Mating Patterns of Red-Eyed Treefrogs, *Agalychnis callidryas* and *A. moreletii*

Venetia S. Briggs

Department of Biology, University of Miami, Coral Gables, FL, USA

Abstract

Deviations from random mating in frogs are often explained by two different size-based patterns. The large-male mating advantage predicts that males found in amplexus with females will be larger on average than non-amplectant males, whereas size-assortative mating predicts that males and females found in amplexus will maintain an optimal size ratio. Both these pairing patterns are consistent with a female mating preference for larger males, or for males of a given size relative to the choosy female. I examined pairing patterns of two species of Neotropical hylids, *Agalychnis callidryas* and *A. moreletii* for three consecutive breeding seasons in Belize, Central America to evaluate whether mating behavior was influenced by either a large-male mating advantage or size-assortative mating. For each species, I compared size traits between amplectant and non-amplectant males, and within amplectant pairs, and I quantified fertilization success for each amplectant pair. For both species I found evidence of deviations from random mating by size, but the nature of the deviations varied between species and among years. The proportion of eggs fertilized was consistently high among years for both species and there was no relationship between fertilization success and the size ratio of amplectant pairs. These data are consistent with female mate preference, but a role for male–male competition cannot be excluded. My findings suggest that mating patterns may be density-dependent and that the nature and intensity of sexual selection may be increased by extreme environmental conditions.

Introduction

Male frogs may mate several times throughout a breeding season; however, energy and time constraints of producing mature eggs severely limit breeding opportunities for a female (Berven 1981; Andersson 1994). Male competition for females is expected because the operational sex ratio is male-biased (Emlen 1976; Kvarnemo & Ahnesjö 1996). Yet anisogamy dictates that females are usually the choosier sex (Maynard Smith 1982) with significant associated fitness costs (Parker 1982; Reynolds & Gross 1990). This differential parental investment is thought to directly affect mating patterns (Trivers 1972; Andersson 1994).

Among anurans, considerable variation exists in mating patterns and some of this variation can be explained by the length of breeding season (Wells 1977; reviewed by Sullivan et al. 1995), in addition to the operational sex ratio (Emlen & Oring 1977). In explosively breeding species that congregate en masse for short periods, the opportunity for intense male–male competition is greater and there is typically little occasion for females to exercise choice. Mating instead may involve a scramble competition among males for females (Emlen & Oring 1977). In this case, deviations from random mating may be expressed as a large-male mating advantage. However, in species with protracted breeding periods the opportunity for females to exercise choice is greater

and may play a greater role in producing deviations from random mating, expressed as a large-male advantage, or size-assortative mating.

Although random mating patterns have been documented in some anuran species (Friedl & Klump 2005), there is substantial evidence for non-random mating in several frog species (reviewed by Sullivan et al. 1995; Halliday & Tejedo 1995; Bastos & Haddad 1996; Tárano & Herrera 2003; Gutiérrez & Lüddecke 2004; Benard 2007). These studies show that a general deviation from random mating in frogs relates to body size, and male body size has been documented as a suitable indicator of male quality (Wilbur et al. 1978; reviewed by Woodward 1982; Halliday & Tejedo 1995). In animals that exhibit indeterminate growth, large body size may signify older age, better resource-accruing capabilities, superior fitness and the ability to survive fluctuating climatic conditions (Sullivan et al. 1995), all of which may be reflections of high genetic quality (Wilbur et al. 1978; Halliday & Tejedo 1995) that may improve offspring survival (Zahavi 1975; Hamilton & Zuk 1982; Heisler 1984; Kodric-Brown & Brown 1984; Andersson 1986; Sullivan et al. 1995). This is especially so in species in which males contribute only sperm to reproduction (Andersson 1994; Shuster & Wade 2003).

There are two hypotheses that propose a size-based female preference (reviewed by Woodward 1982; Halliday 1983; Halliday & Tejedo 1995). A female preference for large body size predicts a large-male mating advantage where males that are successful in fertilizing the eggs of females are larger, on average than unsuccessful males (Wilbur et al. 1978; Berven 1981) and findings show that larger males are more often found in amplexus than smaller males and/or females respond preferentially to vocalizations of large males (Gatz 1981; Lee 1986; Morris 1989; reviewed by Sullivan et al. 1995; Bastos & Haddad 1996; Howard & Young 1998; Benard 2007). Large males may also be more successful in male–male encounters (Arak 1983). Some studies also continue to show that females that mate with large males produce more offspring or superior offspring relative to those that mate with males of smaller size (Maynard Smith 1982; Kodric-Brown & Brown 1984; Semlitsch 1994; V. S. Briggs unpubl. data).

Female preference may also be for males of a given body size relative to their own and the prediction of this hypothesis is that the preference will result in a pattern of size-assortative mating (Licht 1976). The biomechanics of axillary amplexus require the vents of males and females to be in close apposition to ensure effective fertilization. Females are thus expected to choose males of a size relative to their own body size that maximizes fertilization success. Likewise, male mate choice may result in the same pattern by choosing larger females as an indicator of fecundity (Blankenhorn 1977; Lada et al. 1995) or because they are better able to defend a female which is closer in relative body size (Arak 1983). This type of positive size-assortative mating occurs in decidedly fewer anuran species (Davies & Halliday 1977; Lee & Crump 1981; Marquez & Tejedo 1990; Bourne 1992; Tárano & Herrera 2003; Gutiérrez & Lüddecke 2004).

As a first step toward understanding the breeding behavior and the basis of possible female mate choice, I examined pairing patterns in two Neotropical hylid frogs, *Agalychnis callidryas* and *A. moreletii*, closely related phyllomedusine species that share similar life histories (Duellman 2001; Weins et al. 2006; Gomez-Mestre et al. in press). I test for the presence of a large-male mating advantage or size-assortative mating in the breeding behavior of these two species during three consecutive breeding seasons in Belize, Central America. Finally, I compare the fertilization success of amplectant pairs to determine whether there is selection for size-assortative mating in *A. callidryas* and *A. moreletii*.

Methods

Study Species

Red-eyed treefrogs, *A. callidryas* and *A. moreletii* are moderately large Neotropical hylids (subfamily Phyllomedusinae). Males average 45–55 mm and 55–65 mm in snout–vent length (SVL), respectively (Lee 1996). Females are larger than males in both species ranging from 55 to 75 mm in *A. callidryas* and 65 to 85 mm in *A. moreletii* (Lee 1996; Duellman 2001; Savage 2003). The more common *A. callidryas* ranges from central Veracruz, Mexico to western Ecuador (Santos-Barrera et al. 2004). *Agalychnis moreletii*, recently elevated to critically endangered status (2004), is restricted to small areas of south-central Veracruz, Mexico, western Belize (Maya Mountains), north-western Honduras, central Guatemala, and El Salvador (Santos-Barrera et al. 2004). Both species are nocturnal and arboreal, and inhabit humid lowland and lower montane forests. Males become active with the onset of the rainy season and congregate in choruses, calling from vegetation surrounding permanent pools and temporary ponds (Lee 1996). Females appear at ponds later in the

season after heavy rains. Both species can be found simultaneously at ponds (Lee 1996). Gelatinous egg masses are deposited on a range of oviposition substrates, but typically on the underside of leaves overhanging water. Upon hatching, larvae drop into the water where they complete development (Lee 1996). The morphology and vocalizations of both species are well described (Duellman 2001; Ryan 2001; Savage 2003) but there are few data beyond anecdotal reports documenting breeding behavior and mating patterns of either species (Pyburn 1970; Scott 1983; Donnelly & Guyer 1994; D'Orgeix & Turner 1995).

Study Site

I conducted all research at Las Cuevas Research Station (16°43′N, 88°59′W) Cayo District, Belize. The station is located in the Chiquibul Forest Reserve, which lies within a much larger area of protected forest in the Maya Mountains of Belize. The rainy season in the western Cayo district of Belize begins toward the end of May or early June (Belize Weather Bureau 2004). The area averages approximately 3000 mm of rainfall per year (Walker 1973) and has an elevation range of 560–860 m. Vegetation is a mosaic of deciduous semi-evergreen and deciduous seasonal tropical forest punctuated by stands of pine (Penn et al. 2004). There are several seasonally filled ponds and a few permanent pools that serve as breeding sites for several anuran species (Fitzherbert et al. 2001).

Collection and Maintenance

I collected amplectant pairs and calling non-amplectant males of *A. callidryas* and *A. moreletii* at breeding aggregations during 22:00–03:00 hours on seven nights between May 24 and Aug. 3, 2003; on seven nights between May 25 and Aug. 3, 2004; and on 26 nights between Jun. 8 and Aug. 25, 2005. Males began calling at dusk and females arrived at the pond between 21:00 and 22:00 h. I conservatively characterized non-amplectant males as unsuccessful though these males may have been successful in securing a mate prior to or after capture, but I collected non-amplectant males after pairs had been located, thus any differences between amplectant and non-amplectant males are likely to reflect true differences in the sample population (Lee & Crump 1981; Howard et al. 1994; Lee 2001). I measured adult individuals for SVL and mass, and each received a unique toe-clip for later identification.

Females were allowed to extrude the full complement of eggs before being measured and all individuals were returned to the site of capture within 2 d.

I placed amplectant pairs in separate covered 5-gallon plastic buckets with standing-pond water, and suitable perch and egg deposition sites. Non-amplectant males were similarly housed in groups of 10–15 individuals. I counted the number of eggs and misted each clutch daily with pond water to prevent desiccation. I scored fertilization success as the proportion of healthy eggs to total oviposited eggs 12 h after deposition occurred. Unfertilized eggs become pale yellow with dark areas after 12 h, have a dimpled yolk surface, and collapsed egg capsules (Duellman & Trueb 1994; V. S. Briggs pers. obs.).

To test the hypothesis that large males can supplant small males in amplexus, but rarely the reverse, as has been demonstrated for some temperate species of explosively breeding frogs and toads (Lamb 1984; Howard & Kluge 1985), I conducted 15 male-displacement trials in 2003 and 2004, using *A. callidryas*. A non-amplectant male was placed in a covered 5-gallon bucket, housing a recently amplectant pair. Each non-amplectant male differed in SVL from the amplectant male by a minimum of 5 mm and individuals were identified by the natural white spot pattern present on their dorsum. I spot-checked each group with red-light every hour and at the end of a 6-h period, I noted positions of individuals. A successful displacement is scored if the previously non-amplectant male was found in amplexus and data would be consistent with a 'take-over'.

Data Analyses

I measured pond area and estimated population density and operational sex ratio by counting mean maximum number of individuals and the mean ratio of males to females on a nightly basis throughout the breeding season. I conducted a two-tailed multivariate analysis of variance (MANOVA) to investigate the effects that breeding season and mating status, as predictor variables, had on SVL and mass of males as response variables in the model (Zar 1999). I investigated the potential for an interaction effect between breeding year and mating status of males and conducted post hoc comparisons within breeding years to locate differences between males.

To test for large-male mating advantage within each breeding year I compared mean SVL and body mass for amplectant and non-amplectant males using a one-tailed one-way analysis of variance (ANOVA)

and set power to an 80% probability of detecting a difference (Zar 1999). Males that were captured on multiple nights and multiple years (uncommon occurrence) were only used once in the analyses. Wilbur et al. (1978) proposed that the percentage of eggs fertilized in a clutch covaries positively with male body size because larger males presumably produce more sperm (Travis 1980; Emerson 1997) and females should prefer larger males, therefore I used Pearson's product moment correlation analyses between male SVL and number of fertilized eggs.

To test for size-assortative mating, I calculated Pearson's correlations between body sizes (SVL) of males and females found in amplexus. I conducted 1000 random permutations of all possible size ratios (♀ SVL/♂ SVL) to investigate whether actual size ratios differed from random. As deviations from optimum size ratio increases one would predict a non-random pairing pattern that lends support to Licht's (1976) hypothesis of an optimum size ratio that maximizes fertilization success. I used a Pearson's correlation to determine whether body size ratio within pairs affected fertilization success. All data analyses were done using SPSS ver. 15.0, power analyses were computed using STATA ver. 9.2 and random permutations were done using Microsoft Excel 2003.

Results

Large-Male Mating Advantage

Agalychnis callidryas
I captured 153 males on 40 nights for a total of 250 search nights during the 3-yr study period. In 2005 I conducted frog counts per pond. I counted a mean total of 35 frogs at Elegans pond which measured 15×15 m² for a calculated mean population density of 0.16 frogs/m²; Warree pond measured 10×12 m² and 25 frogs for a mean density of 0.21 frogs/m²; Coral pond at 20×25 m² yielded 30 frogs and a density of 0.06 frogs/m² and Aguada pond at 20×20 m² yielded 20 frogs for 0.05 frogs/m². The overall operational sex ratio of *A. callidryas* was 3.4 males to one female when females were present.

There was a significant interaction effect of year × mating status of males on SVL using a MANOVA (Pillai–Bartlett's trace F = 19.005, d.f. = 3, p < 0.001) but not on mass (Pillai–Bartlett's trace F = 0.784, d.f. = 3, p = 0.505). Tests of between-subject effects show significant inter-annual effects on male SVL (Pillai–Bartlett's trace F = 57.195, d.f. = 252, p < 0.001) and Tukey's HSD post hoc comparisons reveal that SVL significantly differed between 2004

and 2003 (p < 0.001), between 2004 and 2005 (p < 0.001) but not between 2003 and 2005 (p = 0.798). There were also significant inter-annual effects on male mass (Pillai–Bartlett's trace F = 5.660, d.f. = 147, p = 0.004) and post hoc comparisons show that mass of males differed between 2003 and 2004 (p = 0.004), 2003 and 2005 (p = 0.014), but not between 2004 and 2005 (p = 0.902).

There was no difference in mean body size of amplectant males versus non-amplectant males in 2003 and 2005 (Table 1); however, low power analyses (15% and 67%, respectively) indicate that a larger sample size may improve results. Males in 2004 were larger than in either of the other two breeding seasons and there was evidence of a large-male mating advantage because mean SVL of amplectant males were significantly larger than that of non-amplectant males (Table 1), despite a power analysis of 58% of detecting an actual difference. There was no difference in mean body mass between amplectant and non-amplectant males for any of the three breeding seasons (Table 1).

In none of the male-displacement trials, did I observe any attempts by non-amplectant males to supplant amplectant males. At each hourly check,

Table 1: Results of one-way analysis of variance of body size traits of *Agalychnis callidryas* and *A. moreletii* between non-amplectant and amplectant males for each breeding season

Size trait	Non-amplectant (n)	Amplectant (n)	F	p
Agalychnis callidryas				
2003				
SVL	46.2 ± 0.04 (24)	47.2 ± 0.10 (10)	1.237	0.274
MASS	3.9 ± 0.12 (22)	3.9 ± 0.10 (5)	0.050	0.825
2004				
SVL	51.0 ± 0.05 (50)	53.1 ± 0.08 (16)	4.818	0.032*
MASS	4.4 ± 0.09 (50)	4.5 ± 0.22 (16)	0.080	0.779
2005				
SVL	47.6 ± 0.10 (13)	46.6 ± 0.20 (40)	1.785	0.080
MASS	4.6 ± 0.10 (14)	4.3 ± 0.11 (41)	1.221	0.227
Agalychnis moreletii				
2003				
SVL	59.7 ± 0.06 (37)	57.0 ± 0.00 (2)	1.250	0.280
MASS	7.5 ± 0.20 (37)	NA (0)	NA	NA
2004				
SVL	63.2 ± 0.07 (24)	66.0 ± 0.07 (10)	5.337	0.027*
MASS	8.5 ± 0.30 (24)	8.3 ± 0.31 (10)	0.237	0.630
2005				
SVL	59.2 ± 0.06 (25)	58.7 ± 0.04 (43)	0.604	0.440
MASS	7.7 ± 0.16 (25)	8.1 ± 0.13 (43)	4.533	0.037*

Values represent mean ± 1 SE for each measure, SVL (mm) and mass (g).
*p < 0.05.

the position of the non-amplectant male was always found in either in a calling or resting position some distance away from the amplectant pair. In 12 of 15 trials, original pairs were still found in amplexus six hours later and in three cases the amplectant male was no longer in amplexus and the female was unpaired at that point.

Agalychnis moreletii

I captured 119 males of *A. moreletii* on 26 nights for a total of 250 nights of the three year study period. In 2005, I estimated population density for Elegans pond at 0.20 frogs/m^2; Warree pond was 0.21 frogs/m^2; Coral pond was 0.10 frogs/m^2 and Aguada pond had a density of 0.07 frogs/m^2. On nights when females were present, operational sex ratio was 4.5 males to each female.

Using a MANOVA, I detected a significant interaction effect of year × mating status on male SVL (Pillai–Bartlett's trace F = 5.811, d.f. = 1, p = 0.017) but not on mass (Pillai–Bartlett's trace F = 2.028, d.f. = 1, p = 0.157). Results of between-subject effects show a significant inter-annual effect on male SVL (Pillai–Bartlett's trace F = 13.391, d.f. = 2, p < 0.001) and Tukey's HSD post-hoc comparisons reveal that SVL significantly differed between 2004 and 2005 (p < 0.001). There was a significant inter-annual effect on male mass (Pillai–Bartlett's trace F = 6.686, d.f. = 2, p = 0.002) between the 2004 and 2005 breeding season (p = 0.024).

In 2003, I collected only two pairs of this species (power = 19%) and was therefore unable to make comparisons. During the 2004 breeding season, male frogs were also larger than in other years and I found evidence of a large-male mating advantage with a power analysis of 80%. Amplectant males were significantly larger than non-amplectant males (Table 1). In 2005, I found no size difference between amplectant and non-amplectant males with an 11% power analysis (Table 1). I detected no difference in mean body mass between amplectant and non-amplectant males in 2003 and 2004. However in 2005, amplectant males were significantly heavier than non-amplectant males (Table 1).

Size-Assortative Mating

Agalychnis callidryas
In 2003, there was a strong positive association between female and male body size within amplectant pairs (Table 2). Larger females tended to be paired with larger males. The mean

Table 2: Results of Pearson's product moment correlations of mean body size traits within males and females found as amplectant pairs of *Agalychnis callidryas* and *A. moreletii*

Size Trait	Female	Male	n	r	p
Agalychnis callidryas					
2003					
SVL	63.3 ± 0.20	47.2 ± 0.10	10	0.868	0.001**
MASS	10.1 ± 1.32	3.9 ± 0.10	5	0.297	0.628
2004					
SVL	67.9 ± 0.06	52.5 ± 0.06	15	0.084	0.766
MASS	10.4 ± 0.17	4.3 ± 0.16	15	0.121	0.669
2005					
SVL	60.2 ± 0.03	46.4 ± 0.04	39	0.114	0.489
MASS	9.7 ± 0.15	4.3 ± 0.11	39	0.021	0.899
Agalychnis moreletii					
2003					
SVL	77.0 ± 0.50	57.0 ± 0.00	2	NA	NA
MASS	NA	NA	–	NA	NA
2004					
SVL	83.4 ± 0.13	66.4 ± 0.06	9	0.515	0.156
MASS	18.3 ± 0.69	8.4 ± 0.32	9	0.112	0.774
2005					
SVL	75.8 ± 0.07	59.0 ± 0.04	43	0.329	0.031*
MASS	19.8 ± 0.39	8.1 ± 0.13	43	0.132	0.394

Values represent mean ± 1SE for each measure, SVL (mm) and mass (g).
*p < 0.05; **p < 0.01.

female:male SVL ratio was 1.34 ± 0.02 SE (n = 10) and differed significantly from random according to permutation results (p = 0.007). In 2004, mean SVL ratio was 1.30 ± 0.02 (n = 15) and I found no evidence of a correlation in SVL between members of amplectant pairs (Table 2). Similarly, in 2005, I found no correlation between SVL within pairs (Table 2) and found the mean SVL ratio to be 1.29 ± 0.01 (n = 39). Permutation results show the size ratios within pairs were not significantly different from random in 2004 or 2005 (p = 0.401 and 0.423, respectively). I did not find evidence for annual body weight correlations between amplectant pairs in neither breeding season (Table 2).

There was no significant correlation between size ratio of amplectant pairs and proportion of eggs fertilized across breeding seasons (Pearson's r = −0.202, n = 56, p = 0.147) and data pooled across seasons show that male SVL was not positively correlated with proportion of eggs fertilized (Pearson's r = −0.019, n = 105, p = 0.787). Fertilization success was less than 100% in only 2 of 56 total clutches which does not differ from observations of clutches produced by amplectant pairs in the field (V. S. Briggs pers. obs.).

Agalychnis moreletii

In 2004, I found no relationship within amplectant pairs for SVL or mass (Table 2). Mean female:male SVL ratio was 1.30 ± 0.02 (n = 9). However, there was a significant positive relationship within pairs in 2005 (Table 2), indicating size-assortative mating for SVL but not for mass. The mean SVL ratio within pairs was 1.30 ± 0.02 (n = 43). Permutation results show that size ratios within pairs differed significantly from random pairings in 2004 (p = 0.05) and 2005 (p = 0.012).

I found no significant correlation between male SVL and proportion of eggs fertilized when data were pooled across breeding seasons (Pearson's r = −0.190, n = 46, p = 0.206) and there was no significant correlation between size ratios of amplectant pairs and proportion of eggs fertilized (Pearson's r = 0.173, n = 46, p = 0.234). Fertilization success was less than 100% in only one of 46 total clutches similar to what I have observed with naturally amplectant pairs in the field (V. S. Briggs pers. obs.).

Discussion

Anuran mating patterns are often non-random with respect to body size (reviewed by Halliday & Tejedo 1995). By investigating natural pairing patterns in *A. callidryas* and *A. moreletii* across three breeding seasons, I demonstrate that both species subscribe to a size-related non-random mating pattern and show considerable among-year variation in pairing patterns. Of the 3 yr of study, amplectant males of both species were significantly larger than non-amplectant males in 2004, consistent with a large-male mating advantage. I also found evidence of size-assortative mating for *A. callidryas* in 2003 and for *A. moreletii* in 2005 because SVLs within amplectant pairs were positively correlated.

My study gives some evidence for mating success biased toward larger males for both species of *Agalychnis* and parallels findings on other species (reviewed by Sullivan et al. 1995; Bastos & Haddad 1996; Howard & Young 1998; Benard 2007). Friedl & Klump (2005) assert that a large-male mating advantage is absent in many hylid species; however, a more recent study of *Pseudacris regilla*, one of the hylids included in Friedl & Klump's (2005) study shows that large males had higher mating success than small males (Benard 2007). My results also provide evidence for size-assortative mating in two species of *Agalychnis*. Females of other species have also been shown to choose males relative to their own

body size, e.g. *Bufo americanus* (Licht 1976), *Bufo bufo* (Davies & Halliday 1977), *Triprion petasatus* (Lee & Crump 1981), and *Hyla labialis* (Gutiérrez & Lüddecke 2004).

Effective fertilization may require that cloacae be properly juxtaposed during amplexus; if a male is too large or too small relative to the female, cloacal apposition is not efficient, and the proportion of eggs fertilized may be reduced and predicts a positive size association within pairs (Licht 1976). My results show that an average size ratio of 1.3 within pairs was apparent for both species; however, fertilization success was independent of body size ratios and does not support Licht's (1976) hypothesis explaining size-assortative mating for *Agalychnis*. A less than optimal size ratio reduces fertilization success in *Hyla elegans* (Bastos & Haddad 1996) and *Ololygon rubra* (Bourne 1992) and several other species (Davies & Halliday 1977; Ryan 1985; Gibbons & McCarthy 1986; Bourne 1993). In this study, there was nearly 100% fertilization success in both species of *Agalychnis* and male size was not correlated with fertilization success as has been suggested in some species (Howard 1978; Kruse 1981; Howard & Kluge 1985; Krupa 1988). This is not an artifact of laboratory conditions because observational field data reveal high fertilization success that yields a near-complete batch of embryos (V. S. Briggs pers. obs.; K. M. Warkentin pers. comm.).

Observed mating patterns in these two *Agalychnis* species may be due to either female choice, male–male competition, or a combination of these (Wilbur et al. 1978). Based on limited evidence in *A. callidryas* female choice is the most likely explanation of my results. Although there is evidence for male–male competition in *A. callidryas* of Gamboa, Panama (V. S. Briggs pers. obs., M. S. Caldwell pers. comm.), frog populations in my study showed no evidence of this. Results of my male-displacement trials demonstrated that non-amplectant males did not attempt to supplant amplectant males from females. Additionally, I recorded no instances of male combat or takeovers in the field. Males were found calling within a few meters of amplectant pairs with no attempt to displace the successful male (V. S. Briggs pers. obs., 2003–2005). Male displacement has been documented in some temperate species (Lamb 1984; Howard & Kluge 1985); however, studies also demonstrate that male displacement behaviour is uncommon in most hylid frogs (Godwin & Roble 1983; Perrill 1984; Marquez & Tejedo 1990; Ritke & Semlitsch 1991; Murphy 1994; Friedl & Klump 2005). Differences in my study may be explained by the effects of density. At higher densities where there is a strong male-biased operational

sex ratio, the intensity of male–male competition is increased (Emlen 1976; Kvarnemo & Ahnesjö 1996; Byrne & Roberts 2004) and female choice may be obscured (Shuster & Wade 2003). There is less evidence for *A. moreletii* because unlike *A. callidryas*, I have observed some male–male competition for perch sites and it is difficult to determine at this time whether female choice or male–male competition is more important.

Mating patterns may be influenced by factors other than selective pressures imposed by female choice (West Eberhard 1983) and male–male competition (Andersson 1994). These include a tradeoff with natural selection (Ryan & Tuttle 1983; Benard 2007) where male traits are also preferred by predators or is in response to fluctuating environmental conditions where some conditions favor one mating pattern over another (Crump 1974). In this study, there is considerable inter-annual variation in male sizes and mating patterns. 2004 is recorded as the driest year and the onset of the rainy season was delayed for over a month (Belize Weather Bureau 2004). The consequences of this were lower densities of frogs, decreased calling rates, smaller aggregations of males per pond, fewer amplectant pairs prior to the first heavy rains, and more disturbance of egg clutches because of desiccation or increased mortality of hatchlings because of a lack of water in temporary ponds (V. S. Briggs, unpubl. data). Reproductive patterns, such as timing and length of breeding seasons of frogs are largely influenced by rainfall and temperature (Crump 1974) and reflect life-cycle adaptations (Duellman & Trueb 1994).

In this study, the lengthy dry period of 2004 may have exerted stress on *A. callidryas* and *A. moreletii* and may have influenced behavioral patterns. Several tropical frog species significantly alter behavior during dry periods such that male vocalization and female response to that vocalization, are depressed (Pough et al. 1983; Prado et al. 2005; Kopp & Eterovick 2006). Under such conditions males of lower quality may be more vulnerable to stress, which may in turn constrain their ability to compete for mates (Duellman & Trueb 1994). The breeding seasons of 2003 and 2005 were considerably wetter than that of 2004 and under such relatively benign conditions, lower quality males would not differ notably in body size or condition in comparison with males of higher quality. Desiccation-related stress on red-eyed treefrogs was likely less in those years. Thus, the magnitude of sexual selection may be altered by harsh environmental conditions and may help to explain why amplectant males were larger

than non-amplectant males during the 2004 breeding season. In 2003 and 2005, uneven sample sizes between amplectant and non-amplectant males may have influenced the results of the data and I failed to observe an effect of mating status on male size. Further investigation is warranted.

My study is one of the few to document mating patterns across multiple breeding seasons and for two different frog species. Results of my study show the complexity of this system and associated mating patterns and leaves room for alternative interpretations. It does however highlight the need for more long-term studies in order to determine true mating patterns. I provide evidence illustrating that though a pattern may be observed clearly in one year, this pattern may be subject to other contributing factors in subsequent years and populations when viewed in the context of a larger timeframe. Future studies aimed to understand the role of sexual selection in mating patterns, including in other taxa, ought to be designed to make observations throughout breeding seasons and across breeding seasons within species. Only then can we gather sufficient data to understand the roles of female choice and male–male competition in mating systems.

Acknowledgements

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