



Do big dads make big babies? Paternal effects on larval performance in red-eyed treefrogs of Belize (*Agalychnis callidryas*, *A. moreletii*)

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Under the good genes model of sexual selection, females prefer males with attributes that signify high genetic quality to improve offspring fitness. This study tested the hypothesis that female mating preferences result in enhanced offspring performance, owing to genetic variation among sires in two species of red-eyed treefrogs (*Agalychnis callidryas* and *A. moreletii*). The study provides evidence for paternal size effects on offspring traits, most notably on hatchling size, larval duration and size at metamorphosis. Large males of both species produced larger hatchlings relative to half-sibs. Larger hatchlings may have immediate growth and survival advantages that propel them through the larval period and suggests the potential for increased post-metamorphic fitness benefits. Large males of *A. callidryas* sired larger froglets and there were positive correlations between sire size and froglet traits for both species. Tadpoles of both species that remained in a longer larval period emerged from metamorphosis at a greater size. The two species share similar life history traits, but considerable inter-specific differences of paternal effects between *A. callidryas* and *A. moreletii* may help to explain the variation in breeding behaviour and tadpole biology within these two species. In this system, females may exercise directional selection for offspring at a larger metamorphic body size that will increase her fitness through enhanced survival and reproductive success.

Key words: *Agalychnis callidryas*, *A. moreletii*, female choice, good genes, sexual selection, tadpole traits

INTRODUCTION

Sexual selection theory suggests that the variation in male characters is largely responsible for shaping female choice patterns and, as a consequence, female fitness (Darwin, 1871; Trivers, 1972; Andersson, 1994). The evolution of female choice has been clearly demonstrated to be driven by the immediate fitness benefits gained by the female. In these systems, males provide direct benefits to ensure the female's survival and/or the survival of her offspring in the form of food, territory, oviposition sites, nuptial gifts and paternal care (reviewed in Andersson, 1994). Females choose males offering superior resources to increase reproductive success. However, in non-resource based systems or those systems that lack conventional forms of paternal provisioning, the maintenance of female preference is less clear because males only contribute sperm. In this case, females can only receive the indirect benefits of genes transmitted to her offspring (Maynard Smith, 1978).

Two models have been proposed to maintain female preference for indirect benefits. The Fisherian process (Fisher, 1930) suggests that female choice is directed toward heritable male traits where the choosiest female mates with the most attractive male and there is fixation

of male trait and female preference alleles (Trivers, 1972). Under the "good genes" or "viability indicator" model of sexual selection, females prefer males with particular fitness characteristics that signify high genetic quality that will improve offspring survival (Zahavi, 1975; Andersson, 1986; Sullivan et al., 1995). Much work has been done to test theories of female mate choice and there is a solid body of empirical evidence for female preference for indirect benefits of enhanced offspring viability across taxa (Woodward, 1987; Moore, 1994; Alatalo, et al. 1998; Bonduriansky & Head, 2007), suggesting that there must be an inherent mechanism to detect quality and ensure the heritability of fitness traits.

Neotropical hylids offer an excellent system to test hypotheses based upon the "good genes" or "viability indicator" model because males of most species do not hold territories, provide no parental care, contribute only sperm to reproduction, and fertilization is external. Frogs undergo indeterminate growth and a large body size may signify high genetic quality and indicate faster growth rate, better competitive ability or longer life expectancy (Wilbur et al., 1978). Previous research documents a large-male mating advantage where larger males are more often found in amplexus relative to smaller males (reviewed in Sullivan et al., 1995; Briggs, 2008) or females respond preferentially to vocalizations of large males

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(reviewed in Sullivan & Kwiatowski, 2007). As a result, females that mate with large males could produce more offspring or superior offspring relative to those sired by males of smaller size (Maynard Smith, 1978).

In anurans, the larval period provides discrete time points and/or tangible anatomical features that can be measured as indicators of growth and development that may be affected by paternity (Altwegg & Reyer, 2003). The larval period is a critical stage where mortality can be catastrophic (Wassersug, 1973) and offspring with enhanced performance traits as larvae may possess survival advantages that carry over to the post-metamorphic stage (Altwegg & Reyer, 2003). These advantages may account in part for variation evident in body size and size-related acoustic variation in the calls of adult frogs. Female mating preferences, then, may be based on male traits that correlate with larval performance measures. Thus, larval performance may be an index of fitness and provide a link to adult reproductive success.

This study tests the hypotheses based on the “good genes” model of a female preference for large males in two species of Neotropical hylids, *Agalychnis callidryas* and *A. moreletii*. Previous work on the mating system of these two species indicate that there is opportunity for female choice in the study population because there is a male-biased sex ratio, there is no scramble competition for females, females move among calling males and initiate amplexus, females choose oviposition sites that are not related to male calling sites, males do not

provision females and there is a seasonally-dependent large-male mating advantage (Briggs, 2008). These species coexist in only a few areas, which provides a unique opportunity to compare two related hylids that share similar life history traits but who differ in time of emergence and in acoustic traits (Briggs, 2008). Tadpoles of both species are found together but depending on the length of the rainy season, one species dominates natal ponds (Briggs, 2008) and may have an effect on growth and metamorphosis. These species offer model systems to investigate female preferences for “good genes” based on body size and the opportunity to investigate whether there are consequences of female choice, and potential differences between both species.

Experiments were conducted on both *A. callidryas* and *A. moreletii* to investigate maternal investment as i) total number of eggs produced, ii) number of eggs produced per sub-clutch or half-sib sired by different males and iii) yolk provisioning measured as ovum diameter. The consequences of female choice for larval performance traits were examined using clutch-based performance measures: i) fertilization success of egg clutch and ii) hatching success of egg clutch. To investigate whether sire body size results in enhanced offspring viability, i) larval growth, ii) larval development, iii) survivorship to metamorphosis, and iv) size at metamorphosis were measured. In a split sib design, tadpole performance measures are investigated for sire size correlates as an indicator of “good genes”.

Table 1. Summary of descriptive data for *Agalychnis callidryas* and *A. moreletii*

Measure	<i>n</i>	Range	Mean±1SE
<i>Agalychnis callidryas</i>			
Female SVL (mm)	45	47.6–65.6	57.3±1.80
Female Mass (g)	45	6.2–11.9	9.7±0.16
Total Egg Clutches	39	87.0–235.0	151.8±8.53
No. of Eggs/clutch for first half-sib	39	19.0–109.0	87.2±4.66
No. of Eggs/clutch for second half-sib	39	45.0–190.0	49.1±2.85
Male SVL (mm)	37	40.9–50.3	46.8±0.26
Male Mass (g)	37	2.0–5.6	4.38±0.08
Hatchling SVL (mm)/half-sib	39	3.8–9.6	6.9±0.05
Hatchling Total Length (mm)/half-sib	39	12.5–25.3	19.5±0.14
Hatchling Mass (g)/half-sib	39	0.02–0.16	0.08±0.002
<i>Agalychnis moreletii</i>			
Female SVL (mm)	34	58.2–85.0	76.8±0.80
Female Mass (g)	34	16.2–30.9	19.6±0.5
Total Egg Clutches	34	93.0–334.0	200.4±9.30
No. of Eggs/clutch for first half-sib	52	12.0–139.0	76.0±3.86
No. of Eggs/clutch for second half-sib	52	18.0–218.0	94.0±6.20
Male SVL (mm)	50	68.0–107.1	79.2±0.08
Male Mass (g)	50	4.9–7.3	5.9±0.37
Hatchling SVL (mm)/half-sib	52	5.6–9.9	7.2±0.03
Hatchling Total Length (mm)/half-sib	52	12.2–25.1	19.1±0.08
Hatchling Mass (g)/half-sib	52	0.03–0.18	0.08±0.001

MATERIALS AND METHODS

Individuals were collected from breeding aggregations at Las Cuevas Research Station (16°43"N, 88°59"W), Cayo District, Belize, during the hours of 2200–0100 on 26 nights between 8 June and 25 August 2005 (*A. callidryas*: 20 amplexant pairs, 7 gravid females (dams), 17 calling non-amplexant males (sires); *A. moreletii*: 27 pairs, 7 gravid females, 23 calling non-amplexant males). Pairs were housed in suitable chambers with standing water and leaflets of *Chaemadora elegans* palm as oviposition sites. Non-amplexant males were measured for body size (snout-vent-length, SVL) and were categorized each as $\pm 1SD$ relative to annual mean male body size. Each non-amplexant male was housed separately.

Amplexant pairs were monitored at 30 minute intervals under red light until the first clutch of eggs was deposited (between 2200–0100 hours similar to natural mating times in the wild). When the female descended to uptake water for the remaining eggs and the pair was separated, the female was rinsed with pond water to remove any remaining sperm, and the original male was replaced with a non-amplexant male of a different relative size. The second pair was left to complete egg oviposition (between 0200–0400 hours). In all cases, females were paired with two males resulting in half-sibs. There were a total of 39 and 52 maternal half-sibs from *A. callidryas* and *A. moreletii*, respectively, for the complete quantitative study.

Individuals were measured and eggs were counted per half-sib. To determine yolk provisioning, egg diameter was measured the morning after oviposition before cleavage begins (Gosner, 1960). Egg clutches were suspended above standing water and misted daily until hatching (6–7 days). Half sibs were housed in separate containers and reared at 15 tadpoles per litre. Tadpoles were fed a standard amount of 0.1 g chow and 2 g of leaf litter per litre of water every other day. Leaf litter was collected from the natal pond and used as supplemental feeding to optimize general growth conditions (Fenolio, 1996). Rearing containers were cleaned regularly.

Maternal investment was measured as the i) total number of eggs oviposited, and a female size-fecundity relationship was calculated using a Pearson's product moment correlation coefficient analysis (Pearson's r). A one-way analysis of variance (ANOVA) was used to detect differences in maternal investment in ii) the number of eggs produced per half-sib and sired by two males. Yolk provisioning was measured as iii) ova diameter for each half-sib and a Pearson's r was used to determine whether there was a relationship between the body size of each female (SVL and mass) and ova diameter.

Fertilization success was scored as the proportion of healthy eggs to total oviposited eggs 12 hours after deposition occurred. Unfertilized eggs become pale yellow with dark areas after 12 hours and have a dimpled yolk surface and collapsed egg capsules (Briggs, 2008).

i) Fertilization success and ii) hatching success were scored and compared for each half-sib using a one-way ANOVA. A Pearson's r was used to examine whether hatchling traits (SVL, total body length, and mass) were

correlated with sire body size (SVL).

Larval performance traits such as: i) larval growth and ii) larval development every 15 days until metamorphic climax were compared between large and small sized sires when mated to the same female. Larval growth was calculated after one week (14 day - 7 day) and after one month (30 day - 7 day); larval development was measured as larval duration, survivorship and size at metamorphosis. Tadpoles ($n=10$ per half-sib) were measured for SVL, total body length and mass and staged for development using the Gosner (1960) anuran staging table. At Gosner stage 40–41, when tadpoles undergoing metamorphosis climbed above the water level each was reared individually henceforth. The number of tadpoles that completed metamorphosis into froglets was scored as a proportion of the original clutch for larval performance traits between sires for iii) survivorship to metamorphosis and measured for iv) size at metamorphosis (SVL, mass). All metamorphosed froglets and remaining tadpoles were returned to natal ponds at Las Cuevas.

A General Linear Model (GLM) two-way multivariate analysis of variance (MANOVA) was used to investigate the overall effects of sire body size (large vs. small), the identity of dam and the interaction effects of sire size within dams on offspring traits (i.e. SVL, total body length, mass). The Pillai-Bartlett's trace criterion is suggested to be the most robust statistic for general protection against departures from univariate normality and homogeneity of variance-covariance matrices (Gill, 2001). Therefore Pillai-Bartlett's trace criterion (V) and its F approximation values are reported. Where main effects were detected, follow-up univariate or between subject-effects F -tests were subsequently conducted to locate the origin of potential differences within maternal half-sibs (Sokal & Rohlf, 1995). All data analyses were conducted using SPSS for Windows v. 19.0 (SPSS Inc., Chicago, IL).

RESULTS

Maternal Investment

Agalychnis callidryas

Adult female body size and resulting egg clutch sizes are listed in Table 1. Females produced entire egg clutches similar to what is reported in the wild (Table 1, Briggs, 2008). However, between half-sibs, egg clutch size differed between the first and second mate independent of sire body size. A greater number of eggs were oviposited with the first mate than with the second mate ($F=48.72$, $df=1$, $p<0.001$; Table 1). There was no female size-fecundity relationship for SVL (Pearson's $r=0.04$, $n=27$, $p=0.86$) or mass ($r=0.29$, $n=27$, $p=0.14$), but there were insufficient clutches measured for ova diameter to warrant analyses of variable yolk provisioning in this species.

Agalychnis moreletii

Adult female body sizes and resulting egg clutches are listed in Table 1. Of half-sibs, mean clutch size differed between first and second mate independent of sire body size ($F=6.05$, $df=1$, $p=0.01$). The second sub-clutch of eggs was larger on average than the clutch sired by the

Table 2. Clutch based paternal effects on half-sibships of *Agalychnis callidryas* and *A. moreletii*

Character	Mean±SE		Test of Between-Subject Effects		
	Small sire	Large sire	MS	F	p
<i>Agalychnis callidryas</i>					
Fertilization success (%)	98.3±1.2 (29)	96.5±1.3 (26)	34.166	0.52	0.911
Hatching success (%)	40.6±5.52 (47)	40.9±5.74 (44)	10.455	0.008	0.931
<i>Agalychnis moreletii</i>					
Fertilization success (%)	100.0±0.08 (35)	100.0±0.08 (32)	0.028	0.14	0.715
Hatching success (%)	32.1±3.79 (55)	36.9±3.75 (56)	793.881	1.129	0.296

first mate. There was no size-fecundity relationship for female SVL (Pearson's $r=0.32$, $n=34$, $p=0.064$) or mass ($r=0.24$, $n=34$, $p=0.177$). Mean ovum diameter was 4.9 ± 0.03 mm ($n=34$ clutches) with a range of 3.8 to 7.7 mm but there was no difference in yolk provisioning in ovum size between first and second mates ($F=0.203$, $n=34$, $p=0.655$). Maternal body size was not correlated with mean ova diameter for SVL (Pearson's $r=0.202$, $n=34$, $p=0.776$) or mass ($r=-0.07$, $n=34$, $p=0.761$).

Larval Performance Traits

Fertilization and Hatching Success

Adult male sizes are listed in Table 1. Male body size (SVL) were compared to the annual mean male body size of 46.7 ± 1.99 mm ($n=54$) for *A. callidryas* and 79.8 ± 0.91 mm ($n=74$) for *A. moreletii* to be categorized as larger or smaller males during pairing trials. Fertilization success of both *A. callidryas* and *A. moreletii* (Table 2) showed no difference in the number of eggs that were successfully fertilized between large and small sized males when mated to the same female. Both species yielded high fertilization rates, with very few clutches not completely fertilized. Similarly, hatching success between maternal half-sibs was not affected by sire size in either *A. callidryas* or *A. moreletii* (Table 2).

Hatchling Traits

Agalychnis callidryas. Hatchling traits (Table 1) were positively correlated with sire body size (Pearson's $r_{svl}=0.198$, $p<0.001$; $r_{total}=0.287$, $p<0.001$; $r_{mass}=0.188$, $p<0.001$). GLM MANOVA results revealed a significant multivariate main effect for sire body size on hatchling traits and a significant interaction effect between sire size and dam identity (Table 3), suggesting that sire size affects hatchling traits within maternal half-sibs. Given the significance of the overall test, follow-up univariate tests also demonstrated significant differences in all hatchling traits (SVL, total length and mass) when maternal half-sibs were compared by sire size to reveal that larger males sired larger, longer and heavier hatchlings (Table 4).

Agalychnis moreletii. Hatchling traits (Table 1) were positively correlated with sire SVL (Pearson's $r_{svl}=0.204$, $p<0.001$; $r_{total}=0.263$, $p<0.001$; $r_{mass}=0.304$, $p<0.001$). GLM MANOVA results revealed a significant multivariate main effect for sire body size on hatchling traits (Table 3) and a significant interaction effect between sire size and dam identity (Table 3). Univariate tests (Table 4) indicate a non-significant trend for large males to sire

bigger hatchlings as measured by SVL and for smaller males to sire significantly longer hatchlings (measured as total length). Hatchling mass was not affected by sire size (Table 4).

Larval Growth, Development, and Metamorphic Climax

Agalychnis callidryas. At one week post-hatching (14 day) MANOVA results showed that there were no paternal size effects on tadpole size, but there was a significant sire size \times dam interaction effect (Table 3). Growth rate within the first week displayed main effects for sire body size and an interaction effect (Table 3). Follow-up univariate tests showed that tadpoles of half-sibs were similar in body size after the first week of growth, but tadpoles sired by small males grew faster than their half-sibs in SVL but not mass, during this first week of development (Table 4). There was a sire size effect and an interaction effect on tadpole growth within the first month (Table 3). Tadpoles of small males were greater in SVL but half-sibs of large males were heavier as seen from the follow-up univariate tests (Table 4).

There were significant paternal effects on offspring traits in newly metamorphosed froglets but there were no statistically significant interactions between sire size and dam (Table 3), suggesting that larger males sired tadpoles that had a significantly longer larval period than tadpoles sired by smaller males when nested by dam (Table 4). Within maternal half-sibs, larger males did not sire a significantly greater number of survivors to reach metamorphosis (Table 4), however, large males tended to sire froglets that were both larger and heavier than their counterparts. Only SVL measurements at metamorphic climax were significantly different between maternal half-sibs (Table 4).

Froglet size and froglet mass were positively correlated with larval duration (Pearson's $r_{svl}=0.300$, $n=336$, $p<0.001$; $r_{mass}=0.325$, $n=336$, $p<0.001$), suggesting that a longer larval period produced bigger froglets at metamorphosis. Froglet traits and larval period were positively correlated with sire body size measured as SVL (Pearson's $r_{svl}^{froglet}=0.275$, $p<0.001$; $r_{froglet\ mass}^{froglet}=0.366$, $p<0.001$; $r_{larv\ per}^{froglet}=0.187$, $n=336$, $p<0.001$, respectively).

Agalychnis moreletii. At one-week post-hatching, sire body size did not affect tadpole body size nor was there a sire size \times dam interaction effect (Table 3); univariate tests for hatchling traits of maternal half-sibs also failed to show any associations with sire size (Table 4). Growth rates within the first week of development were similar between half-sibs and there was no apparent sire size

Table 3. MANOVA summary table - overall effects on larval performance traits of *Agalychnis callidryas* and *Agalychnis moreletii* (*Significant at 0.05 level, ***Significant at 0.001 level).

Effect	<i>Agalychnis callidryas</i>				<i>Agalychnis moreletii</i>			
	Pillai's V	df	F-value	p-value	Pillai's V	df	F-value	p-value
Hatchling Traits								
<i>Sire size</i>	0.081	3, 307	9.031	<0.001***	0.064	3, 475	7.606	<0.001***
<i>Dam</i>	1.121	16, 321	11.961	<0.001***	1.881	2, 493	19.022	<0.001***
<i>Sire size x Dam</i>	0.584	36, 927	6.222	<0.001***	0.427	48, 1431	4.953	<0.001***
Larval Growth Traits								
Tadpole size 14d								
<i>Sire size</i>	0.001	2, 322	0.156	0.856	0.006	2, 85	0.654	0.521
<i>Dam</i>	2.969	16, 281	13.501	<0.001***	0.078	23, 488	0.857	0.082
<i>Sire size x Dam</i>	0.279	30, 646	3.498	<0.001***	0.113	18, 221	1.466	0.098
Growth Rate week 1								
<i>Sire size</i>	0.203	2, 322	41.039	<0.001***	0.012	2, 85	1.594	0.205
<i>Dam</i>	0.012	16, 314	0.134	0.432	0.021	23, 486	0.413	0.215
<i>Sire size x Dam</i>	0.908	30, 644	17.923	<0.001***	0.069	20, 234	0.908	0.577
Growth Rate month 1								
<i>Sire size</i>	0.227	2, 231	33.926	<0.001***	0.001	2, 89	0.111	0.953
<i>Dam</i>	0.003	16, 309	0.094	0.367	0.058	23, 456	0.494	0.087
<i>Sire size x Dam</i>	0.976	20, 464	22.123	<0.001***	0.353	20, 187	6.414	<0.001***
Froglet Traits								
<i>Sire size</i>	0.028	3, 310	2.944	0.033*	0.003	3, 145	0.42	0.739
<i>Dam</i>	0.001	16, 278	0.002	0.146	0.001	23, 393	0.001	0.112
<i>Sire size x Dam</i>	0.132	30, 936	1.434	0.062	0.133	60, 387	1.123	0.245

effect or interaction effects (Table 3). Growth of half-sibs was not significantly different within the first month but there was an interaction effect (Table 3). However, follow-up univariate tests were unable to identify the potential source of the variation (Table 4).

At the end of metamorphosis, surviving froglets were similar between maternal half-sibs. There were no significant paternal effects on offspring traits (Table 4). There was, however, a positive relationship between froglet SVL and froglet mass with larval duration, respectively (Pearson's $r_{svl}=0.440$, $n=52$, $p<0.001$; $r_{mass}=0.517$, $n=52$, $p<0.001$). Froglet traits and larval period were similarly correlated with sire SVL (Pearson's $r_{froglet\ svl}=0.148$, $p<0.001$; $r_{froglet\ mass}=0.160$, $p<0.001$; $r_{larv\ per}=0.279$, $n=527$, $p<0.001$). Survivorship to metamorphosis did not differ between large and small sires (Table 4).

DISCUSSION

The results of this study show that paternal factors, encoded in male body size had a greater effect on offspring size in *A. callidryas*, but these effects were diluted in *A. moreletii*. Studies investigating paternal effects on offspring performance reveal that some variation in larval performance is related to variation in paternal size, but has not been consistent across all offspring performance traits or among all species, or populations (Howard, 1978; Travis, 1981; Woodward, 1986, 1987;

Woodward et al., 1988; Mitchell, 1990; Howard et al., 1994; Semlitsch, 1994; Welch et al. 1998). It may be that sexual selection affects the transfer of both dam and sire effects to offspring, but may act on different phenotypic traits and occur at different time points in an offspring's lifetime (Bonduriansky & Head, 2007).

Maternal investment is usually the result of a trade-off between egg size and number (Kaplan, 1980; Kaplan, 1992). In this study, there was variable maternal investment for number of eggs produced per half-sib but not for yolk provisioning; and neither was affected by the size of dam or sire. Average total clutch size in *A. callidryas* is 200 eggs and 300 eggs for *A. moreletii* (Briggs 2008), thus sub-clutch size may be a function of cryptic female choice where the female adjusts clutch size when presented with multiple partners (Reyer et al., 1999); or sub-clutch size may be a function of time where females drop unfertilized eggs (personal observation) to prevent becoming egg-bound that threatens her survival (Duellman & Trueb, 1986). Maternal investment as yolk allocation has been shown to influence size and growth rates during early stages of development (Crump, 1984; Dzininski & Alford 2005), but these effects have also been shown to diminish during the larval period and have no effect later in offspring growth or in frogs at metamorphic climax (Travis et al., 1987; Newman, 1988; Mousseau & Fox, 1998; Altwegg & Reyer, 2003).

Table 4. Univariate ANOVA summary table - larval performance traits of maternal half-sibs between large and small sized sires of *Agalychnis callidryas* and *A. moreletii*. (* Significant at 0.05 level, ** Significant at 0.01 level, *** Significant at 0.001 level)

Character	Mean±SE		Test of Between-Subject Effects		
	Small sire	Large sire	MS	F	p
<i>Agalychnis callidryas</i>					
Hatchling SVL (mm)	6.7±0.04 (157)	7.3±0.04 (182)	5.774	23.675	<0.001***
Hatchling Total (mm)	18.9±0.13 (157)	19.8±0.12 (182)	48.74	21.019	<0.001***
Hatchling mass (g)	0.075±0.001 (157)	0.088±0.001 (182)	0.004	14.211	<0.001***
SVL 14d (mm)	8.1±0.06 (164)	8.4±0.05 (192)	0.01	0.028	0.868
Mass 14d (g)	0.11±0.002 (164)	0.12±0.002 (192)	0	0.212	0.646
SVL Growth week1 (mm)	1.8±0.07 (164)	1.2±0.05 (192)	27.055	50.226	<0.001***
Mass Growth week 1 (g)	0.04±0.003 (164)	0.04±0.002 (192)	0.001	1.109	0.293
SVL Growth month 1 (mm)	2.4±0.09 (126)	2.0±0.07 (130)	6.139	8.968	0.003**
Mass Growth month 1 (g)	0.08±0.004 (126)	0.09±0.003 (130)	0.01	6.505	0.011*
Larval duration (days)	130.2±3.69 (147)	142.2±2.66 (189)	4789.837	7.477	0.007**
Survivorship (%)	30.2±5.33 (14)	42.5±5.79 (12)	716.919	1.978	0.295
SVL at metamorphosis (mm)	17.0±0.22 (147)	17.8±0.16 (189)	8.984	4.101	0.044*
Mass at metamorphosis (g)	0.44±0.31 (147)	0.68±0.22 (189)	1.415	0.321	0.572
<i>Agalychnis moreletii</i>					
Hatchling SVL (mm)	6.8±0.03 (309)	6.9±0.03 (209)	21.342	3.359	0.067
Hatchling Total (mm)	18.2±0.08 (309)	19.1±0.07 (209)	447.672	10.502	0.001**
Hatchling mass (g)	0.07 ±0.001 (309)	0.08±0.001 (209)	0	1.1	0.295
SVL 14d (mm)	8.5±0.05 (141)	8.4±0.05 (100)	0.381	1.294	0.256
Mass 14d (g)	0.13±0.01 (141)	0.13±0.01 (100)	0	0.032	0.858
SVL Growth week1 (mm)	1.07 ±0.05 (167)	1.11±0.06 (110)	1.267	3.199	0.075
Mass Growth week 1 (g)	0.02±0.01 (167)	0.04±0.01 (110)	0.001	0.253	0.615
SVL Growth month 1 (mm)	2.16±0.05 (264)	2.25±0.05 (250)	0.974	1.43	0.232
Mass Growth month 1 (g)	0.12 ±0.004 (264)	0.12±0.004 (250)	0.001	0.457	0.499
Larval duration (days)	120.1±2.27 (277)	121.3±2.47 (250)	87.168	0.128	0.72
Survivorship (%)	53.7±4.13 (27)	54.6±4.49 (22)	118.265	0.279	0.614
SVL at metamorphosis (mm)	18.4±0.11 (277)	18.3±0.12 (250)	0.183	0.112	0.738
Mass at metamorphosis (g)	0.52±0.34 (277)	1.00±0.37 (250)	13.809	0.898	0.344

Here, because fertilization and hatching success were both very high and there was no effect of sire size, females may be selecting mates to increase post-hatching survival where size is more important (Welch et al., 1998). Tadpoles sired by large males demonstrated an immediate growth advantage by emerging at the hatchling stage at a larger size than their half-sibs in both species. In *A. callidryas*, tadpoles sired by small males grew at a steady rate during the first few weeks of development, whereas tadpoles of *A. moreletii* displayed similar growth rates between half-sibs. Growth patterns may be in response to competitive stress that selects for increased growth rate to maintain similar body sizes of siblings (Relyea & Auld, 2005) in an effort to maintain metamorphic synchrony (Breden & Kelly, 1982). This small size advantage has pronounced effects on subsequent developmental stages, such that larger hatchlings have an immediate growth advantage because they have the ability to acquire more resources (Ficetola & de Bernardi,

2006), potentially avoid gape-limited predators at an earlier age (Relyea & Auld, 2005) and may maintain the headstart in growth and emerge from metamorphosis at a larger size (Altwegg & Reyer, 2003).

Tadpoles sired by large males in *A. callidryas* (but not *A. moreletii*) remained in the larval stage for a longer period and emerged at a greater size at metamorphosis. When presented with stage-specific predators, there is a trade-off in larval duration and size at metamorphic climax (Vonesh & Warkentin, 2006) such that in a favourable environment, a prolonged larval duration provides greater advantages (Berven, 1981; Woodward et al., 1988). Advantages include increased feeding opportunities that will enhance growth, the avoidance of terrestrial predators and the opportunity to emerge from metamorphosis at a larger size which has further fitness advantages (Relyea & Auld, 2005; Ficetola & de Bernardi, 2006). Growth rate may be highly dependent upon local conditions and may not be geared toward producing

the fastest growing tadpoles (Fischer et al., 2004) but with producing larger metamorphosed froglets (Gomez-Mestre & Tejedo, 2003; Ficetola & de Bernardi, 2006).

Large males produced froglets that were larger in *A. callidryas* but not in *A. moreletii*. Size at metamorphosis has been shown to be a reliable indicator trait of post-metamorphic success: larger metamorphosed froglets have been shown to have enhanced locomotion measured by jumping performance (Ficetola & de Bernardi, 2006), and an increased chance of survival than those metamorphosing at a smaller size (Altwegg & Reyer, 2003) and smaller mass (Gomez-Mestre & Tejedo, 2003). This early size difference may translate into advantages into adulthood and have far-reaching fitness consequences. Larger froglets have a greater probability of surviving until the next year as juveniles (Travis, 1981); larger females typically have larger clutches (Kaplan, 1989); and larger males may obtain more matings (reviewed in Sullivan et al., 1995; Briggs, 2008).

Sire and dam effects may not be the only contributing factors to offspring growth. The effects of density was presumably reduced as water volume and food were adjusted across half-sibs, however, there may have been some effect on larval growth and development that obscured the potentially greater effects of genetic factors. The phenotypic and behavioural differences of the two species potentially highlight a response to different selective pressures typically experienced in nature. For example, the larger, more globose tadpoles of *A. moreletii* may have an advantage over other tadpoles and gape-limited predators, whereas the more slender, stream-lined tadpoles of *A. callidryas* may be better suited for predator avoidance (Wassersug, 1973; Relyea & Auld, 2005). There may be a trade-off between growth and predator-avoidance mechanisms. Tadpoles of *A. moreletii* may be more resilient and less affected by crowding and thus, better able to maintain high growth and development rates. This would produce half-sibs that do not have a pronounced difference in offspring traits between sires which were visible in offspring of half-sibs of *A. callidryas*.

Tadpole feeding behaviour may also contribute to growth differences between these two species. Larger, more active schooling aggregates of *A. moreletii* (personal observation) stir up a greater amount of particulate matter in the water column and provide more feeding opportunities that may enhance the competitive advantage (Eterovick, 2000), relative to the more inactive tadpoles of *A. callidryas*, who spend more time suspended in the water column (Vonesh & Warkentin, 2006). Here, increased activity levels may account for the increased growth of *A. moreletii* and potentially diffuse paternal effects in this study. Laboratory conditions may serve to increase the range of genetic expression beyond what is found in nature and substantially reduces the role of selective mortality in response, to natural predators and competitive stress.

Because there is a large-male mating advantage in both species of *Agalychnis* (Briggs, 2008), the results of this study indicate females may be choosing males based on "good genes" for body size which has more

pronounced effects on the latter stages of development. Larval duration and greater size at metamorphosis may be adaptive tadpole traits that increases further reproductive success, particularly since small growth advantages has the potential to affect adult fitness (Howard, 1978; Woodward, 1986, 1987; Altwegg & Reyer, 2003; Ficetola & de Bernardi, 2006; Bonduriansky & Head, 2007). This study illustrates a female preference for larger males result in larger hatchlings that metamorphose at larger sizes when not obscured by competition. Future studies may need to include the necessary forces of competition and predation to determine consistent and predictable paternal effects on individual fitness.

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REFERENCES

- Alatalo, R.V., Kotiaho, J., Mappes, J. & Parri, S. (1998). Mate choice for offspring performance: major benefits or minor costs? *Proceedings of the Royal Society of London Series B: Biological Science* 265, 2297–2301.
- Altwegg, R. & Reyer, H.U. (2003). Patterns of natural selection on size at metamorphosis in water frogs. *Evolution* 57, 872–882.
- Andersson, M. (1986). Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* 40, 804–816.
- Andersson, M. (1994). *Sexual Selection*. Princeton (NJ): Princeton University Press.
- Berven, K.A. (1981). Mate choice in the wood frog *Rana sylvatica*. *Evolution* 35, 707–722.
- Bonduriansky, R. & Head, M. (2007). Maternal and paternal condition effects on offspring phenotype in *Telostylinus angusticollis* (Diptera: Neriidae). *Journal of Evolutionary Biology* 20, 2379–2388.
- Breden, F. & Kelly, C.H. (1982). The effect of conspecific interactions on metamorphosis in *Bufo americanus*. *Ecology* 63, 1682–1689.
- Briggs, V.S. (2008). Mating patterns of red-eyed treefrogs *Agalychnis callidryas* and *A. moreletii*. *Ethology* 114, 489–498.
- Crump, M.L. (1984). Intraclutch egg size variability in *Hyla crucifer* (Anura: Hylidae). *Copeia* 1984, 302–308.

- Darwin, C. (1871). *The descent of man and selection in relation to sex*. London: John Murray.
- Duellman, W.E. & Trueb, L. (1986). *Biology of amphibians*. New York: McGraw-Hill.
- Dziminski, M.A. & Alford, R.A. (2005). Patterns and fitness consequences of intraclutch variation in egg provisioning in tropical Australian frogs. *Oecologia* 146, 98–109.
- Eterovick, P.C. (2000). Effects of aggregation on feeding of *Bufo crucifer* tadpoles (Anura: Bufonidae). *Copeia* 2000, 210–215.
- Fenolio, D. (1996). Captive reproduction of the orange-legged monkey frog (*Phyllomedusa hypocondrialis*) and development of a protocol for Phyllomedusine frog reproduction in the laboratory. *Advances in Herpetoculture* 1, 13–21.
- Ficetola, G.F. & de Bernardi, F. (2006). Trade-off between larval development rate and post-metamorphic traits in the frog *Rana latastei*. *Evolutionary Ecology* 20, 143–158.
- Fischer, K., Zeilstra, I., Hetz, S.K. & Fielder, K. (2004). Physiological costs of growing fast: does accelerated growth reduce pay-off in adult fitness? *Evolutionary Ecology* 18, 343–353.
- Fisher, R.A. (1930). *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.
- Gill, J. (2001). *Generalized Linear Models: A Unified Approach*. Series: Quantitative Applications in the Social Sciences, No. 134. Sage Publications, Thousand Oaks, C.A.
- Gomez-Mestre, I. & Tejedo, M. (2003). Local adaptation of an anuran amphibian to osmotically stressful environments. *Evolution* 57, 1889–1899.
- Gosner, K.L. (1960). A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16, 183–190.
- Howard, R.D. (1978). The evolution of mating strategies in bullfrogs *Rana catesbiana*. *Evolution* 32, 705–716.
- Howard, R.D., Whiteman, H.H. & Schueller, T.I. (1994). Sexual selection in American toads a test of a good-genes hypothesis. *Evolution* 48, 1286–1300.
- Kaplan, R.H. (1980). The implications of ovum size variability for offspring fitness and clutch size within several populations of salamanders (*Ambystoma*). *Evolution* 34, 51–64.
- Kaplan, R.H. (1989). Ovum size plasticity and maternal effects on the early development of the frog *Bombina orientalis* Boulenger in a field population in Korea. *Functional Ecology* 3, 597–604.
- Kaplan, R.H. (1992). Greater maternal investment can decrease offspring survival in the frog *Bombina orientalis*. *Ecology* 73, 280–288.
- Maynard Smith, J. (1978). *The Evolution of Sex*. Cambridge (UK): Cambridge University Press.
- Mitchell, S.L. (1990). The mating system genetically affects offspring performance in Woodhouse's toad (*Bufo woodhousei*). *Evolution* 44, 502–519.
- Moore, A.J. (1994). Genetic evidence for the 'good genes' process of sexual selection. *Behavioral and Ecological Sociology* 35, 235–241.
- Mousseau, T.A. & Fox, C.W. (1998). The adaptive significance of maternal effects. *Trends in Ecology and Evolution* 13, 403–407.
- Newman, R.A. (1988). Genetic variation for larval anuran (*Scaphiopus couchii*) development time in an uncertain environment. *Evolution* 42, 761–773.
- Relyea, R.A. & Auld, J.R. (2005). Predator and competitor-induced plasticity: how changes in foraging morphology affect phenotypic trade-offs. *Ecology* 86, 1723–1729.
- Reyer, H.G., Frei, G. & Som, C. (1999). Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. *Proceedings of Royal Society of London B* 266, 2101–2107.
- Semlitsch, R.D. (1994). Evolutionary consequences of non-random mating: do large males increase offspring fitness in the anuran *Bufo bufo*? *Behavioral and Ecological Sociology* 34, 19–24.
- Sokal, R.R. & Rohlf, F.J. (1995). *Biometry* 3rd edition. W.H. Freeman and Company, New York.
- Sullivan, B.K. & Kwiatowski, M.A. (2007). Courtship displays in anurans and lizards. theoretical and empirical contributions to our understanding of costs and selection on males due to female choice. *Functional Ecology* 2007, 1–7
- Sullivan, B.K., Ryan, M.J. & Verrell, P. (1995). Female choice and mating system structure. In *Amphibian Biology: Social Behavior*. Vol. 2, 469–517. Heatwole H. & Sullivan, B.K. (eds). Chipping Norton: Surrey Beatty and Sons.
- Travis, J. (1981). Control of larval growth variation in a population of *Pseudacris triseriata* (Anura: Hylidae). *Evolution* 35, 423–432.
- Travis, J., Emerson, S.B. & Blouin, M. (1987). A quantitative-genetic analysis of larval life-history traits in *Hyla crucifer*. *Evolution* 41, 145–156.
- Trivers, R. L. (1972). Parental investment and sexual selection. In: *Sexual Selection and the Descent of Man 1871–1971*, 136–179 Campbell B., (ed). Chicago: Aldine-Atherton.
- Vonesh, J.R. & Warkentin, K.M. (2006). Opposite shifts in size at metamorphosis in response to larval and metamorph predators. *Ecology* 87, 55–562.
- Wassersug, R.J. (1973). Aspects of social behavior in anuran larvae. In: *Evolutionary biology of the anurans: Contemporary research of major problems*, 273–297. Vial, J.L. (ed). Columbia (MO): University of Missouri Press.
- Welch, A.M., Semlitsch, R.D. & Gerhardt, H.C. (1998). Call duration as an indicator of genetic quality in male gray tree frogs. *Science* 280, 1928–1930.
- Wilbur, H.M., Rubenstein, D.I., & Fairchild, L. (1978). Sexual selection in toads: the roles of female choice and male body size. *Evolution* 32, 264–270.
- Woodward, B.D. (1986). Paternal effects on juvenile growth in *Scaphiopus multiplicatus* (the New Mexico spadefoot toad). *American Naturalist* 128, 58–65.
- Woodward, B.D. (1987). Paternal effects on offspring traits in *Scaphiopus couchi* (Anura: Pelobatidae). *Oecologia* 73, 626–629.
- Woodward, B.D., Travis, J. & Mitchell, S. (1988). The effects of mating system on progeny performance in *Hyla crucifer* (Anura: Hylidae). *Evolution* 42, 784–794.
- Zahavi, A. (1975). Mate selection - a selection for handicap. *Journal of Theoretical Biology* 53, 205–214.

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