A Quantitative Genetics Study of *Agalychnis callidryas*

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Abstract

Mate choice is based on a combination of traits that may include body size, calls, and territory quality. In Belizean red-eyed treefrog populations, *Agalychnis callidryas,* large males have a mating advantage and they sire larger tadpoles. To determine whether similar conditions exist in other *A. callidryas* populations, we manipulated controlled matings and quantified the resulting offspring. General research objectives were: 1) to determine if a large-male mating advantage exists in Panamanian populations and 2) to investigate maternal and paternal effects on egg clutches and the growth and development of tadpoles. Each female was paired with two males and each male with two females to obtain tadpoles from half-sibships. Individual tadpoles were raised to metamorphosis in cages suspended in the pond. We analyzed maternal and paternal effects on larval performance and growth rate. Amplectant males were significantly larger than non-amplectant males suggesting female preference for large males. Maternal effects were present in the early stages of offspring development in the

form of a female size-fecundity relationship, while paternal effects were evident in later stages of tadpole growth and development.

Introduction

Sexual selection, one of the driving forces of natural selection, also drives the microevolutionary process towards specific genetic traits (Mitchell 1990). Sexual selection can generally be broken down into two categories: intrasexual selection and intersexual selection. Intrasexual selection involves males that compete with each other using weapons such as claws, fangs, or large body size to gain an advantage or access to females. Intersexual selection involves females choosing males based on characteristics such as body size, access to resources, or ornaments (Berven 1981; Andersson 1986). When the female chooses males that do not give her a direct benefit, such as resources or protection, the reasons for her choice of that specific male are less clear (Mitchell 1990; Sheldon et al. 1997; Welch 2003). One hypothesis seeks to explore whether females benefit when they select mates based on characteristics such as ornaments or size. This "good-genes hypothesis" suggests that females select males whose phenotypic characteristics are indicators of genetic quality. The female indirectly benefits because the increased genetic quality and fitness of the males can be passed to offspring and ensure higher survival rates or superior larval performance (Andersson 1986; Woodward et al. 1988; Mitchell 1990; Howard et. al. 1994; Welch 2003).

Anurans exhibit a wide variety of mating preferences and are therefore excellent models for testing the good-genes hypothesis. In many anuran species, there is a large-male mating advantage (Berven 1981; Howard et al. 1994; Tárano & Herrera 2003; Benard 2007; Briggs 2008). Moreover, large males sire large tadpoles (Mitchell 1990; Altwegg & Reyer 2003; Briggs unpubl.), and large metamorphs have an increased chance of

survival (Travis et al. 1987; Mitchell 1990; Altwegg & Reyer 2003; Welch 2003). Also, previous studies show that large male size positively correlates with large metamorph size, a factor that may increase fecundity and the chances of successfully finding a mate (Berven 1987; Travis et al. 1987; Altwegg & Reyer 2003). Females may choose larger mates because large size may indicate more experience, a higher fertilization rate, more access to resources, and an overall increase in survival rate and fitness (Berven 1981; Briggs 2008).

Red-eyed treefrogs, *Agalychnis callidryas*, are hylids that exhibit non-random mating (Briggs 2008). Studies in Belize have found that a large-male mating advantage exists along with little to no male-male competition (Briggs 2008), and that large males sire larger hatchlings and metamorphs (Briggs unpubl.). This project tests the good-genes hypothesis and whether a large-male mating advantage exists in Panamanian populations. We test the effects of a potential female preference for large male size on tadpole growth and development. Measures of egg yolk diameter determine how much energy each female invested in her eggs and help to determine the maternal effects on tadpoles. Testing for paternal effects on tadpoles involved calculating offspring performance between large and small males. The study also compared hatching success and the growth rates and development of the tadpoles.

Methods

Study Organism

Red-eyed treefrogs, *Agalychnis callidryas*, are Neotropical hylids with a habitat ranging from Mexico to western Ecuador (Briggs 2008). An arboreal and nocturnal species, *A. callidryas* are prolonged breeders (Donnelly & Guyer 1994) that become active during the rainy season, which typically lasts from May to December (Warkentin 1999). Males aggregate

around ponds and call to females at the onset of the season (Briggs 2008). Females are larger than males (see results) and typically lay around two hundred eggs in two to three egg clutches in one night. After females lay their eggs, they are not ready to mate again for the following two months. The clutches are oviposited on the underside of leaves that overhang standing pools of water or temporary ponds (D'Orgeix & Turner 1995; Briggs 2008). Tadpoles hatch within six days and reach metamorphosis in two to three months (Warkentin 1999).

Study Site

Research was conducted at the Smithsonian Tropical Research Institute in Gamboa, Panama. The station is located in lowland tropical rainforest on the bank of the Panama Canal and Chagres River, thirty kilometers north of Panama City (Smithsonian Tropical Research Institute). All *Agalychnis callidryas* used in this study were collected and observed at Experimental Pond, Gamboa.

Experimental Design

A half-sibling design was used to test maternal and paternal effects on tadpoles. Twenty *A. callidryas* females and 19 males were collected during the month of July between 21:00 and 1:00. Fourteen amplectant pairs, six gravid females and five lone males were collected. Individuals were paired at random in the laboratory. Each female was paired with two males and each male with two females, making 40 crosses. The pairs were housed in 3-gallon plastic containers with approximately 3 mm of water to allow each female to refill her bladder while laying clutches. Pairs were checked after one hour using a red light filter to minimize disturbance. Once a sub-clutch was oviposited and the female lowered to the water to refill her bladder, the male was removed and replaced with another male.

The following morning, the mass of adult *A. callidryas* was measured

using a Pesola micro-line spring scale, and snout-vent length (SVL) was measured using dial plastic calipers. Eggs of each sub-clutch were counted and then transferred to leaves and placed in cups containing aged tap water. Eggs were misted regularly to prevent desiccation. Ten eggs per sub-clutch were haphazardly chosen and ovum diameter was measured at 8X magnification using a Zeiss Stemi DV4 microscope. Those measurements were the basis for calculating average ovum diameter per female and per pair.

Once each tadpole in a clutch hatched, they were introduced to the pond before they began feeding (1-3 d post-hatching). Tadpoles were individually raised in 4.5 L mesh cages in the pond ($N = 396$). There were ten replicates per sib-ship. The tadpoles were allowed to develop naturally in their cages until metamorphosis. Tadpoles were exposed to the same variables (water temperature, depth, and climate) and fed on phytoplankton that filtered through the cages. To measure growth rate and development, tadpoles were taken out of the pond, photographed using a Nikon D40 digital camera, and SVL and total length were measured every three weeks using NIH ImageJ software. To determine their age, each tadpole was staged using the Gosner (1960) developmental stage.

Results

Size of Males

The mean mass of males was 3.40 ± 0.39 g and mean SVL of males was 44.78 ± 1.78 mm ($N = 235$). The data revealed a strong positive correlation between male mass and SVL (Pearson's rank correlation: $r = 0.602$, $N = 235$, $P < 0.001$). Mean mass of non-amplectant males was 3.39 ± 0.40 g and mean SVL was 44.61 ± 1.85 mm ($N = 158$). Mean mass of amplectant males was 3.41 ± 0.39 g and mean SVL was 45.13 ± 1.57 mm ($N = 77$). Amplectant males were significantly larger than non-amplectant males

based on SVL ($F = 5.56$, $df = 233$, $P = 0.019$) but not based on mass ($F = 0.01$, $df = 233$, $P = 0.912$). Males were conservatively categorized into two categories: larger than the average SVL and smaller than the average SVL. Using a paired t-test we found that the SVL of categorical large males was significantly larger than that of small males ($t = 3.12$, $df = 17$, $P = 0.006$).

Size of Females

Mean mass of females was 7.66 ± 1.03 g and mean SVL was 57.50 ± 2.72 mm ($N = 230$). There was a strong positive correlation between female mass and SVL (Pearson's rank correlation: $r = 0.623$, $N = 230$, $P < 0.001$).

Maternal Effects and Investment

The data revealed a relationship between female SVL and the number of eggs (Pearson's rank correlation: $r = 0.239$, $N = 88$, $P = 0.025$; Figure 1) as well as a relationship between female mass and the number of eggs (Pearson's rank correlation: $r = 0.238$, $N = 76$, $P = 0.038$), indicating a female size-fecundity ratio. Larger females produced more eggs.

The mean ovum diameter per female was 2.54 ± 0.43 mm, and the mean ovum diameter per pair was 2.55 ± 0.45 mm. To investigate whether ovum diameter differed among females or among pairs, a Chi square test showed that no significant difference existed among females or pairs (females: $\chi^2 = 41.74$, $df = 56$; pairs, $P = 0.922$: $\chi^2 = 62.22$, $df = 57$, $P = 0.296$). A one-way ANOVA tested whether the first and second clutch differed in ovum diameter, and revealed no statistical difference between the two clutches ($F = 2.45$, $df = 1378$, $P = 0.870$).

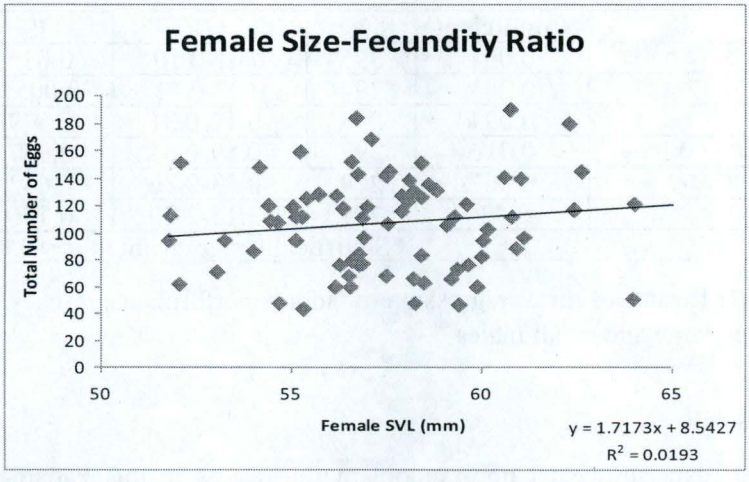

Figure 1: Results of Pearson's correlation of femail size-fecundity relationship.

Paternal Effects

Calculating the ratio of the number of hatchlings to the number of eggs oviposited in each clutch determined the rate of hatching success and allowed comparisons between males. A paired t-test showed that no significant difference in hatching success existed between males ($t = 1.11$, $df = 54$, $P = 0.270$).

Linear regression tested for relationships between the total length and SVL of tadpoles and male size at three points: the hatchling stage, three weeks post-hatching, and six weeks post-hatching (Table 1). Hatchlings of small males had a 0.05 mm increase in size when compared with hatchlings of large males (95% CI: 0.005-0.102, $P = 0.030$, Table 1).

Trait	Coefficient	N	CI	P
Hatchling SVL	0.054	395	0.01-0.10	0.03*
Hatchling total	0.243	394	0.07-0.41	0.005*
SVL wk 3	0.094	297	-0.12-0.31	0.379
Total wk 3	0.016	297	-0.59-0.62	0.957
SVL wk 6	0.067	279	-0.23-0.36	0.652
Total wk 6	-0.430	281	-3.15-2.29	0.756
*Statistically significant at $P < 0.05$				

Table 1: Results of linear regression of tadpole performance traits between large and small males

Discussion

Our data show that non-random mating exists in this Panamanian *Agalychnis callidryas* population because there is evidence for a large-male mating advantage. Amplectant males were larger than non-amplectant males. Although males do compete with each other in Panamanian populations, we hypothesize that there is still opportunity for female choice. Our findings are consistent with the findings of studies focused on other anuran species (Berven 1981; Howard et al. 1994; Tárano & Herrera 2003; Benard 2007).

An exploration of maternal effects in this study was limited to female yolk investment, but data revealed no differences in ovum diameter among females and among pairs. Larger females, however, have an advantage over smaller females because they are more fertile and oviposit more eggs. We focus on potential paternal effects later in tadpole development. Previous studies in anurans have shown that maternal effects are visible during pre-hatchling development (Crump & Kaplan 1979; Kaplan 1989) and tend to decrease with further development. Studies also show that effects of dam and sire may affect different stages of offspring development (Bonduriansky & Head 2007).

The data revealed no significant difference in hatching success between large and small males. In the current study, the eggs developed in the laboratory instead of being exposed to natural conditions. Therefore, all eggs were exposed to the same amount of moisture and light and were protected from predation and climatic factors. Although we did not observe a difference in hatching success, it is possible that if the eggs were exposed to natural conditions, the hatching success would differ between large and small males because of differences in quality.

We found that hatchlings from small males were larger than hatchlings from large males. Although many studies have shown that larger hatchling size is beneficial, small hatchling size does not always mean that the hatchlings are less fit or have a disadvantage. Studies on the common frog, *Rana temporaria,* Laurila & Kujasalo, suggest that anurans exhibit phenotypic plasticity during metamorphosis in response to environmental conditions such as desiccation and predation. The authors conclude that although predator cues in the pond delay tadpole development, they metamorphose later but at a larger size (1999). Further analysis needs to be done on our data to find out whether small hatchling size is advantageous later in development and at metamorphosis.

Results showed no difference between half-siblings at week three and week six measurements, but this may be due to the fact that the tadpoles developed at different individual rates in the pond. Therefore, tadpole length may no longer be an accurate determinant of a tadpole's "age." During the complex life cycle of anurans, tadpoles start out very small, grow into fairly large metamorphs, and then their length is reduced when they become froglets as they resorb their tails (Gosner 1960). Therefore, it is possible that a smaller metamorph may have actually been farther along in development than a larger metamorph if it was closer to becoming a froglet and had begun to resorb its tail. This indicates that further analysis

needs to be done to compare the size of the froglets when they emerged from the pond. The size of the froglet will more accurately determine whether the paternal effects were significant and whether female choice for large males supports the good-genes hypothesis for enhanced offspring performance.

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