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Editor's choice

Original Article

The biparental care hypothesis for the evolution of monogamy: experimental evidence in an amphibian

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Selection for biparental care is considered to be an important factor favoring the evolution of monogamy if the value of exclusive cooperation in care for mutual offspring outweighs the benefits of polygamy for either sex. Support for this hypothesis has come primarily through parent removal experiments in avian taxa. We tested this hypothesis in the first known example of a socially and genetically monogamous amphibian, the mimic poison frog (*Ranitomeya imitator*). Biparental care in *R. imitator* is characterized by egg attendance, tadpole transport, and feeding of tadpoles with unfertilized trophic eggs. Using a male removal experiment, we found lower tadpole growth and lower survival for widowed females compared with control families. We demonstrate that in addition to egg attendance and tadpole transport, male parental care is critical for offspring survival throughout larval development. Previous research has shown the importance of female trophic egg provisioning of tadpoles in *R. imitator*. This, coupled with the results of the present study demonstrating the adaptive value of male care, supports the hypothesis that selection for biparental care has driven the evolution of monogamy in an amphibian.

Key words: **amphibian, biparental care, Dendrobatidae, monogamy, parental investment, Ranitomeya imitator.**

INTRODUCTION

Monogamy is a rare mating system largely restricted to birds; about 90% of avian species are at least socially monogamous (Lack 1968). It occurs infrequently in mammals (Kleiman 1977) and fish (Barlow 1984; Whiteman and Côte 2004) and is exceptionally rare in other vertebrate taxa. Social monogamy refers to the long-term behavioral association between a male and a female, but the definition does not entail exclusive reproduction between 2 individuals (Reichard 2003). Genetic monogamy, in contrast, refers to exclusive mating and often requires genetic parentage analysis for confirmation (Reichard 2003). The use of molecular markers to estimate parentage has revealed that examples of true monogamy, both social and genetic, are much less common than previously thought. For example, genetic monogamy occurs in less than 25% of socially monogamous birds (Griffith et al. 2002). The factors that favor monogamy have long been of interest to evolutionary biologists (Kleiman 1977; Wittenberger and Tilson 1980; Reichard and Boesch 2003) and the evolution of genetic monogamy is of special interest given the prevalence of extrapair mating in many socially monogamous species.

The value of biparental care for offspring success is considered to be an important factor favoring the evolution of monogamy (Lack 1968; Kleiman 1977; Wittenberger and Tilson 1980; Clutton-Brock 1991). When biparental care becomes crucial for offspring survival, monogamy should be favored if parents can achieve higher reproductive success through exclusive cooperation in care for mutual offspring than either could achieve through polygamy (the biparental care hypothesis; Wittenberger and Tilson 1980). Indeed, levels of parental care and parental investment by each sex are important determinants of the mating system of a species (Trivers 1972). Recent theoretical models have revealed complex relationships between parental investment, sex ratios, and sexual selection but confirm the importance of relative parental investment in mating system evolution (Kokko and Jennions 2008).

The biparental care hypothesis has been supported by empirical work, mostly in avian taxa (Møller 2000). In other groups, however, the causes of monogamy are varied. Biparental care may favor monogamy in some fish and empirical support has come from studies on cichlids (Grüter and Taborsky 2004), but a variety of factors may favor monogamy in association with a rich diversity of reproductive modes in this group (Whiteman and Côte 2004).

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In mammals, biparental care has been shown to be important in maintaining monogamy in some species (e.g., Gubernick and Teferi 2000; Wright 2006). It is also likely an important factor maintaining monogamy in some human societies (Marlowe 2000). However, a comparative analysis across all mammals suggests that monogamy is frequently disassociated from biparental care; in fact, monogamy has evolved more often in the absence of paternal care than in its presence (Komers and Brotherton 1997). The mixed support for this hypothesis across vertebrates demonstrates the importance of testing broad evolutionary hypotheses across diverse taxonomic groups. The mimic poison frog, *Ranitomeya imitator*, is the first known example of social and genetic monogamy in an amphibian. It offers a unique opportunity to test the biparental care hypothesis, adding a new class to the range of species included in comparative analyses and enhancing the generality of conclusions about the importance of biparental care.

It is thought that anuran parental care evolved to facilitate terrestrial reproduction in tropical climates, a transition likely driven by aquatic predation pressures on eggs and larvae (McDiarmid 1978; Summers and McKeon 2004). Many tropical anurans lay terrestrial egg clutches and use small temporary pools and phytotelmata (arboreal pools of water in the axils of plant leaves) for tadpole deposition (Wells 2007). Smaller pools have a lower risk of predation (Roth and Jackson 1987; Pearman 1995), but also contain less food and result in increased competition and risk of cannibalism, resulting in a trade-off (Summers 1990, 1999; Twomey et al. 2008). Competition between anuran larvae in small pools presumably only arose later in the transition to terrestrial reproduction, after species using small pools reached high densities. Some anurans have evolved to deposit tadpoles individually in very small pools and consequently experienced a reduction in larval competition (Summers and McKeon 2004; Brown, Morales, et al. 2008). Several of these species have also evolved advanced forms of parental care in which female parents provide unfertilized (trophic) eggs to tadpoles in these small nutrient-poor environments (Brust 1993; Caldwell and de Oliveira 1999; Heying 2001; Brown et al. 2010).

In the family Dendrobatidae (neotropical poison frogs), male-only parental care is ancestral and is typically characterized by attendance of terrestrial egg clutches and tadpole transport to temporary pools or phytotelmata (Summers and McKeon 2004; Summers and Tumulty 2013). Several species in this family exhibit biparental care, and it is the evolution of female care, from male-only care, that led to biparental care (Summers and McKeon 2004; Summers and Tumulty 2013). Female trophic egg feeding in this family is associated with the use of very small nutrient-poor pools (Caldwell and de Oliveira 1999; Brown et al. 2010). Indeed, a comparative analysis across all frogs by Brown et al. (2010) showed a strong negative correlation between parental care and breeding pool size, with biparental care correlating with the use of the smallest pools.

Detailed behavioral studies of the mimic poison frog (*R. imitator*) have revealed that it exhibits biparental care, characterized by female trophic egg feeding and long-term pair bonding (Brown, Morales, et al. 2008; Brown, Twomey, et al. 2008). The use of molecular markers has further revealed that this species is both socially and genetically monogamous (we consider species with a prevalence of monogamy more than 90% as genetically monogamous), with polygyny occurring in only 1 of 12 pairs (Brown et al. 2010). Following courtship, *R. imitator* lay arboreal clutches of 1–3 eggs on understory vegetation. Males attend egg clutches and transport individual tadpoles on their backs to small phytotelmata (Figure 1). It is important to note that egg clutches are not always laid above phytotelmata, so if the eggs hatch without a parent to transport them, they will not necessarily fall into water. This species typically uses phytotelmata of *Dieffenbachia* sp. (Araceae) and *Heliconia* sp. (Heliconiaceae) for tadpole deposition, which contain pools that hold approximately 25 mL of water (Brown, Twomey, et al. 2008). Males guard multipurpose territories consisting of breeding plants (Brown et al. 2009) and survey pools containing their offspring. Throughout tadpole development, males will call to females, lead them to individual phytotelmata, and stimulate them to feed tadpoles with trophic eggs. In particular, males will make tactile contact with females and call continuously within several centimeters of females until they lay one or more trophic eggs (females do not always lay eggs the first time they enter the pool). It also appears that offspring engage in begging behavior; tadpoles were observed to wriggle and nibble at either parent when they entered the water, possibly signaling hunger. Females usually lay 1–2 trophic eggs at a time and tadpoles are fed every 6–10 days (Brown, Twomey, et al. 2008).

Phytotelmata of *Dieffenbachia* sp. and *Heliconia* sp. contain insufficient nutrients for the tadpoles of this species to survive and develop, and trophic egg feeding has apparently allowed *R. imitator* to monopolize these small pools, an otherwise open breeding niche. Brown et al. (2010) confirmed this experimentally, showing that tadpoles denied trophic eggs had lower growth rates than controls, and their growth was not significantly different from zero. This suggests that small pool size is the key ecological trait that drove the evolution of biparental care in this species. Although it is clear that the trophic

Figure 1
A male *Ranitomeya imitator* transporting a tadpole. Males attend egg clutches and transport tadpoles individually to small nutrient-poor pools. Throughout tadpole development, males call to females, lead them to pools containing offspring, and stimulate them to provision tadpoles with unfertilized trophic eggs.

eggs provided by females are important for tadpole growth in these small pools, the adaptive value of male care and its role in orchestrating female provisioning has not been addressed. To test the hypothesis that the importance of biparental care maintains monogamy in this species, we monitored egg feeding and tadpole growth in the field and conducted male removal experiments to evaluate the importance of male care. Specifically, we asked 1) how important is egg feeding for tadpole growth and survival? 2) Is male care necessary throughout the tadpole stage, when tadpoles rely on trophic eggs provided by females? and 3) Is male care necessary for egg attendance and tadpole transport, an important life history transition?

MATERIALS AND METHODS

Experiments were conducted in secondary premontane tropical forest near the town of Chazuta, Peru, over two 4-month field seasons during the rainy seasons of 2010 and 2011. Four sites were chosen based on presence of *R. imitator* and their breeding plants, *Dieffenbachia* sp. We used the methodology of Brown, Twomey, et al. (2008) for monitoring individuals and identifying pairs. Mark-recapture surveys were conducted daily to identify individuals and map territories. We gave each individual adult a unique toe clip and took dorsal photographs; photo ID sheets were created allowing us to sometimes identify individuals visually from a distance without disrupting behavior. Although excessive toe clipping has been shown to have a slight negative effect on survival in other anurans (Mccarthy and Parris 2004), we attempted to minimize these effects by never removing more than 2 toes per individual (Grafe et al. 2011), and we are not aware of any individuals in this study dying as a result of having their toes clipped. Additionally, we used toe clips for both marking individuals and as sources of DNA for genetic parentage analysis (Gonser and Collura 1996). Males were distinguished from females mainly by calling behavior and also by a slight size dimorphism; snout-vent length was 16.3 mm (±0.51 standard deviation [SD]) for males and 18.0 mm (±0.67 SD) for females (Welch's *t*-test, $t_{43.6} = 10.45$, $P < 0.0001$), and males were generally more slender. Males and females that occupied the same territory and were repeatedly observed engaging in courtship and egg-feeding behavior were assumed to be monogamous pairs. Count data (encounter frequencies of adult frogs and number of tadpoles per family) were analyzed using a generalized linear model with a quasipoisson distribution to account for a highly skewed distribution.

Artificial pools were made from ¼-inch PVC pipe with a cap on the bottom end and attached to vegetation throughout all sites. Pools were filled with rainwater and drainage holes were drilled to maintain water volume at a maximum of 25 mL, the mean volume of natural pools used by *R. imitator* at this site (Brown, Twomey, et al. 2008). Adults regularly deposited tadpoles in these artificial pools and used them as refugia. Pools were checked daily for new tadpole depositions and for trophic eggs laid in pools already containing tadpoles; new trophic eggs were usually attached to the side of the pool just below the water line and were easily visible. We were also able to document tadpole mortality through these daily surveys. Once a week, we poured the contents of the pools through a fine mesh to extract the tadpoles and take growth measurements. Total length was measured to the nearest 0.01 mm using dial calipers; mass was measured to the nearest 0.002 g.

Importance of egg feeding

During the second field season of this study (2011), we monitored all tadpoles deposited in artificial pools, not just those included in the removal study, and recorded the number of trophic eggs received by each tadpole. To evaluate the relationship between trophic egg feeding and tadpole growth, we calculated the average growth rate of tadpoles for the first 3 weeks after deposition, as well as the average number of trophic eggs received by each over this time period. We limited this analysis to 3 weeks because larger tadpoles consumed trophic eggs much more rapidly than smaller, younger tadpoles, and we were less confident in our ability to estimate the number of eggs received by tadpoles older than 3 weeks of age (see Results). We calculated average weekly growth rate as the difference in mass between the first and last measurements divided by the number of weeks between measurements. In most cases, this was 3 weeks but some tadpoles died before week 3; using growth rate, as opposed to simply taking the difference in mass, allowed us to include these tadpoles by controlling for the amount of time between measurements. The average number of trophic eggs observed in a tadpole's pool per week was also calculated. We analyzed the relationship between average growth rate and average number of trophic eggs received using linear regression. The trophic egg data were square root transformed to improve linearity. We also analyzed the untransformed data using Spearman's rank correlation and achieved similar estimates of significance; only the results from the linear regression are presented.

Tadpole stage removals

To test the importance of male care while pairs were caring for tadpoles, we monitored the growth and survival of tadpoles in male removal and control families. Because many tadpoles were not fed following deposition in artificial pools (see Results), we limited our experiment to include only families with tadpoles that were receiving care. We monitored 37 tadpoles from 23 families. Families were randomly assigned to either male removal (n = 11) or control (n = 12) treatments when their tadpoles were 3 weeks old (Gosner stages 26–30; Gosner 1960) and were monitored until week 6 (Figure 3A). Removals were conducted at week 3 to allow time to identify the parents of each tadpole and confirm that tadpoles were receiving care, as evidenced by 2 weeks of growth and the periodic presence of trophic eggs. Removed males were kept in captivity and released at the end of the experiment. Opportunistic behavioral observations continued throughout the experimental period.

Because pairs were caring for several tadpoles simultaneously, we included "family" as an effect in our statistical analyses of tadpole growth and survival. Average growth rates were calculated for each tadpole for pre- and postremoval time periods as described above. We compared growth rates between treatments using a repeated measures nested analysis of variance (Anova). The within-subjects repeated measurements were the 2 average growth rate calculations for each tadpole: growth rate preremoval (week 0–3) and postremoval (week 3–6). We included "treatment" as a fixed effect and "family" nested within "treatment" as a random effect. We coded tadpole survival as a binary response for each family: whether or not a family experienced any offspring mortality during the experimental period. We were able to monitor 25 of the tadpoles in this experiment until they either died or completed metamorphosis, so we also coded families according to whether or not any tadpoles completed metamorphosis. These binary data were compared using Fisher's exact tests.

To test whether growth rate differences between treatments were attributable to differences in female trophic egg provisioning, we calculated the weekly provisioning rate for each female; this was the average number of trophic eggs received by all of her tadpoles per week.

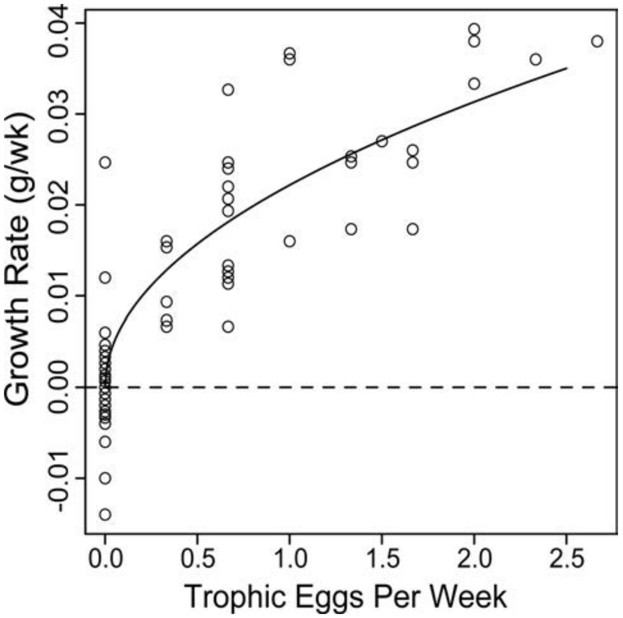

Figure 2
The relationship between growth rate and the number of trophic eggs received by tadpoles per week during the first 3 weeks of tadpole development. The trophic egg data were square root transformed to improve linearity; the data were back transformed for visual representation and plotted with the linear regression line of the transformed data.

We compared provisioning rates between treatments before and after removal using a repeated measures Anova, with the pre- and postremoval provisioning rates as the within-subjects repeated measurements and treatment as a fixed effect.

Confirming relatedness

Tissue samples from all individuals were collected in the field (toe clips from adults and tail clips from tadpoles). DNA was extracted and amplified at 6 polymorphic microsatellite loci using primers (DimiA06, DimiB02, DimiB07, DimiC05, DimiD01, DimiF06) specifically developed for *R. imitator* (Brown, Chouteau, et al. 2009). Alleles were identified using GeneMapper software (Applied Biosystems, Foster City, CA). To ensure accuracy, each locus was run twice for every individual and if the results were not the same, the locus was run a third time. Relatedness between pairs of individuals was analyzed using the program *Kinship* (ver. 1.3.1; Goodnight and Queller 1999). *Kinship* uses genotypes and population allele frequencies calculated from the data set to estimate the likelihood that pairs of individuals share a specific degree of relatedness and calculates significance using simulated comparisons of randomly chosen pairs; we ran 1000 simulated pairs to generate these values.

Egg stage removals

We removed males attending egg clutches to test the hypothesis that tadpole transport is a male-only parental behavior, and thus a potential cost exists to male abandonment of eggs before hatching. We located 12 males attending egg clutches during the early stages of development (Gosner stage < 15; Gosner 1960) and randomly assigned them to removal (n = 6) or control (n = 6) treatments. Clutches were monitored until they either died or hatched and were checked daily for depredation or desiccation and, if they survived, transportation. A clutch was considered depredated if the embryo disappeared from the egg less than 10 days after oviposition (the earliest hatching time of successful clutches is 11 days; Tumulty J, unpublished data) or if the clutch disappeared entirely. A clutch was considered desiccated if the eggs shrank visibly over the course of several days and failed to develop further. All instances of tadpole transport were confirmed by observing one of the parents with a tadpole on its back on the same day the embryo(s) disappeared from the clutch. Clutch survival, and whether or not clutches were transported, was analyzed using Fisher's exact tests. We did not confirm the genetic parentage of egg clutches; males seen attending clutches were presumed to be the fathers.

This experiment spanned 2 field seasons (2010 and 2011) and the data were pooled because there were no differences between years within treatment for any of the comparisons. Statistical analyses were carried out using SPSS version 19.0 (IBM Corp., Somers, NY) or R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Importance of egg feeding

The number of trophic eggs received by tadpoles was quite variable. During the first 3 weeks after deposition, tadpoles received anywhere from 0 to 8 trophic eggs (mean = 1.35 ± 1.95 SD; median = 0; n = 76). For over half of these tadpoles, we did not observe any trophic eggs in their pools (n = 42). The relationship between growth rate and the number of trophic eggs a tadpole received per week was highly significant ($r^2_{74} = 0.78$, $P < 0.001$; Figure 2), and the growth rate of tadpoles that received no trophic eggs did not differ from zero (exact Wilcoxon signed-rank test, $V_{42} = 414$, $P > 0.5$; Figure 2). Additionally, tadpoles that received trophic eggs were more likely to survive to 3 weeks of age (proportion survival with trophic eggs: 0.91 ± 0.05 standard error [SE]; without trophic eggs: 0.67 ± 0.07 SE; Fisher's exact test, $P = 0.013$).

Genetic relatedness

The numbers of alleles identified at each locus in this population were DimiA06–9, DimiB02–19, DimiB07–11, DimiC05–7, DimiD01–13, DimiF06–15. We were unable to obtain tissue samples from every tadpole due to mortality; thus, for families R3 and R4 in the removal treatment and family C4 in the control treatment, we were not able to determine genetic parentage. For families R10 and C9, we did not obtain tissue samples from the putative female parent, and for family C10, we did not obtain a tissue sample from the putative male parent. For all other families, we correctly assigned parentage to both parents for 27 of 29 (93%) tadpoles (see Supplementary Material). More specifically, we confirmed the paternity of all tadpoles in the removal treatment for which we had samples (n = 14); this verified that removed males were the genetic parents of their putative offspring. For the controls, 1 male apparently fathered extrapair offspring, as his tadpole was unrelated to the female he had been observed associating with (family C8). Another tadpole was incorrectly assigned to both parents (family C3). All other control tadpoles (n = 15) were confirmed to be the genetic offspring of all putative parents for which we had DNA samples.

Tadpole stage removal results

The number of tadpoles deposited singly in artificial pools by each pair ranged from 1 to 4, with an average of 1.58 (±0.90 SD) and

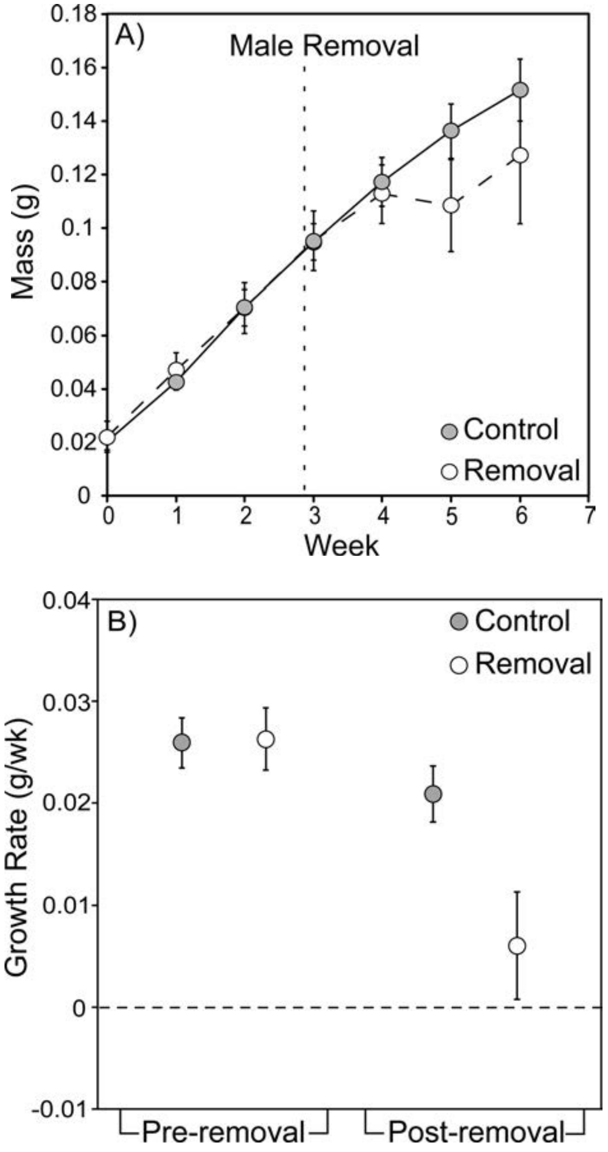

Figure 3
The effect of male removal on tadpole growth. (A) Mean mass of tadpoles in control and removal treatments each week for 6 weeks. Week 0 measurements were taken within 2 days of tadpole deposition in artificial pools. Males in the removal treatment were removed at or just before week 3. (B) Mean growth rate of tadpoles in removal and control treatments for preremoval (week 0–3) and postremoval (week 3–6) time periods. Growth rates were calculated as change in mass per week. For both plots, error bars represent the mean ± 1 SE.

1.64 (±0.92 SD) tadpoles per family in control and removal treatments, respectively. There was no difference in family size between treatments ($t_{22} = 0.139$, $P > 0.5$).

Two tadpoles in the removal treatment died before any postremoval growth measurements were taken and therefore were not included in growth rate comparisons. There was no difference in mass ($F_{1,\ 8.4} = 1.152$, $P = 0.313$) or length ($F_{1,\ 10.5} = 0.109$, $P > 0.5$) at time of removal (week 3; Figure 3A). The results of the repeated measures Anova on mass showed that time affected growth rate ($F_{1,\ 10} = 26.746$, $P < 0.001$) and that the effect of time was dependent on treatment ($F_{1,\ 10} = 6.171$, $P = 0.032$) but not on family within treatment ($F_{18,\ 10} = 1.173$, $P = 0.411$). In other words, the removal tadpoles grew at a slower rate postremoval (week 3–6) than preremoval (week 0–3), whereas the growth rate of control tadpoles did not change (Figure 3B and Table 1). The growth rate, as measured by change in length, was affected by time ($F_{1,\ 10} = 104.605$, $P < 0.001$), but there was no treatment interaction ($F_{1,\ 10} = 2.353$, $P = 0.156$), and there was a borderline significant family effect ($F_{18,\ 10} = 2.796$, $P = 0.050$). Although we did not measure width, the tadpoles in the removal treatment appeared visibly thinner during the postremoval period, suggesting that they were not being fed regularly.

Only 1 control tadpole (out of 19) died during the 3-week experimental period (week 3–6), whereas 9 out of 18 removal tadpoles died. The mean proportion offspring survival within families was 0.96 (±0.06 SE) for controls and 0.41 (±0.15 SE) for removals. Families with removed males were more likely to experience tadpole mortality than control families (Fisher's exact test, $P = 0.009$). Of tadpoles that were monitored until death or metamorphosis ($n = 25$), only 1 of 14 tadpoles in the removal treatment survived to metamorphosis (0.10 ± 0.09 SE) compared with 7 out of 11 controls (0.64 ± 0.18 SE). Control families were more likely to have at least 1 offspring complete metamorphosis than were removals (Fisher's exact test, $P = 0.035$). The difference in metamorphosis between treatments demonstrates an 85% reduction in reproductive success for widowed females.

At least 5 of 11 widowed females did feed tadpoles following removal of their mate, as indicated by the presence of trophic eggs in artificial pools of their offspring. We found no difference between treatments in female provisioning rate ($F_{1,\ 22} = 0.342$, $P > 0.5$); instead, our estimates of provisioning rates decreased for both treatments during the postremoval period ($F_{1,\ 22} = 23.63$, $P < 0.001$). However, opportunistic observations revealed that older, larger tadpoles consumed trophic eggs much faster than younger, smaller tadpoles; very young tadpoles would often take several days to consume a single trophic egg, whereas older tadpoles could consume them within several hours. Most of these provisioning events by widowed females (5 out of 8) took place within the first week after male removal (week 4), and no feeding occurred during week 6. We documented trophic eggs in the pools of 5 control females during week 6 suggesting that egg feeding normally does continue for at least 6 weeks.

Behavioral observations

We recorded 517 encounters for 41 adult frogs in this experiment. There was no difference between sexes in the number of observations per individual ($t_{39} = -0.737$, $P = 0.47$). We classified 19 of these encounters as egg feeding. Although pairs were often encountered in pools together, we only considered the behavior as egg-feeding if the male was calling to a female in a pool we knew to have a tadpole. A few opportunistic, detailed observations of egg feeding confirm the behavioral observations of Brown, Twomey, et al. (2008). Typically, a female was observed following a calling male; the male would call several times, hop a few inches, and then continue calling, sometimes turning to face the female while calling. The male would lead the female to a pool containing a tadpole and continue to call. In the few instances that we had a clear view of the tadpole during these interactions, the tadpole appeared to be wriggling at the cloaca of the female, which was slightly submerged. Males continued to call throughout egg feeding. These behaviors appeared similar to courtship, but unlike fertilized eggs resulting from courtship, which are attached to leaves above the water level, trophic eggs are laid in the water

Table 1

Results of the repeated measures nested Anova of growth rate, as measured by change in tadpole mass and length

Variable	df	MS	F	P
Mass				
Time	1	0.002	26.75	<0.001
Time × Treatment	1	0.001	6.17	0.032
Time × Family (Treatment)	18	<0.001	1.17	0.411
Error	10	8.97×10^{-5}		
Length				
Time	1	34.37	104.61	<0.001
Time × Treatment	1	0.77	2.35	0.156
Time × Family (Treatment)	18	0.92	2.80	0.050
Error	10	0.33		

df = degrees of freedom; MS = mean square. "Time" represents the 2 repeated measurements for each tadpole: growth rate preremoval and growth rate postremoval. "Treatment" compares the effect of male removal with control pairs, and "Family" nested within "Treatment" controls for potentially confounding family effects.

and show no evidence of fertilization. It is unclear what, exactly, differentiates courtship from egg feeding, but the wriggling "hunger" cue given by tadpoles may be important in stimulating egg feeding.

We only observed one occurrence in which we were confident that egg feeding had occurred without male stimulation; we observed a female in a position with her vent slightly submerged and a tadpole wriggling at her cloaca, but there was no male in sight and we did not hear calling. We left to avoid disturbing her and about 30 min later, when we returned, she had moved away and we saw a trophic egg at the location where her vent had been positioned. During the postremoval experimental period, we found no differences between treatments in the number of encounters for each female ($t_{19} = 1.03$, $P = 0.32$) and we did not observe any obvious differences in female behavior between treatments.

Egg stage removal results

There were relatively high rates of mortality due to predation, desiccation, and fungal infection in both the control and removal treatments and there was no difference between treatments in survival (control: 0.50 ± 0.20 SE; removal: 0.33 ± 0.19 SE; Fisher's exact test, $P > 0.5$) or whether or not the tadpoles were transported (control: 0.50 ± 0.20 SE; removal: 0.17 ± 0.15 SE; Fisher's exact test, $P > 0.5$). All of the control egg clutches that hatched ($n = 3$) were transported by the male parent. Only 2 removal egg clutches survived, one of which was transported by the female parent on hatching. Of the clutches that were transported ($n = 4$), mean hatching age was 11.75 days (±0.5 SD), but the clutch that was not transported did not hatch until day 16. All instances of tadpole transport were confirmed visually.

DISCUSSION

Biparental care is thought to play a key role in the evolution of monogamy if the value of exclusive cooperation in care for mutual offspring outweighs the benefits of polygamy for either sex. Our examination of the importance of biparental care in *R. imitator* supports this hypothesis. *Ranitomeya imitator* breed in very small nutrient-poor pools of water and our results agree with those of Brown et al. (2010) by demonstrating the importance of trophic egg feeding—and hence female care—for tadpole growth and survival in artificial pools of the same volume. We also experimentally demonstrate a significant reduction in reproductive success when males were removed after tadpole deposition, establishing the importance of male care for tadpole growth and survival throughout development. Males were regularly observed calling to females, leading them to particular phytotelmata, and continuing to call while females laid trophic eggs. These behaviors indicate that males coordinate feeding events and, as such, the male's absence causes the female to provision offspring less frequently or not at all, as indicated by the decreased tadpole growth and survival in the removal treatment.

We did, however, find evidence that several females fed tadpoles in the absence of a male, indicating that male stimulation is not essential for egg feeding to occur. Furthermore, we did not find any difference in provisioning rate of control and "widowed" females; instead, our estimates of provisioning rate decreased in both treatments during the postremoval period (week 3–6) compared with the preremoval period (week 0–3). Although this seems to indicate that females feed older tadpoles less frequently, we think that instead the provisioning rates of older tadpoles were underestimated because older, larger tadpoles consumed trophic eggs much faster than younger tadpoles. Thus, trophic eggs in the pools of tadpoles during the postremoval period were more likely to be consumed before we could document their presence during site surveys. Egg feeding and tadpole growth rate were tightly correlated during the first 3 weeks and the growth rate of control tadpoles did not change from the preremoval to postremoval periods indicating that they continued to be provisioned regularly. Although these experiments do not conclusively demonstrate an unequivocal link between male care and trophic egg feeding during the postremoval period, all the pieces of evidence taken together strongly suggest that the lower growth and survival of tadpoles in the removal treatment is due to the lack of males stimulating females to provision offspring.

An alternative explanation for the results could be that when males are removed, females must take over some parental behaviors normally performed by males, for example, defending territories and surveying pools, and are more stressed as a result. This switch could also use up time females would normally devote to foraging, and as such, less energy is invested into egg production. Although we cannot discount this hypothesis, we did not observe any obvious differences in female behavior when males were removed, which would indicate that they were taking on typical paternal behaviors. Furthermore, if true, this explanation would still be consistent with the general idea that male removal has a high cost to offspring success and an increased likelihood of offspring abandonment by "widowed" females because of difficulties raising tadpoles alone.

We did not find a significant cost to male removal during the egg stage as similar egg mortality was observed in both treatments. However, these results are based on a very small sample size and it is possible that a cost to male removal would be detected with a larger sample size. Additionally, because we were able to demonstrate a cost to male removal at a later stage of development, if we had conducted egg stage removals and tracked the progress of offspring throughout both egg and tadpole development, we would expect to see an even greater difference between treatments over the entire larval period. This type of experiment is not feasible, though, given the difficulties of tracking the deposition location of each tadpole after it is transported. Perhaps more importantly, females were observed attending egg clutches and transporting tadpoles, both of which were previously thought to be male-only behaviors. In fact, we observed 1 control family in which the male and female alternated turns attending a clutch. Male clutch attendance and tadpole transport were more common, but the occasional participation of females in these activities indicates a close association and cooperation in care between males and females throughout both the egg and tadpole stages. Interestingly, the 1 egg clutch in the removal treatment that was not transported did not hatch until 4 days later than the mean hatching time of transported clutches. Brown, Twomey, et al. (2008) observed males inducing hatching by tearing open eggs with their rear legs; our results suggest that hatching is delayed until induced by a parent, which could be an adaptation to oviposition in nonaquatic habitats.

Why are mimic poison frogs socially and genetically monogamous?

There is little doubt that selection for biparental care can explain social monogamy (Mock and Fujioka 1990; Reichard and Boesch 2003). However, the prevalence of extrapair matings, particularly in birds, indicates that individuals can seldom maximize fitness through genetic monogamy (Griffith et al. 2002). In *R. imitator*, only 1 of 12 males in putatively monogamous pairs was found to be polygynous by Brown et al. (2010). This is a fairly low frequency when compared with other socially monogamous taxa (Møller 2000; Reichard and Boesch 2003). So perhaps the more interesting question is why are *R. imitator* pairs (mostly) genetically monogamous?

We can gain some insight by comparing our results with other removal studies in socially monogamous taxa. Females often provide a majority of care in birds and mammals. The few avian studies that conduct female removals find large fitness costs (Sasvari 1986); thus, researchers are often interested in evaluating the importance of male care. Some experiments on socially monogamous birds show very little cost from male removal (Gowaty 1983; Dunn and Hannon 1992; Møller 2000). Interestingly, many of these species in which male care may be helpful, but not necessary, also have high rates of extrapair paternity (Gowaty and Bridges 1991; Dunn et al. 1994). This inverse relationship between importance of paternal care and occurrence of extrapair paternity was supported by a comparative analysis by Møller (2000), with many species that experience complete brood failure in response to male removal having relatively low rates (<10%) of extrapair mating. The correlation suggests that genetic monogamy may be favored when male care is important for offspring survival. Our results indicate this to be the case in *R. imitator*, which showed an 85% reduction in reproductive success when males were removed, and this species has low rates (8.3%) of extrapair mating (Brown et al. 2010). Thus, the strength of selection for biparental care in this species may be sufficiently strong to make polygamy unprofitable.

Because polygamy does occur occasionally in *R. imitator* (Brown et al. 2010), it is worthwhile to consider how it might arise, and why it is not more common. Polyandry is unlikely; males are highly territorial and aggressive. More importantly, there are limits on the number of tadpoles a female can provision simultaneously. We obtained rough estimates of egg production from clutch sizes and trophic eggs that suggest females can produce up to 3–5 eggs per week. Tadpoles are usually fed 1–2 trophic eggs per provisioning event and are fed every 6–10 days (Brown, Twomey, et al. 2008). This limits pairs to 2–4 tadpoles at a time and 4 tadpoles was our largest family size. Thus, the limits on polyandry are clear: the rate of egg production in this species restricts females to caring for offspring from 1 male at a time. Further research on the energetics of egg production is needed to elucidate these limitations. Finally, due to external fertilization, multiple paternity as a result of females mating sequentially with multiple males does not occur, as it does in some taxa with internal fertilization (Griffith et al. 2002; Avise and Liu 2011).

Polygyny could occur in 1 of 2 ways: a male would have to either 1) monopolize more than 1 female and care for both sets of offspring simultaneously or 2) "trick" his mate into feeding offspring from another female. The first possibility is probably difficult given the high level of parental investment required. Our results show that approximately 7–12 weeks of sustained biparental care is required to rear offspring, and there may be limitations on the number of offspring a single male can successfully manage. Males spend most of the day guarding territories and surveying pools; in contrast, females seem to spend much of their time foraging. Based on our estimates of the limits to egg production, we assume that regular foraging by females is necessary to maintain egg production. Viewed in this light, male territoriality and surveying of pools "frees up" females to forage and invest in egg production; males can then relay information to the female on which tadpoles need to be provisioned. The investment in defending breeding resources, surveying pools, and coordinating feeding events would appear to be large enough to limit polygyny. One male in Brown et al.'s (2010) study was able to manage this polygynous mating strategy, but its rarity suggests that it may be costly. Furthermore, although we did not observe female territorial behavior or aggression, female–female aggression has been observed in captivity and may function as mate guarding (Twomey E, personal communication).

The second scenario (a male tricking his mate into feeding the offspring of another female) is theoretically possible if females cannot detect kin and do not initially know the location of their tadpoles when they are first deposited. However, our observational results of the close association between males and females during egg attendance and the participation of females in tadpole transport indicate that they sometimes know where their offspring are deposited. Further research is necessary to test whether females can recognize their own offspring, and olfactory cues may be important (Schulte et al. 2011). Finally, these frogs breed continually throughout the rainy season (or as long as phytotelmata are filled with water), and pairs are often caring for several tadpoles of different ages from 2 or more separate clutches simultaneously. Thus, the overlapping nature of breeding and care likely prevents pairs from switching partners and maintains pair bonds throughout the breeding season.

The biparental care hypothesis is gaining support from a variety of taxonomic groups and our results indicate that *R. imitator* exhibits a highly cooperative, long-term association between male and female parents in care for mutual offspring. If either parent abandons the other, the widowed parent cannot sufficiently care for the offspring alone because 1) tadpoles require trophic eggs for growth and 2) females appear unable or unwilling to feed frequently enough to sustain growth and survival in the absence of the male. We conclude that selection for long-term biparental care, demanded by the key ecological trait of small pool size, drove the evolution of social and genetic monogamy in *R. imitator*.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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REFERENCES

Avise JC, Liu J-X. 2011. Multiple mating and its relationship to brood size in pregnant fishes versus pregnant mammals and other viviparous vertebrates. Proc Natl Acad Sci USA. 108:7091–7095.

Barlow GW. 1984. Patterns of monogamy among teleost fishes. Arch fur Fischereiwiss. 35:75–123.

Brown JL, Chouteau M, Glenn T, Summers K. 2009. The development and analysis of twenty-one microsatellite loci for three species of Amazonian poison frogs. Conserv Genet Resour. 1:149–151.

Brown JL, Morales V, Summers K. 2008. Divergence in parental care, habitat selection and larval life history between two species of Peruvian poison frogs: an experimental analysis. J Evol Biol. 21:1534–1543.

Brown JL, Morales V, Summers K. 2009. Home range size and location in relation to reproductive resources in poison frogs (Dendrobatidae): a Monte Carlo approach using GIS data. Anim Behav. 77:547–554.

Brown JL, Morales V, Summers K. 2010. A key ecological trait drove the evolution of biparental care and monogamy in an amphibian. Am Nat. 175:436–446.

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

Brust DG. 1993. Maternal brood care by *Dendrobates pumilio*: a frog that feeds its young. J Herpetol. 27:96–98.

Caldwell JP, de Oliveira VRL. 1999. Determinants of biparental care in the spotted poison frog, *Dendrobates vanzolinii* (Anura: Dendrobatidae). Copeia. 1999:565–575.

Clutton-Brock TH. 1991. The evolution of parental care. Princeton (NJ): Princeton University Press.

Dunn PO, Hannon SJ. 1992. Effects of food abundance and male parental care on reproductive success and monogamy in tree swallows. Auk. 109:488–499.

Dunn PO, Whittingham LA, Lifjeld JT, Robertson RJ, Boag PT. 1994. Effects of breeding density, synchrony, and experience on extrapair paternity in tree swallows. Behav Ecol. 5:123–129.

Gonser RA, Collura RV. 1996. Waste not, want not: toe-clips as a source of DNA. J Herpetol. 30:445–447.

Goodnight KF, Queller DC. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Mol Ecol. 8:1231–1234.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16:183–190.

Gowaty PA. 1983. Male parental care and apparent monogamy among eastern bluebirds (*Sialia sialis*). Am Nat. 121:149–157.

Gowaty PA, Bridges WC. 1991. Behavioral, demographic, and environmental correlates of extrapair fertilizations in eastern bluebirds, *Sialia sialis*. Behav Ecol. 2:339–350.

Grafe TU, Stewart MM, Lampert KP, Rödel M-O. 2011. Putting toe clipping into perspective: a viable method for marking anurans. J Herpetol. 45:28–35.

Griffith SC, Owens IPF, Thuman KA. 2002. Extra pair paternity in birds: a review of interspecific variation and adaptive function. Mol Ecol. 11:2195–2212.

Grüter C, Taborsky B. 2004. Mouthbrooding and biparental care: an unexpected combination, but male brood care pays. Anim Behav. 68:1283–1289.

Gubernick DJ, Teferi T. 2000. Adaptive significance of male parental care in a monogamous mammal. Proc R Soc Lond B Biol Sci. 267:147–150.

Heying HE. 2001. Social and reproductive behaviour in the Madagascan poison frog, *Mantella laevigata*, with comparisons to the dendrobatids. Anim Behav. 61:567–577.

Kleiman DG. 1977. Monogamy in mammals. Q Rev Biol. 52:39–69.

Kokko H, Jennions MD. 2008. Parental investment, sexual selection and sex ratios. J Evol Biol. 21:919–948.

Komers PE, Brotherton PNM. 1997. Female space use is the best predictor of monogamy in mammals. Proc R Soc Lond B Biol Sci. 264:1261–1270.

Lack D. 1968. Ecological adaptations for breeding in birds. London: Methuen.

Marlowe F. 2000. Paternal investment and the human mating system. Behav Process. 51:45–61.

Mccarthy MA, Parris KM. 2004. Clarifying the effect of toe clipping on frogs with Bayesian statistics. J Appl Ecol. 41:780–786.

McDiarmid RW. 1978. Evolution of parental care in frogs. In: Burghardt G, Bekoff M, editors. The development of behavior: comparative and evolutionary aspects. New York: Garland STPM Press. p. 127–147.

Mock DW, Fujioka M. 1990. Monogamy and long-term pair bonding in vertebrates. Trends Ecol Evol. 5:39–43.

Møller AP. 2000. Male parental care, female reproductive success, and extrapair paternity. Behav Ecol. 11:161–168.

Pearman PB. 1995. Effects of pond size and consequent predator density on two species of tadpoles. Oecologia. 102:1–8.

Reichard UH. 2003. Monogamy: past and present. In: Reichard UH, Boesch C, editors. Monogamy: mating strategies and partnerships in birds, humans and other mammals. Cambridge: Cambridge University Press. p. 3–25.

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

Roth AH, Jackson JF. 1987. The effect of pool size on recruitment of predatory insects and on mortality in a larval anuran. Herpetologica. 43:224–232.

Sasvari L. 1986. Reproductive effort of widowed birds. J Anim Ecol. 55:553–564.

Schulte LM, Yeager J, Schulte R, Veith M, Werner P, Beck LA, Lötters S. 2011. The smell of success: choice of larval rearing sites by means of chemical cues in a Peruvian poison frog. Anim Behav. 81:1147–1154.

Summers K. 1990. Paternal care and the cost of polygyny in the green dart-poison frog. Behav Ecol Sociobiol. 27:307–313.

Summers K. 1999. The effects of cannibalism on Amazonian poison frog egg and tadpole deposition and survivorship in *Heliconia* axil pools. Oecologia. 119:557–564.

Summers K, McKeon CS. 2004. The evolutionary ecology of phytotelmata use in neotropical poison frogs. Misc Publ Museum Zool Univ Michigan. 193:55–73.

Summers K, Tumulty J. 2013. Parental care, sexual selection, and mating systems in neotropical poison frogs. In: Macedo RH, Machado G, editors. Sexual selection: perspectives and models from the neotropics. Waltham (MA): Elsevier Academic Press.

Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, editor. Sexual selection and the descent of man 1871–1971. Chicago: Aldine Press. p. 136–179.

Twomey E, Morales V, Summers K. 2008. Evaluating condition-specific and asymmetric competition in a species-distribution context. Oikos. 117:1175–1184.

Wells KD. 2007. The ecology and behavior of amphibians. Chicago: University of Chicago Press.

Whiteman E, Côte I. 2004. Monogamy in marine fishes. Biol Rev. 79:351–75.

Wittenberger JF, Tilson RL. 1980. The evolution of monogamy: hypotheses and evidence. Annu Rev Ecol Syst. 11:197–232.

Wright HWY. 2006. Paternal den attendance is the best predictor of offspring survival in the socially monogamous bat-eared fox. Anim Behav. 71:503–510.