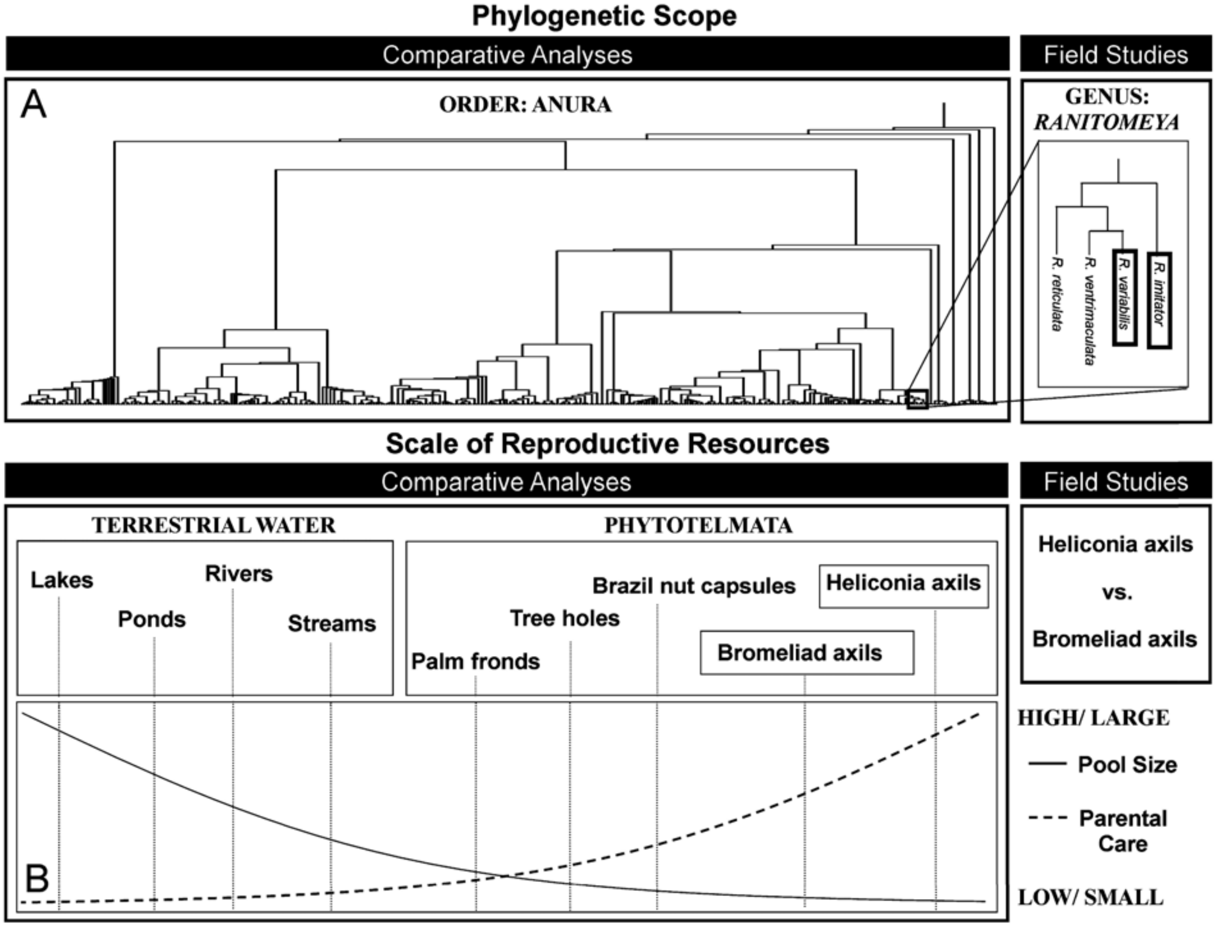

Figure 2: Different scales of this study. Comparative analyses were carried out on a much larger scale than our experimental and genetic analyses both in terms of phylogenetic scope (*A*; looking at the order Anura vs. two closely related species in the genus *Ranitomeya*, family Dendrobatidae) and in the context of type of reproductive resources (*B*; comparing all sizes of reproductive resources being used vs. two small phytotelmata). In general, species that utilize larger bodies of water exhibit lower levels of parental care. See "Results" for comparative analyses.

hood ratio tests, in which 2 times the difference between the log likelihoods of the models was compared with a χ^2 distribution. The degrees of freedom were determined by the difference in the number of parameters being estimated under each model, which in our comparisons was four. Estimates of instantaneous transition rates under a continuous-time Markov model for the analysis of the correlation between breeding site and parental care were also carried out with the Discrete module in BayesTraits. To test the relative likelihoods of alternative pathways, we restricted the two pathways being compared to be equal and reran the maximum likelihood analysis. We then compared the likelihoods of the two dependent models (restricted and unrestricted), using a likelihood ratio test as described above (with df = 1).

We also tested for evolutionary correlations between pairs of traits using the concentrated changes test (Maddison 1990) as implemented in the MacClade program (Maddison and Maddison 2003). In the concentrated changes test, one trait was considered to be the independent variable (e.g., phytotelm breeding), and one was considered to be the dependent variable (e.g., parental care). As a first step, the evolution of the independent trait was reconstructed on the phylogenetic tree, using parsimony (PhytoEvol, PCEvol, available in a zip file in the online edition of the *American Naturalist*). The test used the distribution of the independent variable on the tree to test the hypothesis that the evolution of the dependent variable is significantly associated with the presence (i.e., prior evolution of) the independent variable. The size of the trees involved made it necessary to use simulations to determine the significance of the results (rather than exhaustively evaluating all possibilities). For the simulations, 1,000 replicates were employed, using the Minstate method of optimization for character reconstruction under parsimony. We assumed that phytotelm breeding and egg feeding were derived in the analyses where these were the independent traits (respectively).

Reciprocal Transplant Experiments

To test the importance of biparental care to offspring survival in *Ranitomeya imitator*, we used five treatments in which the tadpoles of each species were placed in natural phytotelmata and allowed to consume the resources within the pools. Tadpoles of both species were placed in larger pools (normally used by *Ranitomeya variabilis*) and in smaller pools (normally used by *R. imitator*). A control treatment contained *R. imitator* tadpoles in small pools: tadpoles were removed and replaced in the same pool (and hence continued to receive trophic eggs).

Tadpoles of *R. imitator* and *R. variabilis* (Gosner stages 22–25) were placed within pools formed in a species of *Aechmea* bromeliad ($n = 8$ and $n = 10$, respectively) and pools within the leaf axils of a species of *Heliconia* ($n = 10$ and $n = 8$, respectively). *Aechmea* bromeliads are frequently used by *R. variabilis* for egg and tadpole deposition, whereas *Heliconia* are used exclusively by *R. imitator* (Brown et al. 2008*b*). A coarse mesh was placed above the surface of the pool, preventing frogs and large insects from entering or from ovipositing.

The fifth (control) treatment consisted of *R. imitator* tadpoles ($n = 9$) in the leaf axils of *Heliconia* sp. (as in the two treatments described above). These tadpoles were removed and then replaced in the same pool. For the duration of this experiment, the tadpoles in this treatment continued to receive trophic eggs from their parents.

Pools were surveyed weekly, and filtered rain water was used to maintain the water levels during the experiments. The tadpoles were weighed and measured (total length, body width, body length) at the start and end of the experiments (after 21 days). Three *R. imitator* and four *R. variabilis* tadpoles in *Heliconia* pools that were not fed died before the end of the experiment.

The mean volume of water ± SD in the *Aechmea* treatment pools was 39.28 ± 6.64 mL (range 20–55 mL, $n = 18$). The pools in *Heliconia* sp. were frequently used by *R. imitator* for tadpole deposition but were not used by *R. variabilis* (Brown et al. 2008*b*). The mean volume of water in the *Heliconia* treatment pools was 17.3 ± 6.64 mL (range 8–28 mL, $n = 27$). There were no significant differences in *Heliconia* pool sizes between treatments ($P = .320$, $\chi^2 = 2.276$, $\mathrm{df} = 2$) or *Aechmea* pool sizes between treatments ($P = .695$, $z = -0.392$, $n = 17$). The *Heliconia* treatment pools were significantly smaller than the *Aechmea* treatment pools ($P < .001$, $z = -4.801$, $n = 45$).

The differences between the final measurement and initial measurements were divided by the initial measurement to standardize the growth rates. Both mass and total length measurements were highly correlated ($P < .001$, $\mathrm{PC} = 0.892$, $n = 76$); thus, we present data only on total length. Because of unbalanced sampled sizes, Kruskal-Wallis and Mann-Whitney tests were used to compare means. Ryan-Einot-Gabriel-Welsch (REGWQ) post hoc tests were performed for pairwise comparisons between treatments.

Genetic Relatedness

We tested for genetic monogamy in *R. imitator* by identifying putatively monogamous breeding pairs, observing them for several months, and collecting tissue from them and from all the tadpoles they fed during the observation period and from neighboring individuals.

Data on individual behavior and associations were obtained from transect surveys of marked individuals in quadrats demarcated with flags. For a detailed explanation of transect methods, see Brown et al. (2008*b*, 2009*b*, 2009*c*). Briefly, between May 19, 2005, and December 1, 2008, we walked transects ($n = 239$) and observed 28 paired *R. imitator* (defined by mutual participation in courtship and parental care) for a total of 3,445 min. In 12 pairs, we were able to collect tissues from both adults and observe the females in these pairs repeatedly feeding at least two tadpoles. We also collected tissues from other individuals and tadpoles from the same populations (particularly from adults captured in areas surrounding the territories of the putatively monogamous pairs and from tadpoles of all phytotelmata surrounding and within each pair's territory).

We then genotyped all the tissues collected, including the 12 putatively monogamous pairs and nearby individuals (total $n = 110$), using six polymorphic microsatellite markers (DimiD04, DimiB07, DimiE03, DimiE02, DimiB02, and DimiC05) specifically designed for *R. imitator* (Brown et al. 2009*a*). To ensure accuracy of our microsatellite results, each locus was run twice for every individual. If the results were not the same, individuals were run a third time.

Relatedness between specific individuals (e.g., putative father-offspring pairs) and averages for classes of comparisons (e.g., all father-offspring comparisons) were calculated with maximum likelihood algorithms implemented in the program Kinship (ver. 1.3.1; Goodnight and Queller 1999). Family relatedness was calculated by taking the mean relatedness of male-offspring, female-offspring, and offspring-offspring for each family. The statistical significance of the level of relatedness between putative parent-offspring pairs and putative sibling pairs (which should be 0.5 on average), relative to average levels of relatedness in the population, was calculated by comparing the likelihood of a model in which the members of the pair were related with an r of 0.5, relative to a model in which they were unrelated. The significance level for each comparison was calculated via a simulation routine implemented in the Kinship program that takes into account population allele frequencies as well as the genotypes of the two individuals under consideration (Goodnight and Queller 1999).

Results

Discrete Trait Evolution

We estimated instantaneous rates of evolutionary change between character states for all four possible pairs of two traits, each with two possible states: terrestrial water body breeding (abbreviated as pond breeding, although this term includes other large terrestrial water bodies, such as streams) or phytotelm breeding; no parental care or parental care. A likelihood ratio test demonstrated a highly significant ($P < .0001$) correlation between the evolution of phytotelm breeding and parental care. The transition rate from pond breeding without parental care to phytotelm breeding without parental care was estimated as approximately twice that of the transition to pond breeding with parental care (fig. 3), but this difference was not statistically significant. In contrast, the transition rate from phytotelm breeding without parental care to phytotelm breeding with parental care was significantly higher ($P < .05$) than that from pond breeding with parental care to phytotelm breeding with parental care (table 1). Overall, our analysis indicates that the most likely evolutionary pathway from ancestral pond-breeding, noncaring species to phytotelm-breeding species with parental care was typically from pond breeding to phytotelm breeding, followed by the evolution of parental care (fig. 3).

Because this method requires the characterization of parental care as a binary character (presence/absence), it actually underestimates the effect of the transition to phytotelm breeding on the evolution of parental care. In several cases, although the evolution of some parental care preceded the evolution of phytotelm breeding, the evolution of phytotelm breeding then drove the evolution of higher levels of parental care (e.g., dendrobatid frogs; Summers and McKeon 2004). A test of the evolutionary correlation between phytotelm breeding and trophic egg feeding was also highly significant. A third test revealed a highly significant association between the evolution of trophic egg feeding and biparental care (table 1).

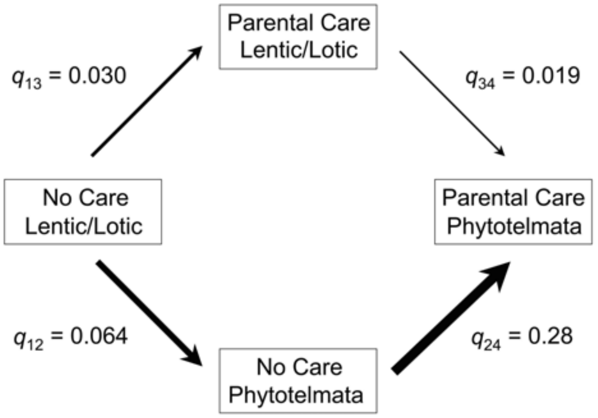

Figure 3: Evolutionary pathways from the ancestral condition of terrestrial water body (lentic/lotic) breeding without parental care to phytotelm breeding with parental care. Each value of q represents the rate estimated (via maximum likelihood) for that particular transition from our phylogenetic tree (Supertree [Nexus], available in a zip file in the online edition of the *American Naturalist*). Simultaneous changes in both characters are not allowed under the maximum likelihood model employed in the method. Likelihood ratio tests reveal that the most likely evolutionary pathway involves the evolution of phytotelm breeding followed by the evolution of parental care.

Concentrated Changes Test

Using the concentrated changes test (Maddison 1990), we found that the relationship between the evolution of phytotelm breeding (assumed to be the independent variable) and parental care (assumed to be the dependent variable) across all frogs was highly significant ($P < .003$): clades that evolved phytotelm breeding were likely to evolve parental care, relative to taxa without phytotelm breeding. A second test revealed that phytotelm breeders are significantly more likely ($P < .0001$) to have egg feeding. Finally, a third test revealed that the evolution of egg feeding is significantly ($P < .0001$) associated with the evolution of biparental care (fig. 4). PhytoEvol and PCEvol (available in a zip file in the online edition of the *American Naturalist*) show the reconstruction (via parsimony) of phytotelm breeding and parental care evolution on the phylogenetic supertree.

Hence two different methods of comparative analysis, carried out on data from species distributed across the order Anura, support the claim that the evolution of phytotelm breeding is associated with the evolution of parental care in general and with the evolution of more intensive forms of parental care (egg feeding). Furthermore, the evolution of egg feeding is associated with the evolution of biparental care.

Table 1: Log likelihoods of correlated and uncorrelated models of character evolution

Comparison	Log likelihood of the model: Independent	Log likelihood of the model: Dependent	2dLnLike	*P*
PHYT-PC	−291.253078	−274.216795	34.073	<.0001
Restrict $q_{12} = q_{13}$		−275.188447	1.943	NS
Restrict $q_{34} = q_{24}$		−278.900387	9.367	<.01
PHYT-EGG	−169.532513	−153.807415	15.725	<.01
EGG-BIPA	−79.423064	−51.438367	55.969	<.0001

Note: Each character has two states (presence/absence). Each row represents models that include a specific pair of characters, as listed. The independent model shows the log likelihood for a model in which the two characters evolve independently. The dependent model shows the log likelihood for a model in which the two characters evolve in a correlated manner. The models are compared using a likelihood ratio test (with df = 4). The heading 2dLnLike indicates two times the difference in the log likelihoods between the two types of models. The distribution of this statistic approximates a χ^2 distribution. *P* shows the statistical significance level for each comparison (NS = not significant). The restrict categories refer to the first comparison (PHYT-PC) and compares models in which specific transition rates have been constrained to be equal with those in which they have not (see fig. 3). In this case, the log likelihoods are compared between dependent models with and without the restriction (with df = 1).

Ecological Experiments

Tadpole growth was significantly different between treatments ($P < .001$, $\chi^2 = 26.868$, df = 4). Individuals of both species placed in small pools (with no egg feeding) grew at significantly slower rates than those placed in larger pools (fig. 5). Four (of eight) *Ranitomeya variabilis* tadpoles and three (of 10) *Ranitomeya imitator* placed in small pools (with no egg feeding) died before the end of the experiment. We observed no mortality in any of the other treatments. These results demonstrate that small phytotelmata contain insufficient nutrients for the growth and survival of tadpoles from either species. In contrast, large phytotelmata contain sufficient nutrients to support the growth and survival of either species without egg feeding (fig. 5). Thus, a complete transition to small pool use favored the evolution of biparental care from uniparental male care.

Genetic Analysis of Monogamy

Maximum likelihood estimates of average relatedness and pairwise relatedness (Goodnight and Queller 1999) between the parents and their putative offspring were consistent with complete monogamy (behavioral and genetic) for 11 out of 12 families (fig. A1 in the online edition of the *American Naturalist*). In these 11 families, average relatedness was estimated as 0.534 ± 0.153 between fathers and their putative offspring ($n = 40$), 0.538 ± 0.157 between mothers and their putative offspring ($n = 40$), and 0.641 ± 0.244 between putative siblings ($n = 78$). In contrast, average relatedness was estimated as −0.030 ± 0.235 between putatively unrelated individuals (all other individuals in the population) and offspring ($n = 2{,}089$). For the individual comparisons, 40 out of 40 father-offspring comparisons, 37 out of 40 mother-offspring comparisons, and 73 out of 78 offspring-offspring comparisons showed significant evidence of relatedness at the 0.5 level. In those few cases where the evidence for relatedness at the 0.5 level was not statistically significant, the point estimates were still consistent with a relatedness of 0.5. The twelfth pair, however, showed a clear pattern of polygyny, in which three clutches of embryos deposited over 2 months showed mixed maternity between two resident females (fig. A2 in the online edition of the *American Naturalist*).

While we were unable to carry out equivalent genetic analyses of parentage in *R. variabilis* (because of the difficulty of connecting individual adults to specific pools and offspring), a comparison of pairwise relatedness between tadpoles and younger larvae associated with the same pool indicated a wide range of relatedness, consistent with our observations of high levels of promiscuity (Brown et al. 2008*b*, 2009*c*).

Discussion

The ecological and social factors influencing the evolution of parental care have been the subject of contention for decades (Trivers 1972; Maynard Smith 1977; Westneat and Sherman 1993). Debates concerning this issue continue (e.g., Burley and Johnson 2002; Wesolowski 2004). It has proven difficult to connect specific ecological or social variables to the evolution of parental care (e.g., Ah-King

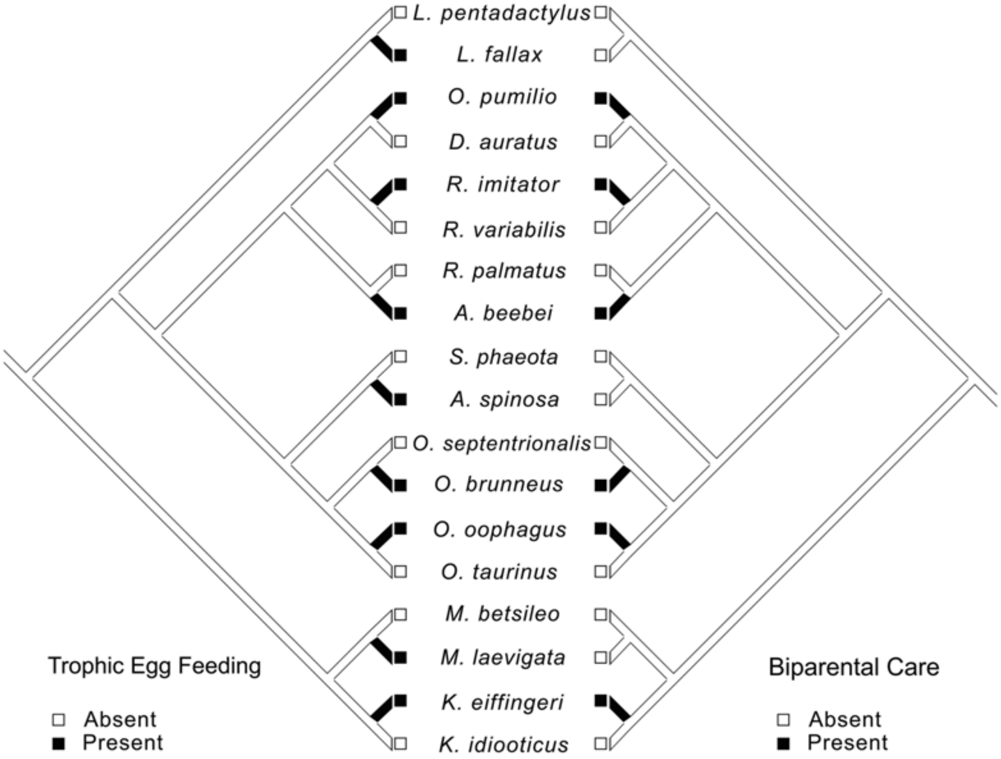

Figure 4: Mirror tree including only those species with egg feeding and one closely related taxon, illustrating the concentrated changes test of an association between egg feeding and biparental care (see Dryad data [http://hdl.handle.net/10255/dryad.1093]; Breeding Site Refs, Supertree [Nexus], PhytoEvol, and PCEvol are available in a zip file in the online edition of the *American Naturalist*).

et al. 2005), although progress has been made in some taxa (e.g., Gonzalez-Voyer et al. 2008).

Using comparative analyses across the order Anura, we have provided evidence that the evolution of phytotelm breeding drove the evolution of parental care, including a particularly intensive form of parental care (trophic egg feeding). In turn, the evolution of trophic egg feeding is associated with the evolution of biparental care.

Our previous research on *Ranitomeya imitator* and *Ranitomeya variabilis* revealed that these two species provide a unique opportunity to investigate the effect of ecology on the evolution of parental care and mating systems (Brown et al. 2008*a*, 2008*b*, 2009*b*, 2009*c*). These species show a major ecological difference associated with breeding strategy: they use breeding pools that differ substantially in size (Brown et al. 2008*b*). A key prediction of the hypothesis that this difference in pool size drove the evolution of egg feeding and biparental care is that the small pools used by *R. imitator* will not support tadpole growth and development unless trophic egg feeding is provided. The results of our reciprocal transplant experiments confirm this prediction.

Theoretically, we expect the evolution of biparental care to lead (in some cases) to the evolution of social and genetic monogamy, since the mutual dependence required for parents to provide the investment necessary to successfully produce offspring can favor mutual commitment and fidelity (Kleiman 1977; Wittenberger and Tilson 1980). However, this prediction has been difficult to test empirically.

Our long-term behavioral observations on *R. imitator* revealed pair bonding and strict social monogamy in this species, in contrast to *R. variabilis*, which is highly promiscuous (Brown et al. 2008*b*). Previous research on the sister species of *R. imitator*, *Ranitomeya vanzolinii*, demonstrated that this species also has pair bonding and social monogamy (also in association with the use of very small breeding pools [Caldwell 1997; Caldwell and de Oliveira 1999]). However, these studies did not address the issue of genetic monogamy. In fact, genetic monogamy has not been demonstrated in any species of amphibian. In one species (of salamander) where social monogamy had been demonstrated, genetic analyses of relatedness demon-

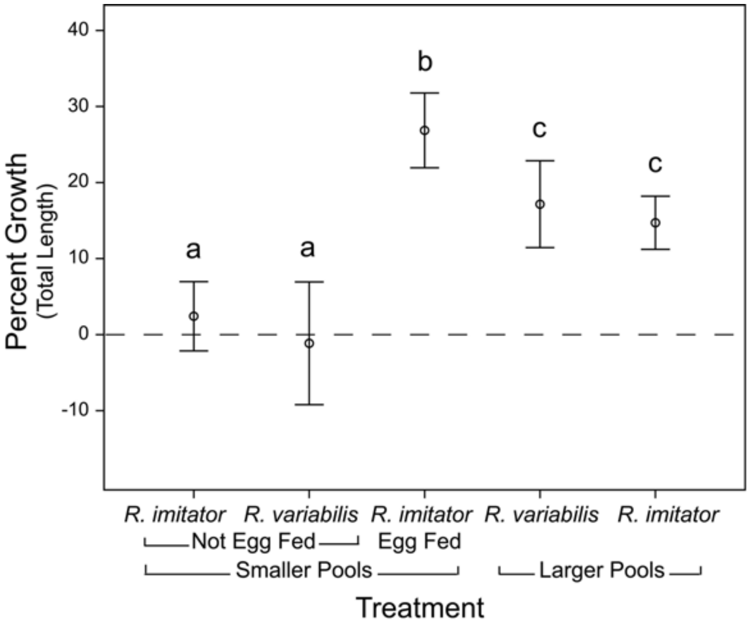

Figure 5: Reciprocal transplant experiments. We used five treatments in which the tadpoles of each species were placed in natural phytotelmata and allowed to consume the resources within the pools. The tadpoles were weighed and measured at the start and end of the experiment. Error bars = 2 SEs.

strated that the genetic mating system was highly promiscuous (Liebgold et al. 2006).

Our microsatellite analyses reveal the first example of genetic monogamy in an amphibian. Eleven out of the 12 pairs we investigated showed genetic as well as social monogamy. In one case, social monogamy did not correlate with genetic monogamy, in that the male was found to have mated with another female that resided near the territory of the putatively monogamous pair. It is not clear whether the primary female was aware of the extrapair mating carried out by her mate, since neither she nor her social mate was observed to interact with the secondary female. Although genetic monogamy appears to be the "rule" in *R. imitator*, these results are based on a small sample of families, and the prevalence of extrapair mating warrants additional study.

Previously, we have shown that the biparental care hypothesis for the evolution of social monogamy is consistent with the results of ecological and behavioral research on *R. imitator*, whereas other hypotheses are not (Brown et al. 2008*b*). Some authors have stressed the distinction between the mate-guarding hypothesis and the biparental care hypothesis as alternatives to explain the evolution of monogamy (Matthews 2003). In the case of *R. imitator*, at least some males apparently can profit from polygyny, and mate guarding by females appears to occur (and has been observed in captivity). Hence, mate guarding is likely to be important in this system, yet it is unlikely to provide a sufficient explanation for monogamy. Intense mate guarding by females occurs in at least two species of poison frogs (*Dendrobates auratus* and *Dendrobates leucomelas*) with uniparental male care, and yet this has not resulted in monogamy (Summers 1989, 1992*a*, 1992*b*). Thus, it is the importance of biparental care to offspring survival that is likely the key factor driving the evolution of monogamy in *R. imitator*.

In summary, we have provided comparative, observational, experimental, and molecular genetic evidence for an evolutionary chain of causation linking change in a specific ecological factor (breeding pool size) to evolutionary changes in parental care and mating system, culminating in biparental care and social and genetic monogamy. It is likely that patterns of parental care and mating strategies coevolved in response to changes in pool availability in *R. imitator*.

As pointed out in "Introduction," our results span two different scales of analysis (fig. 2). Our comparative analysis addressed evolutionary correlations, but the mechanisms that connect pool size to parental care and mating system across species remain to be investigated in detail. Our experimental and genetic analyses clarify these mechanisms within phytotelm-breeding poison frogs by comparing two closely related species that differ in breeding pool size, but it would be useful to replicate these results across multiple taxa in a phylogenetic context.

Similar ecological shifts to nutrient-poor "nurseries" may have led to similar evolutionary transitions in other lineages of frogs (e.g., *Anomaloglossus beebei*; Bourne et al. 2001) and more distantly related taxa (e.g., cockroaches: Bell et al. 2007), but further investigation is required to test this hypothesis.

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Literature Cited

Ah-King, M., C. Kvarnemo, and B. S. Tullberg. 2005. The influence of territoriality and mating system on the evolution of male care: a phylogenetic study on fish. Journal of Evolutionary Biology 18: 371–382.

Arnold, S. J., and D. Duvall. 1994. Animal mating systems: a synthesis based on selection theory. American Naturalist 143:317–348.

Bell, W. J., L. M. Roth, and C. A. Nalepa. 2007. Cockroaches: ecology, behavior, and natural history. Johns Hopkins University Press, Baltimore.

Bickford, D. P. 2004. Differential parental care behaviors of arboreal and terrestrial microhylid frogs from Papua New Guinea. Behavioral Ecology and Sociobiology 55:402–409.

Bourne, G. R., A. C. Collins, A. M. Holder, and M. L. McCarthy. 2001. Vocal communication and reproductive behavior of the frog *Colostethus beebei* in Guyana. Journal of Herpetology 35:272–281.

Brown, J. L., V. Morales, and K. Summers. 2008*a*. Divergence in parental care, habitat selection and larval life history between two species of Peruvian poison frogs: an experimental analysis. Journal of Evolutionary Biology 21:1534–1543.

Brown, J. L., E. Twomey, V. Morales, and K. Summers. 2008*b*. Phytotelm size in relation to parental care and mating strategies in two species of Peruvian poison frogs. Behaviour 145:1139–1165.

Brown, J. L., M. Choteau, T. Glenn, and K. Summers. 2009*a*. The development and analysis of twenty-one microsatellite loci for three species of Amazonian poison frogs. Conservation Genetics Resources 1:149–151, doi:10.1007/s12686-009-9036-5.

Brown, J. L., V. Morales, and K. Summers. 2009*b*. Home range size and location in relation to reproductive resources in poison frogs: a Monte Carlo approach using GIS data. Animal Behaviour 77: 547–554.

———. 2009*c*. Tactical reproductive parasitism via larval cannibalism in Peruvian poison frogs. Biology Letters 5:148–153.

Burley, N. T., and K. Johnson. 2002. The evolution of avian parental care. Philosophical Transactions of the Royal Society B: Biological Sciences 357:241–250.

Caldwell, J. P. 1997. Pair bonding in spotted poison frogs. Nature 385:211.

Caldwell, J. P., and V. R. L. de Oliveira. 1999. Determinants of biparental care in the spotted poison frog, *Dendrobates vanzolinii* (Anura: Dendrobatidae). Copeia 1999:565–575.

Clutton-Brock, T. H. 1991. The evolution of parental care. Princeton University Press, Princeton, NJ.

Crump, M. L. 1974. Reproductive strategies in a tropical anuran community. University of Kansas Museum of Natural History Miscellaneous Publication no. 61.

Duellman, W. E., and L. Trueb. 1986. Biology of amphibians. McGraw-Hill, San Francisco.

Frost, D. R. 2009. Amphibian species of the world: an online reference. Version 5.3. American Museum of Natural History, New York. http://research.amnh.org/herpetology/amphibia/.

Frost, D. R., T. Grant, J. Faivovich, R. H. Bain, A. Haas, C. F. B. Haddad, R. O. De Sa, et al. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History no. 297.

Gonzalez-Voyer, A., J. L. Fitzpatrick, and N. Kolm. 2008. Sexual selection determines parental care patterns in cichlid fishes. Evolution 62:2015–2026.

Goodnight, K. F., and D. C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Molecular Ecology 8:1231–1234.

Grant, T., D. R. Frost, J. P. Caldwell, R. Gagliardo, C. F. B. Haddad, P. J. R. Kok, D. B. Means, B. P. Noonan, W. E. Schargel, and W. C. Wheeler. 2006. Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatanura: Dendrobatidae). Bulletin of the American Museum of Natural History no. 299.

Gubernick, D. J., and T. Teferi. 2000. Adaptive significance of male parental care in a monogamous mammal. Proceedings of the Royal Society B: Biological Sciences 267:147–150.

Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? Biological Reviews 72:21–64.

Kleiman, D. G. 1977. Monogamy in mammals. Quarterly Review of Biology 52:39–69.

Kokko, H., and M. D. Jennions. 2008. Parental investment, sexual selection and sex ratios. Journal of Evolutionary Biology 21:919–948.

Liebgold, E. B., P. R. Cabe, R. G. Jaeger, and P. L. Leberg. 2006. Multiple paternity in a salamander with socially monogamous behaviour. Molecular Ecology 15:4153–4160.

Maddison, D. R., and W. P. Maddison. 2003. MacClade 4: analysis of phylogeny and character evolution. Version 4.06. Sinauer, Sunderland, MA.

Maddison, W. P. 1990. A method for testing the correlated evolution of two binary characters: are gains or losses concentrated on certain branches of a phylogenetic tree? Evolution 44:539–557.

Magnusson, W. E., and J. M. Hero. 1991. Predation and the evolution of complex oviposition behavior in Amazon rainforest frogs. Oecologia (Berlin) 86:310–318.

Matthews, L. M. 2003. Tests of the mate-guarding hypothesis for social monogamy: male snapping shrimp prefer to associate with high-value females. Behavioral Ecology 14:63–67.

Maynard Smith, J. 1977. Parental investment: a prospective analysis. Animal Behaviour 25:1–9.

McDiarmid, R. W. 1978. Evolution of parental care in frogs. Pages 127–147 *in* G. M. Burghardt and M. Bekoff, eds. The development of behavior: comparative and evolutionary aspects. Garland STPM, New York.

Pagel, M. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. Proceedings of the Royal Society B: Biological Sciences 255:37–45.

Reichard, U. H., and C. Boesch. 2003. Monogamy: mating strategies and partnerships in birds, humans and other mammals. Cambridge University Press, Cambridge.

Santos, J. C., L. A. Coloma, K. Summers, J. P. Caldwell, R. Ree, and D. C. Cannatella. 2009. Amazonian amphibian diversity is primarily derived from Late Miocene Andean lineages. PLoS Biology 7:e56.

Summers, K. 1989. Sexual selection and intra-female competition in the green poison dart frog, *Dendrobates auratus*. Animal Behaviour 37:797–805.

———. 1992*a*. Dart-poison frogs and the control of sexual selection. Ethology 91:89–107.

———. 1992*b*. Mating strategies in two species of dart-poison frog: a comparative study. Animal Behaviour 43:907–919.

Summers, K., and D. J. Earn. 1999. The cost of polygyny and the evolution of female care in poison frogs. Biological Journal of the Linnean Society 66:515–538.

Summers, K., and C. S. McKeon. 2004. The evolutionary ecology of phytotelmata use in poison frogs. Miscellaneous Publications of the Museum of Zoology, University of Michigan 193:55–73.

Summers, K., C. S. McKeon, and H. Heying. 2006. The evolution of parental care and egg size: a comparative analysis in frogs. Proceedings of the Royal Society B: Biological Sciences 273:687–692.

Summers, K., C. S. McKeon, H. Heying, J. Hall, and W. Patrick. 2007. Social and environmental influences on egg size evolution in frogs. Journal of Zoology (London) 271:225–232.

Touchon, J. C., and K. M. Warkentin. 2008. Reproductive mode plasticity: aquatic and terrestrial oviposition in a treefrog. Proceedings of the National Academy of Sciences of the USA 105: 7495–7499.

Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136–179 *in* B. Campbell, ed. Sexual selection and the descent of man, 1871–1971. Aldine-Atherton, Chicago.

Wells, K. 2007. The ecology and behavior of amphibians. University of Chicago Press, Chicago.

Wesolowski, T. 2004. The origin of parental care in birds. Behavioral Ecology 15:520–523.

Westneat, D. F., and P. W. Sherman. 1993. Parentage and the evolution of parental behavior. Behavioral Ecology 4:66–77.

Westneat, D. F., and I. R. K. Stuart. 2003. Extra-pair paternity in birds: causes, correlates and conflict. Annual Review of Ecology, Evolution, and Systematics 34:365–396.

Weygoldt, P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). Zeitschrift für Zoologisch, Systematik und Evolutionsforschung 25:51–67.

Wilson, E. O. 1971. Insect societies. Harvard University Press, Cambridge, MA.

Wittenberger, J. F., and R. L. Tilson. 1980. The evolution of monogamy: hypotheses and evidence. Annual Review of Ecology, Evolution, and Systematics 11:197–232.

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Left, *Ranitomeya imitator*, a species of poison frog that uses tiny pools for tadpole deposition and exhibits biparental care (where the female feeds tadpoles food eggs) and monogamy. *Right*, *Ranitomeya variabilis*, a species of poison frog that uses larger pools for tadpole deposition and does not exhibit biparental care or monogamy. Photographs by Jason L. Brown.