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Testis size variation in frogs: testing the alternatives

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Abstract While sperm competition may be a major factor affecting relative testis size in vertebrates as a whole, additional hypotheses have not been given much attention in frogs. This is important because sperm competition is relatively uncommon in frogs and relative testis size varies in frogs that do not have multiple-male breeding systems. This paper tests two additional hypotheses for differences in relative testis size among frogs: relative clutch size (number of eggs/snout vent length) and androgen level. Testis size was measured in 90 species of frogs belonging to five families. Relative testis size was found to be positively correlated with relative clutch size in species that lack sperm competition. Mean androgen levels of species also positively covaried with relative testis size. However, there was no correlation between relative testis size and level of male agonistic behavior among species, despite other work indicating that testosterone levels are positively correlated with agonistic behavior in at least some species. These findings suggest that a number of factors in addition to sperm competition are important in the evolution of testis size in male frogs.

Key words Sperm competition · Testis size · Frog

Introduction

Testis size variation

The considerable variation in relative testis size across vertebrates has generally been ascribed to sperm com-

petition (e.g., Andersson 1994). A positive relationship has been demonstrated between relative testis mass and the number of males a female is likely to copulate with in a reproductive cycle for a wide diversity of birds (Møller 1991; Birkhead and Møller 1992), fish (e.g., Warner and Robertson 1978), and mammals (Harcourt et al. 1981; Kenagy and Trombulak 1986), but also a few frog species (Kusano et al. 1991; Jennions and Passmore 1993). While sperm competition may be a major factor affecting relative testis size in vertebrates as a whole, additional hypotheses have not been given much attention in frogs. This is important because sperm competition is relatively uncommon in frogs (Halliday and Verrell 1984), and relative testis size also varies in frogs that do not exhibit multiple males breeding simultaneously with a single female (Fig. 1; S.B. Emerson, personal observations). The purpose of this paper is to expand the study of testis size in anurans by examining additional factors, besides sperm competition, that might account for interspecific differences in the relative size of the testis.

The vertebrate testis has two major functions: (1) hormone (androgen) production, and (2) sperm production. These two functions provide the basis for generating the additional hypotheses regarding relative testis size in anurans. Some breeding baseline level of androgens appears essential in amphibians for expression of male reproductive behavior, spermatogenesis, and development of secondary sexual characteristics (Houck and Woodley 1995 and references therein). In addition, however, a number of field studies point to wide interspecific variation in androgen levels above the breeding baseline (e.g., Licht et al. 1983; Orchinik et al. 1988; Emerson et al. 1993; Houck and Woodley 1995; Emerson and Hess 1996; Harvey et al. 1997). For example, androgen values can range over two orders of magnitude in male frogs when they are calling. Vocalizing male spadefoot toads, *Scaphiopus couchii*, have a combined testosterone (T) and dihydrotestosterone (DHT) level of 250 ng/ml (Harvey et al. 1997) while vocalizing male Asian toads, *Bufo asper*, have androgen

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levels of around 2.5 ng/ml (Emerson and Hess 1996). Variation in androgen levels has also been observed in other vertebrates, and such differences prompted Wingfield et al. (1990) to propose an explanation termed the "challenge hypothesis". This model posits that variation in circulating androgen levels is related to socially unstable situations, male-male aggression, and male parental care. One hypothesis, besides sperm competition, that could account for differences in relative testis size in frogs is that they are related to interspecific variation in androgen concentration, which, in turn, is correlated with variation in levels of male agonistic behavior. In this scenario, frog species with high levels of direct male-male aggression, independent of sperm competition, would be expected to have higher androgen levels that, presumably, result from relatively larger testes.

Another hypothesis that might account for differences in relative testis size in frogs is variation in clutch size. In frogs, literally thousands of eggs can be laid in a single clutch (Duellman and Trueb 1986). There is wide interspecific variation in egg size and the numbers of eggs in a single clutch (e.g., Crump 1974). Egg size is inversely related to clutch size (Salthe and Duellman 1973; K. Wells, personal communication). Given the considerable interspecific variation in female reproductive effort (e.g., Crump 1974), it seems possible that sperm production may be related to clutch size as well as to competition for fertilization opportunities. A clutch size hypothesis predicts that males of species with larger clutches (i.e., more eggs to fertilize) will have relatively larger testes independent of sperm competition.

The comparative approach

In this study, hypothesis testing takes the general form of comparing relative testis size in frog species with different breeding systems, clutch sizes, androgen levels, and different degrees of androgen-mediated behavior. The three hypotheses are not necessarily mutually exclusive. For example, species with sperm competition could also have male-male aggression and high androgen levels and/or species with relatively large clutches could also have sperm competition. However, every effort was made to compare species that varied in only one attribute or where the alternative hypotheses would make different predictions regarding relative testis size. Cases where that was not possible are further considered in the Discussion.

The sperm competition hypothesis

Previous work (Kusano et al. 1991; Jennions and Passmore 1993) has established that species in the family Rhacophoridae exhibiting sperm competition have relatively large testes compared to other frogs (see also Fig. 1). Multiple-male breeding has also been reported

for the New World hylid species, *Agalychnis callidryas*, *A. saltator* and *Pachymedusa dacnicolor* (Pyburn 1970; Roberts 1994), but their testis size has not been measured. Recently, multiple paternity has been confirmed in one of those species, *A. callidryas*, using DNA fingerprinting (D'Orgeix and Turner 1995). The sperm competition hypothesis predicts that *A. callidryas*, *A. saltator*, and *P. dacnicolor* should have relatively large testes compared to other hylid species lacking multiple-male breeding.

The androgen level hypothesis

The biological significance of variation in androgen levels among frogs is largely unstudied (see Houck and Woodley 1995 for a review), but some initial work suggests that at least part of the variation may be correlated with agonistic behavior among males and male parental care (Mendonca et al. 1985; Townsend and Moger 1987; Orchinik et al. 1988; Emerson et al. 1993). Higher androgen concentrations associated with higher levels of male-male aggression and lower androgen concentrations in conjunction with male parental care in frogs are consistent with the predictions of the challenge hypothesis (Wingfield et al. 1990). This hypothesis has provided strong explanatory power for the variation in androgen levels in other vertebrates, especially birds (Wingfield et al. 1990).

The androgen level hypothesis predicts that if variation in relative testis size in frogs is related to hormone production and higher androgen levels are related to agonistic behavior, species with direct male-male physical aggression would be expected to have higher androgen levels and larger relative testis size than species with males lacking this behavior. [For the purposes of this paper, agonistic behavior refers to those actions on the part of males that involve actual physical contact (after Wells 1977)]. This hypothesis builds on the knowledge that the interstitial tissue of the testis is the major site of androgen production in frogs (Jorgensen 1992). It assumes that at least part of androgen production is correlated with the amount of interstitial tissue in the testis, and so species with the highest androgen levels will have the relatively largest testes. At least part of that assumption can be tested directly by measuring androgen levels in species of frogs whose testis mass is known while controlling for the percentage of interstitial tissue in the testis.

The behavioral prediction can best be tested in the family Hylidae. Males of a number of hylid species are known to approach and contact calling males (Table 2 in Wells 1977), and direct male-male physical aggression is particularly pronounced in the gladiator frogs (Kluge 1981). Male gladiator frogs possess a sharp pre-pollical spine, and fighting between males often ends in serious injury or death (Kluge 1981). Gladiator frogs do not have sperm competition (Kluge 1981). The androgen level hypothesis predicts that (1) hylid frogs with direct

male-male aggression will have relatively larger testes than other hylid species lacking male-male aggression and (2) gladiator frogs will have the relatively largest testes.

The clutch size hypothesis

Most comparative studies are concerned with removing phylogenetic effects by examining independent samples and/or making appropriate corrections (Felsenstein 1985; Pagel and Harvey 1988; Garland et al. 1992). The simplest, most direct test of the clutch size hypothesis involves taking advantage of a known phylogenetic effect. Clutch size in frogs varies between families (Salthe and Duellman 1973). Generally, ranid frogs have smaller clutch sizes (and larger eggs) than bufonid frogs even when reproductive mode is controlled for (Salthe and Duellman 1973; K. Wells, personal communication). If this is the case, then the clutch size hypothesis predicts that bufonid males should have relatively larger testes than ranid males to fertilize the larger female clutches. This hypothesis would be more strongly supported if it could also be demonstrated that those particular species with the largest testes also had the largest clutches. While those data are not available for the bufonid species, it can be examined in a subset of the ranid species. Even though the ranids generally have smaller clutches than bufonids, there is considerable variation in clutch size within the family (Table 1).

Methods

Testis size was measured in 90 species of frogs in the collection of the Field Museum of Natural History (Appendix 1). Species were members of five families: Bufonidae (2 genera, 17 species), Ranidae (7 genera, 28 species), Hylidae (11 genera, 25 species), Pelobatidae (2 genera, 2 species), Microhylidae (4 genera, 4 species), and Rhacophoridae (5 genera, 13 species). Relatively large numbers of genera were sampled in each family (except the Bufonidae) to minimize the problem of non-independence of species data points (Felsenstein 1985). Although it was not possible to sample many different genera of bufonids, a comparison of the species chosen with a recent phylogenetic hypothesis of relationship for the family (A. Graybeal, personal communication) confirms that the species were drawn from different species groups within the family.

Only males with nuptial pads, vocal sacs and throat pigmentation, well-developed secondary sexual characteristics that occur during breeding, were included in the study. This was done because testis mass can vary seasonally (e.g., Kanamadi et al. 1983) with peak mass occurring during the reproductive period. The left testis was dissected from each individual and weighed after being blotted dry to remove excess alcohol. Two to six frogs were dissected for each species. The maximum relative testis size for each species was used in all analyses. Testes were weighed twice, 1 week apart, to confirm the repeatability of the measurements, and the mean of the two measurements was used in analyses.

For five Old World tropical species (from four families) blood samples were taken from live males in the field as they were vocalizing (Emerson and Hess 1996). Species include *Occidozyga laevis*, *Amolops larutensis*, *Bufo asper*, *Megophrys nasuta*, and *Polypedates* sp. Males of the sixth species, *Rana blythi*, do not vocalize. In this species blood was taken from breeding males along the stream who

were not engaged in either male-male aggression or male parental care. Samples were collected at Danum Valley Research Centre, Sabah, Malaysia and University of Malaya Gombak Research Center, Selangor, Malaysia. Comparisons are restricted to tropical species as recent work has suggested that temperate and tropical species of frogs may vary in androgen levels (Emerson and Hess 1996). Blood samples were obtained by cardiac puncture with heparinized syringes or by heparinized capillary pipettes within 2 min of capture. The animals were then sacrificed, the left testis removed and fixed in Histochoice (Amresco). Subsequently the testis was weighed, paraffin embedded and sectioned for histological examination. The cross-sections of the testes were measured in order to ascertain whether there was interspecific variation in the proportion of sperm producing and interstitial tissue in the testis. This was determined using a line intercept sampling method (Brower and Zar 1977). A transect of standard length was laid out on the cross-section of a testis and the percentages of the total length of the transect occupied by interstitial tissue and seminiferous tubules were measured. Transect measures were repeated 20 times on each testis cross section at randomly chosen sites. Differences in percentages of interstitial tissue and seminiferous tubules across species were compared using a *G*-test (Sokal and Rohlf 1988).

Blood samples were analyzed for dihydrotestosterone (DHT) and testosterone (T) using standard radioimmunoassay techniques (see Emerson and Hess 1996 for details of methodology). For each species blood samples were collected from between 2 and 56 individuals (see Emerson et al. 1993; Emerson and Hess 1996), but the testis was not dissected and weighed for each individual. The average value of androgen recorded for a species and the average relative testis mass for the species were chosen to represent the relationship between androgen level and testis for that species. Because androgen levels are known to show considerable within-individual variation over time (e.g., Mendonca et al. 1985) and the maximum androgen level might be more meaningful, a second analysis was also done. In this case the individual frog of a species that had the highest androgen level and whose testis had been weighed was used in the analysis. There was no difference in the results between the two analyses and only the first will be discussed.

Testis residuals were obtained as deviations from a least squares regression of log testis mass against log snout vent length for the six species. Androgen levels were not normally distributed (Shapiro-Wilk test, $W = 0.7925$, $P < 0.04$), and a Spearman rank correlation test was used to analyze the relationship between hormone level and the residual of relative testis size.

Statistical analyses were performed with the JMP Software Program (SAS Institute 1989). All *t*-tests, comparing testis size between groups, involved the use of residuals to avoid the potentially confounding effects of allometry.

Results

Figure 1 shows the relationship between log testis mass and log snout vent length for all species that were measured from preserved material. The equation describing the relationship is $\log y = -6.5182 + 2.67 \log x$ ($r^2 = 0.61$). The slope of the least squares regression (2.67 ± 0.50 95% CI) is not significantly different from 3.0, which in this case would be isometry. This graph confirms initial observations that there is wide interspecific variation in relative testis size across frogs.

Figure 2 plots the relationship between testis mass and snout vent length for species belonging to the family Hylidae. A least squares regression was calculated for the data excluding the species of phyllomedusine frogs thought to have multiple male mating and sperm competition. The equation for the regression is

Fig. 1 Plot of log testis mass (mg) and log snout vent length (mm) for 90 species of frogs. Families are identified by different symbols

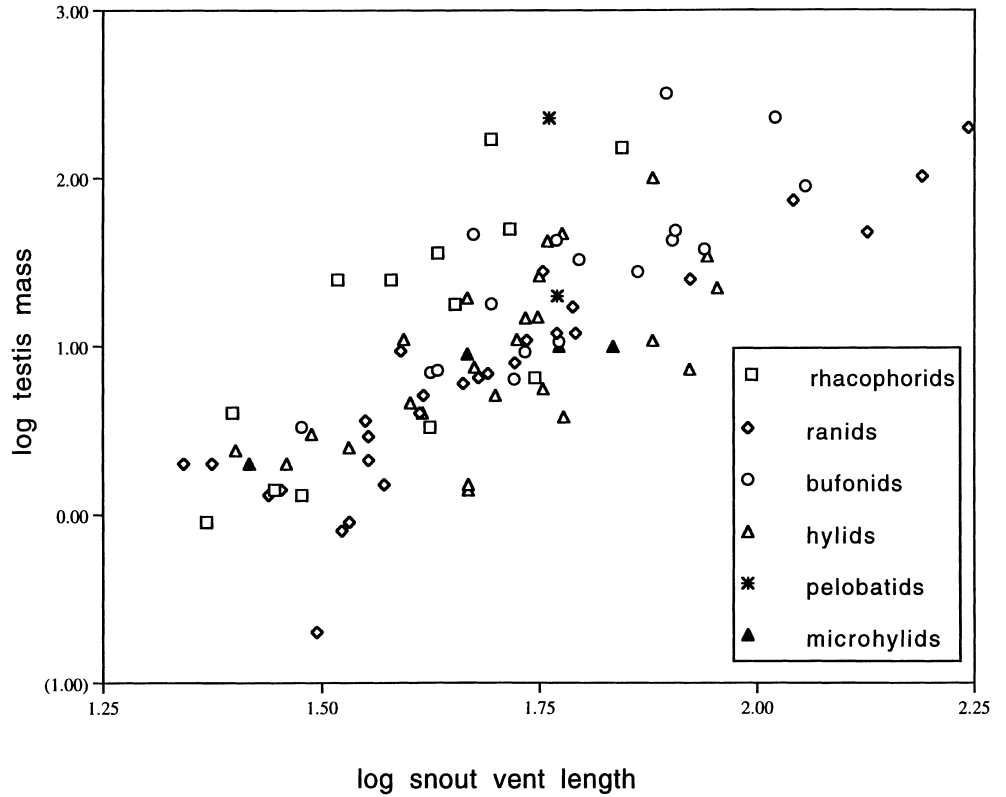
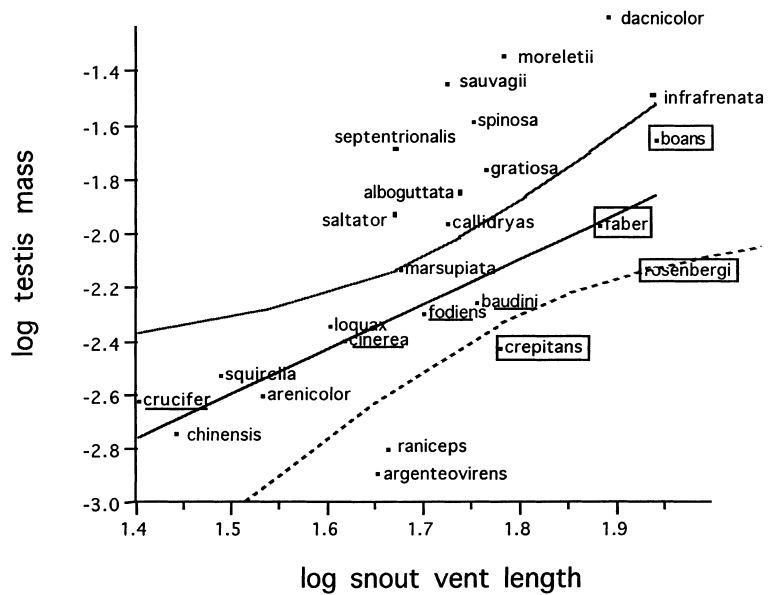


Fig. 2 Plot of log testis mass (g) and log snout vent length (mm) for 25 species of the family Hylidae. Curved lines represent 95% confidence intervals for the relationship calculated by least squares regression. Animals are identified by species names. Species underlined have been reported to show some male-male aggression (Wells 1977). Species in boxes are the gladiator frogs



$\log y = -5.1068 + 1.67 \log x$, ($r^2 = 0.394$). The testes of *Agalychnis callidryas*, *A. saltator*, *A. moreletii*, *Pachymedusa dacnicolor*, *Phyllomedusa savaugii*, *Anotheca spinosa*, *Hyla gratioiosa*, *Osteopilus septentrionalis*, *Litoria infrafrenata* and *L. albobuttata* lie outside the upper 95% confidence interval of the calculated regression. *Hyla crepitans*, *H. raniceps*, and *Gastrotheca argenteovirens* lie outside the lower 95% confidence interval of the calculated regression (Fig. 2). As predicted by the sperm

competition hypothesis, the frogs with multiple male breeding have significantly larger testes than frogs without multiple mating (unpaired one-tailed *t*-test, $t = 3.6$, $n = 24$, $P = 0.0015$).

Those species of hylids where males have been reported to have shown agonistic behavior (but lack sperm competition) are underlined and boxed in Fig. 2. None of those species lies above the 95% confidence interval of the calculated hylid regression. Furthermore, those

species have testes no larger than those of same-sized species in other families that do not exhibit male-male aggression (Fig. 1). Finally, there is no significant difference in testis size between hylid frogs with and without agonistic behavior (unpaired one tailed t -test, $t = 1.59$; $n = 20$; $P = 0.123$). In fact, the (nonsignificant) trend is for species with agonistic behavior to have smaller testes.

Figure 3 plots the relationship between mean androgen level and the residual of testis mass. There is a significant positive correlation between relative testis size and androgen level (Spearman $\rho = 0.8286$, $P < 0.04$). The comparison of testis morphology among species indicates that the interstitial tissue makes up 8–17% of the testis. There were no significant differences among species in the percentage of interstitial tissue ($G = 5.6$, $P > 0.50$).

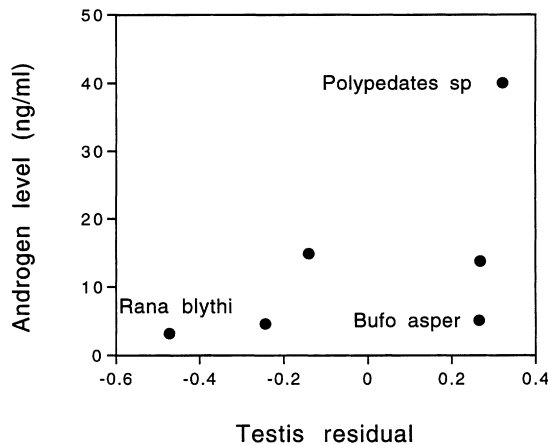


Fig. 3 Plot of mean androgen level (ng/ml) and testis size residual for six species of tropical frogs

Fig. 4 Plot of log testis mass (mg) and log snout vent length (mm) for bufonid (closed squares) and ranid (open circle) frogs

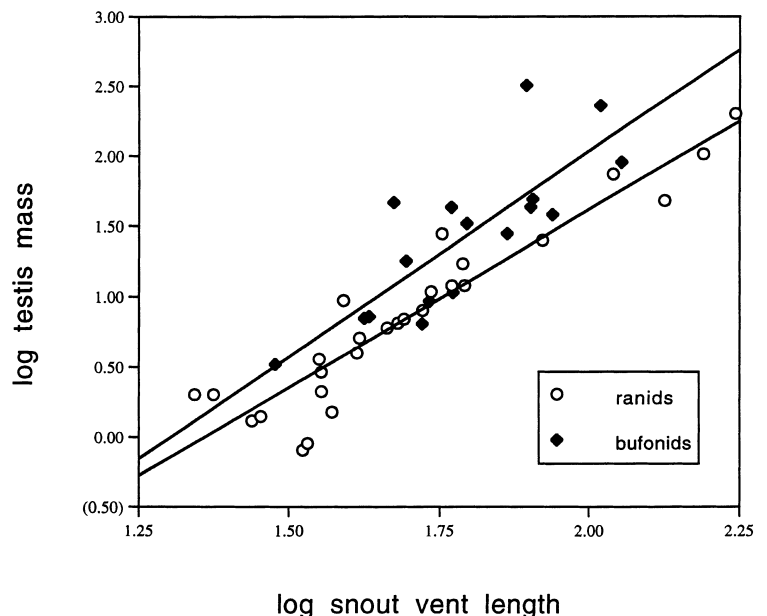


Figure 4 plots the relationship between testis mass and snout vent length for species of the families Bufonidae and Ranidae. Preliminary analysis showed no significant interaction effect between family and snout vent length. Results of an ANCOVA indicate that there is a significant effect of both snout vent length ($F_{1,44} = 237.6$, $P < 0.0000$) and family ($F_{1,44} = 10.77$, $P < 0.002$) on testis size. Bufonids have relatively larger testes than ranids, (adjusted means, SE: bufonids = 0.0165 ± 0.0012 g, ranids = 0.0086 ± 0.0011 g). For the 11 species of ranids where both clutch size and testis size data are available (Table 1), the residuals of clutch size and testis mass are correlated (ANOVA $F_{1,9} = 4.9$, $P = 0.05$), which indicates that the species with the largest clutches also produce the most sperm.

Discussion

The differences in relative testis size among frogs appear to be related to variation in sperm production rather than with androgen correlated agonistic behavior. Similar results have been found in fishes (e.g., Brantley et al. 1993 and references therein). Courting males, despite exhibiting marked agonistic behavior, have relatively smaller testes than satellite-sneaker males in a number of different species. Frog data support both the sperm competition and the clutch size hypotheses. Those phyllomedusine hylid frogs that are known to have multiple-male mating have relatively larger testes than hylid frogs that do not multiply mate. Additionally, two other members of the same subfamily, *Agalychnis moreletii* and *Phyllomedusa savaugii*, whose reproductive biology is not well known, also have relatively larger testes. Phyllomedusine frogs have relatively small clutches and large egg size (Salthe and Duellman

Table 1 Clutch and testis size in ranid frogs (SVL snout-vent length)

Species	Female clutch size	Female SVL (mm)	Male testis size(mg)	Male SVL (mm)	Source
<i>Phrynobatrachus natalensis</i>	650	25.5	2.0	23.7	Wager 1965
<i>Ptychadena grandisonae</i>	1100	41.5	2.9	35.8	S.B. Emerson, unpublished work
<i>Pyxicephalus adspersus</i>	3500	85.0	200.0	170.0	Stewart 1967
<i>Rana blythi</i>	2400	120.0	103.0	155.0	Inger and Bacon 1968
<i>R. brevipoda</i>	1695	74.0	17.2	61.5	Kuramoto 1978
<i>R. cancrivora</i>	2000	55.0	61.0	78.0	R.F. Inger, unpublished work
<i>R. catesbeiana</i>	15000	137.0	74.0	110.0	Wright and Wright 1949
<i>R. chalcinota</i>	1000	60.0	2.0	35.8	R.F. Inger, unpublished work
<i>R. clamitans</i>	3000	67.0	28.0	56.8	Wright and Wright 1949
<i>R. hosei</i>	2636	100.0	6.0	46.0	Inger and Bacon 1968
<i>R. limnocharis</i>	1244	52.0	10.0	54.4	Kuramoto 1978
<i>R. sylvatica</i>	1750	51.0	6.9	49.1	Duellman and Trueb 1986

1973; K. Wells, personal communication) so the large testis size in the species of this group is the opposite of what is predicted by the clutch size hypothesis.

Two hyline hylids, *Anothea spinosa* and *Hyla gratiosa*, also have relatively large testes. *A. spinosa* is a tree-hole breeding species (Duellman 1970) and little is known about its reproductive behavior. However, a recent report indicates that an individual male separately fertilizes small numbers of eggs at a time from a single female over a period of two to four hours (Jungfer 1996). The female deposits three to five eggs at a time, after which the male lifts his cloaca over the eggs and releases a visible amount of fluid. This procedure is repeated until the entire clutch (~160 eggs) is laid. Sperm competition has never been reported in *H. gratiosa* (Murphy 1994), but they do have relatively large clutches (K. Wells, personal communication). *H. gratiosa* also lays its eggs a few at a time (Wright and Wright 1949). It is possible that the type of fertilization where eggs are laid singly or in small groups requires a larger sperm production than the usual situation where the entire clutch is fertilized by a single sperm ejaculate. Data from birds and mammals indicate that relatively larger testes produce not only relatively larger numbers of sperm per ejaculate but also a relatively larger sperm reserve (Møller 1991). If this were also the case for frogs, it could account for why the males of these two species have relatively large testes.

As predicted by the clutch size hypothesis, bufonid species generally have relatively larger testes than ranids. Presumably these differences are related, at least in part, to the larger number of eggs that a male bufonid has to fertilize during a single mating (Salthe and Duellman 1973). For the ranid frogs, where data were available, there was a positive correlation between clutch size and testis size. Given the negative correlation between egg size and clutch size in frogs (Salthe and Duellman 1973), it is possible that smaller eggs may require more sperm for fertilization. At this point there are no data to distinguish whether it is egg number or egg size which might determine the amounts of sperm required.

At the organ level, there is an obvious relationship between androgen level and testis size. Yet, in most other studies examining a diversity of factors in relation to differences in relative testis size, androgen levels have not been compared (e.g., Møller 1991). Frogs without testes produce negligible amounts of androgens, and animals that have been unilaterally castrated show a reduction in androgen level (e.g., Rastogi et al. 1986). It is not surprising therefore that in this study there appears to be an interspecific correlation between mean androgen level and relative testis size (Fig. 3). On the other hand, the sample size is very small, and intraspecific studies of male fishes with very different gonadosomatic indices have not revealed a relationship between androgen level and testis size (Brantley et al. 1993). More data on androgen levels and testis size in frogs are necessary before the correlation found in this analysis can be generalized, but the results of this study suggest that such a relationship should be examined more closely.

Increased androgen levels are often associated with heightened aggression in vertebrates (Wingfield et al. 1990). *Polypedates* sp. and *Rana blythi*, two of the frogs for which hormone levels are known, show male agonistic behaviors, but one of them has the highest mean androgen level while the other has among the lowest mean androgen levels (Emerson et al. 1993; Emerson and Hess 1996; and Fig. 3). The frog, *Polypedates* sp., that has the highest absolute androgen level and relatively largest testis, exhibits male-male aggression in conjunction with sperm competition (Feng and Narins 1991). In this rhacophorid, multiple males gather at the foam nest that the female has constructed around the egg mass and deposit sperm into the foam. Males fight with each other as they jockey for position around the female and her nest (Feng and Narins 1991). For this study, hormone levels were measured in the males of this species while they were vocalizing, not when they were actually engaged in sperm competition and direct male-male aggression. Yet, males of this species still had high androgen levels relative to vocalizing males of other species (Emerson and Hess 1996). In contrast, in

R. blythi, with the relatively smallest testis and lowest androgen level, the males have fangs and fight with each other over territories but do not have sperm competition (Emerson 1992). They exhibit *relatively* high androgen levels when they are in unstable social situations and involved in male-male aggression but have absolutely low androgen levels at other times, probably related to the fact that they exhibit male parental care (Emerson 1992; Emerson et al. 1993; Emerson and Hess 1996). The *highest* androgen level ever recorded for a male *R. blythi* (22 ng/ml, $n = 56$) involved in physical aggression is much lower than the maximum androgen level found in *Polypedates* sp. (68.82 ng/ml, $n = 9$) when vocalizing. The point is that aggression may be related to *relative* androgen levels (compared to some breeding baseline) rather than absolute androgen levels (Wingfield et al. 1990). The absolutely high androgen levels in vocalizing male *Polypedates* sp. suggest the possibility that other behavioral factors besides physical male-male aggression may be associated with androgen level in frogs (Emerson and Hess 1996; Marler and Ryan 1996). The fact that *Bufo asper*, another frog with a relatively large testis, had a very low androgen level (Fig. 4) indicates that higher androgen levels are not a necessary consequence of larger testis size. Relative testis size may determine the maximum androgen level that can be produced, but it does not appear to dictate the minimum concentration.

In summary, these data suggest that while testis size may be correlated with maximum level of androgen production, differences in relative testis size among frogs seem more likely to be the result of selection for sperm production than for androgen related reproductive behaviors such as aggression. In the future, it would be interesting to compare relative testis size in frogs with inguinal and axillary amplexus while controlling for variation in the pattern of egg laying. Position of amplexus is an additional reproductive behavior that may be related to differences in sperm production and relative testis size. Unfortunately, until information becomes available on the ecology, behavior, and endocrinology of additional frog species, it will be impossible to sort out completely the several variables potentially correlated with testis size. However, this study has made clear that sperm competition is not the only factor influencing relative testis size in frogs.

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Appendix I Testis mass and snout vent length (SVL) of anuran species

	SVL (mm)	Testis mass (g)
Family Rhacophoridae		
<i>Chirixalus effingeri</i>	29.7	0.0016
<i>Chiromantis xerampolina</i>	49.5	0.1700
<i>Philautus hosii</i>	42.1	0.0033
<i>P. mjobergi</i>	23.4	0.0009
<i>Polypedates colletti</i>	50.8	0.0062
<i>P. macrotis</i>	45.0	0.0179
<i>P. otitophus</i>	70.0	0.1525
<i>Polypedates</i> spp.	44.0	0.0360
<i>Rhacophorus appendiculatus</i>	29.2	0.0012
<i>R. dulitensis</i>	38.0	0.0250
<i>R. gauni</i>	25.0	0.0040
<i>R. pardalis</i>	52.0	0.0500
<i>R. schlegelii</i>	34.0	0.0300
Family Ranidae		
<i>Amolops larutensis</i>	39.0	0.0094
<i>Occidozyga laevis</i>	22.0	0.0020
<i>Phrynobatrachus dendrobates</i>	28.4	0.0014
<i>P. natalensis</i>	23.7	0.0020
<i>Ptychaedena grandisonae</i>	35.8	0.0029
<i>Pyxicephalus adspersus</i>	170.0	0.2000
<i>Rana areolata</i>	80.4	0.0260
<i>R. blythi</i>	155.0	0.1030
<i>R. brevipoda</i>	61.5	0.0172
<i>R. cancrivora</i>	78.0	0.0610
<i>R. catesbeiana</i>	110.2	0.0740
<i>R. chalconota</i>	35.8	0.0021
<i>R. clamitans</i>	56.8	0.0280
<i>R. cyanophlyctis</i>	61.9	0.0120
<i>R. erythraea</i>	41.0	0.0040
<i>R. finchi</i>	33.3	0.0008
<i>R. hascheana</i>	31.2	0.0002
<i>R. hosei</i>	46.0	0.0060
<i>R. kuhlii</i>	52.7	0.0080
<i>R. laticeps</i>	41.4	0.0051
<i>R. limnocharis</i>	54.4	0.0109
<i>R. luctosa</i>	37.3	0.0015
<i>R. palavanensis</i>	27.4	0.0020
<i>R. pileata</i>	58.9	0.0120
<i>R. plicatella</i>	34.0	0.0009
<i>R. sylvatica</i>	49.1	0.0069
<i>Staurois latopalmaris</i>	47.9	0.0065
<i>S. natator</i>	35.5	0.0036
Family Bufonidae		
<i>Ansonia longidigita</i>	42.2	0.0070
<i>Bufo asper</i>	104.8	0.2290
<i>B. biporcatus</i>	49.5	0.0180
<i>B. boreas</i>	62.5	0.0330
<i>B. bufo</i>	58.9	0.0430
<i>B. coccifer</i>	54.1	0.0093
<i>B. cognatus</i>	79.7	0.0430
<i>B. debilis</i>	43.0	0.0072
<i>B. garapiensis</i>	78.6	0.3200
<i>B. haematiticus</i>	47.2	0.0465
<i>B. maculatus</i>	52.4	0.0112
<i>B. marinus</i>	113.6	0.0900
<i>B. melanostictus</i>	87.0	0.0382
<i>B. parva</i>	30.0	0.0033
<i>B. spinulosus</i>	87.9	0.0796
<i>B. typhonius</i>	52.6	0.0064
<i>B. valliceps</i>	59.3	0.0107
<i>B. woodhousei</i>	80.6	0.0490
Family Hylidae		
<i>Agalychnis callidryas</i>	53.0	0.0110
<i>A. moreletti</i>	62.0	0.0450
<i>A. saltator</i>	39.3	0.0110
<i>Anothea spinosa</i>	56.2	0.0264

Appendix I (continued)

	SVL (mm)	Testis mass (g)
<i>Gastrotheca argenteovirens</i>	44.7	0.0013
<i>G. marsupiata</i>	47.4	0.0075
<i>Hyla arenicolor</i>	33.9	0.0025
<i>H. boans</i>	87.0	0.0224
<i>H. chinensis</i>	28.8	0.0020
<i>H. cinerea</i>	41.3	0.0040
<i>H. crepitans</i>	60.0	0.0038
<i>H. faber</i>	76.0	0.0109
<i>H. gratiosa</i>	56.0	0.0216
<i>H. loquax</i>	40.0	0.0046
<i>H. raniceps</i>	46.6	0.0015
<i>H. rosenbergi</i>	83.7	0.0073
<i>H. squirella</i>	30.8	0.0030
<i>Litoria alboguttata</i>	54.2	0.0148
<i>L. infrafronata</i>	87.7	0.0345
<i>Osteopilus septentrionalis</i>	46.5	0.0194
<i>Pachymedusa dacnicolor</i>	76.0	0.1012
<i>Phyllomedusa sauvagii</i>	57.4	0.0425
<i>Pseudacris crucifer</i>	25.2	0.0024
<i>Pternohyla fodiens</i>	50.1	0.0051
<i>Smilisca baudini</i>	56.8	0.0056
Family Pelobatidae		
<i>Pelobates varaldi</i>	57.7	0.2270
<i>Scaphiopus couchii</i>	59.0	0.0200
Family Microhylidae		
<i>Asterophrys</i> spp.	68.4	0.0102
<i>Breviceps adspersus</i>	26.1	0.0020
<i>Kaloula pulchra</i>	59.3	0.0100
<i>Phrynomerus bifasciata</i>	46.5	0.0090

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