

CALLING IS AN HONEST INDICATOR OF PATERNAL GENETIC QUALITY IN POISON FROGS

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Abstract.—Several competing hypotheses have been put forward to explain why females of many species mate preferentially with males possessing the most conspicuous signals (e.g., ornaments, displays, or songs). We performed a laboratory experiment using two species of poison frogs, *Dendrobates leucomelas* and *Epipedobates tricolor*, to test the hypothesis that male calling performance is an honest indicator of parental quality. Our analyses are based on data from behavioral observations of mating activities of captive-reared individuals (and their offspring) that were housed in terraria for four consecutive breeding seasons. Male mating success increased with male calling rate and chirp duration in both species, suggesting that females preferred males with more elaborate calls. Because calling performance improved with age in *D. leucomelas*, female poison frogs that prefer males with more elaborate calls in the wild may end up mating with older males that have already proven their ability to survive. Females that mated with good callers obtained higher quality offspring. Eggs fertilized by males with high calling rates and long chirp durations had higher hatching success and produced tadpoles that were more likely to metamorphose into surviving frogs. As a consequence, females that mated with males with high calling performance obtained more surviving offspring per egg, compared to females that mated with poor callers. Collectively, our findings comply with the notion that female poison frogs prefer to mate with good callers because calling performance is a reliable predictor of offspring quality. The possible influence of maternal allocation and reasons for the strong effect size compared to previous studies are discussed.

Key words.—*Dendrobates*, *Epipedobates*, good genes, maternal allocation, offspring viability, sexual selection.

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Many studies have shown that female mating preferences are influenced by variation in male advertisement displays (e.g., ornaments, displays, or calls; Andersson 1994). Several competing hypotheses have been put forward to explain why females of some species mate preferentially with males possessing the most conspicuous displays (Kokko et al. 2003). The “good genes” hypothesis suggests that male advertisement displays function as a reliable signal of genetic quality. By selecting males with extravagant secondary sexual characters (i.e., of high genetic quality) females may obtain fitness benefits mediated via enhanced offspring viability (Zahavi 1975, 1977; Andersson 1982; Ryan et al. 1982; Kirkpatrick and Ryan 1991; Petrie and Williams 1993; Proehl and Hodl 1999; Doty and Welch 2001; Kokko et al. 2003).

In support of this theory there is a large body of evidence for benefits (i.e., fitter offspring) to females for choosing a male based on secondary sexual characters (Hamilton and Zuk 1982; Hillgarth 1990; Potts et al. 1991; Moore 1994; Hasselquist et al. 1996; Sheldon et al. 1997; Alatalo et al. 1998; Hoikkala et al. 1998; Welch et al. 1998; Wilkinson et al. 1998; Møller and Alatalo 1999; Sandvik et al. 2000; Barber et al. 2001; Kotiaho et al. 2001; Wedekind et al. 2001; Sheldon et al. 2003). In their meta-analysis of published studies of good genes effects in sexual selection Møller and Alatalo (1999) found a weak but significant correlation between the expression of male secondary sexual traits and offspring survival, a finding in accordance with the hypothesis that females may obtain genetically based viability benefits from their choice. More recently, some authors have argued that many tests of good genes effects might be confounded by common environment effects and differential maternal allocation in response to perceived male quality (reviewed in

Sheldon 2000). This criticism has prompted experiments that test for good genes effects while controlling for differential allocation (by means of artificial insemination or artificial fertilizations of eggs of externally fertilizing taxa) and post-fertilization parental care (by rearing offspring artificially) (e.g., Welch et al. 1998; Barber et al. 2001; Doty and Welch 2001; Wedekind et al. 2001; Kokko et al. 2003; Evans et al. 2004). But the criticism that good genes effects need to be separated from maternal allocation effects also has been disputed for several reasons (Sheldon 2000). For instance, it has been argued that demonstrations of females sacrificing their future reproductive value when mating with attractive males to invest in current offspring can be regarded as evidence in support of good genes. This argument assumes that there is a genetic effect (i.e., that differential allocation is adaptive). Moreover, differential maternal allocation can simply be regarded as an additional effect of parental genes expressed in offspring, because if a female allocates more resources to offspring of attractive males, those offspring may become more attractive due to the actions of their mother (Sheldon 2000).

For a signal to be a reliable indicator of genetic quality it must incur a cost upon the male (Zahavi 1975, 1977; Ryan et al. 1982; Kotiaho 2000), unless the expression of the trait is reliably associated with age. Production of sound is one of the most costly activities in animals (Steven and Josephson 1977). For example, vocalization has been shown to incur considerable metabolic cost in birds (Thomas 2002), insects (Hoback and Wagner 1997; Reinhold et al. 1998; Reinhold 1999), and frogs (Grafe 1996; Grafe and Thein 2001). Features of the frog call that determine energetic costs include calling rate, call duration, and sound pressure level (Pres-

twich 1994). In addition to being energetically costly, conspicuous sexually selected acoustic signals may also be exploited by other species and incur predation costs (Ryan et al. 1982; Zuk and Kolluru 1998). Calling in frogs may therefore be an honest signal of genetic quality (Welch et al. 1998). Male poison frogs like most other anurans use advertisement calls to attract females (Summers 1992; Gerhardt 1994; Proehl 2003). Calling in poison frogs has been shown to affect behavior and metabolic brain activity (Zimmermann and Rahmann 1987), suggesting potential energetic costs (Roithmair 1992, 1994; Graves et al. 2005). Here we test the prediction from the good genes theory that females should prefer males with the most elaborate (costly) calls (i.e., males that have longer calls and higher calling rate) in two species of poison frogs. Observations of wild poison frogs have reported that females prefer males with high rates of vocalization (Proehl 2003). We also explore the level of (indirect) fitness benefits of female choice on male calling. We rear offspring of females mated to different males artificially (to remove possible confounding effects of parental care) and examine whether paternal calling performance reliably predicts offspring viability. Our analyses are based on repeated measures of individuals over four consecutive breeding seasons. This allows us to compare between- and within-male variation and to test for age-related changes in calling performance.

MATERIALS AND METHODS

Study Species

The poison frogs (Dendrobatidae) are a group of Neotropical anurans known for their bright, aposematic coloration and extremely potent skin toxins (Daly 1978, 1995; Daly and Myers 1983; Summers and Clough 2001; Hagman and Forsman 2003). For our study we used *Dendrobates leucomelas* and *Epipedobates tricolor*. These species exhibit male parental care and breed readily in captivity. The males produce loud calls, making them suitable for the study. *Dendrobates leucomelas* is a black and yellow species that occurs in Venezuela and Guyana. Adults reach 31–38 mm in snout-to-vent length (SVL, females usually grow a little larger than males). *Epipedobates tricolor* is variable in coloration but typically dark red to brown with three yellow to white longitudinal stripes along the back and sides. This species is smaller than *D. leucomelas* (16.5–26.5 mm SVL) and occurs in Ecuador. Both species are diurnal and live mainly on the forest floor and in the lower vegetation where they feed on small insects such as springtails, small beetles, and ants (Heselhaus 1992; Walls 1994; Bartlett and Bartlett 1996). The animals we used were captive-reared, sexually mature animals of approximately the same age (14 ± 2 months at the beginning of the study).

Animal Husbandry

The study animals were kept in glass terraria (measuring $60 \times 40 \times 50$ cm) in groups of two males and four females, with five replicates (i.e., 10 males and 20 females) for each species. We kept the two different species separate. Individual males were distinguished by differences in color patterns.

It was not possible, however, to recognize individual females. The terraria were climate controlled (photoperiod 12L:12D, temperature 26°C, relative humidity 75–90%, see also below) and contained a substrate of *Sphagnum* moss, live plants, branches, and a small water dish. Each terrarium also contained four egg-laying sites consisting of coconut halves with a small entrance hole placed over a plastic petri dish. The four coconut halves were arranged adjacent to each other in the form of a square in one end of the terrarium. Other than the egg-laying sites the two parts of the terrarium did not differ and had approximately the same amount of plants, hiding places, and display spots. The frogs were fed calcium and vitamin-dusted fruit flies (*Drosophila melanogaster*), springtails (*Collembola* spp.), and occasionally newly hatched crickets (*Acheta domestica*) ad libitum. The terraria were misted daily (early morning and late afternoon) by means of an automatic sprinkling system. An electronic computer fan placed above the ventilation screening on top of each terrarium managed circulation of air. For heat we placed a thermostatically controlled heating pad (24 W) under each terrarium. The frogs were also able to thermoregulate by moving in and out of water, by seeking shade or by moving up (closer to the lights) and down the different levels provided by branches and plants. We cleaned the glass and plants weekly and replaced the *Sphagnum* moss on a monthly basis. Water was changed daily. The frogs were maintained in captivity and monitored for a period of four years (1996–1999) under a breeding cycle of nine breeding months and three resting months, following (with minor modifications) recommended procedures for the breeding of poison frogs in captivity (Heselhaus 1992; Walls 1994; Bartlett and Bartlett 1996; De Vosjoli and McKeown 1997, 1998a,b,c; Kelley 1998). The frogs were stimulated to breed by maintaining a high relative humidity of 90%. To allow the animals to recuperate, we annually reduced relative humidity until breeding stopped.

We incubated eggs in plastic petri dishes with aged tap water at approximately 22°C. Eggs generally hatched within two weeks of incubation. Tadpoles were kept solitary in plastic cups (150 cl) with aged tap water that we changed weekly. These cups were labeled and placed randomly on shelves. The temperature was approximately 22°C and no special light was provided. Every second day we fed the tadpoles with five defrosted bloodworms (*Chironomidae* spp.) or fish flakes. Tadpoles metamorphosed within approximately 11 weeks.

Calling Behavior

To allow for quantification, comparison, and analysis of repeatability of male calling performance (calling rate and chirp duration), we recorded the calls of each individual male using satellite microphones connected to Sony portable tape recorders. The males were usually positioned well away from one another. Therefore, we placed one microphone at each end of the terrarium. During the recordings we closely monitored the frogs to ensure that we did not confuse the males (e.g., if the males swapped calling sites). Occasionally our recordings picked up the calls of both males in the terrarium. However, they were easily distinguished because the calls from the male closest to the microphone sounded much louder

than the calls from the distant male. Recordings were done daily for two consecutive hours (between 09 00 h and 11 00 h) for three weeks during each of the four breeding periods. Calling rate was measured as the number of chirps made by each male per hour. The duration of each separate chirp was measured with a stopwatch and the mean duration of chirps made by a male in a given hour was computed.

Male Mating Success and Offspring Performance

We monitored frog behavior daily throughout the study period. To allow quantification and comparison of male mating success, the number of successful matings (i.e., when females oviposited) was recorded for each individual male. We assigned eggs to a particular male *E. tricolor* by directly observing amplexus (and oviposition) between the male and a female. Male *D. leucomelas* do not grasp the female during courtship. However, males of this species place themselves in an independent amplexic position (the male sits with his vent close against the female's vent; Duellman and Trueb 1986). Thus, we were able to assign eggs to a particular male *D. leucomelas* by directly observing oviposition. Importantly, in anurans fertilization occurs externally at the time of oviposition (Duellman and Trueb 1986). Hence we assumed that the male that was present fertilized the eggs. We removed all egg clutches from the terrarium within one day following oviposition. To allow quantification and comparison of performance or quality of offspring sired by different males we recorded the number of eggs, the number of hatched eggs, and the number of metamorphosed tadpoles for each male in each of the four breeding seasons. We also monitored individual frog survival for a minimum of five weeks postmetamorphosis. This information was then used to compute hatching success (number of hatched eggs/number of eggs $\times 100\%$), percentage successfully metamorphosed hatchlings (number of metamorphosed tadpoles surviving as frogs for five weeks/number of tadpoles $\times 100\%$), and percentage frogs per egg (number frogs surviving for five weeks/number of eggs $\times 100\%$).

Analyses

Repeatability of differences among individuals in calling performance was estimated based on repeated measurements of individuals using one-way ANOVAs as $S_B^2/(S_B^2 + S_W^2) \times 100\%$, where $S_B^2 = (MS_B - MS_W)/k$, $S_W^2 = MS_W$, k = number of measurements per individual, and MS_W and MS_B are the mean squares obtained within and between individuals, respectively (Sokal and Rohlf 1981). Repeatability was estimated for three different time scales: between two consecutive hours (data for day 11 in the first breeding season); among five days within a breeding season (data for the first hour of days 1, 5, 10, 15, and 20 in the second breeding season), and among the four breeding seasons (data on mean across the 21 days within each breeding season).

The statistical significance of the association between male calling performance and male and offspring fitness components was assessed using correlation analyses (nonparametric analysis was used when data were in the form of proportions). The results presented below are based on pooled data for all four breeding seasons. Separate analyses of data for each of

the four breeding seasons yielded results that were qualitatively similar to those obtained for pooled data, particularly for the associations between calling performance and male fitness components (i.e., the number of matings, eggs, and number of surviving frogs). Not all associations between calling performance and the estimates of offspring performance/fitness were statistically significant in each of the four breeding seasons. This was largely because not all males obtained eggs in each separate breeding season, resulting in reduced sample size (and hence lower statistical power) in some years. Again, however, the overall results were qualitatively similar to those obtained for pooled data.

For *D. leucomelas* the hypothesis of a difference in mating success and offspring performance between males differing in calling performance was assessed also using paired comparisons *t*-tests. In this analysis, the two males sharing the same terrarium were treated as one observation, yielding a total of five paired comparisons. Because of the high correlation between calling rate and chirp duration (see below), the male in each pair with the highest calling rate invariably also had the longest chirp duration. For all dependent variables, paired comparison analyses based on differences in either calling rate or chirp duration therefore yielded identical results. Paired comparison analysis was not performed on data for *E. tricolor* because one of the males did not obtain any matings during the entire study, yielding no more than four paired comparisons for the estimates of offspring fitness. However, the trends for paired comparisons were similar to those of *D. leucomelas*.

RESULTS

Calling Behavior

Mean calling rate (based on pooled data across the four years) was 41 chirps/h (range 29–61) in *D. leucomelas* and 45.5 chirps/h (range 14–66) in *E. tricolor*. Mean chirp duration was 8.0 sec (range 5–10.5) in *D. leucomelas* and 5.4 sec (range 4–7) in *E. tricolor*.

Differences in calling rate and chirp duration among individuals were highly persistent over time (i.e., between two consecutive hours on the same day, among days within a breeding season, and among the four breeding seasons) in both species, with repeatability estimates ranging between 78.8 and 99.9% (Table 1).

In *D. leucomelas*, calling behavior did not vary significantly among days 1, 5, 10, 15, and 20 within a breeding season (repeated measures ANOVA, effect of day, calling rate: $F_{4,6} = 1.29$, $P = 0.37$; chirp duration: $F_{4,6} = 0.83$, $P = 0.55$). However, both calling rate and chirp duration varied among the four breeding seasons, increasing with increasing age (repeated measures ANOVA, effect of year, calling rate: $F_{3,7} = 6.25$, $P < 0.05$; chirp duration: $F_{3,7} = 9.07$, $P < 0.01$; Fig. 1). In *E. tricolor*, calling behavior also did not vary significantly among days 1, 5, 10, 15, and 20 within a breeding season (calling rate: $F_{4,6} = 2.13$, $P = 0.19$; chirp duration: $F_{4,6} = 0.71$, $P = 0.61$). Calling rate but not chirp duration varied among the four breeding seasons (calling rate: $F_{3,7} = 6.38$, $P < 0.05$; chirp duration: $F_{3,7} = 3.83$, $P = 0.065$), but the increase in calling rate with increasing age was not as pronounced as in *D. leucomelas* (Fig. 1).

TABLE 1. Repeatability of calling rate (chirps/h) and chirp duration (sec) in *Dendrobates leucomelas* and *Epipedobates tricolor*. Repeatability was estimated in the short (between two consecutive hours), intermediate (among five five-day intervals), and long (among four consecutive breeding seasons), time scales. Analyses were based on data for 10 individuals of each species. Repeatability was estimated from one-way ANOVAS (see text for details).

| Time scale | df | Calling rate | | | Chirp duration | | |
|----------------------|------|---------------|--------|---------|----------------|-------|---------|
| | | Repeatability | F | P | Repeatability | F | P |
| <i>D. leucomelas</i> | | | | | | | |
| Hour | 9,10 | 99.2 | 256.93 | <0.0001 | 90.7 | 20.66 | <0.0001 |
| Day | 9,40 | 90.3 | 47.40 | <0.0001 | 85.6 | 30.64 | <0.0001 |
| Year | 9,30 | 78.8 | 15.85 | <0.0001 | 83.3 | 21.09 | <0.0001 |
| <i>E. tricolor</i> | | | | | | | |
| Hour | 9,10 | 99.9 | 2589 | <0.0001 | 94.9 | 38.24 | <0.0001 |
| Day | 9,40 | 99.6 | 1214 | <0.0001 | 90.5 | 48.52 | <0.0001 |
| Year | 9,30 | 99.6 | 1022 | <0.0001 | 89.6 | 35.43 | <0.0001 |

Summary Statistics of Male Mating Success and Offspring Performance

The average cumulative number of matings obtained by males during the entire study period (i.e., over four breeding seasons) was 73 (range across different males = 12–180) in *D. leucomelas* and 64 (range 0–144) in *E. tricolor*. Mean number of eggs per mating (measured across the four years) was 5.0 (SD = 0.49, range 4.1–5.9) for *D. leucomelas* and 7.1 (SD = 2.37, range 2.3–9.7) for *E. tricolor*. Mean hatching success of eggs was 53% (range 38–65%) in *D. leucomelas*

and 61% (range 47–72%) in *E. tricolor*. The proportion of tadpoles that metamorphosed successfully and survived as frogs for one month was 38% (range 0–70%) in *D. leucomelas* and 31% (range 0–47%) in *E. tricolor*. The proportion of eggs that developed into surviving frogs was 21% (range 0–41%) in *D. leucomelas* and 20% (range 0–32%) in *E. tricolor*.

Association between Calling Performance and Male Mating Success

Mean calling rate and chirp duration were positively and significantly correlated with male mating success (i.e., cumulative number of copulations, eggs and surviving frogs) in both species (Table 2, Fig. 2). In *D. leucomelas* the higher mating success of males with higher calling rates and longer chirp durations was evident also in the results from the paired comparisons *t*-tests (Fig. 3).

Association between Male Calling Behavior and Offspring Fitness

Our results uncovered positive associations between male calling performance and offspring performances in both species. Eggs fertilized by males with high calling rates and long chirp durations had higher hatching success and produced tadpoles that were more likely to metamorphose into surviving frogs (Table 2, Fig. 4). As a consequence, females that mated with males with higher calling performance obtained more surviving juveniles per egg, compared to females that mated with males with lower calling performance (Table 2, Fig. 4). All associations (with the exception of that between chirp duration and the proportion of tadpoles metamorphosing) were statistically significant.

In *D. leucomelas*, the superior quality of offspring sired by males with higher calling rates and longer chirps was evident also in the results from the paired comparisons between the male with the highest and lowest calling performance within each terrarium (Fig. 3). Thus, eggs fertilized by males with higher calling rates and longer chirp durations had higher hatching success and were more likely to result in surviving juveniles.

Maternal Allocation

By allocating resources to reproduction differentially (for instance by modifying the number and/or size of eggs) in

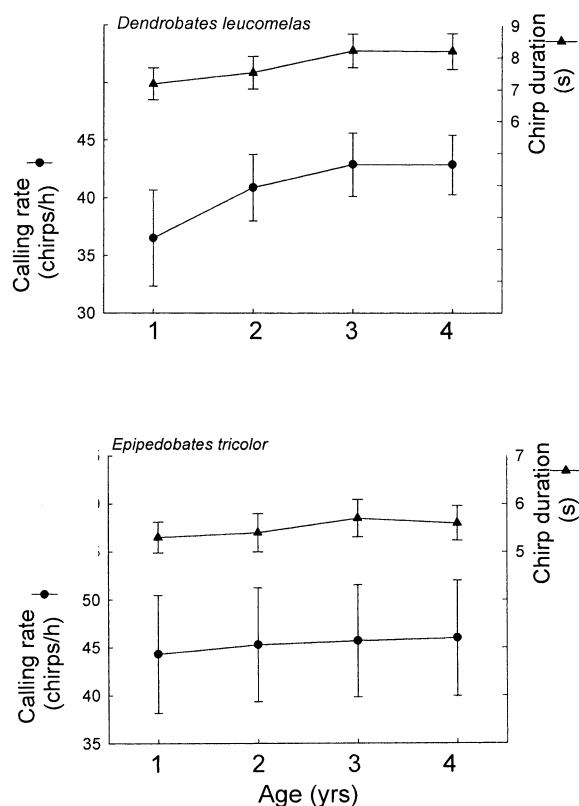


FIG. 1. Increase in calling performance with age in male *Dendrobates leucomelas* (top panel) and *Epipedobates tricolor* (bottom panel). Calling performance was measured as calling rate (chirps/h) and chirp duration (sec). Figure shows means \pm 1 SE for 10 males of each species.

TABLE 2. Associations between calling behavior (i.e., calling rate [chirps/h] and chirp duration [sec]) and fitness components in *Dendrobates leucomelas* and *Epipedobates tricolor*. Analyses are based on mean values across four years for 10 individuals of each species. Table shows correlation coefficients and associated *P*-values. Associations with hatching success, metamorphosed hatchlings, and frogs per egg were examined using nonparametric correlation analysis (Spearman) and the remaining variables with Pearson correlation coefficients.

| Fitness component | <i>n</i> | Calling rate | Chirp duration |
|------------------------------|----------|--------------|----------------|
| <i>D. leucomelas</i> | | | |
| Number of matings | 10 | 0.93, 0.0001 | 0.88, 0.0007 |
| Number of eggs | 10 | 0.92, 0.0002 | 0.90, 0.0003 |
| Hatching success (%) | 10 | 0.77, 0.0092 | 0.60, 0.065 |
| Metamorphosed hatchlings (%) | 10 | 0.73, 0.016 | 0.66, 0.036 |
| Number of frogs | 10 | 0.96, 0.0001 | 0.83, 0.0032 |
| Frogs per egg (%) | 10 | 0.84, 0.0022 | 0.69, 0.027 |
| <i>E. tricolor</i> | | | |
| Number of matings | 10 | 0.90, 0.0004 | 0.96, 0.0001 |
| Number of eggs | 10 | 0.84, 0.0026 | 0.95, 0.0001 |
| Hatching success (%) | 9 | 0.68, 0.041 | 0.64, 0.063 |
| Metamorphosed hatchlings (%) | 9 | 0.94, 0.0002 | 0.90, 0.0008 |
| Number of frogs | 10 | 0.81, 0.0049 | 0.92, 0.0002 |
| Frogs per egg (%) | 9 | 0.96, 0.0001 | 0.94, 0.0002 |

response to perceived male quality, females may increase (or mask) differences among offspring fathered by different males (Sheldon 2000; Qvarnström and Price 2001). However, we found no association between maternal allocation (measured as mean number of eggs per clutch) and male calling performance in *D. leucomelas* (calling rate: $r = 0.012$, $P = 0.97$, $n = 10$; chirp duration: $r = 0.09$, $P = 0.80$, $n = 10$) nor in *E. tricolor* (calling rate: $r = 0.05$, $P = 0.89$, $n = 10$; chirp duration: $r = 0.32$, $P = 0.41$, $n = 10$).

DISCUSSION

Collectively, our findings suggest that female poison frogs prefer to mate with good callers because calling performance is a reliable predictor of offspring quality. Male mating success increased with male calling rate and chirp duration in both species, suggesting that females preferred males with more elaborate calls. This result is consistent with Proehl and Hodl (1999) who suggested that females of the strawberry poison frog (*D. pumilio*) are selective and that male mating success is correlated with calling activity. Importantly, courtship in our captive *D. leucomelas* was similar to that of animals in the field (e.g., males were calling while leading females to oviposition sites; females were stroking, nudging, and climbing males; Summers 1992), suggesting that our results based on behavior in captivity may reflect behavior in natural conditions. Summers' (1992) field-investigation of courtship behavior also reported that female poison frogs were selective and frequently rejected or ignored courting males.

Our study revealed great variation among individual males in calling performance. Importantly, differences in calling rate and chirp duration were highly repeatable within individuals over time (i.e., between two consecutive hours on the same day, among days within a breeding season, and among four different breeding seasons) in both species, with repeatability estimates ranging between 79% and 99%. This result is qualitatively similar to calling performance in strawberry poison frogs (*D. pumilio*; Proehl 2003) and Túngara frogs (*Physalaemus pustulosus*; Ryan and Rand 2003), al-

though repeatability of calling behavior is somewhat higher in our poison frogs.

We cannot exclude the possibility that females in our study copied each other's mate choice (Andersson 1994; Brown and Fawcett 2005). However, this seems unlikely; furthermore, copying does not necessarily exclude selection. Our experiments had five replicates for each species and spanned four successive breeding seasons. Thus, (considering the highly significant effect of different sires on offspring fitness, Table 2 and Fig. 3) if copying indeed did occur, the "original" females in all replicates must have sampled and chosen the "right" male.

Most tests of good genes sexual selection have concentrated on effects of sires on characteristics of offspring early in their development (e.g. Hoikkala et al. 1998; Welch et al. 1998; Johnsen et al. 2000; but for an exception see Promislow et al. 1998). Our study adds further support to the notion that mate choice can have long-term fitness consequences. Notably, mate choice in our study affected offspring viability in several life-history stages (i.e., egg, tadpole, juvenile frog). Eggs fertilized by males with high calling rates and long chirp durations had higher hatching success and produced tadpoles that were more likely to metamorphose into surviving frogs. As a consequence, females that mated with males with high calling performance obtained more surviving offspring per egg, compared to females that mated with poor callers.

The huge effect sizes in our study suggest that female poison frogs who prefer males with superior calling performance may enjoy major viability benefits to their offspring. For example, the correlation coefficient between male calling rate and the proportion of eggs that developed into surviving frogs was 0.84 in *D. leucomelas* and 0.96 in *E. tricolor* (Table 2). By comparison, Møller and Alatalo (1999) reported an average correlation between male trait and offspring survival of 0.122 in their meta-analyses of good genes effects in sexual selection. However, because we did not use a half-sibling design, our estimates are potentially confounded by the possible influence of differential maternal allocation in response

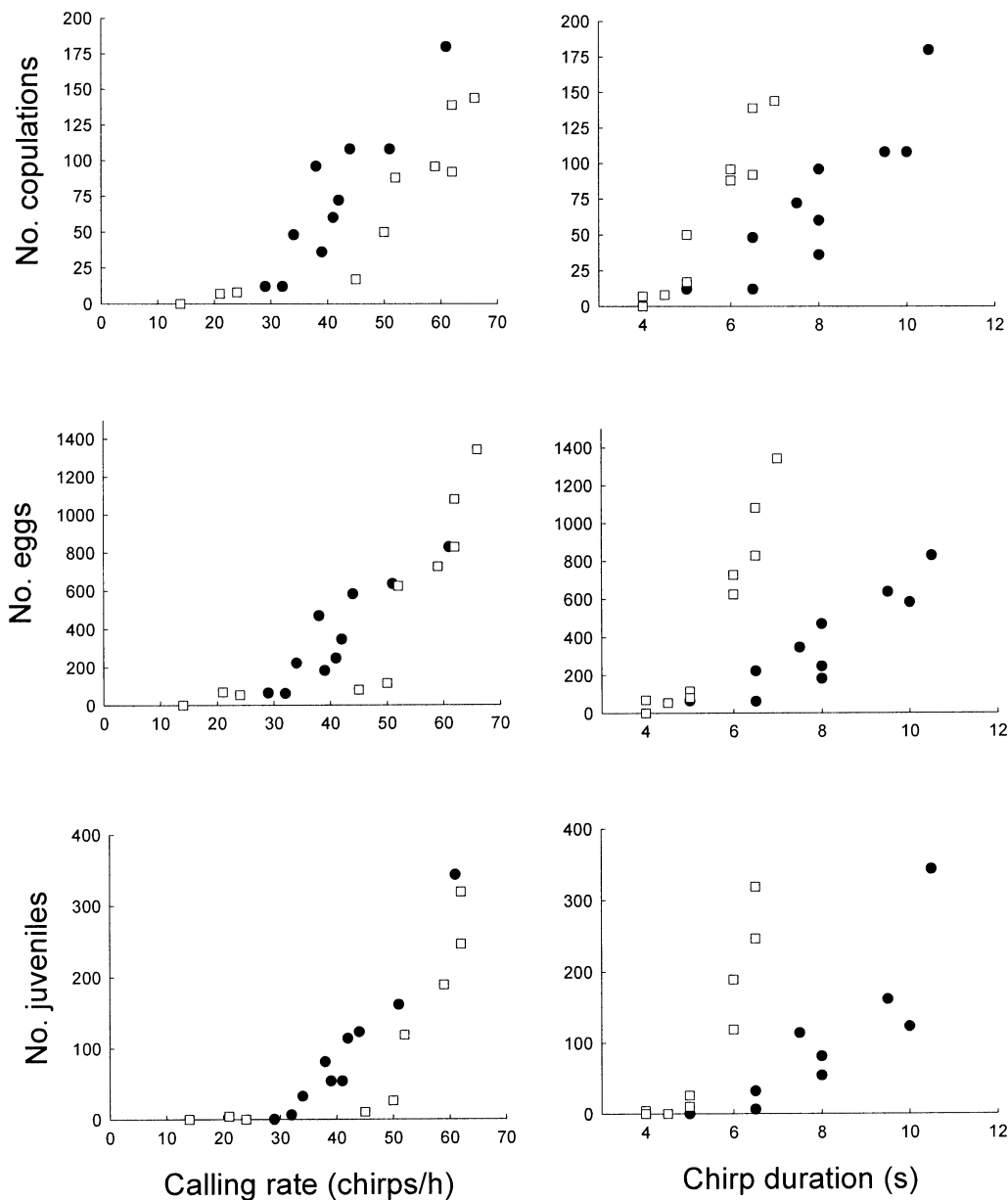


FIG. 2. Associations between calling performance and reproductive success in male *Dendrobates leucomelas* (black dots) and *Epipedobates tricolor* (empty squares). The figure shows the cumulative number of juveniles, eggs, and copulations obtained over four breeding seasons for each male as a function mean calling rate (left panels) and chirp duration (right panels).

to perceived male quality (Sheldon 2000). It has been previously shown that male attractiveness may influence the number of eggs in frogs (Reyer et al. 1999). In our poison frogs, the number of eggs produced per reproductive bout was not correlated with male calling performance. However, we cannot exclude the possibility that female differential allocation is manifested instead as differences in the size or quality of eggs (e.g., Cunningham and Russell 2000). If, for the sake of argument, we attribute one half (0.45) of our total average effect size to good genes and the other half (0.45) to maternal allocation, this implies that male calling performance explains at least 20% of the variance in offspring viability. Clearly, the magnitude of this influence is remarkable compared to that detected in other studies (cf. 1.5% on

average in the 22 studies included in the meta-analysis by Møller and Alatalo 1999; see also Sheldon 2000).

What may be the reason for the huge effect size in our study? Our analyses of repeatability of calling performance (Table 1) show that within-male variation is trivial compared to the magnitude of variation among different males. This temporal stability of the relative calling rate and chirp duration of individuals indicates that calling performance is little influenced by environmental sources of variation and reflects instead inherent male quality. It further enables females to discriminate among males of different quality with a very high precision. In the wild, however, any condition dependence of calling performance (e.g., Kotiaho 2000) may blur the association between the genetic quality of males and

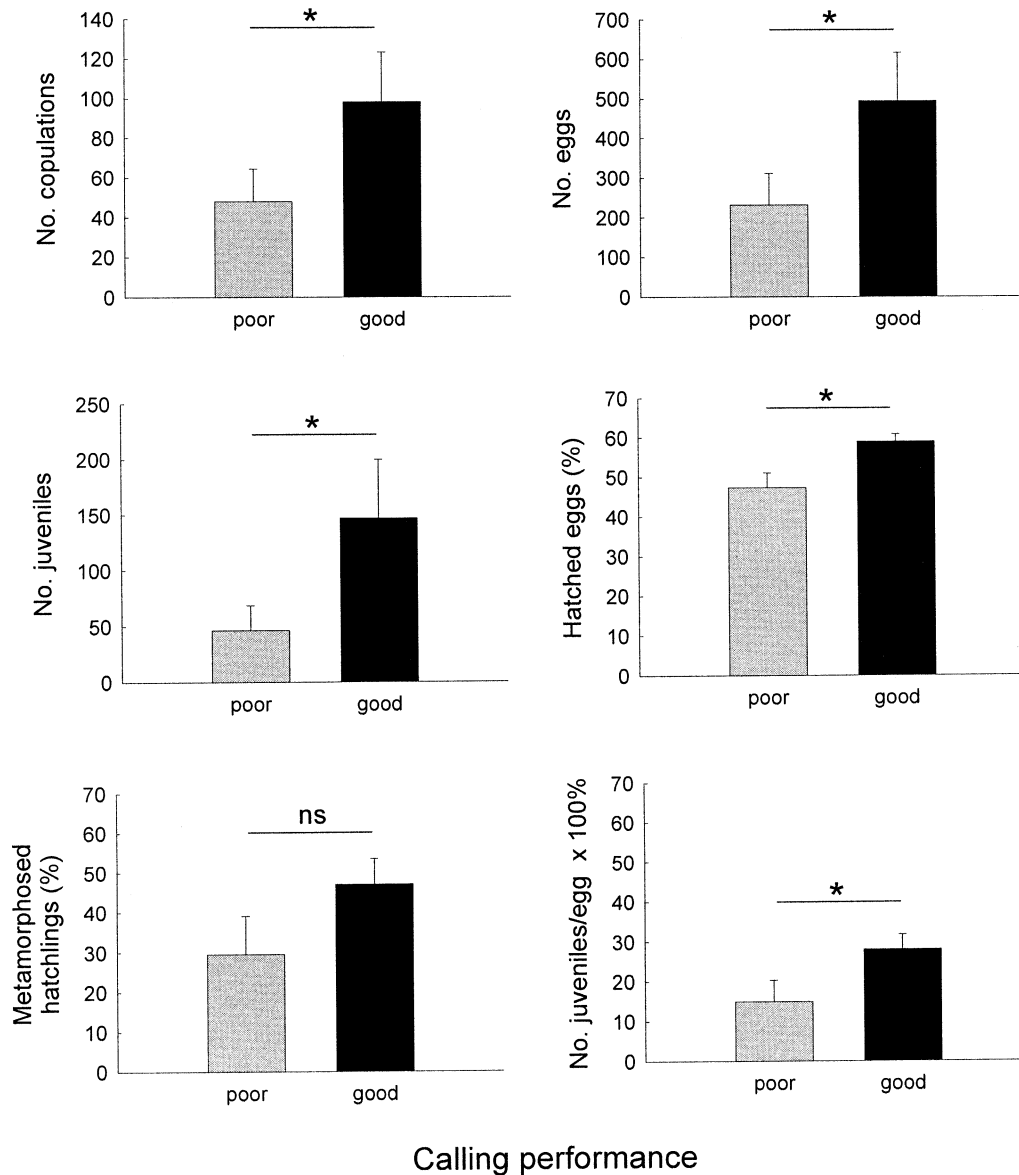


FIG. 3. Comparisons of offspring fitness components between pairs of *Dendrobates leucomelas* males sharing the same terrarium but differing in calling performance (calling rate and chirp duration). Horizontal bars indicate results from paired comparisons *t*-tests. Asterisk indicates $P < 0.05$; ns indicates $P > 0.10$.

the expression of their calls (unless the variation in condition has a genetic basis, as demonstrated by David et al. [2000] and Kotiaho et al. [2001]). Another reason for the large effect size is that our analyses are based on repeated measures of reproductive performance of individuals over four consecutive breeding seasons. The relative influence of random error/environmental noise on the variation in offspring viability among groups of siblings sired by different males is therefore smaller in our study than in studies based on data for a single breeding season. This can be exemplified by the fact that the average correlation between male calling rate and the proportion of eggs that result in live frogs, based on separate estimates for each of the four different breeding seasons, was 0.58 (range 0.39–0.72) in *D. leucomelas* and 0.53 (range 0.42–0.68) in *E. tricolor*. These values are considerably lower

(in both species) than our reported estimates based on pooled data for all seasons (Table 2). To make our findings comparable to the results of the meta-analysis by Møller and Alatalo (1999), we performed a meta-analysis of our eight separate estimates. This yielded an average effects size for proportion live offspring/egg of $z = 0.646$; this value is equivalent to $r = 0.57$ (95% confidence interval 0.45 to 0.67, $n = 8$) and differs significantly from no effect ($t_7 = 9.79$, $P < 0.0001$).

In both our study species the male attends his developing eggs in the leaf litter and then carries the tadpoles on his back to small pools of water (Zimmerman and Zimmerman 1980; Summers 1992; Bartlett and Bartlett 1996). However, our study males were freed of parental care and we raised the tadpoles under solitary identical conditions. Because any

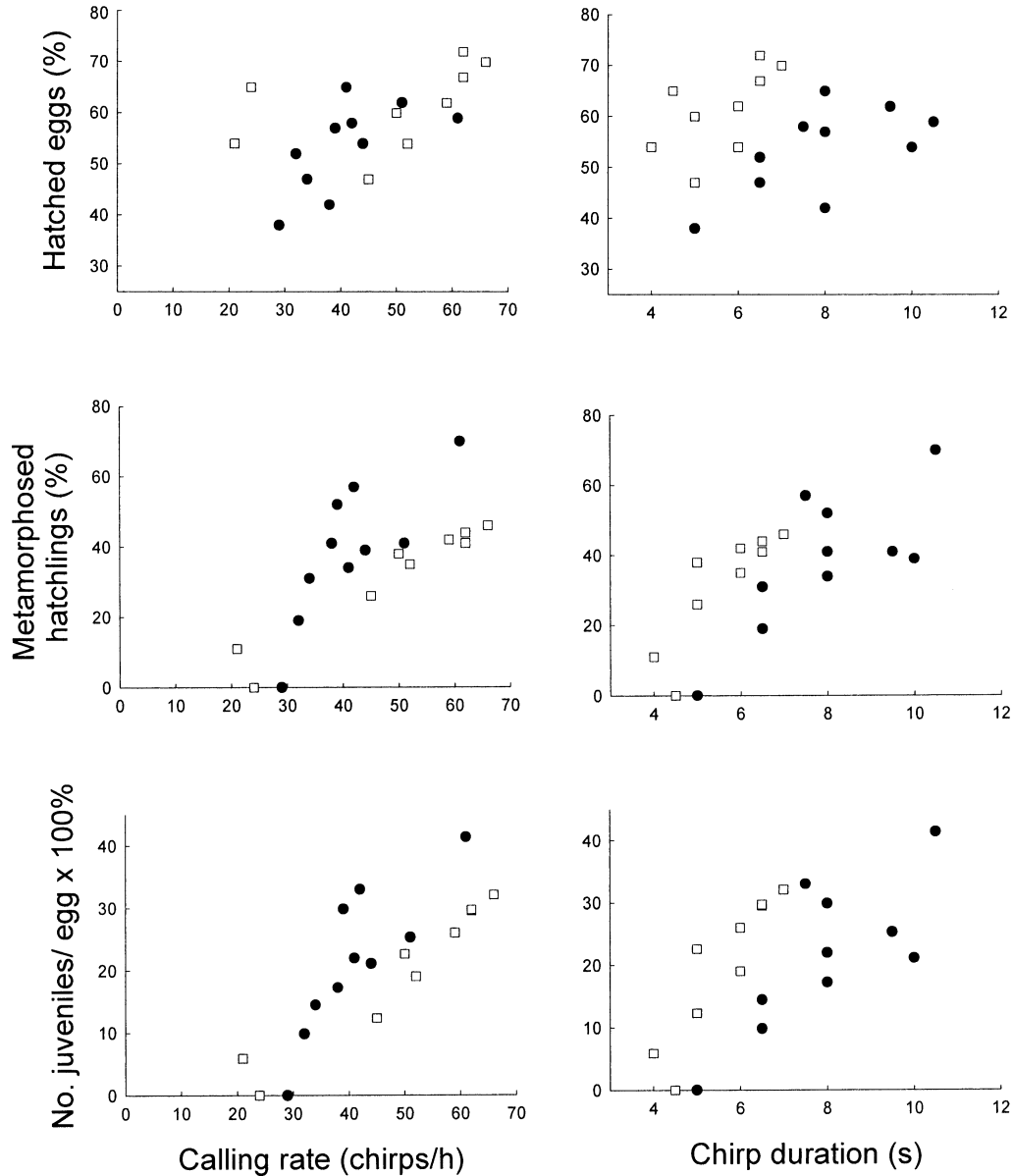


FIG. 4. Associations between male calling performance and offspring fitness components in *Dendrobates leucomelas* (black dots) and *Epipedobates tricolor* (empty squares). The figure shows hatching success, proportion of tadpoles that metamorphosed, and proportion of eggs that resulted in juveniles that survived for at least one month as a function of mean calling rate (left panels) and chirp duration (right panels).

effects mediated via variation among males in quality of parental care are excluded, our results may either under- or overestimate the fitness benefit of mating with high calling performance males. On the one hand, this raises the possibility that additional viability effects of female choice may occur in the wild, particularly if calling performance is positively correlated with the quality of paternal care. Conversely, paternal care may mask intrinsic good genes effects if the amount or quality of care is negatively correlated with male attractiveness. There is some evidence suggesting that polygyny may reduce offspring fitness in poison frogs with male parental care. For example, in a study by Summers (1990), polygynous males of the green poison frog (*D. auratus*) deposited more than one tadpole in the same pool of water. The

resulting increase in tadpole density had a strong negative effect on tadpole growth and survival. The fitness benefit of mating with high calling performance males may thus be reduced, if these males provide poorer paternal care (e.g., as a cost of polygyny). However, the variation in calling performance among years suggests that calling improved with age (although effects of year, rather than age, cannot be disregarded), at least in *D. leucomelas* (Fig. 1). This raises the possibility that female poison frogs who prefer males with more elaborate calls in the wild may end up mating with older males that not only have already proven their ability to survive but also may be more experienced and better providers of care (see also Proehl 2003).

In conclusion, our results are consistent with the notion

that female poison frogs prefer to mate with good callers because calling performance is a reliable predictor of offspring viability. We have no information on heritability of calling characteristics of poison frogs. However, given the strong positive phenotypic correlation between calling rate and chirp duration, it is unlikely that these two aspects of calling performance will evolve independently. Selection thus should favor evolution of both higher calling rate and longer call duration. That we cannot partition good genes effects and maternal effects does not affect this conclusion. Differential maternal allocation can be regarded as an additional effect of parental genes expressed in his offspring that will act as an amplifier of good genes (Sheldon 2000) and thus enhance evolutionary modifications of calling characteristics in male poison frogs in response to female behavior.

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