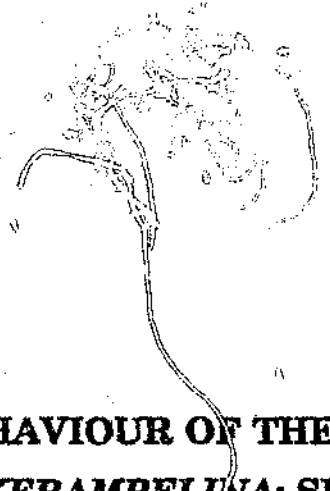

BREEDING BEHAVIOUR OF THE FOAM NEST FROG, *CHIROMANTIS XERAMPELINA*: SPERM COMPETITION AND POLYANDRY

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Thesis submitted to the Faculty of Science,
University of the Witwatersrand, Johannesburg
in fulfilment of the requirements for the
degree of Master of Science.

JOHANNESBURG

June 1992

I declare that this thesis is my own, unaided work unless specifically acknowledged in the text. It has not been submitted before for any degree or examination in any other university.

M.A. Jennings

2 June 1992

Dedicated to my family

Pauline, Richard and David

ABSTRACT

Breeding was observed in the foam nest frog, **Chiromantis xerampelina**, over three breeding seasons The mating pattern was characterized by an extended breeding season with a male-biased operational sex ratio and asynchronous and unpredictable female arrival. At more than 90% of nests, from one to seven unpaired males ('peripheral males') gathered around the amplexing pair during nest construction. Those peripheral males closest to the pair competed with each other, and with the amplexing male, to position their cloacae against the female's cloaca during oviposition bouts. In a detailed study of a single population, over 80% of males were observed as peripheral males, and 57% of males were observed both in amplexus and as peripheral males. Male mating success and participation at nests was unrelated to size or weight. Chorus participation was the best predictor of male mating success and participation at nests.

The most plausible explanation for the presence of peripheral males was a sperm competition hypothesis; namely that peripheral males compete with the amplexing male for fertilizations by shedding sperm into the nest. I collected data on body mass and testis mass for 18 African anurans. Using additional published data on 19 Japanese anurans, an allometric relationship between body mass and testis mass was calculated using 16 genera as independent data points. This revealed that **C. xerampelina** have testes fourteen times heavier than predicted on the basis of body mass. This is consistent with a trend seen in several taxa where testis size is related to the intensity of sperm competition. An additional experiment, in which the amplexing male was prevented from shedding sperm into the nest, showed that peripheral males are capable of fertilizing eggs. I conclude that peripheral males are engaged in an opportunistic alternative mating tactic involving sperm competition. More than half the observed females bred polyandrously, some mating with up to three males. This was the result of amplexing males dismounting between nesting sessions, and males displacing one another from amplexus.

PREFACE

Sperm competition has been a 'hot-issue' for biologists since the early 1980's and several book length treatments of the topic are now available. Despite being documented in almost every well-researched animal group (mammals, birds, fish, insects, millipedes and spiders) it has not been demonstrated in anuran amphibians (frogs and toads) *. This study was initiated to investigate the possibility that sperm competition occurs in the foam nest frog **Chiromantis xerampelina**.

Using observational and morphological evidence and by performing a simple experiment, I was able to demonstrate that sperm competition does occur in **C. xerampelina**. I also documented additional aspects of breeding behaviour which were unusual, or rare, for a frog with a prolonged breeding season. These phenomena included: the presence of polyandrous females; high rates of chorus participation by males; minimal male weight loss; strong call site fidelity; rudimentary female parental care; male mobility; and sporadic, subdued male advertisement calling.

The data presented here are far fewer than I originally intended. As an undergraduate, I conducted a pilot study in December 1989 which alerted me to the potential for experimentation and I had a series of experiments planned. Unfortunately, the breeding season in December 1990 only lasted for three weeks, and this time was devoted to a study of mating success and natural behaviour in the field. During the 1991-1992 field season, there was almost no breeding due to a particularly severe drought. Consequently, few of the planned experiments were conducted. The experiments I intended to conduct, and additional data I had wished to gather included: investigation

[* When I registered for this thesis in February 1991, there was only a single review of sperm competition in frogs (Halliday & Verrell 1984), and no recent papers. In the last year, however, three papers that deal directly with this issue have appeared in major journals: Fukuyama (1991), Kusano et al. (1991) and Feng & Narins (1991)].

of sperm depletion; estimates of fertilization rates for amplexing and peripheral males; effects of female parental care on hatching success; investigation of the cues that males use to locate gravid females; manipulations to determine the method that females use to relocate their nests.

In the course of this thesis, I carried out work that was tangential to the main thrust of my study. During the 1991-1992 drought, I collected data on nocturnal body temperatures of **C. xerampelina** and **Hyperolius marmoratus**. Although not directly related to breeding in the former, I have included this work in Chapter Five. In Appendix I have included an additional short paper reporting field work carried out in the Republic of Panama. It deals with predator-prey interactions between frogs and bats, and the effect of group size on anti-predator behaviour.

I would like to acknowledge the assistance of my supervisor, Professor Neville Passmore. I thank him for his support during this study and in the broader scheme of things. His kindness and generosity is much appreciated.

For counting tadpoles with teaspoons and staying awake most of the night I thank Lorraine & Vince Egan, Marc de Fontaine and Janice Tooley. For help with accommodation, research permits and rainfall reports I thank: the Natal Parks Board and their staff at Mkuzi Game Reserve, especially Peter Goodman; Tamar, Dave and Ruth for accommodation at Mkuzi; Jerry & Hesta Theron for accommodation at Swadini; Les Minter; Craig Ferguson and the owners of Vienna Game Farm for permission to conduct research and the provision of accommodation; and Debbie Osberg for her hospitality, bath oil and patience in Venda.

There are many people to thank in the Zoology Department. For help with equipment I thank Moses Banda, Dave Amm and Phil Bishop. For actually thinking aloud in the tearoom gratitude goes to Al, Andy, Ara, Martin, Sarah, Tom & Wacky. Robin Crewe, Barry Fabian and Neil Caithness are thanked

for letters of reference which have kept me in the gravy. Lindsy, Adele and Janie fielded phone calls, ran messages and helped me master the fax. Rob Veale did a sterling job as the grim reaper. Marcus Byrne loaned me his buckets. Graham Alexander let me go on three Zoo II Fieldtrips, despite my continued ineptitude. Margaret sorted and posted my mail and kept the kettle boiling. The tea room regulars introduced me to the heady world of children, triathlons and home maintenance. Thank you all.

Berndt Weissenbacher's enthusiasm kept me in science, and I thank him for his time, patience and altruism. For their friendship and encouragement I am most grateful to Mandy Dyson, Peter Henzi, Phoebe Barnard, Rob Simmons, Brendon Babb, the Garlands and assorted Backwells. They put things in context, induced double vision and made me laugh. Most of all I wish to thank four people. My parents, Richard and Pauline; my brother, David; and my best friend, Patricia.

I am grateful for the financial support provided by Ian & Jean Garland; the Research Incentive Scheme; two bursaries each from the Foundation for Research Development and the University of the Witwatersrand; and an E.P. Bradlow/John Lemmer Scholarship. Animal ethics clearance was provided by the Animal Ethics Screening Committee (# 90-157-3).

Various papers reporting the findings detailed in this thesis have been published; or are in review. They include:

Jennions, M.D., P.R.Y. Backwell, and N.I. Passmore. (in press). Breeding behaviour of the African frog **Chiromantis xerampelina**: multiple spawning and polyandry. Animal Behaviour.

Jennions, M.D. & P.R.Y. Backwell. (in press). Chorus size influences on the anti-predator response of a neotropical frog. Animal Behaviour.

Jennions, M.D. & N.I. Passmore. Direct and Comparative evidence for sperm competition in an African anuran: testes size and a 'sterile male' experiment. (submitted to Biological Journal of the Linnean Society).

Jennions, M.D., A.A. Turner, and N.I. Passmore. Nocturnal body temperatures in the painted reed frog (**Hyperolius marmoratus**) and the foam nest frog (**Chiromantis xerampelina**). (submitted to Herpetologica).

Papers were also presented orally at the following conferences:

* Second Ethology Group Meeting (ZSSA). Stellenbosch University. January 1990.

* African Amphibian Working Group:7th Meeting. Bulawayo, Zimbabwe. January 1991.

* Third Ethology Group Meeting (ZSSA). Golden Gate National Park. January 1992.

ABBREVIATIONS USED IN THE TEXT

C	degrees Centigrade
cm	centimetre
dB	decibel
df	degrees of freedom
E	east
ESS	Evolutionary stable strategy or state
Fig.	figure
hr	hour
KHz	kilohertz
km	kilometre
Lat.	latitude
Long.	Longitude
m	metre
min	minute
mm	millimetre
N	sample size
NS	non-significant ($P > 0.05$)
P	probability
S	south
SD	standard deviation
SE	standard error
s	second
SPL	sound pressure level
SVL	snout to vent length
x	mean

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 PREAMBLE

Sexual differences in morphology, size and behaviour are well known in animals. One need only think of the ornamental tail of the peacock, the imposing horns of an adult male kudu, or the contrast between the devoted attention to parental duties by lionesses and the infanticidal behaviour of male lions to appreciate the extent of this variation. What is most impressive about these differences is that they occur within a single gene pool. Speciation generates biological diversity, but in many cases it leads to little more than variation on a theme. In the case of sibling species, phenotypic differences are confined almost entirely to mating signals, and different species may be strikingly simi. morphologically. In contrast, in a number of species the sexes are so different in appearance that they were originally placed in separate taxa before the mistake was tified!

There are three potential sources of selection that may account for sexual dimorphism (Hedrick & Temeles 1989). First, there is selection for divergence due to food competition between the sexes (Peters & Grubb 1983). If a breeding pair partition available resources between the male and female, especially when they forage together or share a feeding territory, this will potentially increase their fecundity or viability. Tests of this hypothesis in a Galapagos finch (**Geospiza fortis**), however, have failed to show any correlation between reproductive success and the magnitude of the beak dimorphism of breeding pairs (Price 1984). At present, evidence in support of the food competition hypothesis is lacking.

Second, there is the possibility that unique natural selection pressures act on males and females because of the reproductive roles they play ('dimorphic niche' hypothesis). Evidence for this type of divergent selection is more readily available. In a marine isopod (**Ideotea baltica**), for example, males actively seek females, moving through light-coloured algal in the process. Females are predominantly stationary and brood eggs on darker algal substrate. Natural selection for crypsis, which reduces predation, has resulted in sexual dimorphism in colour patterns and differences in habitat preferences (Schuster 1990). A seemingly ever more obvious example of dimorphic niche selection, are the pronounced morphological differences between the sex organs of males and females. Surely these differences are the result of natural selection for effective gamete production and parental care? The answer, surprisingly, is not always clear cut. Certain sex-specific features, like the uterus and the placenta, are implicated in parental care and are thus viewed as products of natural selection for fecundity. But what about the amazing diversity of male and female genitalia found across taxa? Is the only function of these complex organs and appendages to ensure successful fertilization? Or is there, perhaps, some other selective force at work (Eberhard 1990)? A potential explanation to this conundrum invokes another selection pressure - sexual selection.

The concept of sexual selection was developed by Charles Darwin in 1871 in an attempt to account for bizarre and extravagant features, found primarily in males, that are likely to decrease the viability or classic fitness of the owner. Characters of this ilk are incompatible with the concept of natural selection for increased survivorship. Darwin suggested that elaborate characters and patterns of behaviour that reduce survivorship evolve as a consequence of the advantage they conferred to the bearer in exclusive relation to competition for mates. Although Darwin made a distinction between those features that evolved through sexual selection as opposed to natural selection, it is often difficult to distinguish between these two forces [see Arnold (1983); cf. Grafen (1987)]. This is especially so when natural and

sexual selection both favour similar evolutionary trajectories for a trait (eg. larger body size in males).

Darwin divided sexual selection into two components. First, intra-sexual selection which produces traits that confer an advantage when members of the same sex compete directly for access to the opposite sex (male-male or female-female competition). In many ungulates, for example, males fight each other for access to females using elaborate horns and antlers (Clutton-Brock et al. 1980; Packer 1983). These weapons increase the fighting success of combatants, but they probably carry a survival cost as well. This is indicated by Darwin's own observation that these weapons are not carried by females and, in the case of deer, that antlers are shed at the end of the breeding season. Similarly, male birds often moult out of their bright breeding plumage and assume more cryptic coloration at the end of the breeding season. Both observations suggest that, in the absence of benefits from intra-specific competition for mates, possession of these traits is disadvantageous.

In spite of theoretical predictions, empirical studies have failed to show that male traits advantageous in fighting are costly in terms of viability and, in some cases, the reverse has been found (Harvey & Bradbury 1991). A major problem is that environmental differences during early life may confound the analysis. For example, males that receive plentiful food during maturation may develop larger weapons and survive longer. Excess food can be invested in other traits affecting survivorship. Uneven distribution of resources during ontogeny may obscure the true relationship between traits. The cost of developing weapons may also be reflected in higher mortality during early life history stages. This cost is often neglected by researchers, not least because it is difficult or impossible to calculate the expected magnitude of an adult trait from a dead juvenile.

The second component of sexual selection that Darwin proposed was inter-sexual selection, which results in the evolution of traits that confer an

advantage when members of one sex choose between individuals of the other sex on the basis of these self-same traits (mate choice). Peahens, for example, show a strong preference for peacocks with large tails that bear more eye-spots (Petrie et al. 1991). The fact that these preferred traits are only expressed by males is taken as evidence that they carry survivorship costs. Female European swallows (**Hirundo rustica**) prefer males with longer tail feathers, but tail length is negatively related to survival (Møller 1988a, 1989a). There is also good evidence from crickets (Cade 1979; Hoy 1991) and frogs (Tuttle & Ryan 1981) that displaying to attract females increases predation risk. Perhaps the most general cost incurred by males is an energetic one. Numerous field studies have shown that behavioural traits have the greatest effect on male mating success. These preferred male displays are energetically costly to perform and may expose males to increased risks of starvation or susceptibility to predation (Halliday 1987).

Sexual selection by mate choice remains one of the most controversial areas in behavioural ecology (Krebs & Davies 1991). Strong empirical evidence from field studies show that mating success in a wide range of taxa is correlated with specific male traits (eg. Ryan 1985; Breden & Stoner 1987; Trail & Adams 1989; Andersson 1989; Luyten & Liley 1991; Petrie et al. 1991). One technical problem, however, is that the trait correlated with mating success may not be the preferred trait, but rather a trait correlated with the preferred trait. Experimental manipulation of single traits has been used to address this issue directly (eg. laboratory: Basolo 1990; Millinski & Bakker 1990; Gerhardt 1991; field: Andersson 1982; Borgia 1985; Diamond 1988; Møller 1988a; Höglund et al. 1990). Even so, in some cases the possibility of male-male competition being influenced by the manipulation, and thereby affecting mating success still can not be ruled out (eg. Barnard 1990).

The evolutionary mechanisms generating female preferences remain obscure (Bradbury & Andersson 1987). This is especially so when scientists can see no direct benefit to the female spending time and effort discriminating

between males (Kirkpatrick 1987). The classic 'paradox of the lek' is that females continue to discriminate between males even when the sole resource provided by males is sperm (Kirkpatrick & Ryan 1991; but see Reynolds & Gross 1990). Even on leks, however, correlations between male traits and mating success do not conclusively show that female choice is operating; or phrased more conservatively, that it is the major determinant of male success. The potential for male-male competition to affect mating success still exists (Arak 1988a; Clutton-Brock 1992). In general, teasing apart the extent to which male-male competition and female choice have influenced the evolution of traits remains a difficult task (Harvey & Bradbury 1991).

Sperm competition provides an interesting testing ground where theories about female choice and male-male competition can be tested. Sperm competition occurs when the sperm of two or more males compete to fertilize a female's eggs (Parker 1970). Initially, sperm competition was viewed solely from the masculine perspective. Analysis went little further than arguing that males should produce motile, competitive sperm in large numbers, and that these sperm should then compete to fertilize eggs. Of late, however, increased emphasis has been placed on the role the female plays in the process (Birkhead 1989). Females may determine the conditions that allow sperm competition to occur, as well as its relative intensity. Multiple mating, for example, can be viewed as a choice procedure that allows females to discriminate between males on the basis of their sperm, or precopulatory and copulatory behaviour (Madsen et al. 1992). Females may also set the rules of the game by adjusting the weighting given to different male's sperm. In dunnock (**Prunella modularis**), for example, females may extrude ejaculates in response to cloacal pecking by competing males (Davies 1985). In some cases, sperm competition may actually circumvent female choice - especially if females can not influence the competitive process, as is likely in species with external fertilization. In two labrid fish (**Symphodus tinca** and **S. ocellatus**), for example, small peripheral males release sperm into the water during spawning. Females respond to the presence of peripheral males by

decreasing their reproductive output, suggesting that they prefer to be fertilized by the larger territorial males (van den Berghe et al. 1989).

Frogs have proved an ideal group for studies into sexual selection processes (Ryan 1990). Experimental work is comparatively easy because females are attracted to males on the basis of their advertisement calls. Females also respond readily to synthetic acoustic stimuli in the laboratory. This allows variables to be manipulated individually so that the traits females prefer can be unambiguously identified. The possibility of male-male competition is also ruled out, because females are tested in the absence of males. In addition, most male frogs form leks where resources are not defended and the only benefit derived from female choice lies in indirect genetic gains for progeny [but see Robertson (1990) for a cautionary tale]. Finally, corroborative field studies are also possible because individuals are easily handled and marked, allowing individual reproductive success to be calculated.

Most workers would have added to the above list the additional fact that frogs have external fertilization which allows for certainty when assigning paternity (see quotes in Arak 1983a and Howard 1988a). This statement, however, is patently false. For example, many fish species with external fertilization have mating systems where sperm competition occurs [eg.salmon (Gross 1985), blue-headed wrasse (van den Berghe & Warner 1989), blue-gilled sunfish (Dominey 1980; Gross 1982)]. The potential for sperm competition clearly exists in species with external fertilization. To date, however, there are few studies that show any real potential for sperm competition occurring in frogs. There seem to be important differences between fish and frogs limiting the evolution of sperm competition in the latter. It would be illuminating to discover what these constraints are.

1.1.1 Anuran Mating Patterns

There are more than 3400 extant frog species (Duellman & Trueb 1986). Despite this biological diversity, the majority of species have mating patterns that are relatively easily characterized. The mating patterns of populations (intra-specific variation exists) can be described as falling on a continuum between explosive breeders and prolonged breeders (Wells 1977a). The primary feature influencing this dichotomy is the duration of the breeding season (typically explosive breeders finish breeding in under a week) which determines the spatial and temporal distribution of females and males. In turn, this affects the operational sex ratio (Emlen & Oring 1977), male density, the opportunity for female choice, and the intensity and form of male-male competition. Together these variables reflect the form and intensity of sexual selection pressures and may also be used to characterize mating patterns. Strictly speaking, mating patterns are described in terms of mate-acquisition, pair bond characteristics and the sexual pattern of parental care (Davies 1991). In frogs, however, parental care is comparatively rare (<10% of species) and pair bonds are absent. Most patterns can thus be described solely in terms of mate-acquisition. [Note: I use the term 'mating pattern' in preference to 'mating system' (T.R. Halliday, unpublished)].

Prolonged breeders exhibit either resource-based territoriality or form leks. Males tend to call from fixed positions within the chorus and maintain inter-male spacing through vocal or physical interactions (Telford 1985; Wilczynski & Brenowitz 1988; Backwell & Passmore 1990a). Females are attracted to choruses by conspecific male advertisement calls. The mate-locating tactic is one of female-attraction, and male-male competition is primarily indirect, involving attempts to out call other males by producing more intense or more frequent calls (Arak 1988a; Gerhardt 1988). Active female choice is most likely to occur in prolonged breeders because of the lack of direct male competition for females. Indeed, the best documented examples of female choice based on male call attributes are all from prolonged breeders [eg.

Physalaemus pustulosus (Ryan 1985), **Hyperolius marmoratus** (Passmore et al. unpublished), **Uperolia laevigata (rugosa)** (Robertson 1986, 1990), **Bufo woodhousei** (Sullivan 1987a), **Hyla chrysoscelis** (Morris 1989), **Bufo calamita** (Arak 1988a), **Eleutherodactylus coqui** (Lopez & Narins 1991)].

In prolonged breeders, female arrival is unpredictable and extends over a lengthy time period. It has been suggested that males should spread the energy they invest into reproductive activities (primarily calling) over a portion of, or even the entire, breeding season to increase their likelihood of mating multiply (Arak 1983a). This assumption is not based on a formal model. In many prolonged breeders a large proportion of males fail to mate at all, despite repeated attendance at the chorus. When considering the optimal strategy for males, three factors should be considered. First, female arrival is unpredictable and males can not risk investing all energy into calling on only one or two nights. Second, female choice usually selects for calls containing more energy (Gerhardt 1988). To mate successfully, then, males can not rely on calling weakly every night. To be chosen as a mate they must engage in loud and repetitive calling behaviour and bear large energetic costs. Third, the nightly likelihood of mating will vary with the operational sex ratio. Males do not appear to be able to accurately assess when females will breed. Although there is often a correlation between male and female numbers, the value of r^2 is usually very low (Henzi et al. unpublished). Males should thus use male density as the best predictor of the intensity of competition (ie. the number of competitors the female may choose between). There is evidence that all three factors influence calling strategies.

Males do not call consistently throughout the breeding season. In most species, males are present on a very small percentage (<10%) of the available nights, even though the number of nights a male is present is strongly correlated with his mating success (frogs: Fellers 1979; Goodwin & Rohle 1983; Gerhardt et al. 1987; Green 1990; Salvador & Carrascal 1990; Dyson

et al. in press; birds: Hill 1991). Calling by male anurans is one of the most energetically expensive activities recorded for ectothermic vertebrates (Prestwich et al. 1989) and males may invest so heavily in calling that they are unable to call every night of the season. The low rate of presence at breeding sites indicates that males do not spread their energetic investment evenly over the breeding season. It has been suggested that to maintain calling behaviour in the long term, males must sacrifice some nights at the breeding site in order to forage (Woolbright & Stewart 1987). Green (1990) has tested the energetic constraint hypothesis in **P. pustulosus** where he found no evidence to support it. He suggests that male presence and absence is related to fluctuations in the predation risk. Ryan (1991), however, has reported decreased calling in **P. pustulosus** when food availability is reduced (Marler, unpublished study). In another study, Turner (1991) reported no effect of food availability on calling in **Hyperolius marmoratus**. Finally, it has been suggested, and there is some supporting evidence, that calling in bouts in **Hyla microcephala** is associated with reducing the energetic costs of calling (Schwartz 1991).

Investing considerable effort in calling, even if this reduces the number of nights a male participates in choruses, may be a profitable strategy. Female choice usually selects for calls that contain more energy and males that do not invest heavily in calling when present at the breeding site are unlikely to mate (Gerhardt 1988). We might predict, however, that males should also adjust calling effort in relation to competition, as indicated by the number of other males calling. There is good evidence supporting this prediction. In **Hyla microcephala** males increase calling effort and energy used as chorus size increases (Wells & Taigen 1989). Call rate (and presumably energy used) increases with density in **Rana catesbeiana** (Emlen 1968), **Hyla ebraccata, Hyla phlebodes** (Wells 1988), **Hyla crucifer** (Rosen & Lemon 1974) and **Uperolia rugosa** (Robertson 1984). Bucher et al (1982) have reported increased energetic costs as call rate increases in **Physalaemus pustulosus**. Increased energetic costs as call rate rises have also been

documented in **Hyla cinerea, H. gratiosa** and **H. squirella** (Prestwich et al. 1989)]. Call complexity also increases with chorus size in several species [eg. **Physalaemus pustulosus** (Rand & Ryan 1981); **Smilisca sila** (Tuttle & Ryan 1982); **Geocrinia victoriana** (Littlejohn & Harrison 1985); **Philautus leucorhinus** (Arak 1983b); **Hyperolius tuberilinguis** (Pallett & Passmore 1988)], but the energetic costs of these changes are unknown. In **P. pustulosus** call complexity does not affect males' metabolic rate (Bucher et al. 1982).

In **Hyla versicolor** males' call rate decreases and call duration increases as chorus density rises, so that calling effort remains approximately constant (Wells & Taigen 1986). At present there is no satisfactory explanation for this phenomenon (Wells & Taigen 1989, p21). A final factor complicating the issue of calling strategies is the effect of life history parameters (Elmberg & Lundberg 1991). For example, in short-lived species where survivorship away from the breeding site is low, the best strategy may be to divert all available energy into breeding attempts, regardless of breeding season length. In explosive breeders, males employ very different mate-localization tactics to those of prolonged breeders. Female arrival is more predictable, the operational sex ratio is usually less male-biased and male density is high. Because the breeding season is brief, a male is unlikely to mate more than once. Given this, all available energy should thus be placed into acquiring a mate as soon as possible. This leads to intense and direct competition between males, and female choice is unusual. Males actively search for females and calling seldom occurs, because high male densities increases the likelihood of interception by searching males (Fairchild 1984). Evidence that male density affects calling is provided by studies of populations where male density is low and calling is observed, in species that usually show searching behaviour (eg. **Bufo bufo**, Höglund & Robertson 1988).

Mating patterns are a consequence of the way that individuals interact. Sometimes authors have written about mating systems as though they

determine individual behaviour. Focusing on the adaptive strategies individuals should pursue under various demographic, energetic and life history schedules provides the best framework for understanding mating patterns. In most frogs, the breeding season, because it affects the spatio-temporal distribution of females, will determine how males behave (Davies 1991). In some cases, however, additional aspects of the species biology may mask, or alter, the form of the mating system. For example, male parental care is an additional resource for female which may affect their distribution (Davies 1991) or behaviour (Summers 1990). Similarly, sperm competition may also affect mating patterns by altering the costs and benefits associated with mating and mate-acquisition tactics.

1.1.2 Anuran Mating Tactics

Before the development of behavioural ecology in the mid-1970s, workers sought to discover behaviour patterns that were characteristic of the species; for example, the fixed action pattern of classic ethology (Waltz & Wolf 1984). Despite these attempts, it soon became clear that considerable intra-specific variation exists, not all of which is attributable to measurement error. In the last fifteen years considerable effort has been invested in explaining the underlying mechanisms and the functional significance of behavioural variation (Caro & Bateson 1986). Variation in behaviour related to mating has been particularly well investigated because mating success is unambiguously related to evolutionary fitness. A multitude of studies investigating mating strategies and tactics have thus been conducted (Austad 1984).

Most alternative mating tactics appear to 'parasitize' the dominant tactic (van den Berghe 1988). The pressures leading to alternative mating tactics differ from those causing variation in other functional behaviours (eg. foraging, anti-predation) because they are primarily a consequence of intra-specific competition. They occur more commonly in males, because females

are the limiting sex in most species (Clutton-Brock & Vincent 1991). There is thus stronger selection on males than females for access to mates and male-male competition drives the evolution of alternative mating tactics (Telford & Dyson 1988). Even in those frogs where males provide parental care, the rate at which males can care for additional clutches is usually higher than the rate at which females can produce them (Clutton-Brock & Vincent 1991; Summers 1989, 1990). In the absence of competition, males should utilize the tactic conferring the highest fitness payoffs [by definition the dominant tactic; but see van den Berghe (1988)]. As competition escalates, the number of males that can employ the dominant tactic becomes a subset of the total population. Those males unable to pursue the dominant tactic are thus forced to switch to alternative methods of mate acquisition. These alternative tactics are usually less successful, but in rare cases may be equally rewarding (Gerhardt et al. 1987; Schuster & Wade 1991).

In many taxa the mating tactic an individual expresses appears to be fixed in the medium to long term. This is especially true when expression of tactics is conditional on size or age (Hughes 1985). In some cases, tactics may be directly correlated with underlying genetic variation and are fixed for life (Gross 1985; Ryan & Causey 1989; Schuster & Wade 1991). Frogs are unusual because switching between tactics is relatively common and occurs over short time periods. There is considerable flexibility in male behaviour, with males responding to short-term changes in the social environment (Arak 1988b). Fixed tactics, especially genetically determined ones, are unknown in frogs.

In frogs, the duration of the breeding season partially determines the intensity of male-male competition and hence the types of alternative mating tactics that are possible. There are clear differences in both the frequency and types of alternative mating tactic exhibited in prolonged and explosive breeders, even when they are different populations of the same species (Tejedo 1988). In prolonged breeders, territoriality may place an upper limit

on the number of males that can call. Excess males often pursue a tactic of satelliting, whereby they remain motionless near a calling male and attempt to intercept females attracted to the caller (Perrill et al. 1978). Similar tactics of interception have been documented in insects (Cade 1979), birds (van Rhijn 1973) and mammals (Clutton-Brock et al. 1982). In a literature review, I found that eleven of thirteen frog species with satellite males were prolonged breeders. In explosive breeders, the main alternative mating tactic is amplexus displacement. Unpaired males attempt to wedge themselves between an amplexing pair and, if successful, proceed to dislodge the amplexing male (Lamb 1984). Competition is direct and appears to be independent of female choice (Davies & Halliday 1979). Fifteen of nineteen species where displacement has been documented in the field are explosive breeders (unpublished review). A third tactic 'opportunistic calling' by non-territorial males in **Rana catesbeiana** (a species displaying resource-based territoriality) has been proposed by Howard (1984). I suggest, however, that these males should simply be described as short term territory holders.

1.1.3 Sperm Competition

Sperm competition is defined as the competition between sperm of different males to fertilize the egg(s) of a single female (Parker 1970; Birkhead 1989). In some mating systems, it is an important source of sexual selection pressures and affects the behaviour and morphology of both sexes. With a few notable exceptions (eg. Townsend et al. 1981), fertilization in frogs is external and males shed sperm over eggs as they are extruded. The potential for sperm competition exists if additional, unamplexed males shed sperm during, or immediately after, eggs are deposited. The amplexing male, however, has three potential advantages over additional males. First, he has direct cues regarding the timing of oviposition that are provided by the female. Second, he has the greatest proximity to unfertilized eggs. Three, he is attached to the female and can thus remain with her should she move. If these advantages confer a very high percentage of fertilizations to the amplexing

male, there will be little opportunity for sperm competition to occur.

Available evidence for sperm competition in species with external fertilization has been acquired almost solely from fish. Only recently has strong evidence accumulated that sperm competition also occurs in frogs (see Preface). Coe (1967, 1974) documented unpaired males alongside amplexing males in two foam-nesting rhacophorids, **Chiromantis rufescens** and **C. petersii**. His description suggested that males were co-operating in the construction of the nest. These observations were noted by E.O. Wilson (1975) in his classic text Sociobiology: the modern synthesis, where he suggested that kin selection might explain the males' co-operative behaviour. An alternative explanation provided by Halliday & Verrell (1984) was that additional males in **Chiromantis** are competing with the amplexing male to fertilize eggs. Until my study, however, there was no data that clearly distinguished between these two hypotheses.

1.2 STUDY SPECIES

Chiromantis xerampelina is a large frog (male snout vent length = 50.5 - 69.4 mm and female 67.8 - 82.9 mm) (Fig. 1.1). Adults are slender with long, thin limbs. The toes and fingers are elongated and webbed and each bears a terminal adhesive disc (Passmore & Carruthers 1979). The two inner fingers are opposable to the outer two which allows them to grasp securely to thin twigs, much akin to a chameleon. The body colour ranges from a cryptic grey speckled with brown patches, to a chalky white when they are exposed to sunlight (Pienaar et al. 1976). They are distributed throughout the low-lying regions of South Africa, Swaziland and Mozambique. They also occur in Botswana, the Caprivi strip, Zimbabwe, Malawi and Tanzania, extending into the coastal areas of Kenya and Somalia (Coe 1974). There are only two congenerics - **C. rufescens** which occurs in West and central Africa and **C. petersii** which inhabits the dry savannah of East Africa. **Chiromantis** is one of ten genera in the family **Rhacophoridae** (186 species). The other

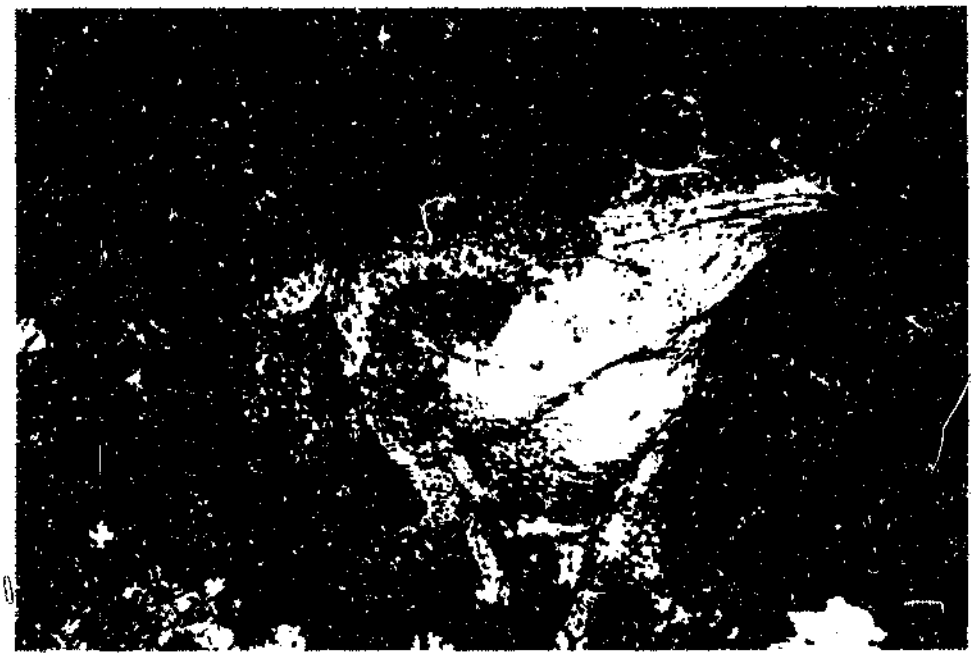

Figure 1.1 Photographs of (A) a calling male and (B) a gravid female **Chiromantis xerampelina.**

genera occur in the Asian tropics, subtropical India, Sri Lanka, the Phillipines, Japan, the Greater Sunda Islands and Madagascar (Duellman & Trueb 1986).

Chiromantis xerampelina usually breeds on emergent vegetation, tree branches or other structures overhanging temporary water sources. They may, however, also breed over slow-flowing rivers and streams (personal observation, Mkuzi). Males call from elevated places that are potential oviposition sites. Breeding occurs in the summer months (October to March), but is normally discontinuous because (a) surface water is often absent; and (b) females seem to require rain as a trigger for ovulation. Females construct foam nests by releasing an oviducal secretion which they then churn into a white foam. Eggs are deposited directly into the foam. Nest construction is characterized by the presence of additional, unpaired males that gather around the amplexing pair. Prior to this study, the only available information on breeding was that of Wager (1926, 1957, 1965), Stewart (1967), Taylor (1971a, 1971b), Coe (1974) and Pienaar et al. (1976). Much of this literature was anecdotal and, in some cases, the information appears to be factually inaccurate.

1.3 STUDY AREAS

Three different study sites were used during this study (Fig. 1.2). Between December 1989 and January 1990, research was conducted at Mkuzi Game Reserve in Northern Natal (latitude 27°36' 50" S; longitude 32°12' 3" E). The site was approximately 2.5 km south-west of Mantuma Camp, on the eastern side of the main road, about 15 m from the verge. It consisted of a small natural pan with a maximum depth of approximately 1 m and a surface area of 200 m^2. The surrounding vegetation was primarily **Acacia** thicket. Trees that overhung the water and were subsequently used as nest sites were **Acacia luderitzii, Euclea sp.,** and **Spirostachys africanus.** Other frog species that called at the pan included **Ptychadena anchietae,**

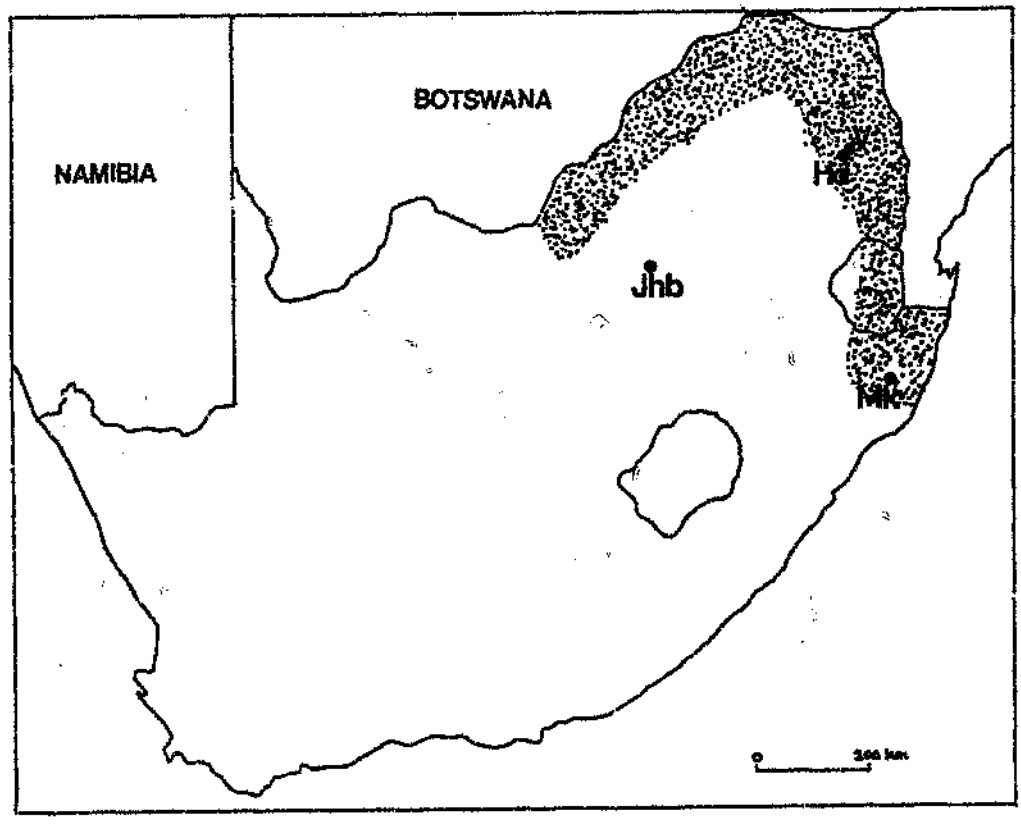

Figure 1.2a Map showing the distribution of **Chiromantis xerampelina** in South Africa, as well as the location of the three study sites (Symbols: Mk = Mkuzi Game Reserve; Hd = Hoedspruit; V = Vienna Game Farm; Jhb = Johannesburg).

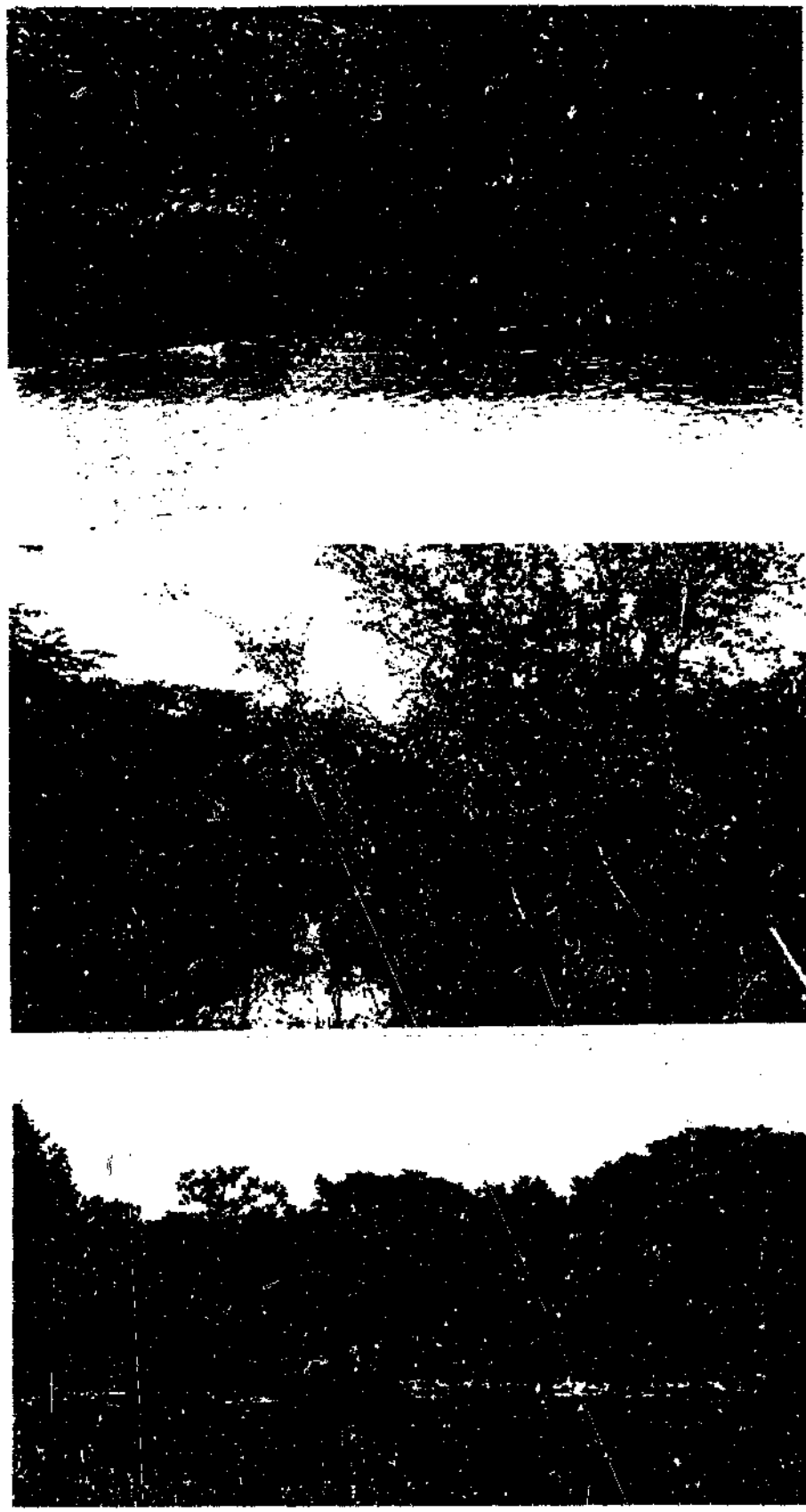

Figure 1.2b Photographs of: (A) The study site at Mkuzi: (B) The main study site at Hoedspruit; (C) the dam at Mariepsig. There is no photograph of Vienna.

P.mossambicus, P. porosissima, Phyrnomerus bifasciatus, Phyrnobatrachus natalensis, Cacosternum nanum, Leptopelis mossambicus and **Afrixalus aureus.**

In December 1990, research was conducted in the Hoedspruit district of the Eastern Transvaal. The site was on the R531 road approximately 2.5 km from the Swadini turn-off (latitude 24°27' 15" S; longitude 30°52' 20" E). It was an artificial pan fed by a drainage ditch and was ±500 m^2 with a maximum depth of 1.2 m. Several small **Acacia spp**, sedges and flooded grasses were used as nest sites. Other frog species breeding here included **Ptychadena anchietae, Phrynomerus bifasciatus, Pyxicephalus adspersus, Xenopus muelleri, X. laevis, Schismaderma carens, Phrynobatrachus natalensis, P. mababiensis, Hyperolius marmoratus** and **Kassina senegalensis**. Additional observations were also made at two farm dams at Mariepsig Caravan Park, 800m south-east of the main study site.

In December 1991 and January 1992, research was conducted on Vienna Game Farm 5km north of Hoedspruit. The study site was a large dam (Snare dam) approximately 25 m by 40 m (latitude 24°16' 44" S; longitude 30°58' 26" E). Nests were constructed along the main embankment on **Acacia spp** and on dead branches placed in the water. Other frog species breeding here included **Ptychadena anchietae, Hemisus marmoratus, Tomopterna cryptotis, Xenopus laevis, X. muelleri, Hildebrandtia ornata, Kassina senegalensis** and **Rana angolensis.**

All three study sites are surrounded by bushveld vegetation (Acock 1988). Rainfall occurs primarily during the summer months (October - February), with approximately 500-700 mm falling per annum. During the summer, daytime temperatures are usually above 30 C. At night, temperatures drop into the middle to low 20's. More detailed accounts of vegetation and climate can be found in Acock (1988) and Tyson (1986).

1.4 SCOPE AND OBJECTIVES

This thesis is concerned primarily with the potential for sperm competition in **Chiromantis xerampelina**. Observational, morphological and experimental techniques were used to address this issue. The findings are placed in the broader context of breeding behaviour.

The major questions addressed in this work are:

1) Does sperm competition occur in **C. xerampelina**?
2) Does its occurrence affect male spacing, calling and reproductive physiology?
3) Do females show any morphological or behavioural traits that influence the intensity of, or bias, sperm competition?

Additional topics addressed are:

(1) Does basking and low evaporative water loss lead to elevated nocturnal body temperatures: a test using **Chiromantis xerampelina**?

(2) Does chorus size affect the anti-bat-predator response of the Neotropical frog, **Physalaemus pustulosus**?

CHAPTER TWO

BREEDING BEHAVIOUR, REPRODUCTIVE BIOLOGY AND MATING SUCCESS

2.1 INTRODUCTION

2.1.1 Sperm competition and mating patterns

Anurans are an ideal group for studies of mating behaviour and variation in mating systems because individuals are easily marked, handled and observed (Arak 1983a). Many reviewers also note that external fertilization in frogs allows for accurate quantification of the reproductive success of individuals (eg. Howard 1988a). External fertilization does not, however, ensure that paternity is known with certainty. For example, in several fish species with external fertilization, group spawnings occur and paternity is divided amongst the males present. Workers either assume that fertilizations are shared equally among the males present (van den Berghe et al. 1989), or that fertilization success is related to male proximity to the female (Gross 1985). Of course, estimates of reproductive success are most accurate when fertilization rates are calculated using data from biochemical paternity tests (Westneat et al. 1987).

The dominant male reproductive tactic in anurans is stationary calling. The most frequently documented alternative tactics are: *satellite behaviour*, where non-calling males attempt to intercept females attracted to calling males (Perrill et al. 1978); *amplexus displacement*, where unpaired males attempt to displace amplexing males (Telford & Van Sickle 1989); and *active searching* by silent males who clasp any individual they encounter during

their searches (Woolbright et al. 1990).

Alternative reproductive tactics are usually a consequence of male-male competition for a limited number of females. Competition may limit the number of males that can employ the dominant mating tactic, forcing some males to adopt alternative tactics with lower reproductive success (Kodric-Brown 1986). Generally, alternative reproductive tactics yield lower pay-offs than the dominant tactic, although tactics with equal pay-off (Gerhardt et al. 1987) and even higher pay-off (van den Berghe 1988) have also been reported. Tactics may be reversible or fixed, and either opportunistic, conditional or part of a mixed Evolutionary Stable Strategy/State (Austad 1984). In most anurans alternative tactics are conditionally employed by smaller males (Howard 1978; Loman & Madsen 1986; Arak 1988b; Krupa 1989). Fixed tactics have not been recorded in anurans, but when there is considerable intra-population variation in male size, and tactics are size-based, males may be limited to a single tactic for an entire breeding season (Howard 1984).

In this chapter I present behavioural evidence for the occurrence of an 'opportunistic' reproductive tactic rarely recorded in anurans. I suggest that multiple spawning and sperm competition are common features of the mating system of **Chiromantis xerampelina**. This suggestion is corroborated by additional sources of evidence presented in Chapter Three.

2.1.2 Parental Care

The evolution of parental care remains one of the few topics in evolutionary biology where unifying principles are lacking. Parental care is formally defined as parental behaviour that is likely to increase the fitness of the parent's offspring (Clutton-Brock & Godfray 1991). This definition is, however, sometimes difficult to apply (Gwynne 1991). In certain crickets, for example, males produce spermatophores that contain nutrients. Females are

more likely to mate with males producing spermatophores which, when consumed, increase the survival rate of the offspring. Should spermatophores be considered a male investment in mating, and products of sexual selection by female choice (Wickler 1985; Sakaluk 1986)? Or investment in offspring arising from natural selection for parental care (Parker & Simmons 1989)? Clutton-Brock (1991) in a recent book-length review of parental care focused on five main questions. The questions which are best understood are: What are the costs and benefits of parental care? [Answer: the main benefit is increased offspring survival, growth and breeding success, hence increased fitness for parents]; Why does the extent of parental care fluctuate between species? [Answer: it varies in relation to the magnitude of the harshness of the environment, and the degree of intra-specific competition]. Less well understood are the questions: What explains male, female and biparental care? Is parental care adjusted in relation to the costs for parents and the benefits for offspring? How is parental care divided between sons and daughters?

Parental care has been reported in a number of frogs (McDiarmid 1978; Wells 1981; Townsend 1986; Summers 1989). Most reports are from terrestrial breeders in the tropics (Wells 1981). Both males and females have been implicated in care. In fact, frogs are unusual in that male parental care is relatively widespread. There are two possible factors that make male parental care more likely to occur in frogs than in other vertebrates. First, the costs of parental care are usually low (Wells 1981). Second, eggs or tadpoles are often deposited in the male's territory so that he can provide care for them and continue to attract additional females (but see Townsend 1986). Parental care may take several forms including; guarding eggs or tadpoles against predation (Kluge 1981); preventing desiccation (Townsend et al. 1984); maintaining access to water (Kok et al. 1989); and carrying tadpoles, either to water (Summers 1990) or until development is completed (Ingram et al. 1975).

2.1.3 Clutch size

In anurans, as body size increases within a taxon (eg. genus or family), so does ovum size and clutch size (Salthe & Mecham 1974 and references therein). A similar trend is observed when clutch size in species with a given mode of reproduction are compared (eg. eggs in foam nests, eggs carried, eggs in water) (Crump 1974). According to Salthe & Mecham (1974), virtually all studies at the time of their review showed that, within a given species, larger females produce more eggs than smaller females. In contrast, Crump (1974) found a significant correlation between body size and fecundity in only 27% of the 41 species she examined. Inter-specifically, however, those species that constructed foam nests showed a significant positive relationship between size and fecundity. It has been suggested that clutch size within the genus **Chiromantis** is related to the aridity of the environment (Coe 1974). The forest-dwelling **C. rufescens** has a smaller clutch size than the woodland-inhabiting **C. xerampelina**, which, in turn, has a smaller clutch size than the savannah-dwelling **C. petersii** (Coe 1974).

2.1.4 Polyandry

A female mating with more than one male on the same or successive nights has only been reported in one species of anuran, the leaf-folding frog, **Afrixalus delicatus** (Backwell & Passmore 1990b). Multiple-mating with more than one partner is usually adaptive for males because it is likely to increase the number of offspring sired. For females, however, the benefits of multiple-mating are less clear. At first glance, it seems unlikely to raise a female's reproductive success. Potential adaptive explanations for multiple-mating by females include; (a) an increase in offspring viability. By allowing sperm competition to take place, females can 'select' for the sperm of the most viable male if sperm viability and male viability are related (for a

fascinating example involving adders (**Vipera berus**) see Madsen et al. 1992; but also see Parker 1992); (b) it may increase the genetic diversity of the progeny and raise the likelihood of survival in a fluctuating environment (Stacey 1982); (c) it may ensure that females receive sufficient sperm to fertilize all their eggs; (d) females may benefit directly from nutrients associated with ejaculates (Gwynne 1984); (e) it may result in more males providing parental care (Davies 1985); (f) it may reduce sexual harassment (Svard & Wiklund 1986); (g) it may guard against genetic aberrations arising from sperm storage. There are also some non-adaptive explanations for multiple-mating by females; (a) it may be a non-adaptive consequence of selection on males for an increased tendency to mate. The trait then arises in females as a genetic correlate of selection on males (Halliday & Arnold 1987) [A recent review of data relating to this explanation can be found in Arnold & Halliday (1992)]; and (b) some males may be selected to leave females before completion of oviposition (in species with external fertilization) if the rate of fertilization drops such that a 'fresh' female would yield a higher mean rate of fertilization (Backwell & Passmore 1990b).

2.1.5 Construction of foam nests

Frogs that produce foam nests are known in 19 of 301 anuran genera (Hödl 1990; Haddad et al. 1990). This behaviour seems to reflect convergent evolution and it has evolved independently in at least four families (Rhacophoridae, Leptodactylidae, Myobatrichidae and Hyperoliidae) [Duellman & Trueb 1986]. A recent report suggests that foam nests are also built by the hylid **Hyla rizibilis**, adding a fifth family (Hylidae) to the above list (Haddad et al. 1990). In the rhacophorids, females beat oviducal fluid into a frothy mass with their hind legs. In the other families, both sexes may co-operate in nest building, and the forelimbs may be used to beat the foam. It has been suggested that the chief benefit of a foam nest is to reduce the risk of desiccation (Ryan 1985). An additional benefit may be reduced predation

on eggs and early tadpole stages, especially that arising through conspecific cannibalism (Hödl 1990).

2.2 METHODS

2.2.1 Behavioural Observations and Mating Success

Observations of marked, breeding frogs were made in December 1990, in the Hoedspruit district, Transvaal, South Africa (24°27' S, 30°52' E). The study pond was approximately 500 m^2, with a maximum depth of 1.2 m. The closest neighbouring breeding site was 800 m away.

Thirty two males were collected individually by hand, measured, weighed, marked and released at the point of capture within 15 minutes. Snout-vent length (SVL) of all males was measured to the nearest 0.1 mm. Males were then hydrated and weighed to the nearest 0.1 g. Males were individually marked by heat branding on the dorsum. Size measurements were also taken for 34 females collected at the main study pond and the two neighbouring ponds at Mariepsig.

Observations were made on 21 nights over a 22 night period between 2000 and 0730 hours. Only two nests were constructed in the three weeks prior to the study and no males were present at the pond on termination of the study. Each night a census of the population was undertaken. I located twelve regions in the pond where males were consistently present (mean region size = 1.9 m^2 , range = 0.4-4.9 m^2). Seven of these regions were partially submerged trees, four patches of emergent grass and one a reedbed. The identity of males, the region they were in, and their behaviour were all noted. An effort was made to census the pond hourly, but this was not always possible due to observations of nesting. All males present at nests were identified. I located females that were either unpaired in the water, or already in amplexus out of the water, and observed them throughout nest

construction. Early each evening I checked the pond for nests not observed the previous night. This allowed for accurate correction of undercounts of female presence. Video recordings were made of the construction of six nests. These were used to determine the duration of the first 10 periods of quiesence between activity bouts for each nest session.

In the description of nesting, I refer to unpaired males on the lateral aspect of the branch with their legs in the foam, or that were immediately alongside another male whose legs were in the foam as 'peripheral males'. Any individual touching the foam nest is described as being 'on the nest'. Males are described as 'mated' if they were in amplexus during oviposition.

2.2.2 Male weight change

Male weight change was calculated for nine males at Hoedspruit (1990). They were re-weighed three to 19 days (mean = 14 days) after their initial capture. Male weight change was also calculated for nine males at Vienna (1991-92). They were re-weighed between two and 16 days after their initial capture (mean = 8.4 days). Weight loss (or gain) was calculated as the percentage change per day:

$$\frac{\text{(Final mass - Initial mass)}}{\text{Initial mass}} \times \frac{100}{1} \times \frac{1}{\text{Days since initial capture}}$$

2.2.3 Calling Behaviour

Data on calling behaviour were collected at Hoedspruit in December 1990 during the 22 day study. Male vocalizations were recorded on a Sony TCD-5M tape recorder using a Sony EMC-16T microphone. Recordings were made at air temperatures of 20 - 24 C. Air temperature was measured using a Bat-12 Bailey Instruments digital thermometer. Recordings were made opportunistically because males were frequently silent for extended periods -

focal sampling was thus unfeasible. Fifteen of the 30 males present at the site for two or more nights were recorded. Call rate was measured using a Uniscan II sound spectrum analyzer. Details of spectral and temporal call parameters (dominant frequency, pulse rate etc.) are presented in Appendix II. Peak sound pressure levels (SPL) of calls were measured in the field using a Brüel & Kjaer 2209 sound level meter. The microphone was held horizontally 25 cm in front of the caller. Measurements were collected for two males (25 calls and 30 calls). Sound pressure levels were converted from the relative decibel scale to absolute units (microbars) for statistical analysis.

2.2.4 Parental Care

Observations of female parental care were made at all three study sites (Mkuzi, Hoedspruit and Vienna). The additional foam produced by returning females was examined by hand for eggs. It was clearly distinguishable from the foam produced on the first night, because the surface of the latter had already hardened by the second night.

2.2.5 Clutch Size

The number of emerging tadpoles and clutch size were calculated for nests at Mkuzi and Vienna. Plastic buckets were suspended beneath nests and tadpoles fell into the bucket on hatching. Tadpoles were counted by hand, using a teaspoon to transfer live individuals. At Mkuzi, nests were not examined for unfertilized or desiccated eggs. At Vienna, nests were examined for eggs and these were then counted after dissolving the nest in water.

2.3 RESULTS

2.3.1 Sexual Size Dimorphism

At Hoedspruit there was pronounced sexual size dimorphism with a

female/male size ratio of 1.21. Mean female size was 76.1 mm (SD = 3.2 mm, range = 67.8-81.3 mm, N = 34). The average male size was 63.2 mm (SD = 3.3 mm, range = 55.1-68.9 mm, N = 30) with a mean mass of 13.2 g (SD = 2.6g, range = 8.7-17.5 g, N = 26). [For more details on sexual dimorphism see Chapter Four]. There was a strong positive correlation between male SVL and mass ($r = 0.88$, N = 26, $P < 0.0001$).

2.3.2 Male weight change

At Hoedspruit, male weight loss as a percentage of initial body weight was only 0.003% per day (N = 9 males; mean interval between weighing = 14.0 days). At Vienna, mean male weight loss was 0.23% per day (N = 9 males, mean interval between weighing = 8.4 days). At Vienna, however, only three males lost weight, while six gained weight. The greater value for mean weight loss at Vienna was largely due to a single male who lost 4.3% of his body weight in six days. If the data from both sites are pooled, mean weight change is 0.12% per day (N = 18 males) (Fig. 2.1). If the outlier is excluded, the mean loss per day was 0.08%.

2.3.3 Demographics and male behaviour

At Hoedspruit, the breeding season lasted 22 nights (December 5-26) during which a total of 32 males were marked and 26 females were present. If the two nests found before the study are included, the sex ratio was 1 to 1.1 (female: male). From 7 to 28 males (mean = 19.6) and 0 to 6 females (mean = 1.2) were present each night. The operational sex ratio (OSR) ranged from 0 to 0.29 (mean = 1:14) with no correlation between the number of males and females present per night ($r = 0.26$, N = 21, $P > 0.2$) (Fig. 2.2). There was no correlation between male weight and date of arrival at the pan ($r = 0.25$, N = 32, $P = 0.16$). With one exception, absent males were not found at neighbouring ponds and are unlikely to have been engaged in reproductive activities elsewhere. On average, individual males were present on 13.7

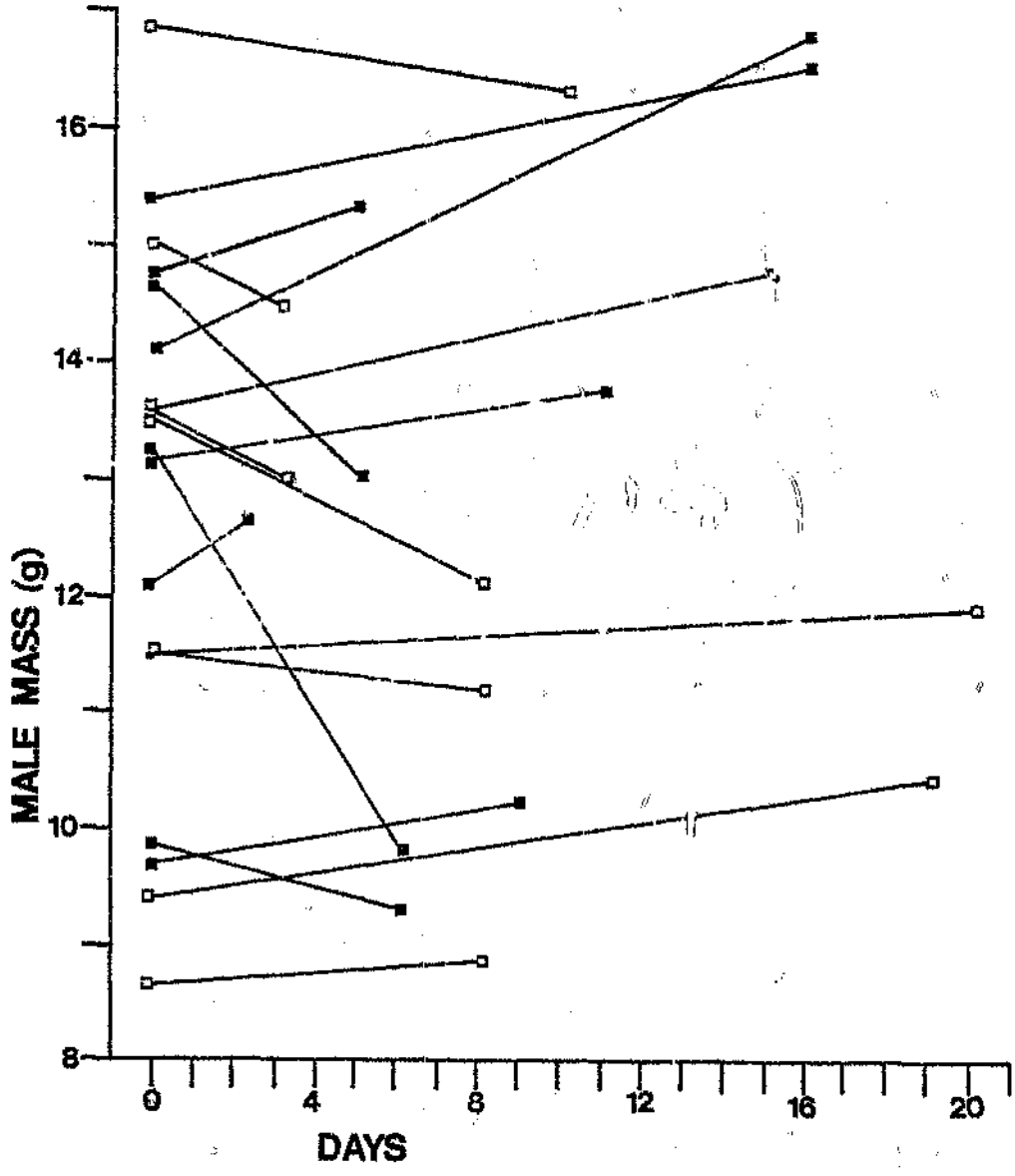

Figure 2.1 Male weight change for 18 males from Hoedspruit and Vienna. 'Days' refers to days since initial capture. Eight males lost weight and 10 males gained weight. The mean weight loss per day was 0.12% per day (N = 18 males).

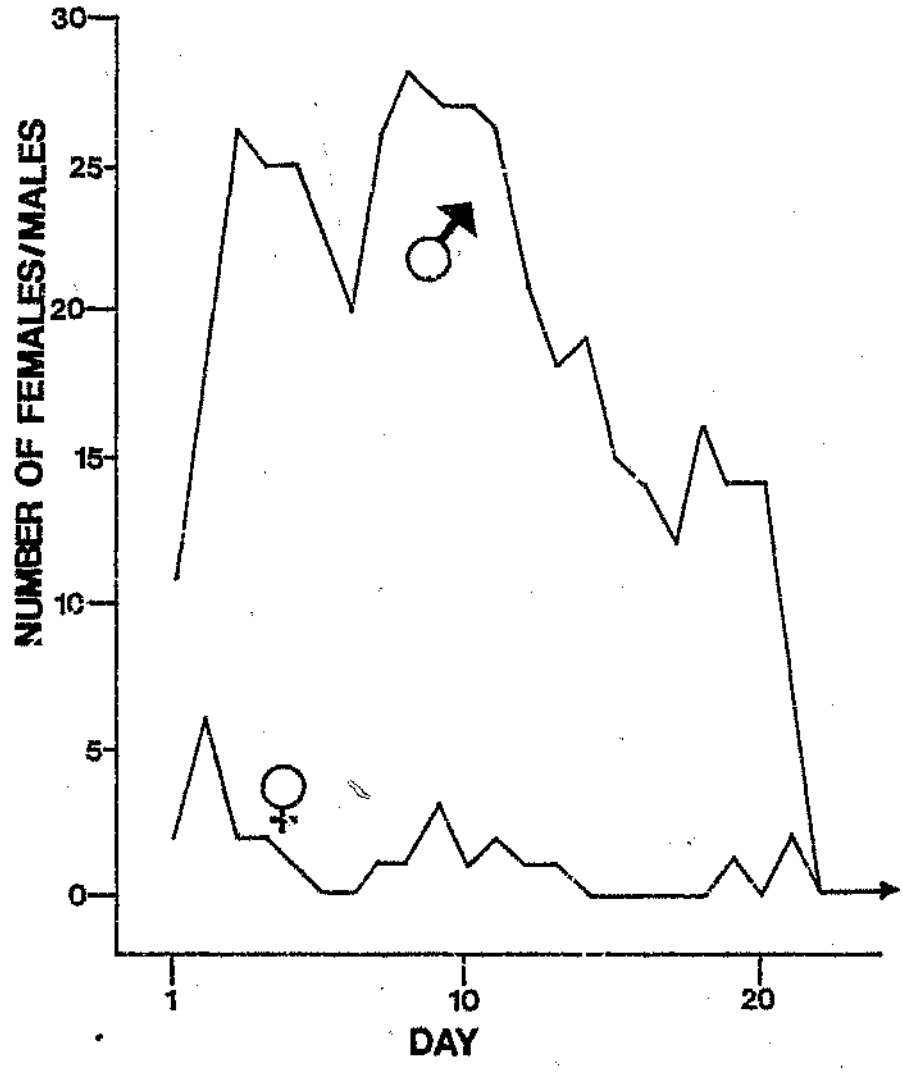

Figure 2.2 The nightly pattern of male and female presence at Hoedspruit over a 22 day breeding season. No census was conducted on night 21.The figure shows the same number of males present on night 21 as on night 20. The operational sex ratio was always male-biased. Night 1 = December 5, 1990.

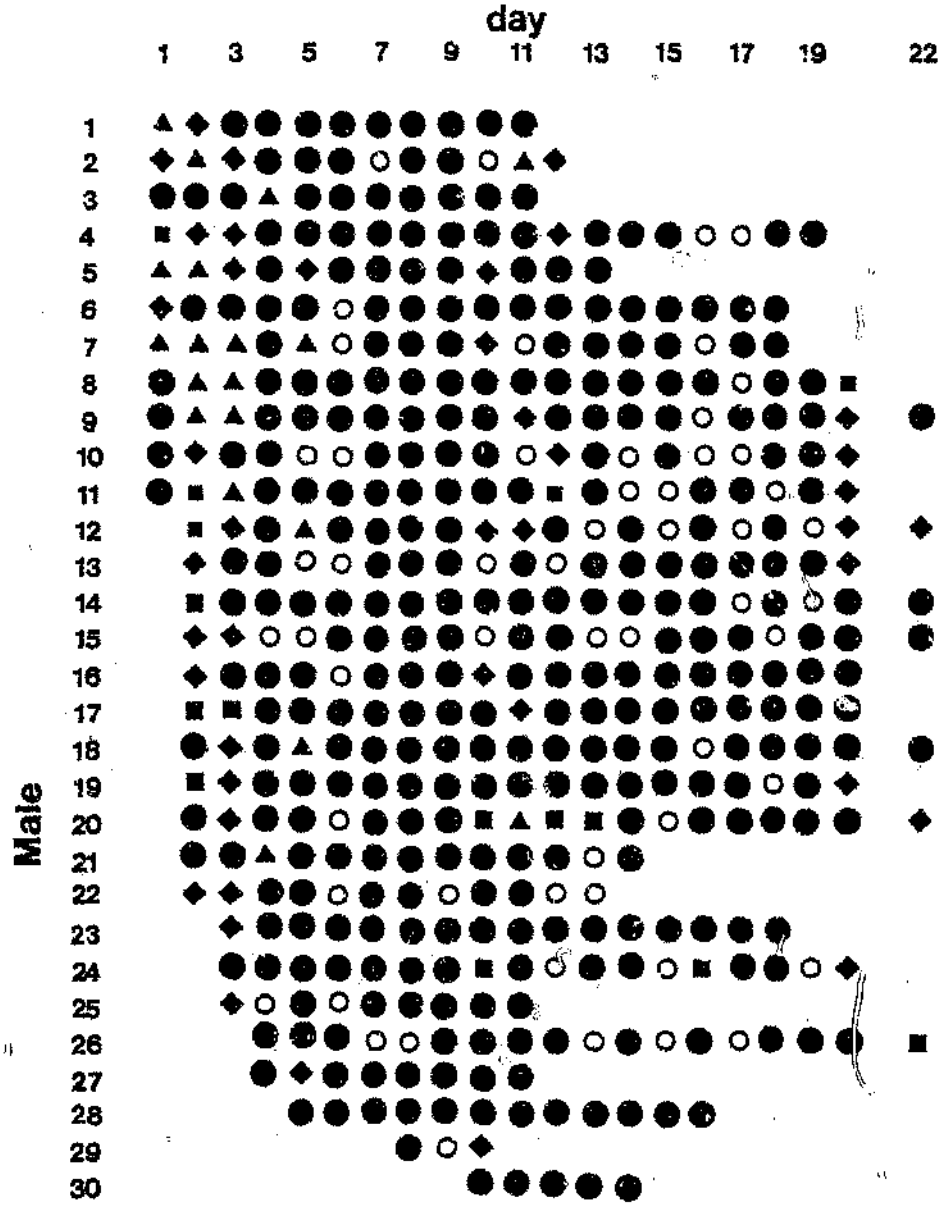

Figure 2.3 Data on individual presence for 30 males at Hoedspruit over the breeding season (22 nights). No data is available on male behaviour on night 21. Only 69% of the gravid females present at the breeding site were observed, and some nests were not observed from the start of construction. Hence, the actual reproductive activity of males was greater than recorded here. Night 1 = December 5, 1990 (Symbols: ■ = amplexed; ◆ = peripheral only; ▲ = amplexed and peripheral; ● = present but not seen at a nest; ○ = not present).

nights (65% of the season) (SD = 4.5, range = 2-20) (Fig 2.3). The total number of 'frog-nights' was 408 (see Ryan 1985). Two males are excluded from all subsequent analyses of mating and nesting success as they were only present for a single night.

Individual males showed strong preferences for certain regions of the pond. The maximum number of nights a male was observed in a single region (one of the 12 delimited) was expressed as a percentage of the total number of nights the male was present at the pond. Mean 'regional fidelity' was 65.3% (range = 29-95%, N = 30) (Fig. 2.4). If presence at the favoured region and the next nearest region are considered together, mean fidelity rises to 74.8%. Although males showed this high level of fidelity they were not invariably stationary. Ninety five percent of males (N = 27 of 30) moved between regions on at least one of the nights they were present. This movement was associated both with joining nests in other regions as an amplexing or peripheral male, and what appeared to be exploratory forays. Movement within a region was characteristic. For example, a male on a tree tended to move from branch to branch within that tree.

2.3.4 Calling behaviour

Males called from elevated sites over water and defended neither active calling spaces nor territories. There were no agonistic interactions between unpaired males away from nests. Males continued to produce advertisement calls even when positioned on top of each other. All 30 males were observed calling, but there was considerable inter-individual variation in calling effort. Satellite males were not observed (sensu Perrill et al. 1978).

Attempts were made to record all 30 males, but due to the sporadic nature of calling, only 15 males were recorded. There was no significant size difference between recorded and unrecorded males (Mann-Whitney U-test,

Two-tailed, P = 0.38), suggesting that variance in calling behaviour is unrelated to size or weight. For the fifteen males that were recorded (recordings were 1 - 5 minutes in duration), the mean call rate was 10.6 calls per minute (range = 2.3 - 28.8 calls per minute; N = 15 males; 1 - 4 recordings per male). These data reflect maximum call rate, because recordings were made non-randomly during bouts of high calling activity.

In total, three different calls were produced by males. Unpaired males produced two calls similar in frequency range, but with different pulse rates (slow and fast calls) (see Appendix II). These two calls types were not linked in a set sequence. Of 925 calls recorded from 15 males, 65 (7.0%) were slow calls. There was no apparent correlation between differential production of the two call types and changes in the social or acoustic environment. Males in amplexus continued to produce these two calls, especially in the period immediately subsequent to pairing. A third call was produced during nesting. It was a very soft 'whirring' sound accompanied by visible vibrations of the body. It was produced both by the amplexing male and by peripheral males, especially those closest to the mating pair. I also saw this call produced by amplexing males in the absence of peripheral males. My subjective impression, however, was that this call was produced more frequently in response to the competitive cloacal juxtapositioning of other males on the nest. Sperm release in some anurans is accompanied by visible vibrations of the body wall. The sound produced may thus have been an incidental effect of ejaculation. This might also account for increased 'call' production as the number of peripheral males rose. The calls of males were very subdued and this made measurement of call intensity difficult because the background SPL was often louder than that of calls. Measurements were only made for two males. Mean peak call intensity was 92.4 and 93.6 dB at 25 cm (N = 2 males). There was, however, considerable intra-individual variation in call intensity for both males. For example, the smaller range in SPL was from 81.5 dB to 100 dB (N = 30 calls). This eight-fold range in loudness was not due to slow and fast calls having different intensities.

2.3.5 Nesting Behaviour

Of twenty six females that bred at Hoedspruit, 18 (69%) were observed in amplexus and initial pair formation was observed for 11 females (42%). Nests were built in 2-4 sessions. A nest session consisted of egg-laying/churning bouts and periods of relative immobility. Bouts lasted about one to six seconds. The duration of the interval between bouts was significantly different for the first three sessions (ANOVA $F(2, 146) = 36.4$, $P < 0.0001$). For the first session, the mean inter-bout duration (period of quiescence) was 56.4 s (SD = 33.4, N = 59); for the second session it was 88.6s (SD = 71.8, N = 60); and for the third session, it was 216.1s (SD = 153.4 N = 30). The inter-bout duration in the third session was significantly longer than that in the first or the second (Scheffe's Test, Two-tailed, $P < 0.01$). There was no significant difference between the inter-bout durations of the first and second sessions (Scheffe's Test, Two-tailed, NS). Females left the nest between sessions and descended to the pond where they took up water. The mean duration of a session was 74.3 min (SD = 24.0, N = 16), whilst the period between sessions averaged 52.2 min (SD = 8.2, N = 12). Nest building occurred between 2000 hours and 0630 hours. One nest took over 7 hours to construct. The following description of behaviour prior to and during nesting is based on detailed observations at 14 nests.

Females approached males from the water and then proceeded to climb the vegetation or tree trunk towards a group of males. Females did not move between regions, and any potential for female choice was limited to that between males within one of the twelve regions. There was a noticeable increase in male movement but no apparent change in calling behaviour (cf. Coe 1974) and males did not descend to meet the female. Although females approached regions where males were calling, they did not appear to select a male. Initiation of amplexus did not involve a female making physical

contact with a male. It occurred when a male jumped onto a female. On at least two occasions the male jumped from 30-40 cm above the female. The amplexing male was not necessarily one that the female had been facing, nor one she had been approaching towards. Males clasped the females in a modified form of axillary amplexus. Their forearms were not in the female's axilla, but rather on her 'shoulders'. Sexual size dimorphism resulted in a 2-3 cm separation of the pair's cloacae.

At 93% of nests, single males were also present in the vicinity of the pair during the period between amplexus and the start of nest building. Some of these males moved up to 12 m to reach and then follow the amplexing pair. The mated pairs moved 0.3-2 m from the initial point of amplexus before assuming a position on the side of a branch. Unpaired males then positioned themselves on either side of the pair. They clung to the branch, but held on lower down than the female, so that their cloacae were approximately in line with that of the female.

Nest construction began when the female released an oviducal secretion and churned it into a white foam with movements that fully extended her hind legs. Egg laying began approximately 15 minutes after the start of foam production. Bouts of oviposition always occurred in conjunction with female churning. Males remained motionless between bouts, while females occasionally moved their hind legs (but rarely extended them fully). When oviposition and female churning occurred, the amplexing male scraped his feet down the female's dorsum from the region of his cloaca to the area where the eggs emerged. I suggest this action serves to distribute semen over extruded eggs.

Peripheral males also responded to the female's churning motions. An 'active' peripheral male would swing his lower body away from the branch then, with a jabbing motion, position his cloaca adjacent to that of the female (Fig 2.5). Cloacal proximity was maintained for 1-3 seconds. During bouts of

oviposition, peripheral males jostled each other and the amplexing male as they attempted to maintain cloacal proximity with the female. Peripheral males competed to slide their lower bodies underneath the amplexing male, while the amplexing male attempted to continue making leg movements down the female's dorsum. After the female halted churning activity the peripheral males and the pair returned to their motionless pre-oviposition state. Attempts to maintain cloacal proximity were displayed primarily by the two males immediately adjacent to the amplexing pair, and less commonly by peripheral males further from the pair. Males further from the pair tended not to move during oviposition. I describe them as 'inactive' peripheral males.

Of the 23 nests constructed at the study site, two were communally built (one involved three females, the other two females). The behaviour of both sexes at these nests was identical to that at nests involving a single female. The number of males present at a nest varied. Between and during nesting sessions new males usually arrived and join in as peripheral males. Occasionally, a male left the nest before the female had finished ovipositing. The mean number of peripheral males per session was 3.5 (SD = 1.7, N = 33 sessions), with a non-statistically significant trend towards increased numbers of peripheral males in later sessions. The average number of males participating in a given nest was 5.5 (SD = 2.8, N = 15 nests) and the mean number of males per female at a nest was 4.9 (SD = 2.6, N = 15 nests).

2.3.6 Polyandry and amplexus displacement

Female departure at the end of a session was observed 20 times and in 11 cases (55%) the amplexing male dismounted leaving the female to descend to the water on her own. On the other 9 (45%) occasions the male remained in amplexus. Unpaired females amplexed while returning to the nest for the start of the next session. The originally amplexing male only amplexed successfully in one of nine observed female returns (11.1%). During the

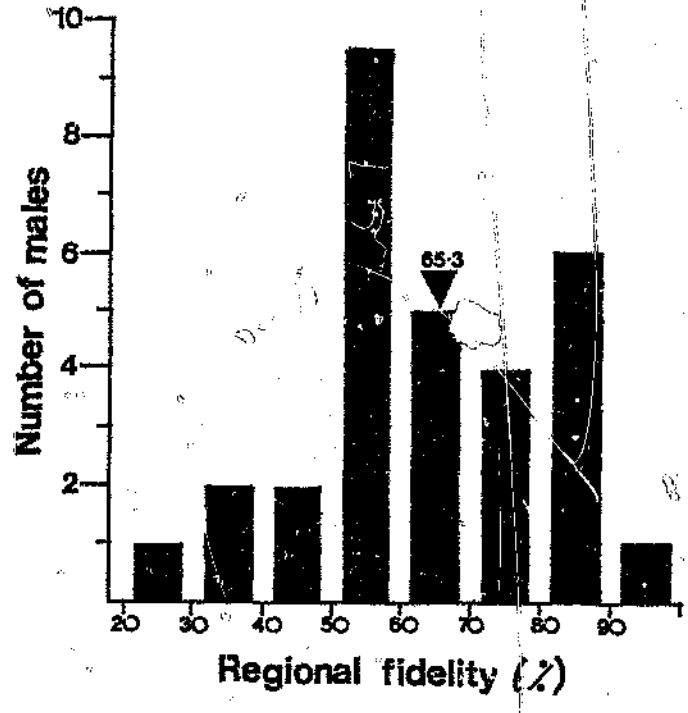

Figure 2.4 A histogram of male 'regional fidelity' for 30 males at Hoedspruit. Regional fidelity was defined as the percentage of nights present at the breeding site spent in one of twelve demarcated areas. Mean fidelity was 65.3%.

Figure 2.5 A photograph showing two peripheral males and an amplexing pair during nest construction. The peripheral male on the left can be seen juxtaposing his cloaca with that of the female.

Figure 2.6 A photograph showing three peripheral males waiting on the nest for the return of the female. The male in the middle is the original amplexing male.

Mating success and nest participation were analyzed at four scales of interaction: nest sessions, nests, nights and breeding season (see Morris 1989). The first two scales are most likely to reveal female choice or direct male-male competition. There was no significant size difference between mated and peripheral males during nesting session (Sign Test, NS, N = 32 sessions). Neither were mated males larger than the average peripheral males at the nests significantly more often than expected by chance (Sign Test, N = 15 nests, NS). There was no significant pattern of mated males being larger than non-nesting males on a significant number of nights (Sign Test, N = 12 nights, NS). There was also no significant size difference between males that did and did not mate during the breeding season (Mann-Whitney U-test, N = 30, P = 0.24). The only significant correlate with the number of nests a male participated in was the number of nights he was present at the pond ($r = 0.45$, $P < 0.01$). Nights present was an even stronger predictor of mating success (number of females amplexed) ($r = 0.6$, $P < 0.001$). There was no significant correlation between size and number of nights present ($r = -0.1$, N = 30, $P > 0.5$) but there was a significant correlation between nights present and regional fidelity ($r = 0.49$, $P = 0.008$).

2.3.8 Communal nesting

At the Mkuzi study site, communal nest building involving two females was observed on one occasion (1 of 24 nests observed = 4.2%). There was no apparent limit on suitable nest sites. On another occasion, two gravid females built nests less than 1 m apart, despite having encountered each other prior to the start of nesting. In Hoedspruit in December 1991, three nests were built communally over a farm dam at Mariepsig. One of these nests was truly enormous (Fig. 2.9) and I estimate that a minimum of 40 females were involved. Although suitable nest sites were limited, there were still a sufficient number of sites available to exclude space constraints as an explanation for communal nests. Almost all breeding at this pan occurred on a single night which coincided with the first rains of the season. On the

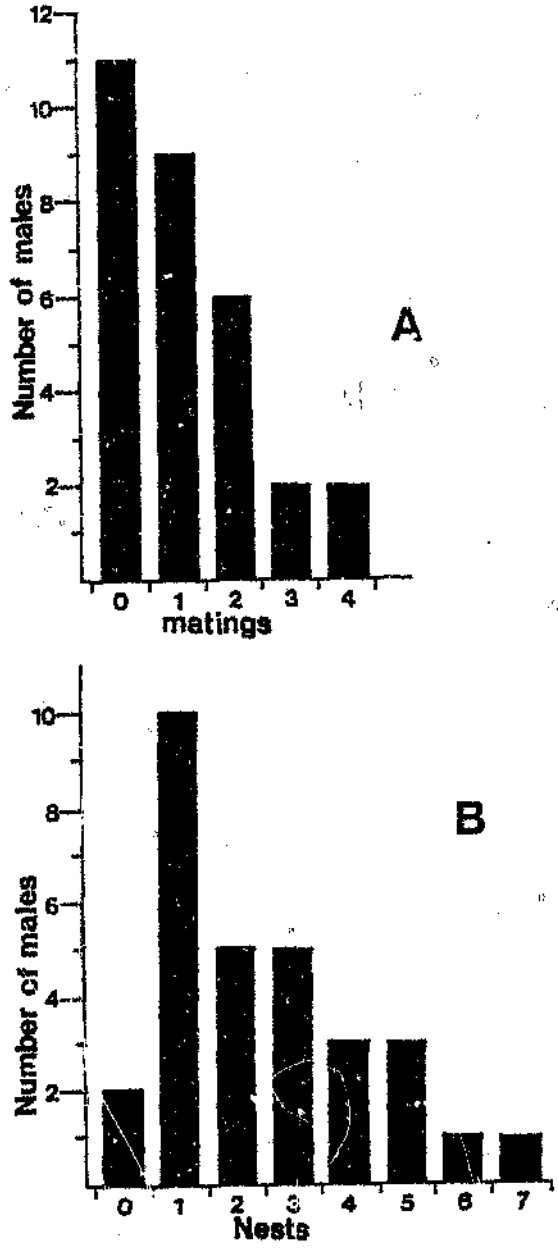

Figure 2.8 Frequency histograms showing the distribution of observed reproductive behaviour for 30 males at Hoedspruit over a 22 night period. (A) the number of gravid females amplexed by a male during oviposition (matings) and (B) the number of nests males were seen at, either as peripheral or amplexing males or both (nests).

following nights, only one other gravid female bred at this dam. At the main study site at Hoedspruit, two of the 23 nests were built communally (8.7%). One involved three females, the other two females. Communal nests were not built at Vienna.

2.3.9 Parental Care

In **C. xerampelina** females return to the breeding site on the night following initial nest construction. They then proceed to add an additional layer of foam to the nest (Fig 2.10). At Mkuzi, 33 of 34 nests had additional foam added. At Hoedspruit, 25 of 26 nests were enlarged. At Vienna all 8 nests were enlarged. Overall, 97% of nests were enlarged. Females do not deposit eggs on the second night. At Mkuzi I observed males amplexing with returning females, and peripheral males were even present. These males, in most cases, remained only briefly at the nests. In one case, however, two peripheral males and an amplexing male were present throughout nest enlargement (> 3hr) - despite the female being non-gravid. In general though, males at Mkuzi appeared to be capable of distinguishing between gravid females and females providing parental care. At Hoedspruit and Vienna, males were never seen amplexing with non-gravid females.

Females probably remain in the vicinity of the nest on the day between oviposition and addition of extra foam. This was most clearly demonstrated by the large number of females seen in the day time at the Mariepsig site following the building of an enormous communal nest. Even if females remain near the nest, however, there may still be other nests in the area which the female must distinguish from her own nest. How the female recognizes her nest is unknown. Possible explanations, which should be tested empirically, are (a) individual-specific chemical cues; and (b) memorizing location.

2.3.10 Clutch Size

Tadpoles emerged 4.5 - 6 days after oviposition (N = 24 nests). The mean number of tadpoles per nest at Mkuzi was 537 (SD = 189, N = 17 nests). At Vienna, the mean number of tadpoles emerging was 820 (range = 637-985; N = 4 nests). Mean clutch size (tadpoles + eggs in nest) was 1203 (range = 827-1670; N = 7 nests). Of the total clutch laid, 85.8% (range = 72.9 - 98.2%; N = 4 nests) emerged as tadpoles. All data are for single-female nests.

2.4 DISCUSSION

2.4.1 Communal nesting

There is no clear explanation for the seasonal pattern of communal nest building. Five of the six communal nests were built at the start of the breeding season on nights when the total number of gravid females present at the breeding sites was higher. Communal nesting appears to be more common at the start of the breeding season (D. Gaynor, personal communication). A partial explanation for this pattern is that females are more likely to encounter one another when female density is high. However, observations that females do not always build nests communally, even when the potential exists, suggest that this is an incomplete explanation.

The functional significance of communal nest building is unknown. In the foam-nesting, neotropical frog **P. pustulosus**, communal nest building is common and unrelated to limited nest sites. It has been suggested that communal nesting decreases the surface to volume ratio and consequently reduces the risk of desiccation (Ryan 1985). A similar explanation may also account for communal nesting in **C. xerampelina**. Waldman (1981) has shown that communal oviposition in the wood frog (**Rana sylvatica**), which breeds in a temperate environment, elevates the temperature of eggs,

just water to nests. Additional water is involved, because females still descend to the pond between nest sessions as water appears to be necessary for foam production. The benefit of the female adding foam on the second night probably lies in a reduced risk of desiccation for tadpoles. Extra foam will probably decrease water loss, and may also influence nest temperature by insulating the nest. There is little opportunity for male paternal care in **Chiromantis** because the tadpoles are hidden in the foam nest. The foam nest is also unpalatable (I only saw millipedes feeding on dried up nests) and this may be a sufficiently strong deterrent to predators). Parental care is rarely reported in frogs living in arid environments.

2.4.4 Overall Breeding Patterns

Chiromantis xerampelina may be considered a prolonged breeding frog (sensu Wells 1977). At Hoedspruit, male and female presence was not synchronized by rainfall, female arrival was unpredictable and the OSR was always strongly male biased. As in most other studies conducted over the major part of the breeding season, I found that chorus attendance is the best predictor of male mating success [eg. **Bufo americanus** (Gatz 1981a); **B. calamita** (Arak 1988a);**Bufo rangeri** (Cherry, in press); **B. woodhousei** (Woodward 1982a); **Centrolenella fleischmanni** (Greer & Wells 1980); **C. prosoblepon** (Jacobson 1985); **Hyperolius marmoratus** (Dyson et al. in press); **Hyla chrysoscelis** Goodwin & Roble 1982); **H. cinerea** (Gerhardt et al. 1987); **H. rosenbergi** (Kluge 1981); **Physalaemus pustulosus** (Ryan 1985); **Rana virgatipes** (Given 1988)]. The percentage of nights present in the chorus was high relative to that found in many other anuran species (eg. Gerhardt et al. 1987; Sullivan 1987; Green 1990; Salvador & Carrascal 1990; Cherry in press).

The comparative stability in male body weight is interesting because it contrasts with the results of several other studies (see review in Halliday 1987; Given 1988). It is rather unexpected, given that (a) males repeatedly

I suggest that subdued, intermittent calling and male movement can all be related to the peripheral male tactic. Males do not need to be in amplexus to fertilize eggs. Males that are not chosen by females may still fertilize their eggs by being peripheral. Successful alternative mating tactics reduce selection on traits associated with the dominant tactic (Halliday & Verrell 1986). This may have resulted in lowered benefits to a high call rate, which is generally the only trait that, within a night, is correlated with mating success (eg. Lopez & Narins 1991). Calling is energetically expensive and males may thus have diverted energy from calling and used it to be present on a greater number of nights. Male movement is almost certainly related to seeking out females. This is normally an ineffective strategy for a prolonged breeder because of the high operational sex ratio. In **C. xerampelina**, however, males benefit if they encounter unpaired or paired females because both are potential mates, so the 'effective' OSR is lowered.

2.4.6 Peripheral Males

The most unusual aspect of mating in **Chiromantis xerampelina** is the presence of peripheral males. Coe (1967) suggested that unpaired males helped in nest construction in **C. rufescens**. Observations of **C. xerampelina** do not support this conclusion. The data provide strong evidence that peripheral males are fertile males exhibiting an alternative reproductive tactic. They show that: (1) fifty seven percent of amplexing males also acted as peripheral males. These males are almost certainly fertile because all investigated nests had high fertilization rates. One male mated without peripheral males being present during nest construction and this nest produced tadpoles. This male was also observed as a peripheral male; (2) peripheral males only competed with the amplexing male for cloacal proximity to the female during oviposition; (3) peripheral and amplexing males were not observed helping to churn the foam.

Figure 2.5 A photograph showing two peripheral males and an amplexing pair during nest construction. The peripheral male on the left can be seen juxtaposing his cloaca with that of the female.

Figure 2.6 A photograph showing three peripheral males waiting on the nest for the return of the female. The male in the middle is the original amplexing male.

period of female absence peripheral males remained within about 1 m of the nest, usually remaining on the nest itself (Fig. 2.6).

I observed amplexus displacement attempts on 14 occasions. They occurred either before nesting had started, or at the start of a subsequent nesting session. Seven (50%) of these 14 attempts were successful. There was no significant advantage to large male size, either as attacker or defender (Sign Test, NS) (Fig. 2.7).

The combination of amplexing males dismounting between sessions and successful amplexus displacement meant that females were not necessarily in amplexus with the same male in each nesting session. Of those females observed for two or more nesting sessions, 66% (N = 10 of 15) amplexed with more than one male. Two females amplexed with at least three males. Thus a minimum of 66% of egg clutches were fertilized by more than one male due to multiple-mating.

2.3.7 Male Reproductive Success

In **Chiromantis xerampelina** the fertilization success of peripheral and amplexing males is unknown so data on variation in reproductive success are unavailable. Of the 30 males present at the pond for more than one night, 28 (93.3%) participated in at least one nest, 26 (83.3%) were seen as peripheral males, 18 (60.0%) amplexed at least once and 17 (56.7%) were observed as both peripheral and amplexing males. The average number of nests that males were observed to participate in was 2.5 (SD = 1.8, N = 30 males), whilst the average number of females that males amplexed with was 1.2 (SD = 1.2, N = 30 males) (Fig. 2.8). The above data underestimate male reproductive behaviour, however, because only 69% of the gravid females present at the pond were observed. When the data is extrapolated to include all 26 females present, the mean number of nests participated in is 3.8 and the mean number of females amplexed per male is 1.8.

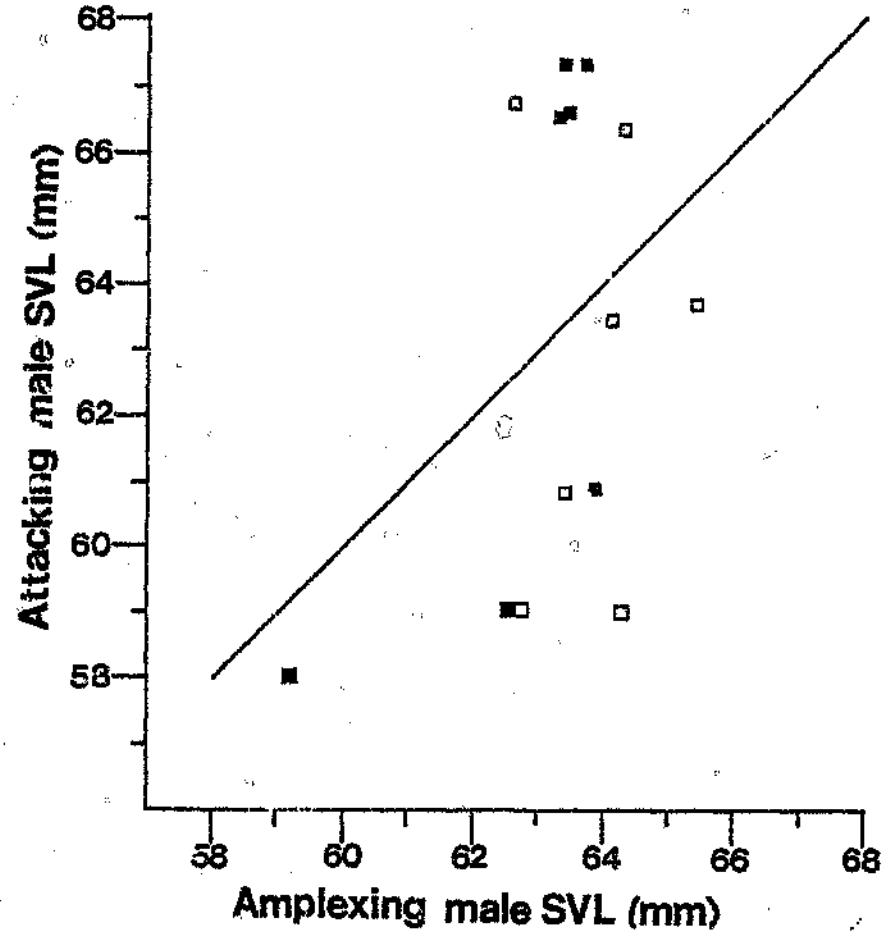

Figure 2.7 The relationship between male snout-vent-length (SVL) and success during amplexus displacement attempts for 14 displacement attempts at Hoedspruit. The line represents equal-sized contestants (symbols: ■= successful displacement; □= unsuccessful displacement attempt).

Figure 2.9 A photograph showing an enormous communal nest built at Mariepsig. The photograph shows the nest prior to the addition of foam on the following night!

Figure 2.10 A photograph showing a solitary female adding foam to her nest on the night following oviposition. The leg movement of the female is typical of that during a churning bout.

especially those in the centre of the egg mass. This elevated temperature leads to faster growth rates and increased hatching success. By analogy, communal nest building in **C. xerampelina** may *lower* the temperature inside the nest if foam acts as an insulator. Both explanations, however, are mere conjecture and should be tested empirically.

2.4.2 Clutch Size

The clutch sizes I recorded were significantly larger than the figure of 150 usually quoted (eg. Coe 1974) which is attributable to Wager (1965). Clutch size in **C. xerampelina** is significantly larger than that in the two congenerics [**C. xerampelina** = 800-1700 eggs; **C. rufescens** = 113 - 201 eggs; **C. petersii** = 300-350 (Coe 1974)]. The difference from **C. rufescens** clutch size is probably attributable to body size. Females **C. rufescens** are, on average, only 60mm in length (Coe 1974), and there is an allometric relationship between body size and clutch size in frogs (Salthe & Mecham 1974). The hatching success of nests is comparatively low. Most frog species have fertilization rates close to 100% (Kruse 1981a). Given the protected environment of a foam nest, one would expect clutch size and emergent tadpole number to be similar. The difference between these two measures is probably attributable to desiccation. The outer layer of the nest dries rapidly and any eggs deposited here are unlikely to develop. Even so, unfertilized eggs are also found deeper in the nests which is surprising given the confined space in which males shed sperm. Perhaps sperm mobility in the foam is limited so that eggs need to be fertilized shortly after oviposition?

2.4.3 Parental Care

Conflicting reports have been presented regarding the occurrence of parental care by female **C. xerampelina**. It has been suggested that females remain at the nest and moisten it (Rose 1962), although this notion was discounted by Wager (1965). In this study, I found that females are adding foam, and not

struggle to remain in proximity with the female's cloaca; (b) they are visibly slowing down towards the end of nest building; and (c) they participate in so many nests, sometimes over several successive nights. Possibly, reduced calling effort may partially offset these costs. The high percentage of nights males are present at the breeding site is also consistent with a relatively low rate of weight loss suggesting reduced energetic costs of reproductive activity. At all scales of analysis, from nest sessions to the entire breeding season, there was no evidence for non-random mating with respect to body size. Mated males were no larger or heavier than unmated males. There was also no size-assortative mating. This suggests that there is neither female choice of large males, nor a large-male advantage in male-male competition. The distribution of matings among males was far more equitable than that usually recorded for anurans. At least 60% of males amplexed gravid females.

2.4.5 Calling Behaviour

Several aspects of male behaviour prior to amplexus are unusual. First, the call SPL was very low. Passmore (1981) measured sound levels in 17 species of African anurans. Only one species (**Phrynobatrachus mababiensis**) had an equally soft call (92dB). Furthermore, Passmore noted that the calls of African anurans were louder than those reported from Australian and North American species. Second, calling was highly sporadic for a prolonged breeder. The maximum call rate for **C. xerampelina** was more reminiscent of the mean call rate of other prolonged breeders. Third, males continued to call in amplexus. This has also been reported in **Phyllomedusa hypochondrialis** (Pyburn 1971), but its significance is unknown. Four, males showed very high call site fidelity. Five, in spite of call site fidelity, males did move around the breeding site, especially at Mkuzi (see Chapter Four). Male movement is also unusual for a prolonged breeder, where males usually call from stationary sites.

I suggest that the most plausible explanation for the presence of these males is that they compete with amplexing males for fertilizations, and that those peripheral males that actively compete to place their cloacae against the female's cloaca during oviposition are ejaculating. If so, their actions should lead to sperm competition. My evidence is comparable to that for group spawning in several fish species where similar circumstantial evidence has led to the acceptance of a sperm competition hypothesis (Dominey 1980; Taborsky et al. 1987). It is more difficult to interpret the behaviour of inactive peripheral males. They may simply be waiting for an opportunity to get closer to the pair, or they may also be releasing sperm into the foam.

If active peripheral males are pursuing an alternative reproductive tactic it is a fairly sophisticated one. For it to have evolved the following adaptive changes in behaviour were required; (1) the ability to release sperm without being in amplexus; (2) the ability to recognize the cue for oviposition without being in amplexus: (3) the capacity to locate females from a distance. Most anuran species show little ability to recognize females at a distance (Wells 1977a; Robertson 1986); and (4) the ability to juxtapose cloacae with the female from a variety of positions and to maintain cloacal proximity while other males are jostling.

Several workers who have examined group spawning in fish have shown that males obtaining 'sneak' fertilizations are a subset of the total male population. They are usually smaller and/or younger than dominant males and, in the short term, are largely limited to this sneaking tactic (eg., Dominey 1980; Gross 1985; Taborsky et al. 1987; van den Berghe & Warner 1989). In frogs, males pursuing alternative tactics are, on average, also smaller (Gerhardt et al. 1987). The small size of 'sneakers' may be the result of; (a) direct selection for small body size because it decreases conspicuousness (Gross 1985); (b) 'sneakers' being younger than dominant males (van den Berghe et al. 1989); (c) genotypic differences between males

(Gross 1985; Ryan & Causey 1989); (d) a response to an active female preference for larger males (Ryan & Causey 1989).

In contrast to the above studies I did not find that peripheral males were smaller than amplexing males. Size and age are related in anurans because of indeterminate growth, suggesting that peripheral males are no younger than amplexing males (but see Halliday & Verrell 1988). Individuals of all sizes were found in amplexus and acting as peripheral males. The alternative reproductive tactic of peripheral males does not appear to be a 'best-of-a-bad-job' tactic resulting from phenotypic constraints, but rather a reversible (sensu Austad 1984), 'opportunistic' or 'side-payment' tactic (Davies 1982). At present it is impossible to determine the relative fitness of the tactic because fertilization rates are unknown. Hence the possibility of a mixed evolutionary stable strategy or state occurring remains open. Explanations (a) - (c) do not account for the peripheral male tactic in **C. xerampelina**. The data do not support a hypothesis that the tactic is a response to female mate choice, because the tactic was exhibited by almost all males. There was also no evidence for female choice, although it may have existed in the past.

In **C. xerampelina** the alternative reproductive tactic of amplexus displacement was also documented. The data are unusual in that there was no large-male mating advantage (cf. Telford & Van Sickle 1989). I did not measure the forearm length of males and it is possible that this influenced displacement success (Lee 1986). The rate of successful displacement (50%) is also the highest recorded in an anuran species (Telford & van Sickle 1989). Amplexus displacement is comparatively rare in prolonged breeders (Telford & Dyson 1988).

2.4.7 Polyandry

Polyandrous behaviour whereby more than one male fertilizes eggs in a single clutch has only been documented in one other anuran, **Afrixalus**

delicatus (Backwell & Passmore 1990b). Here I have reported that at least 66% of females were polyandrous. The main cause of polyandry is the dismounting of amplexing males between nesting sessions. The data indicated that males only have an 11% probability of re-amplexing. Polyandry is often described as an adaptive female strategy (reviewed in Halliday & Arnold 1987), but I can not construct a plausible adaptive explanation at present. Increasing the genetic diversity of progeny, or ensuring fertilization, is especially implausible if one accepts that multiple spawning is occurring. Further research is necessary to account for the proximate and ultimate mechanisms that results in males dismounting.

2.4.8 Sperm Competition in Anurans?

Sperm competition as a result of group spawning has been reported in many fish species with external fertilization. Sperm competition, however, has not yet been demonstrated in an anuran (Halliday & Verrell 1984). My initial observations suggest that it probably occurs in **C. xerampelina**. If so, it is also likely to occur in the two congenerics. Peripheral males that gather around the spawning pair have also been noted in the foam-nesters **Polypedates dennysi** (Duellman & Trueb 1986), **P. leucomystax** (Feng & Narins 1991), **Rhacophorus schlegelli** and **R. arboreus** (Fukuyama 1991). Pyburn (1970) documented the presence of unpaired males at leaf nests of **Phyllomedusa dacnicolor** and **Agalychnis callidryas**, but he also noted that these males were attempting to displace the amplexing male. The functional significance of these males' presence remains obscure. Interestingly though, in both species females descend to the water and build nests in sessions. At present, speculation about the evolution and significance of the peripheral male tactic is constrained by the absence of data on the relative fertilization success of amplexing and peripheral males. Preliminary electrophoretic work shows that there is almost no protein polymorphism in the study population (Jennions, W.S. Grant and N.I. Passmore, unpublished data).

CHAPTER THREE

DIRECT AND COMPARATIVE EVIDENCE FOR SPERM COMPETITION

3.1 SUMMARY

An allometric relationship between body mass and testis mass was calculated using data from 37 African and Japanese frogs (16 genera). This showed that testis size in **Chiromantis xerampelina, Rhacophorus arboreus** and **R. schlegelli** is unusually large. All three species have multi-male breeding and sperm competition appears to have selected for increases sperm production in all three species. Additional evidence that sperm competition occurs in **C. xerampelina** is provided by a 'sterile male' experiment. It showed that peripheral males are capable of fertilizing eggs when the amplexing male is prevented from so-doing.

3.2 INTRODUCTION

Why does testis size vary so widely among species within a taxa? The first researcher to investigate this problem was R.V. Short (1977, 1979, 1981) who, looking specifically at the great apes, noted that males in multi-male groups tended to have relatively large testes. He suggested a likely explanation for this relationship, namely, that multiple mating by females leads to sperm competition for fertilization of ova (Parker 1970; Harvey & May 1989). This relationship was latter confirmed using the comparative method to control for body size effects (Harcourt et al. 1981). More generally, inter-specific comparative studies of birds and mammals have shown a similar positive

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relationship between testis size and the number of males a female is likely to copulate with in a single reproductive cycle (mammals: Kenagy & Trombulak 1986; primates: Harcourt et al. 1981; Harvey & Harcourt 1984; Møller 1988b; birds: Møller 1988c, 1991). In butterflies of the Pieridae and Satyridae families, there is a direct relationship between the mass of ejaculate and polyandry, as well as the production rate of ejaculate and polyandry (Svard & Wiklund 1989).

In mammals, having corrected for the effect of body size, larger testis size is associated with: higher sperm production rates; larger sperm reserves; and more sperm per ejaculate (Møller 1989b). Similarly, in birds there is a positive relationship between testis size and ejaculate parameters like sperm concentration, ejaculate volume and number of sperm per ejaculate (Møller 1988c). Sperm competition mechanisms are largely unknown (Birkhead & Hunter 1990), but theoretical models suggest that numerical superiority is an adaptive strategy for sperm competition (Parker 1990a, 1990b; Gage 1991). The situation has been compared to a lottery or raffle, where one's probability of winning (fertilizing ova) is directly related to the number of tickets purchased (sperm ejaculated). The comparative data clearly support this analysis.

Intra-specific studies also suggest that numerical superiority is selected for under conditions of sperm competition. In species where phenotypically distinct mating morphs exist, those morphs pursuing a tactic involving sperm competition tend to have larger testes than other morphs [eg. blue-headed wrasse, **Thalassoma bifasciatum** (van den Berghe & Warner 1989); Salmon, **Oncorhynchus kisutch** (Gross 1985); blue-gilled sunfish, **Lepomis macrochirus** (Dominey 1980; Trivers 1985)]. Although there are benefits to increased sperm production in terms of fertilization success, sperm are also energetically costly to produce (Dewsbury 1982; Nakatsuru & Kramer 1982). We might thus predict that males will adjust ejaculate size (sperm production) in relation to the risk of sperm competition. Indeed, this

relationship has been found within several species, again suggesting that the level of sperm competition is positively associated with selection for numerical superiority in terms of sperm per ejaculate [eg. humans, **Homo sapiens** (Baker & Bellis 1989); mealworms, **Tenebrio molitor** (Gage & Baker 1991); Mediterranean fruit fly, **Ceratitis capitata** (Gage 1991)].

Sperm competition has not been conclusively demonstrated in any species of amphibian (Halliday & Verrell 1984). Some urodeles (newts & salamanders) possess internal fertilization, store sperm and are inseminated by more than one male (Verrell 1989). This is clearly a situation in which sperm competition may arise, and there is strong circumstantial evidence for its occurrence in some urodeles (Verrell 1989). In frogs, however, the potential for sperm competition appears to be limited. In most species a single male clasps a female and remains in this position, known as amplexus, throughout spawning. Fertilization is almost always external (cf. Townsend et al. 1981) and the amplexing male releases sperm in synchrony with female oviposition. Sperm competition is only likely to evolve if unpaired males can release sperm which have a reasonable chance of fertilizing eggs before the sperm of the amplexing male. Given the amplexing male's proximity to the female this is probably a rare occurrence.

In a few species, multi-male breeding has been observed. In the African foam-nesting rhacophorids **Chiromantis xerampelina**, **C. petersii** and **C. rufescens** (Coe 1974; Jennions et al. in press) and the Japanese foam-nesting rhacophorids **Rhacophorus arboreus** and **R. schlegelli** (Kasuya et al. 1987; Fukuyama 1991) unpaired peripheral males gather around amplexing pairs. Peripheral males insert their cloacae into the foam during spawning and, in the case of **C. xerampelina**, even attempt to juxtapose their cloacae with that of the female during bouts of oviposition (Jennions et al. in press). Peripheral males in these species are almost certainly shedding sperm, although direct confirmation of this is lacking. In this chapter I

provide direct experimental evidence that male **C. xerampelina** can fertilize eggs from a peripheral position.

In the Central American leaf frogs, **Phyllomedusa dacnicolor** and **Agalychnis callidryas** (Fyburn 1970, D'Orgeix personal communication), and the Malaysian treefrog **Polypedates leucomystax** (Feng & Narins 1991) more than one male may clasp the female during spawning. However, the behaviour preceding multi-male breeding is often characterized by displacement attempts. It is thus unclear whether these additional males are attempting to displace the amplexing male, or whether they are displaying a genuine alternative reproductive tactic.

Simultaneous release of sperm by more than one male in species with external fertilization may lead to intense sperm competition. In species with internal fertilization, mechanisms of sperm precedence and sperm removal can be selected for (Smith 1984). In species with external fertilization, however, the only mechanisms whereby males can raise their fertilization success are (a) to increase the number of sperm released and (b) to maintain proximity to the female (Gross 1985). There will thus be strong selection for increased sperm production, leading to relatively large testes. Here I examine testis size in eighteen species of African frogs. Kusano et al. (1991) collected data on nineteen species of Japanese frogs. They reported relatively large testes in two multi-male breeding rhacophorids. The allometric relationship between body mass and testes mass was, however, calculated using species as independent data points. This may lead to false conclusions because of the confounding effect of phylogeny (Harvey & Purvis 1991). Here I combine my data and those of Kusano et al. and recalculate the allometric relationship using sixteen genera as independent data points. I show that the testes of three species with confirmed multi-male breeding are larger than expected for their body size.

3.3 METHODS

3.3.1 A 'Sterile Male' Experiment

In **Chiromantis xerampelina** peripheral males gather around the amplexing pair during oviposition. The female releases an oviducal secretion which she churns into a foam nest. Oviposition occurs during bouts of churning (for more details see Chapter Two). Nests are built on branches, or any other solid structure, overhanging water.

I located nesting pairs shortly before oviposition commenced and placed the amplexing male's lower body in a plastic bag (20 cm x 20 cm). The bag was then attached to the tree branch with tape. The bag was replaced when the female returned after each nest session. Peripheral males position themselves on either side of the amplexing pair, and were able to move underneath the bag and remain in contact with the female's cloaca. Two control trials were conducted, during which all peripheral males were removed from the nest. One trial was conducted in which four peripheral males were present. Plastic buckets were suspended beneath nests to collect emerging tadpoles. Tadpoles were counted by hand. Nests were then dissolved and unfertilized eggs were also counted.

3.3.2 Testis size

I collected specimens during the peak breeding season (December to January). Males were collected during the 1990-1991 and 1991-1992 breeding seasons. They were kept in captivity and starved for one to two days to minimize the effect of stomach content on body mass measurements. The wet mass of the body was then measured to the nearest 0.01 g on an electronic balance. Testes were then dissected out and their combined mass measured to the nearest 0.1 mg. Testes Mass refers to the combined mass of both testes.

Comparative studies are often confounded by the effects of phylogeny which may obscure or create misleading associations between measured variables. It is thus inadvisable to treat species as independent data points. For example, Höglund (1989a) re-investigated a positive correlation between sexual dimorphism and lekking in birds. When he examined the relationship using genera as independent data points, no correlation was found. Recommended comparative methods are either directional comparisons, where ancestral traits are compared with descendant traits; or non-directional comparisons, where traits of immediate descendants of a common ancestor are compared with each other (Felsenstein 1985; Pagel & Harvey 1988; Harvey & Purvis 1991). Unfortunately, good cladistic studies of African anuran phylogenies are still in their infancy and are effectively unavailable. In the absence of a reliable phylogeny, an alternative approach is to use the evolutionary classifications (which arrange species into a hierarchy of taxa such as genera, families and orders) as an approximate evolutionary tree (Harvey & Purvis 1991). Here I pursue this approach, examining the relationship between testes mass and body mass using data from sixteen genera.

Organ size usually scales allometrically with body size (Calder 1984) and I therefore analyzed the testes mass/body mass relationship using a linear regression of log-transformed data. Generic points were calculated using the means of the logs of both variables. There are, however, measurement errors for both variables and a reduced major axis analysis is thus recommended (Harvey 1982). The size of measurement error is, however, unknown and a Model I regression is thus equally acceptable (Møller 1991). The correlation coefficient between the variables is high, so the results should converge on those from a reduced major axis regression (Møller 1991).

3.4 RESULTS

3.4.1 The 'Sterile Male' Experiment

In both controls where peripheral males were excluded from the nest, fertilization success was zero and no tadpoles emerged. Unfertilized egg counts for the control nests were 1670 and 1516. In the experimental trial where peripheral males were present, tadpoles did emerge. Five hundred and twenty tadpoles left the nest, whilst 896 eggs were unfertilized (hatching success = 36.7%). The plastic bag was an effective condom, so these tadpoles were sired by peripheral males.

3.4.2 Testis Size in African Anurans

I measured the testis size of 18 species, from ten genera and four families. Body mass, testes mass (combined mass of both testes) and testes mass as a percentage of body mass are shown in Table 3.1. The range of values for testes mass as a percentage of body mass was relatively small (0.1% - 0.6%) across 17 of the species, despite a thirty-fold difference in body size between the smallest and largest species. **Chiromantis xerampelina** males had exceptionally large testes that were, on average, 7.8% of body weight (range = 4.9 -11.3%, N = 12). There was a significant positive relationship between body mass and testes mass ($r = 0.80$, N = 18 species, $P = 0.0001$) (Fig. 3.1). When **C. xerampelina** was excluded from the data, the relationship was even stronger ($r = 0.92$, N = 17 species, $P < 0.0001$).

3.4.3 Body size and Testis Size

I plotted the relationship between the mean log of body mass and the mean log of testes mass for 16 genera (Fig. 3.2). The Model I regression equation was:

Log [testes mass (mg)] = - 4.18 (SE = 1.08) + 1.46 (SE = 0.29) log [body mass (mg)]

[$F(1,15) = 1213.0$, $P < 0.001$, $R^2 = 64.8$ %]

To remove the effect of body size, I calculated the relative testes size for individual species, which was the ratio of the observed testes mass to that predicted by the above regression (Harvey & Mace 1982; Harvey & Harcourt 1984) (Table 3.1). Mean relative testes mass for **Chiromantis xerampelina** was 14.55, for **Rhacophorus arboreus** it was 10.30 and **R. schlegelli** was 3.81.

3.4.4 Testis Size in Foam-nesting Rhacophorids

There are three species of rhacophorid in Africa, and data are unavailable for **Chiromantis petersii** and **C. rufescens**. I thus used data from five species of Japanese foam-nesting rhacophorid (raw data provided by T. Kusano). The mean relative testes size for foam-nesting rhacophorids was 5.86 (SD = 5.30, N = 6), and for the other species it was 0.76 (SD = 0.50, N = 31). This difference is highly significant (Mann-Whitney U-test, Two-tailed, P = 0.0002). I also plotted the individual testes mass of a male against his body mass after log-transformation of variables (Fig 3.3). Kusano et al. (1991) showed that the regression was not significantly different in slope or elevation for four species (**Rhacophorus schlegelli**, **R. viridis**, **R. owstoni** and **Polypedates leucomystax**) and a single regression line is drawn for the pooled data of these four species. The regression line for **C. xerampelina**, did not differ significantly from that of **R. arboreus** in slope (Homogenity of Slope test, F = 0.49, P = 0.49). The elevation of the slope for **C. xerapelina** was almost significantly greater than that of **R. arboreus** (ANCOVA, F = 3.89, P = 0.058). **Chiromantis xerampelina** had the largest relative testes size of the 37 species examined.

TABLE 3.1 Body mass and testes mass for 18 species of African anuran. Mean and standard deviation are shown. Relative testes size is the ratio of observed mass to that predicted on the basis of the allometric regression for 16 genera.

Families and species	N	Body mass (g)	Testes mass (mg)	Testes/body (%)	Locality	Relative testes size
Hyperoliidae						
Afrixalus fornasinii	11	2.56 ± 0.49	3.8 ± 0.8	0.15 ± 0.03	Zululand	0.60
Hyperolius marmoratus	20	1.35 ± 0.37	2.9 ± 1.4	0.21 ± 0.07	Hoedspruit	1.16
H. tuberilinguis	18	1.41 ± 0.27	5.1 ± 2.5	0.35 ± 0.12	Zululand	1.96
H. argus	20	2.00 ± 0.22	4.1 ± 1.1	0.21 ± 0.06	Zululand	0.95
Kassina senegalensis	3	5.00 ± 1.31	32.1 ± 17.6	0.62 ± 0.22	Hoedspruit	1.92
K. maculata	11	15.57 ± 2.69	70.6 ± 27.6	0.44 ± 0.12	Zululand	0.82
Rhacophoridae						
Chiromantis xerampelina	12	13.43 ± 2.69	1030 ± 340.7	7.79 ± 2.38	Hoedspruit	14.55

TABLE 3.1 /....CONTINUED

Families and species	N	Body mass (g)	Testes mass (mg)	Testes/body (%)	Locality	Relative testes size
Bufonidae						
Bufo maculatus	11	9.81 ± 1.27	37.3 ± 11.9	0.39 ± 0.14	Venda	0.84
B. gutturalis	9	38.70 ± 6.48	118.7 ± 38.1	0.31 ± 0.08	Venda, Hoedspruit	0.36
B. garmani	5	42.71 ± 5.73	55.5 ± 19.6	0.13 ± 0.06	Hoedspruit	0.17
Ranidae						
Hemisus marmoratum	8	3.16 ± 0.50	3.9 ± 1.5	0.13 ± 0.04	Hoedspruit	0.46
Tomopterna cryptotis	3	6.34 ± 0.50	18.0 ± 9.0	0.27 ± 0.15	Hoedspruit	0.72
Leptopelis natalensis	3	4.70 ± 0.76	9.1 ± 2.0	0.19 ± 0.04	Zululand	0.60
Ptychadena anchietae	10	5.63 ± 0.85	15.2 ± 1.9	0.28 ± 0.05	Hoedspruit	0.77
P. [illegible]hynchus	4	14.85 ± 3.18	31.3 ± 4.3	0.22 ± 0.04	Zululand	0.39
P. pororsissima	3	5.49 ± 0.36	9.5 ± 8.5	0.17 ± 0.15	Zululand	0.50
P. mascareniensis	3	10.40 ± 0.89	28.4 ± 0.1	0.19 ± 0.04	Zululand	0.48
Phrynobatrachus natalensis	3	2.58 ± 0.17	2.8 ± 0.5	0.11 ± 0.02	Zululand	0.44

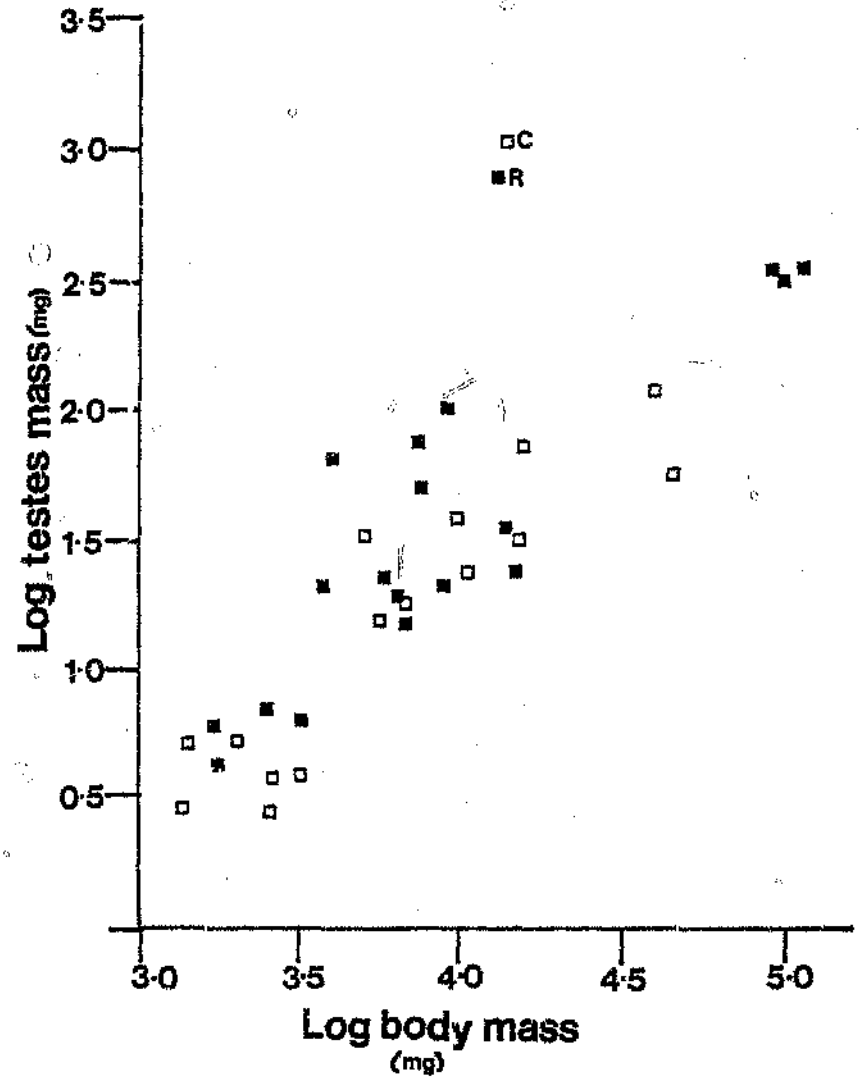

Figure 3.1 The allometric relationship between body mass and testes mass for 37 species of anurans [symbols: ■ = Japanese anurans - data collected by (Kuseno et al 1991); □= African anurans - data collected in this study; C = **Chiromantis xerampelina**; R = **Rhacophorus arboreus**).

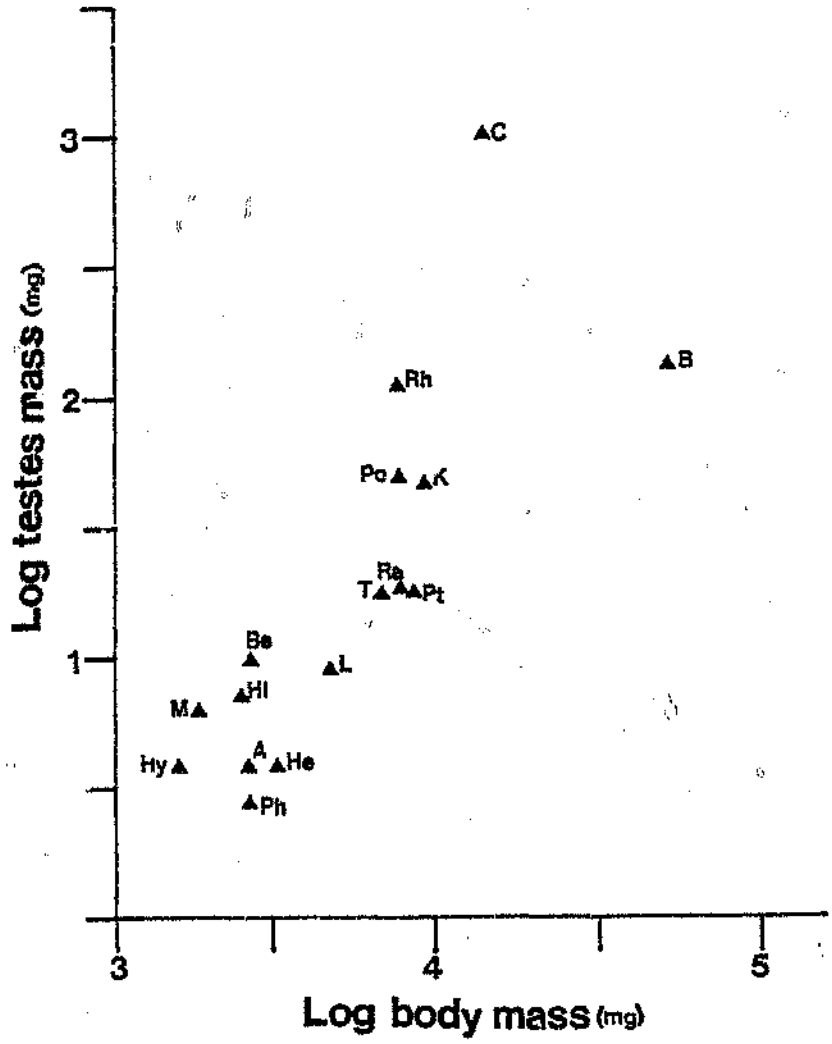

Figure 3.2 The allometric relationship between body mass and testes mass for 16 genera of anurans. The regression equation is: Log (testes mass) = -4.18 + 1.46.Log(body mass). [F (1,15) = 1213.0, P < 0.001, R^2 = 64.8%]. (Symbols: A = **Afrixalus**; Be = **Buegeria**; B = **Bufo** ; C = **Chiromantis** ; He = **Hemisus** ; Hl = **Hyla**; Hy = **Hyperolius** ; K = **Kassina** ; L = **Leptopelis**; M = **Microhyla** ; Ph = **Phrynomerus** ; Po = **Polypedates** ; Pt = **Ptychadena** ; Ra = **Rana** ; Rh = **Rhacophorus** ; T = **Tomopterna**).

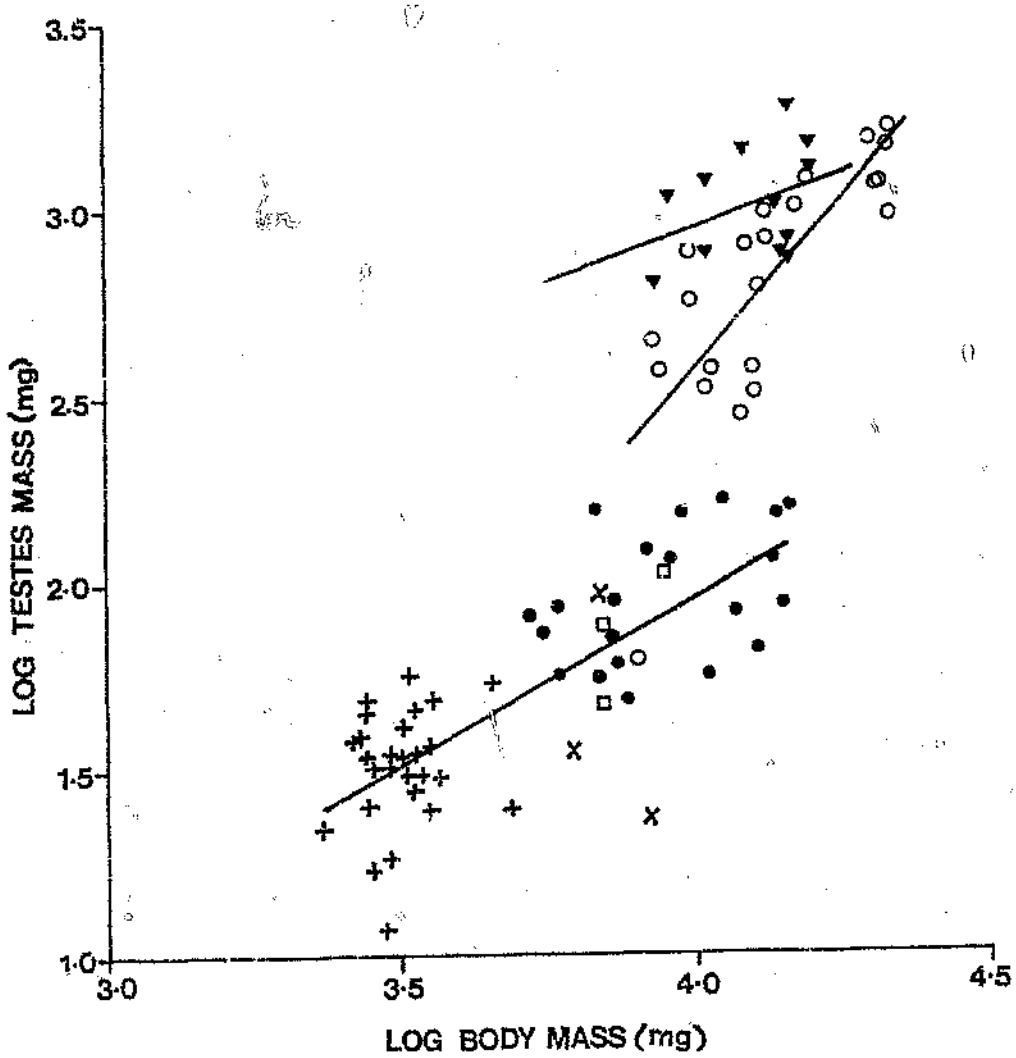

Figure 3.3 The allometric relationship between body mass and testes mass for six foam-nest building rhacophorids. Each symbol indicates an individual male. The regressions for **Chiromantis xerampelina** and **Rhacophorus arboreus** do not differ significantly in slope, but differ in elevation at the 0.06% level. The regressions are: Y = 1.81X - 4.65 for **R. arboreus** (○); Y = 0.57X + 0.63 for **C. xerampelina** (▾) and Y = 0.88X - 1.57 for the other four species [**R. schlegelli** (·), **R. viridis** (●); **R. owstoni** (□) and **Polypedates leucomystax** (✗)].

TABLE 3.2 Variation in the frequency and intensity of multi-male breeding in four foam-nesti

Species	Frequency of multi-male breeding (N = females)	Mean number of males participating in a spawning	Maximum number of males participating in a spawning	Testes body (%)
C. xerampelina	92.3% (N = 39)	5.5 (SD = 2.8)	11 a	7.8% (N
R. arboreus	81.4% (N = 97)	3.4 (SD = 1.9)	12 b	5.2% (N
R. schlegelli	44.4% (N = 9)	1.9 (SD = 1.3)	4 c	1.1% (N
P. leucomystax	3 males (N = 1)	?? e	?? e	0.7% (N

(a) Jennions, Backwell and Passmore (In Press)

(b) Toda (1989)

(c) Fukuyama (1991)

(d) Kusano et al. (1991)

(e) Feng & Narins (1991)

3.5 DISCUSSION

3.5.1 Sperm Release by Peripheral Males

To my knowledge, this is the first study of an anuran to document successful fertilization of eggs by peripheral males. The data conclusively show that peripheral males are releasing sperm. Multiple-paternity due to sperm competition, however, may still be absent if the amplexing male has complete priority in access to unfertilized eggs. This is unlikely, but further research is needed. The significance of the low hatching success is unclear. The plastic bag may have affected the rate at which peripheral males came into close contact with the female's cloaca. Even so, this should not have affected their ability to release sperm directly into the foam. This suggests that the juxtapositioning of cloacae with the female by peripheral males (see Chapter Two) may increase their fertilization success. Future studies should examine the mobility of sperm in the foam nest.

3.5.2 Inter-specific Variation in Testis Size

My study confirmed the trends that Kusano et al. (1991) noted for Japanese anurans. Eleven of the 19 species they examined were in two genera, however, so the potential for a confounding effect of phylogeny was fairly strong. Combining data from both studies, a regression of testes mass on body mass was performed using generic data. The slope of the regression was significantly larger than two-thirds (ANOVA, $F(1,14) = 7.66$, $P = 0.015$) but did not differ significantly from unity (ANOVA, $F(1,14) = 2.59$, $P = 0.13$). Even when the regression was recalculated excluding the two genera with enlarged testes (**Chiromantis** and **Rhacophorus**) the slope remained greater than two-thirds (ANOVA, $F(1,12)$, $P = 0.009$). This result is unexpected, as a scaling factor of two-thirds is commonly found for body organs (Calder 1984).

Chiromantis xerampelina have larger testes than expected on the basis of the regression of testes mass on body mass. In fact, their testes are relatively speaking even larger than those of **Rhacophorus arboreus**. Kusano et al. (1991) have already outlined an argument for the existence of sperm competition in **R. arboreus**. There is no evidence that male testes size is related to secretion of substances used in nest construction. In **C. xerampelina** females add additional foam to the nest the night following spawning and this does not require the assistance of males (Chapter Two). There is also no evidence that males help to churn the foam in either **C. xerampelina** or **R. arboreus** (Kusuya et al. 1987). The simplest explanation for the presence of peripheral males is provided by a sperm competition hypothesis.

3.5.3 Testis size and the Frequency of Multi-male Breeding

If sperm competition does account for variation in testes size, there should be a positive relationship between the frequency of multi-male breeding, the average number of males participating in a spawning and testis size. Table 3.2 shows these data for four species with multi-male breeding. These data indicate that multi-male breeding occurs more often in **Chiromantis xerampelina** than in **Rhacophorus arboreus** (92.3% versus 81.4; although this difference is not statistically significant: Chi-square test, $\chi^2 = 2.5$, $P > 0.05$). Multi-male breeding is also more intense in **C. xerampelina** (5.5 males versus 3.4 males at a spawning). Multi-male breeding occurs significantly more often, and is more intense, in **R. arboreus** than in **R. schlegelli** (Kusano et al. 1991). Relative testes size is positively related to the frequency of multi-male breeding and the number of males participating at nests (testes mass in: **C. xerampelina** > **R. arboreus** > **R. schlegelli** > **Polypedates leucomystax**) (Table 3.2). This relationship provides additional support for the sperm competition hypothesis as an explanation of testis size in anurans.

Several factors have been implicated in the high level of multi-male breeding in **R. arboreus** (Kusano et al. 1991), and by implication **C. xerampelina**: a) arboreal nests are more conspicuous and easily located; b) spawning takes a long time c) the sex ratio is strongly male-biased; d) foam-nesting allows sperm to be contained in the vicinity of the eggs. However, many anurans with conspicuous foam nests, prolonged amplexus and strongly male-biased sex ratios, do not exhibit multi-male breeding. At best, the combination of these factors may increase the likelihood that multi-male breeding occurs.

Of the eight documented cases of multi-male breeding in anurans, six are from foam-nesting rhacophorids. Furthermore, in the two hylids (**Agalychnis callidryas** and **Phyllomedusa dacnicolor**) the frequency of multi-male breeding is very low, and may simply be a consequence of extended attempts at amplexus displacement (personal observations of **A. callidryas**). In the absence of a phylogeny it is impossible to say whether multi-male breeding in rhacophorids is a shared derived character or has evolved on more than one occasion. Those aspects of their breeding behaviour, or physiology, which have resulted in the evolution of a reproductive tactic involving sperm competition need to be investigated further, preferably in conjunction with a good phylogeny.

3.5.4 Sperm Depletion

Sperm depletion provides an alternative explanation for large testis size (Catar 1985). Van den Berghe and Warner (1989) have documented large testes size (>5% body mass) in group-spawning male coral reef fish (**Thalassoma bifasciatum**). They noted that sperm competition among group-spawning males appears to limit the number of effective matings a male can engage in.

Kusano et al. (1991) possess no evidence that multi-male breeding Japanese rhacophorids participate in spawning more frequently than other species

without multi-male breeding systems. My study of **Chiromantis xerampelina**, however, showed that males may participate in a mean of 3.8 spawnings over a three week period (Chapter Two). One male participated in at least seven nests. More importantly, several males were involved in spawnings for three or four consecutive nights. A brief survey of the literature on anuran mating systems suggests that this is an exceptionally high level of reproductive activity for an anuran. Sperm depletion has not been documented in naturally breeding anurans [although it has been reported in a laboratory experiment (Smith-Gill & Berven 1980)], but it may be a limiting factor for **C. xerampelina**. Sperm depletion due to increased opportunities to engage in breeding combined with intense sperm competition may both select for enlarged testis size in **C. xerampelina**.

Acknowledgements - I thank P. Bishop, M. Banda, S. Davies, L. Egan, D. Osberg, A. Turner, M. Villet and R. Veale for field and logistic assistance.

CHAPTER FOUR

INTER-POPULATION VARIATION IN MATING PATTERNS AND SEXUAL SIZE DIMORPHISM

4.1 INTRODUCTION

4.1.1 Mating Pattern Variation in Anurans

Variation in anuran mating patterns is commonly recorded when researchers investigate more than one population, or observe the same population over several years. My survey of the literature (Table 4.1) shows that 21 of 28 species (75%) have both random and non-random mating patterns with respect to male snout-vent-length (or, in a few cases, another morphological criterion); 17 of 22 species (77%) showed both a large-male mating advantage and random mating. More than half of the species examined by Olsen et al. (1986), in which size-assortative or large-male mating advantage patterns occurred, also showed variation within these patterns. It is important that conclusions about the potential mating patterns of frog species are not reached on the basis of a study of a single-population (Sullivan 1987a).

Multi-season studies are also preferable to single-season studies because the effects of environmental fluctuations on mating can be investigated. Six of thirteen (46%) multi-season, single-site studies showed different mating patterns between years (Table 4.1). Finally, there is a danger that researchers, even if they study many populations, will select populations on the basis of their ease of study. This is likely to bias the observed mating patterns. Ideally, populations and sites should be selected at random. For

example, few researchers working in southern Africa would choose to study populations of a given species in arid areas if the species' range also covered wetter areas. Data collection is easier when the breeding season is extended. Non-random selection of sites may, however, lead to an inaccurate estimate of the variability in mating patterns and breeding behaviour within the species. Similarly, workers tend to choose populations of a 'manageable' size so that male mating success can be calculated, even though population size may have important implications for female mate choice (Telford et al. 1988).

By examining numerous sites, or monitoring the same site over several years, a variety of benefits are acheived. First, it is possible to directly assess the effects of environmental variables on mating patterns (Sullivan 1989). The confounding effects of phylogeny are controlled for because the same species is involved in each study (Clutton-Brock & Harvey 1984). Experimental manipulations can then be implemented to investigate the relative importance of environmental factors, as well as working out the underlying mechanisms causing variation in mating patterns (eg. Höglund 1989b; Woolbright et al. 1990; Elmberg & Lundberg 1991). Second, when dealing with a single site, the effects of ecological variation are partially controlled and variation in mating patterns can to related to 'conventional wisdom' indices of sexual selection intensity such as the operational sex ratio or male density (Emlen & Oring 1977; Arak 1983a; but see Sullivan 1989). Third, by documenting variation, one can determine the comparative strength of selective forces. If female choice, for example, only affects male mating success in a small percentage of populations then gene flow between populations will tend to reduce its effectiveness as a mechanism of evolutionary change. Likewise, within a single population, if female choice rarely affects male mating success its relative importance must be reassessed. Four, the effectiveness, frequency and form of alternative mating tactic employed can be related to environmental and social factors (eg. Fairchild 1984; Höglund & Robertson 1988; Tejedo 1988). Five, with respect to mate choice theory, examining variation in female choice may be

particularly interesting (Barnard 1991). For example, Fisherian runaway models predict inter-population variation in the mean female preference (Kirkpatrick 1987; Kirkpatrick & Ryan 1991). A correlation between mean female preference and mean magnitude of the preferred male traits is also predicted (Ryan & Wilczynski 1987; but see Houde & Endler 1990 and Balmford & Read 1991).

Proximate explanations for variation in mating patterns are based primarily on the effects of temporal parameters (Wells 1977a). The duration of the breeding season has a strong effect on the operational sex ratio and male density. These, in turn, are thought to affect patterns of mate choice (Wells 1977a; Arak 1983a), the frequency and type of alternative mating tactics (Howard 1984; Fairchild 1984; Tejedo 1988), body size and sexual dimorphism (Woolbright 1983, 1985; Sullivan 1984), and calling behaviour (Höglund & Robertson 1988). Breeding season duration may also affect the mating pattern by altering the time available for male-male competition (Höglund 1989b; Elmberg 1991). There are, of course, additional factors which may influence breeding behaviour such as the time available to acquire food resources (eg. Elmberg & Lundberg 1991) and the presence of sperm competition.

4.1.2 Sexual Size Dimorphism

There are three main explanations proposed that account for sexual dimorphism (Hedrick & Temeles 1989): selection for reduced food competition between the sexes; natural selection for reproductive specialization ('dimorphic niche hypothesis'); and sexual selection. [More details are provided in Chapter One]. In frogs, females are usually larger than males [90% of species (Shine 1979); 96% of species (Fukuyama & Kusano 1989)]. The most likely explanation for this pattern invokes a combination of sexual selection and dimorphic niche selection. First, in almost all species where sample sizes are large enough to detect trends, female fecundity increases

TABLE 4.1 Observed patterns of random and non-random mating in 43 species of anurans. Species that have been studied in a single population for a single season, the same population for several seasons, or more than one population are all included. This table is compiled from reviews by Arak 1983; Howard & Kluge 1985; and Olsen et al. 1986. Additional data were obtained from the primary literature. Asterisks indicate a multi-year, single-site study. Large male advantage usually refers to a size difference in SVL between mated and unmated males. However, in a few studies it also refers to other morphological measures (eg. forearm length or a principal component score based on morphological criteria).

Species	Large male advantage	Size assortative mating	Breeding season	Reference
Afrixalus delicatus	No	No	Prolonged	Backwell 1991
Buergeria buergeri	No	No	Prolonged	Fukuyama & Kusano 1989*
	No	No		Fukuyama & Kusano 1989*
	No	No		Fukuyama & Kusano 1989*
Bufo americanus	No	No	Explosive	Kruse 1981b
	No	No		Wilbur et al. 1978
	No	Yes		Licht 1976
	Yes	No		Gatz 1981a *
	Yes	No		Gatz 1981a *
	Yes	No		Gatz 1981a *
	Yes	No		Howard 1988 **
	Yes	No		Howard 1988 **
	No	No		Howard 1988 **
Bufo boreas	Yes	Yes	Explosive	Olsen et al. 1986
	Yes	Yes/No		Olsen et al. 1986
	No	No		Olsen et al. 1986
Bufo calamita	Yes	No	Prolonged	Arak 1983a, 1988a
	Yes	No		Arak 1983a, 1988a

TABLE 4.1 /....CONT

Species	Large male advantage	Size assortative mating	Breeding season	Reference
Bufo bufo	No	No	Explosive	Arak 1983a
	-	Yes		Davies & Halliday 1977
	Yes	-		Davies & Halliday 1979
	No	No		Arak 1983a
	No	No		Höglund & Robertson 1987*
	No	Yes		Höglund & Robertson 1987*
	Yes	No		Loman & Madsen 1986
	Yes	No		Reading & Clarke 1983
Bufo canorus	Yes	No	Explosive/	Kagarise Sherman 1980 *
	No	No	Prolonged	Kagarise Sherman 1980 *
	Yes	Yes		Kagarise Sherman 1980 *
	Yes	No		Kagarise Sherman 1980 *
Bufo cognatus	No	No	Explosive	Sullivan 1982
Bufo exsul	Yes	Yes	Explosive	Kagarise Sherman 1980 *
	No	No		Kagarise Sherman 1980 *
Bufo gutturalis	Yes	No	Explosive	Telford & van Sickle 1989
Bufo houstonensis	Yes	-	Prolonged/	Hillis et al. 1984
	Yes	-	Explosive	Hillis et al. 1984
Bufo marinus	Yes	No	?	Lee 1986

TABLE 4.1 /....CONT

Species	Large male advantage	Size assortative mating	Breeding season	Reference
Bufo periglenes	No	No	Explosive	Jacobson & Vandenberg 1991
	No	Yes		Jacobson & Vandenberg 1991
Bufo quercicus	Yes	No	Explosive?	Wilbur et al. 1978
	Yes	No		Wilbur et al. 1978
	No	No		Wilbur et al. 1978
Bufo terrestris	No	No	Explosive	Wilbur et al. 1978
	Yes	No		Lee 1986
Bufo typhonius	No	No	Explosive	Wells 1979
Bufo valliceps	No	No	Explosive	Lee & Salzberg 1989
	No	No		Lee & Salzberg 1989
	Yes	No		Lee & Salzberg 1989
Bufo woodhousei fowleri	Yes	-		Fairchild 1981
Bufo woodhousei	No	No		Sullivan 1982 **
	No	No		Sullivan 1983 **
	No	No		Sullivan 1987 **
	No	No		Sullivan 1987 **
	No	No		Sullivan 1987 **
	No	No		Sullivan 1989 ***
	No	No		Sullivan 1989 ***
	Yes	-		Woodward 1982b

TABLE 4.1 /....CONT

Species	Large male advantage	Size assortative mating	Breeding season	Reference
Bufo woodhousei	Yes	No	Explosive	Fairchild 1981
	Yes	No	(at times	Woodward 1982a *
	Yes	No	protracted)	Woodward 1982a *
	Yes	No		Woodward 1982a *
Centrolenella colymbiphyllum	Yes	-	Prolonged	McDiarmid 1978
Centrolenella fleischmanni	No	-	Prolonged	Jacobson 1985
Centrolenella prosoblepon	No	-	Prolonged	Jacobson 1985
Chiromantis xerampelina	No	No	Prolonged	Jennions et al. in press
Eleutherodactylus altamazonicus	-	No	?	Crump 1974
Eleutherodactylus coqui	No	No	Prolonged	Lopez & Narins 1991
	Yes	-		Woolbright 1989
Eleutherodactylus croceoinguinis	-	No	Prolonged	Crump 1974
Eleutherodactylus variabilis	-	No	Prolonged	Crump 1974
Hyla arborea	Yes	Yes	Prolonged	de Orense et al. 1990

TABLE 4.1 /....CONT

Species	Large male advantage	Size assortative mating	Breeding season	Reference
Hyla chrysoscelis	Yes	No	Prolonged	Morris 1989 *
	No	No		Morris 1989 *
	Yes	No		Morris 1989 *
	No	No		Morris 1989 *
	No	No		Ritke & Semlitsch 1991
	No	No		Godwin & Roble 1983
Hyla cinerea	No	No	Prolonged	Gerhardt et al. 1987 *
	No	No		Gerhardt et al. 1987 *
	No	No		Gerhardt et al. 1987 *
Hyla garbei	-	No	Prolonged	Crump 1974
Hyla gratiosa	No	No	Prolonged	Murphy (pers comm)
Hyla marmorata	Yes	No	Prolonged	Lee & Crump 1981
Hyla pseudopuma	No	No	Explosive	Crump & Townsend 1990
	No	No		Crump & Townsend 1990
	No	No		Crump & Townsend 1990
	No	No		Crump & Townsend 1990
Hyla regilla	No	No	Prolonged	Perrill 1984
Hyla rosenbergi	No	No	Prolonged	Kluge 1981

TABLE 4.1 /....CONT

Species	Large male advantage	Size assortative mating	Breeding season	Reference
Hyla versicolor	Yes	No	Prolonged	Gatz 1981b
	No	No		Gatz 1981b
	No	No		Fellers 1979
Hyperolius marmoratus	No	No	Prolonged	Passmore & Telford 1983
	No	No		Dyson et al. in press
	No	No		Passmore et al. unpubl.
Physalaemus pustulosus	Yes	No	Prolonged	Ryan 1980 *
	Yes	No		Ryan 1983 *
Pseudacris crucifer	Yes	No	Prolonged	Gatz 1981b
	No	No		Forester & Lykens 1986
Rana arvalis	Yes	-	?	Arak 1988c
Rana catesbeiana	Yes	-	Prolonged	Howard 1978 *
	Yes	Yes		Howard 1978 *
	Yes	Yes		Howard 1983 *
	Yes	No		Howard 1983 *
	Yes	Yes		Howard 1983 *
Rana clamitans	Yes	-	Prolonged	Wells 1977 **
	No	-		Wells 1977 **
Rhacophorus leucomystax	No	-	Prolonged	Arak 1988c

TABLE 4.1 /....CONT

Species	Large male advantage	Size assortative mating	Breeding season	Reference
Rana sylvatica	Yes	Yes	Explosive	Berven 1981
	Yes	Yes		Berven 1981
	Yes	No		Berven 1981
	Yes	Yes		Howard & Kluge 1985 *
	Yes	-		Howard & Kluge 1985 *
	Yes	-		Howard 1980
Rana temporaria	Yes	Yes	Explosive	Arak 1983
	No	No		Arak 1983
	No	No		Ryser 1989
	No	No		Elmberg 1991
Scaphiopus bombifrons	No	No	Explosive	Woodward 1982a
Scaphiopus couchi	Yes	No	Explosive	Woodward 1982a
	No	No		Woodward 1982a
	No	No		Woodward 1982a
	No	No		Woodward 1982a
Scaphiopus multiplicatis	Yes	-	Explosive	Woodward 1982a
	No	-		Woodward 1982a
Triprion petasatus	No	Yes	Explosive	Lee & Crump 1981
Uperolia laevigata	Yes	Yes	Prolonged	Robertson 1986 *
	-	Yes		Robertson 1990 *
	-	Yes		Robertson 1990 *

with body size (Davies & Halliday 1977; Reading 1986; Banks & Beebeee 1986; Gibbons & McCarthy 1986; but see Crump 1974). There is thus the potential for strong natural selection for increased growth by females. If this growth involves delayed reproduction or increased mortality costs, however, selection for increased body size may be weaker than initially predicted. Second, male size may have an effect on mating success. Studies of prolonged breeders show that a large-male mating advantage and size-assortative mating (which is advantageous for larger males when female size is related to fecundity) occur (Table 4.1). In only four species **Physalaemus pustulosus** (Ryan 1980, 1983, 1985), **Rana catesbeiana** (Howard 1978, 1988), **R. clamitans** (Wells 1977b, 1978) and **Uperolia laevigata** (formerly **U. rugosa**) (Robertson 1986, 1990) are these non-random mating patterns associated with active female choice based on traits associated with male size. In explosive breeders there is often a stronger correlation between male mating success and body size, because male size is positively related to success during amplexus displacement attempts (Davies & Halliday 1977, 1978; Lamb 1984; Telford & van Sickle 1989).

There are several possible explanations for non-random mating with respect to body size. Briefly, they are: (1) male-male competition via displacement, competition for resources, or the production of louder/more frequent calls; (2) female choice for larger males, optimum-size males, better territories, or the active avoidance of smaller satellites; and (3) incidental effects such as different size classes arriving at the pond at different times, or pooling data from populations with different age/size structures (Arak 1983a). In general, however, in spite of claims that active female choice for larger males should be widespread (Wilbur et al. 1978; Ryan 1980), it is a rarely documented phenomenon. In addition, even though larger males are usually more successful at displacing smaller males from amplexus, the association between male size and reproductive success is often weak (Howard 1988b). In contrast, the relationship between female size and fecundity is usually strong (Arak 1988c; Howard 1988b; Fukuyama & Kusano 1989).

Arak (1988c) has developed a simple mathematical model which predicts the magnitude of sexual size dimorphism. The model assumes (there is no empirical evidence to test this assumption) that the standard deviations and optima of the functions relating survival to body size are the same for both male and female frogs. If true, the difference in body size between the sexes is proportional to the difference in their reproductive selection gradients. The reproductive selection gradient is the regression coefficient of a regression of reproductive success on body size. Empirical data from nine species showed that size dimorphism was proportional to the difference in the reproductive success gradients ($r = 0.87$, $P < 0.002$) (Arak 1988c). However, the predicted direction of sexual dimorphism was incorrect! That is, there was a stronger effect of male size on reproductive success than female size in each of the nine species, yet females were larger than males. (Note that Arak's data on selection gradients appear unusual in this regard).

There are several possible explanations for the anomalous results of Arak's model. (1) The age structure of populations is rarely known. There is some evidence that females delay reproduction (Howard 1981), so the size difference between the sexes when considering same-age individuals may actually be less than estimated. It is also possible that age may have a direct effect on reproductive success so that body size is simply a correlate of age. (2) Data on reproductive gradients are usually based on one season. Ideally, lifetime reproductive success should be calculated. (3) There may be different body-size/survival functions for males and females. Predation may be higher on larger males (Howard 1981; Halliday & Verrell 1986). There is also evidence that larger males lose body weight faster than smaller males (Wells 1978). Males invest heavily in reproduction (Wells & Taigen 1989) and smaller size may be advantageous if it allows more rapid replacement of depleted energy reserves. (4) Male growth may be constrained by breeding activity. The small size of males may simply be a consequence of higher investment in reproductive activities and limited opportunity for growth (Woolbright 1983, 1989). (5) Perhaps most important of all, frogs exhibit

indeterminate growth. It may thus be wiser to consider selection on growth rate and size at maturity rather than size itself (Arak 1988c). High growth rates, especially in males, often carries costs in terms of increased pre-reproductive mortality (Clutton-Brock et al. 1985).

What of empirical evidence pertaining to these five explanations? In **Eleutherodactylus coqui**, females are larger than males. Males benefit more from increased body size than do females and there is no evidence that larger male size carries a cost in terms of increased predation. Why then are males smaller? Woolbright (1989) found that when males were unable to breed in a laboratory setting, they continued to grow at the same rate as females in the field. This is, perhaps, the best empirical support for one of the five explanations presented above. Even so, possible longer [illegible] effects of large body size on male survival are unknown and these may also contribute towards limited increase in male body size.

Shine (1990) in a recent review of ectothermic vertebrates (fish, frogs and reptiles) has shown that there is a strong positive correlation between the magnitude of sexual size dimorphism at maturity and at adult body size. This suggests that sexual differences in pre-maturation growth and/or age at maturity are the primary determinants of size dimorphism; and that post-maturation factors such as differential survival and growth between the sexes are less important. This is in agreement with (5) above, and suggests that (2), (3) and (4) may be less important areas to investigate if we wish to explain sexual size dimorphism. Finally, Cherry and Halliday (unpublished manuscript) have shown that a large percentage (74%) of the variation in male and female size is attributable to differences between the sexes in age of maturity (first breeding) and longevity. Cherry and Francillion-Vieillot (in press) have also shown that female growth rates in anurnas usually exceed those of males (13 species: female growth > male growth; four species: female growth = male growth; one species: female growth < male growth). Increased growth rates may result in higher mortality rates (Clutton-Brock et al. 1985).

4.2 METHODS

Observations of mating behaviour in **Chiromantis xerampelina** were made at all three study sites (Mkuzi, Hoedspruit and Vienna). Details of the December 1990 study at Hoedspruit can be found in Chapter Two. Additional observations at Hoedspruit were also made at Mariepsig dam.

At Mkuzi, observations were made on an unmarked population on 23 nights over a 25 night period (December 12, 1989 - January 5, 1990). The nightly number of males present at the pan were calculated by repeatedly circling the pan and counting the number of males in each tree. The largest total for a single circuit was taken as the number of males present for that night. The number of females present per night was calculated by examining the site each day for nests built the previous night. One instance of communal nesting was observed and the number of females involved is known. Communal nests are clearly distinguishable from single-female nests because of the exaggerated size of the former. The operational sex ratio and the number of males present at the pan was calculated for the first nine nights of the study when surveys were conducted most thoroughly. Thereafter, my main focus was on documenting behaviour during nest building.

At Vienna, opportunistic observations were made from December 16, 1991 to January 5, 1992. Due to a severe drought, unusually low levels of breeding occurred. In addition, experimental manipulations were carried out when possible. Data from Vienna are thus anecdotal. Some of the results presented here overlap those of Chapter Two which can be referred to for additional information.

4.3 RESULTS

The main inter-population differences are summarized in Table 4.2.

4.3.1 Body size

At Mkuzi in 1989, mean male **Chiromantis xerampelina** snout-vent-length was 58.7 mm (SD = 3.7, N = 58, range = 50.5 - 66.9). Mean female size was 76.2 mm (SD = 3.7, N = 22, range = 69.0 - 82.9). The size dimorphism ratio (Female SVL/male SVL) was 1.30.

At Hoedspruit in 1990, data from the main study site and Mariepsig dam were pooled because there were no significant differences in the data from the two populations (Mann-Whitney U-tests). Mean male size was 62.8 mm (SD = 3.6, N = 43, range = 50.6 - 69.4). Mean female size was 76.1 mm (SD = 3.2, N = 34, range = 67.8 - 81.3). The size dimorphism ratio was 1.21. At Vienna in 1991, mean male size was 61.9 mm (SD = 3.4, N = 27, range = 56.3 - 67.4). Mean female size was 74.8 mm (SD = 4.4, N = 13, range = 67.8 - 83.0). The size dimorphism ratio was 1.21.

There was no significant difference in female size at the three sites (ANOVA, $F(2, 66) = 0.804$, $P = 0.45$) (Fig. 4.1), but there was a significant difference in male size between sites (ANOVA, $F(2, 125) = 17.37$, $P < 0.0001$). Multiple-comparison tests showed a significant difference between Mkuzi and Hoedspruit and Mkuzi and Vienna (both: Scheffe's test, two-tailed, $P < 0.05$). There was no significant difference in male size between Vienna and Hoedspruit (Scheffe's test, two-tailed, NS) (Fig. 4.2). Pooling data from all three sites, the mean female size was 75.9 mm (SD = 3.6, N = 69, range = 67.8 - 83.0). Pooling male size data from Hoedspruit and Vienna, mean male size was 62.5 mm (SD = 3.5, N = 70, range = 50.6 - 69.4). The mean size dimorphism ratio for Vienna/Hoedspruit was 1.21 (Table 4.1). Females were significantly larger than males at all three sites (All sites: Student's t-tests,

TABLE 4.2 Inter-population variation in **Chiromantis xerampelina**. [* = calculated for 9 nights]

Study Site:	Mkuzi	Hoedspruit	Vienna
Nights of observation	25	21	21
Males per night	28.4 *	19.6	-
Females per night	1.36	1.20	0.41
Mean OSR	1:15 *	1:14	-
Nests with peripheral males	92% (N = 22)	93% (N = 14)	88% (N = 7)
Displacement attempts	None	Yes (N = 14)	Yes
Amplexing males Dismounting	Every time a session ended (N = 22 nests)	55% of observed departures (N = 11 of 20)	60% of observed departures (N = 6 of 10)
Probability of dismounting male re-amplexing	-	9% (N = 1 in 11)	25% (N = 1 in 4)
Female size	76.2 mm (N = 22)	76.1 mm (N = 34)	74.8 mm (N = 13)
Male size	58.7 mm (N = 58)	63.2 mm (N = 30)	61.9 mm (N = 27)
Female/Male Size Ratio	1.30	1.21	1.21

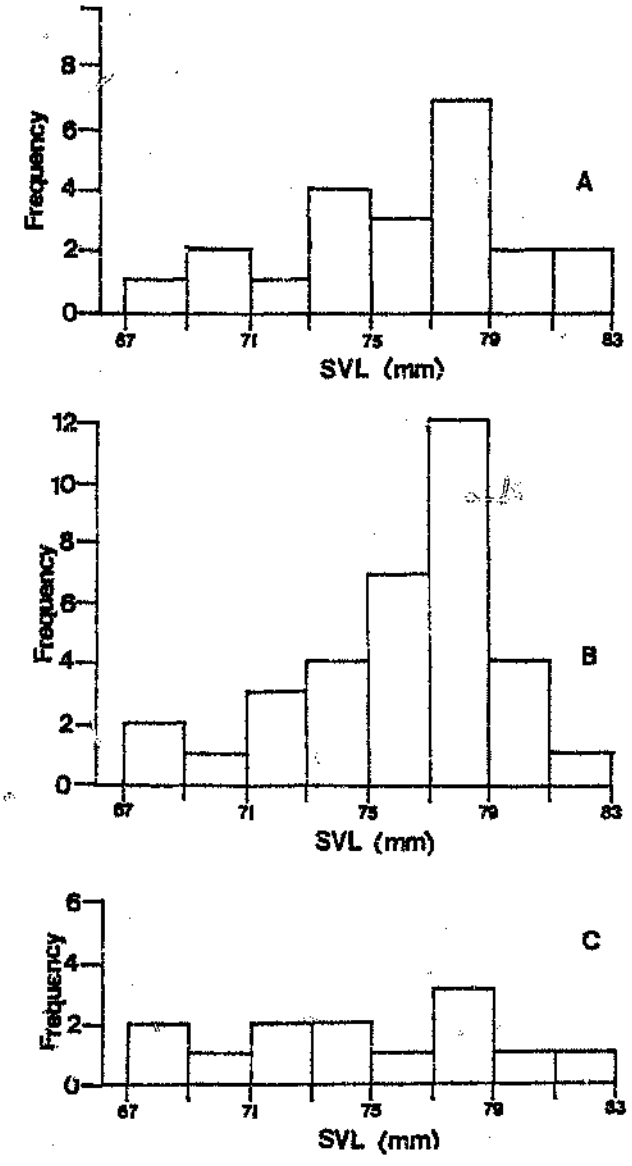

Figure 4.1 Histograms showing the distribution of female size in three populations: A = Mkuzi (1989-1990); B = Hoedspruit (1990-1991); C = Vienna (1991-1992). There was no significant difference in size between sites.

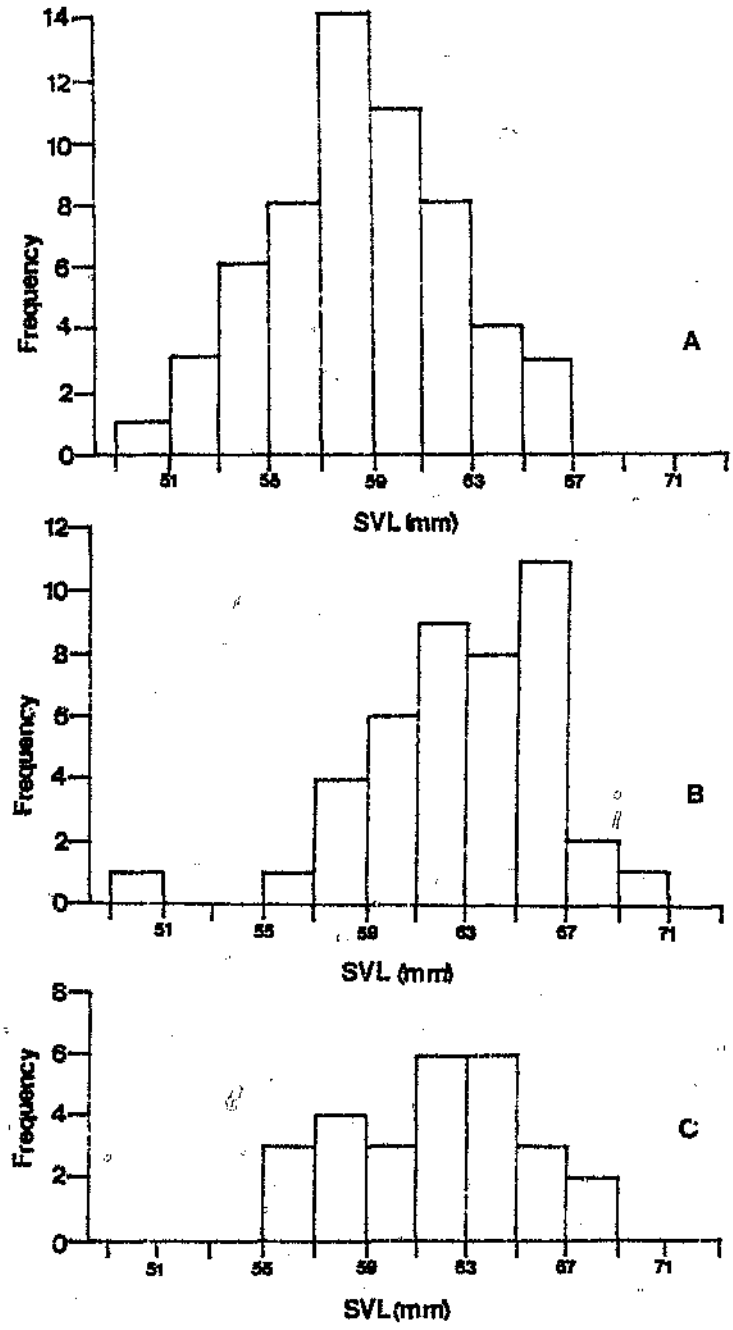

Figure 4.2 Histograms showing the distribution of male size in three populations: A = Mkuzi (1989-1990); B = Hoedspruit (1990-1991); C = Vienna (1991-1992). There was a significant size differences between Mkuzi and Hoedspruit and Mkuzi and Vienna (Scheffe's test, two-tailed, $P < 0.05$ both).

two-tailed: Mkuzi, $t = 18.84$, $P < 0.0001$; Hoedspruit $t = 16.75$, $P < 0.0001$; Vienna, $t = 10.19$, $P < 0.0001$).

4.3.2 Demographics

At Mkuzi, breeding was observed from December 12, 1989 until it ceased on January 5, 1990. However, several nests had been constructed before the study began and the season was thus longer than the 25 day period of observation. At Hoedspruit, only two nests were constructed in the three weeks prior to the study. The study ran over 22 nights, from December 5 to 26, 1990 when breeding stopped. At the Mariepsig dam on December 5, 1990, a very large number of gravid females (40+) and males (100+) were present at the dam and built nests. In the nights that followed, only a single additional female bred at the dam. There were never more than one or two males present on subsequent nights. Breeding shifted to a second dam at Mariepsig, approximately 60 m from the nesting sites at the first dam. At Vienna, breeding was monitored from December 16, 1991 to January 5, 1992. Four dried out nests were already present. Over the study period, only eight nests were built, whilst 27 males were marked. One or two additional nests were built during January (C. Ferguson, pers comm).

The mean number of males per night at Mkuzi was 28.4 (N = 9 nights), while the mean number at Hoedspruit was 19.6 (N = 21 nights). At Mkuzi, the mean number of females per night was 1.20 (range = 0 - 5 females, N = 25 nights). At Hoedspruit the mean was 1.36 females per night (range = 0 - 6 females, N = 22 nights). Mean operational sex ratio at Mkuzi was 1:15 (range 0 - 0.14, N = 9 nights), at Hoedspruit it was 1:14 (range 0 - 0.29, N = 21 nights). At Mkuzi 92% of nests (22 of 24 nests) and at Hoedspruit 93% of nests had peripheral males (14 of 15 nests). At Vienna, 8 gravid females were observed. In some cases, I manipulated male numbers at nests. However, at 6 of 7 nests (88%), peripheral males were, or would have been, present. There

was no significant difference in the frequency of multi-male nests at the three sites (Chi-square test, df = 2, χ^2 = 0.75, P > 0.5).

4.3.3 Male and Female Behaviour

At Mkuzi, amplexus displacement attempts were never observed. At Hoedspruit, however, 14 attempts were observed. More nest building and mating was observed at Mkuzi than at Hoedspruit, therefore this difference is not an artifact of sample size. Data from Vienna are not presented because most nests were artificially manipulated but attempts at displacement were also recorded.

At Mkuzi, each and every time the female descended to the water between nest sessions, the amplexing male dismounted. Because the population was unmarked, it was not possible to establish whether or not the originally amplexing male amplexed with the female when she returned. At Hoedspruit, the amplexing male dismounted in 11 of 20 female descents (55%) . The amplexing male only re-amplexed in 1 of 11 observed returns (9.1%). At Vienna, the amplexing male dismounted in 6 of 10 (60%) observed female descents and re-amplexed in 1 of 4 observed female returns (25%). Pooling the Hoedspruit/Vienna data, 56.7% of males (17 of 30) dismounted with 13.3% (2 in 15) re-amplexing when the female returned to the nest.

Although recordings of vocalizations were not made at Mkuzi, the amount of calling at Mkuzi and Hoedspruit appeared to be similar. Calling at Vienna, however, appeared to be more sporadic. This is reflected in the absence of tape recordings from Vienna, despite attempts to record males. Equal effort was made to obtain recordings at Hoedspruit and Vienna, but the percentage of males recorded at the two sites was significantly different (Vienna: 0 of 27 males; Hoedspruit 15 of 30 males; Chi-square test, df = 1, χ^2 = 7.35, P < 0.01).

Male movement between call sites that was unrelated to female presence (ie. not just males moving towards a gravid female) occurred in all three populations. However, the males at Mkuzi were far more active. They were regularly seen to climb up a large tree, drop into the water and then swim to the base of the tree and climb up it again. This behaviour was not observed at Hoedspruit or Vienna. At Mkuzi the number of males on a given tree also varied between censuses, due to male movement, while the number remained fairly constant per tree/call site at Hoedspruit and Vienna.

4.4 DISCUSSION

4.4.1 Body Size

In male, but not female, **Chiromantis xerampelina** body size varied between populations. There was a significant difference in male size at Mkuzi versus the other two sites. There was no difference between the Vienna and Hoedspruit site. Given that the latter are 23 km apart, with no habitat or topological discontinuities acting as barriers to movement, they probably form a continuous population in terms of gene flow. The similarity in body size for December 1990 and 1991 at Hoedspruit/Vienna, despite the very different environmental conditions (severe drought in 1991-1992), suggests that the size difference between Mkuzi and Hoedspruit/Vienna males is not solely due to phenotypic plasticity, but has an underlying heritable component. Additional support for this suggestion is provided by the similar size of females at all three sites. If environmental or dietary factors were responsible for difference in male size, one would expect to see a similar inter-population difference in female size (eg. Sullivan 1987). The sex-specific nature of the inter-population size difference suggests a selective force acting on males. Perhaps the existence of the amplexus displacement tactic in the Hoedspruit/Vienna population, which usually selects for larger body size (Telford & van Sickle 1989), and its absence from the Mkuzi population, contributes towards the difference in male size?

4.4.2 Sexual Size Dimorphism

The degree of sexual size dimorphism for **Chiromantis xerampelina** varied between 1.21 and 1.30. The mean for the two populations was 1.26. An inter-population range in the dimorphism ratio of 0.09 falls well within the known degree of variation. In a review of dimorphism in Japanese anurans, the mean range recorded was 0.10 (range = 0.02 - 0.31, N = 6) (Fukuyama & Kusano 1989). The magnitude of size dimorphism is fairly large. Based on a review of size dimorphism in 56 populations of 45 species, size dimorphism in the Hoedspruit/Vienna population was in the top 28%, while Mkuzi was in the top 14% (Fukuyama & Kusano 1989).

How can sexual dimorphism be explained? First, the age structure of the population is unknown, hence the possibility that females are on average older than males can not be discounted, although frog researchers have tended to overlook this possibility. What of selection for large male body size? At Hoedspruit, there was no evidence for non-random mating with respect to body size. There was also no evidence for female choice of larger males. Although amplexus displacements were observed, there did not appear to be a large male mating advantage (Chapter Two). However, because inter-population variation in mating patterns does occur (Table 4.1), further data are needed. Even so, behavioural observations suggest a large male advantage is unlikely. The dismounting of males between nest sessions reduces variation in mating success (observed in all three populations) and tends to minimize any effects of direct male-male competition (observed in two of three populations). Similarly, the manner in which amplexus was initiated argued against female choice (Chapter Two). The same pattern of amplexus initiation was seen in all three populations. In sum, the reproductive selection gradient for male size is likely to be close to zero. Two points should be noted though. First, if males were larger they would benefit when in amplexus by reducing the distance between their cloaca and extruded eggs. However, because peripheral males probably fertilize eggs this

reduces any selective force relating to amplexus (Halliday & Verrell 1986); second, the strongest selection is probably for increased testis size (Chapter Three). If testis size can not be selected for independently of body size, then body size might be larger than expected as a correlated response to selection for large testis size.

For female **C. xerampelina**, body size is likely to be related to fecundity (clutch size) as with other anurans. Different benefits of size to males and females do not, however, explain sexual dimorphism (Höglund & Saterberg 1989). If there is no cost associated with increased size, then male size should increase as a correlated response to selection for increased female size. Selective forces acting against increased male size are unknown. Possibly food constraints may select for smaller body size. Finally, given the comparatively stable weight of males during breeding (Chapter Two), there is less evidence that male size is constrained by energetic factors than for other species (eg. Given 1988). In most frogs males *lose* weight during the breeding season (Halliday 1987).

4.4.3 Demographics

Breeding in African frogs is strongly dependent on rain, especially in the drier parts of the country where surface water only remains for short periods in the absence of replenishing rains. Generally speaking, **Chiromantis xerampelina** is a prolonged breeding species. Breeding begins with the first heavy rains of the summer and persists at a very low rate even in the absence of rain. Despite being a prolonged breeder, however, the intensity of breeding fluctuates widely. There appears to be an initial period of one or two nights when large numbers of females breed. This coincides with the first good rains of the season, or rain following a dry spell. Demographically the initial breeding period resembles that of an explosive breeder - high densities and a lower operational sex ratio. Thereafter, breeding extends over a few weeks and resembles that of most prolonged breeders - female arrival is

asynchronous and unpredictable and the operational sex ratio is heavily male-biased. Under natural conditions, the termination of breeding would probably coincide with the disappearance of surface water, but with farm dams and boreholes breeding may be artificially prolonged. Analyses of mating patterns and correlates of mating success in **C. xerampelina** would need to consider two factors: (1) the percentage of breeding occurring during the first and second nights of breeding, and (2) whether trends relating to mating should be considered across the entire season, or whether a distinction should be made between the initial burst of breeding and the prolonged season that follows. For example, imagine a situation where no female choice occurs on the first few nights due to high male density and strong male-male competition. If 80 % of matings occured at this initial stage, then even if female choice was a strong force for the rest of the season, it might be difficult to detect (see also Howard 1988b).

4.4.4 Male and Female Behaviour

Peripheral males were seen at all three study site, with approximately the same frequency (92%, 93% and 88% of nests). Most alternative mating tactics in anurans are density-dependent or vary in relation to the operational sex ratio (eg. Höglund & Robertson 1988; Arak 1988b). The peripheral male tactic in **Chiromantis xerampelina** is related to male density and the OSR in a rather obvious manner. Males that do not amplex become peripheral males. The chances of finding another gravid female are usually such that the best tactic a male can pursue is simply to amplex with the first female he encounters, or to follow the first pair he encounters and join in as a peripheral male. Hence, the percentage of males pursuing the peripheral male tactic at any given time could be expressed as:

$$f(P) = \frac{K.[N(T) - N(F)]}{N(T)} \times \frac{100}{1}$$

where: f(P) = Percentage of males that are peripheral

N(T) = Total number of males present

N(F) = Number of gravid females

K = Probability of finding a pair and becoming peripheral

and where K is proportional to N(F)

[Note: the percentage of males employing the peripheral male tactic *per night*, will depend on the rate of amplexus displacement, the number of males dismounting between sessions and the probability that a dismounting male will re-amplex].

As N(F) increases, the chances of finding a pair increase. So, for a given male density, the frequency of the peripheral male tactic should be positively related to the OSR. If male density is very high, however, it may lower the frequency of the peripheral male tactic. The number of males that can participate in a nest is finite and there are reduced benefits to joining a nest as the number of peripheral males increase [van den Berghe & Warner (1989) have shown that in a tropical reef fish, males adjust their presence at group spawnings in relation to the level of sperm competition from other males]. In **C. xerampelina**, there was some evidence that male density may reach levels that effect f(P). For example, some males left nests before the end of spawning (Chapter Two). This was only seen at nests with large numbers of peripheral males. Unfortunately, data on this phenomena was not collected systematically, and no conclusions can be drawn.

Male calling behaviour was similar at all three sites, at least to the extent that calling was sporadic and subdued. Calling at Vienna, however, was especially intermittent, as reflected by the absence of sound recordings.

Males probably cue on rainfall as an general indicator of the likelihood of female presence. Given the drought conditions, and consequent reduced female presence, this may explain their decreased calling effort. The main inter-population difference in behaviour was the presence or absence of amplexus displacement attempts. No displacement attempts were seen at Mkuzi, while displacement attempts, and successful displacement, were seen at Hoedspruit and Vienna. The reason for this inter-population variation is unclear. According to theory, the intensity of direct male-male competition is related to three factors. First, the operational sex ratio (OSR) (Emlen & Oring 1977; Tejedo 1988). As the OSR becomes more male-biased the number of unpaired males encountering pairs increases, hence the chance of displacement attempts increases (Tejedo 1988). If the OSR was more male-biased at Hoedspruit, this might have explained the results. In fact, the OSRs was almost identical (1:14 versus 1:15). Second, male density may also effect male-male competition. Again, as density rises, the chances of unpaired males encountering pairs rises. There was no conspicuous difference in male density between the Mkuzi and Hoedspruit populations. This explanation is particularly unlikely to explain the situation in **C. xerampelina**, because males encountered pairs at the same rate in both populations, as estimated by the frequency of multi-male nests. Third, inter-population variation in the amplexus displacement tactic may be related to the duration of the pre-spawning period. Höglund (1989) in an experimental study with **Bufo bufo** found that the likelihood of displacement increases as the pre-spawning period increases. This has also been reported in **Rana temporaria** (Arak 1982; Elmberg 1991). Again, however, this explanation is unlikely to account for the situation in **C. xerampelina** where we are dealing with the total absence of the tactic from a population. Besides, variation in the pre-spawning duration between Mkuzi and Hoedspruit is minimal (on the order of minutes) and displacements occured throughout the spawning process.

In **R. temporaria**, inter-population variation (including total absence in some populations) in the tactic of amplexus displacement was related to the

OSR, male density and the duration of the pre-spawning period (Elmberg 1991). None of these explanations account for the absence of displacement attempts from the Mkuzi population. The answer may lie in the benefits derived from being in amplexus. Perhaps the larger male size in the Hoedspruit population increases the fertilization success of amplexing males relative to that of peripheral males, making amplexus more advantageous? This could be tested by examining the fertilization success of different-sized amplexing males in the presence of peripheral males.

CHAPTER FIVE

NOCTURNAL BODY TEMPERATURE IN THE PAINTED REED FROG, *HYPEROLIUS MARMORATUS* AND THE FOAM NEST FROG, *CHIROMANTIS XERAMPELINA*

5.1 SUMMARY

An earlier study by Passmore & Malherbe (1985) reported that the nocturnal body temperature of painted reed frogs (**Hyperolius marmoratus**) is higher than dry-bulb air temperature. It was suggested that this difference in temperature was due to the combined effects of diurnal basking and a low rate of evaporative water loss. I present additional data showing a smaller difference between body temperature and air temperature than originally observed. However, the general trend towards elevated body temperature was confirmed. I also report that the nocturnal body temperature of the foam nest frog (**Chiromantis xerampelina**) is not elevated, even though it is known to bask and has low evaporative water loss. The significance of elevated body temperature is discussed in relation to female choice.

5.2 INTRODUCTION

In many frogs, temporal and sometimes spectral mating call parameters are influenced by temperature. If variation in body temperature affects call parameters that females use as cues during mate choice, it is possible that males may thermoregulate to increase the attractiveness of their calling (Fairchild 1981). Perhaps more importantly, the performance capacity of anurans rises with increasing body temperature (Putnam & Bennett 1981). Males with elevated body temperatures are thus capable of sustaining a

higher rate of calling. In **Hyperolius marmoratus**, mating success is positively correlated with call rate (Passmore et al. unpublished) and call rate, in turn, is positively related to temperature.

In most studies of anurans, it is assumed that dry-bulb or wet-bulb air temperatures are acceptable substitutes for direct measurements of body temperature. In some arboreal anurans, however, reduced rates of evaporative water loss are predicted to result in higher body temperatures than those found in typical anurans (Wygoda 1984; Wygoda & Williams 1991). This trend has been confirmed in **Hyla cinerea** (Weygoda 1988a, 1988b). Passmore and Malherbe (1985) found that nocturnal body temperatures of **H. marmoratus** males were consistently higher than dry-bulb air temperature ($\bar{x}$ = + 3.1 C, N = 81). These frogs bask diurnally and have low evaporative water loss (Withers et al. 1982). Passmore & Malherbe (1985) suggested that these two phenomena might account for elevated body temperatures. I tested this hypothesis by examining nocturnal body temperatures in the foam nest frog **Chiromantis xerampelina**. Foam nest frogs bask diurnally and have remarkably low rates of evaporative water loss (Stinner & Shoemaker 1987; Kaul & Shoemaker 1989). According to the evaporative water loss hypothesis, they should have elevated nocturnal body temperatures. I also re-examined the relationship between ambient and body temperatures in **H. marmoratus**.

5.3 METHODS

Data on **Hyperolius marmoratus** were collected during January 1992 from a small pond on the farm Twinstreams, Mtunzini, Zululand (latitude 28°51' S; longitude 31°46' E). For a detailed description of the study site see Telford (1982). I used a Bailey Instruments BAT-12 digital thermometer equipped with a PT-6 thermocouple to measure temperature. Males and females were captured individually from perches. Conductive heat transfer between the frog and the hand of the researcher was minimized by capturing frogs by

their hind feet. Frogs were then held by the feet on a plastic surface while the thermocouple was inserted into the mouth. Body temperature measurements were complete within 30-40s of capture. Body temperature (Tb) did not increase during handling as indicated by the stable reading from the thermometer. Dry-bulb air temperatures (Ta) were then taken within 5 cm of the site of capture. Male snout-vent length (SVL) was then measured to the nearest 0.1 mm using vernier calipers. The time of night, water temperature (Tw) and male mass (±0.01 g) were also recorded. All measurements were made between 2000 and 2230 hours.

Data on **Chiromantis xerampelina** were collected between November 1991 and January 1992 at Snare dam on Vienna Game Farm, Hoedspruit, Transvaal (latitude 24°17' S; longitude 30°58' E). Methods followed those for **H. marmoratus**, except that body temperature was measured by inserting the probe through the cloaca into the rectum.

5.4 RESULTS

5.4.1 Nocturnal Body Temperature of *Chiromantis xerampelina*

The air temperature measured at the perches of males and females ranged from 17.0 - 27.9 C ($\bar{x}$ = 23.1 3.0 SD). Body temperature ranged from 17.3 - 25.8 C ($\bar{x}$ = 22.6 2.2 SD). There was no significant difference between body temperature and ambient temperature (Matched-pair Student's t-test, One-tailed, t = 0.64, N = 26, P > 0.5). There was a significant correlation between body temperature and dry-bulb air temperature (r = 0.84, P < 0.0001) (Fig. 6.1). There was no significant difference between male and female body temperatures (Mann-Whitney U- test, Two-tailed, P = 0.24).

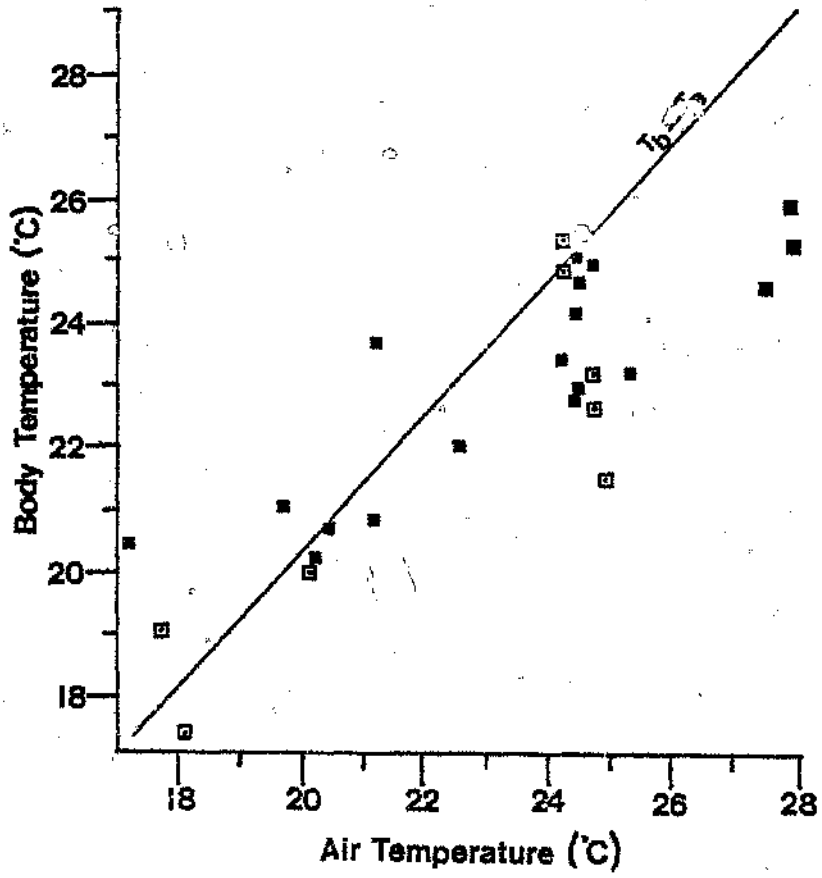

Figure 5.1 The relationship between dry-bulb air temperature and body temperature (cloacal) for 26 **Chiromantis xerampelina** (symbols: ■ = male; □= female). The line represents equality of body temperature and air temperature.

5.4.2 Nocturnal Body Temperature of *Hyperolius marmoratus*

The air temperature (Ta) measured at the perches of males ranged from 17.5 - 25.4 C ($\bar{x}$ = 22.7 1.7 SD). Body temperature (Tb) ranged from 19.6 - 28.0 ($\bar{x}$ = 23.5 1.7 SD). Although there was a significant correlation between Tb and Ta ($r = 0.84$, $P < 0.0001$) (Fig. 6.2), there was also a significant difference between the two temperatures (Tb - Ta: x = 0.8 1.0 SD; range = -1.7 C to +3.4 C; Matched-pair Student's t-test, Two-tailed, $t = 6.87$, $N = 78$, $P < 0.0001$) (Fig. 6.3). Two of the six measured variables were correlated with (Tb - Ta): body temperature ($r = 0.39$, $P = 0.0005$) and ambient temperature ($r = -0.23$, $P < 0.05$) (Figs. 6.4 & 6.5). There was no significant correlation between (Tb - Ta) and time of night ($P = 0.75$), size ($P = 0.83$), mass ($P = 0.76$) or Tw ($P = 0.88$).

Despite the correlation between Tb, Ta and (Tb - Ta), the two variables explained a very small percentage of the variance in (Tb -Ta) in model I linear regressions:

$$(Tb\text{-}Ta) = 4.48\ (SE = 1.51) - 0.16.Ta\ (SE = 0.07)$$

$$[F\ (1,76) = 5.99,\ P = 0.017,\ R^2 = 7.3\%]$$

and

$$(Tb - Ta) = -3.78\ (SE = 1.51) + 0.19.Tb\ (SE = 0.06)$$

$$[F\ (1, 76) = 9.14,\ P = 0.003,\ R^2 = 10.7\%]$$

There was no significant difference in the value of (Tb - Ta) for males and females (Mann-Whitney U-test, $N_1N_2 = 78,7$, $P = 0.29$).

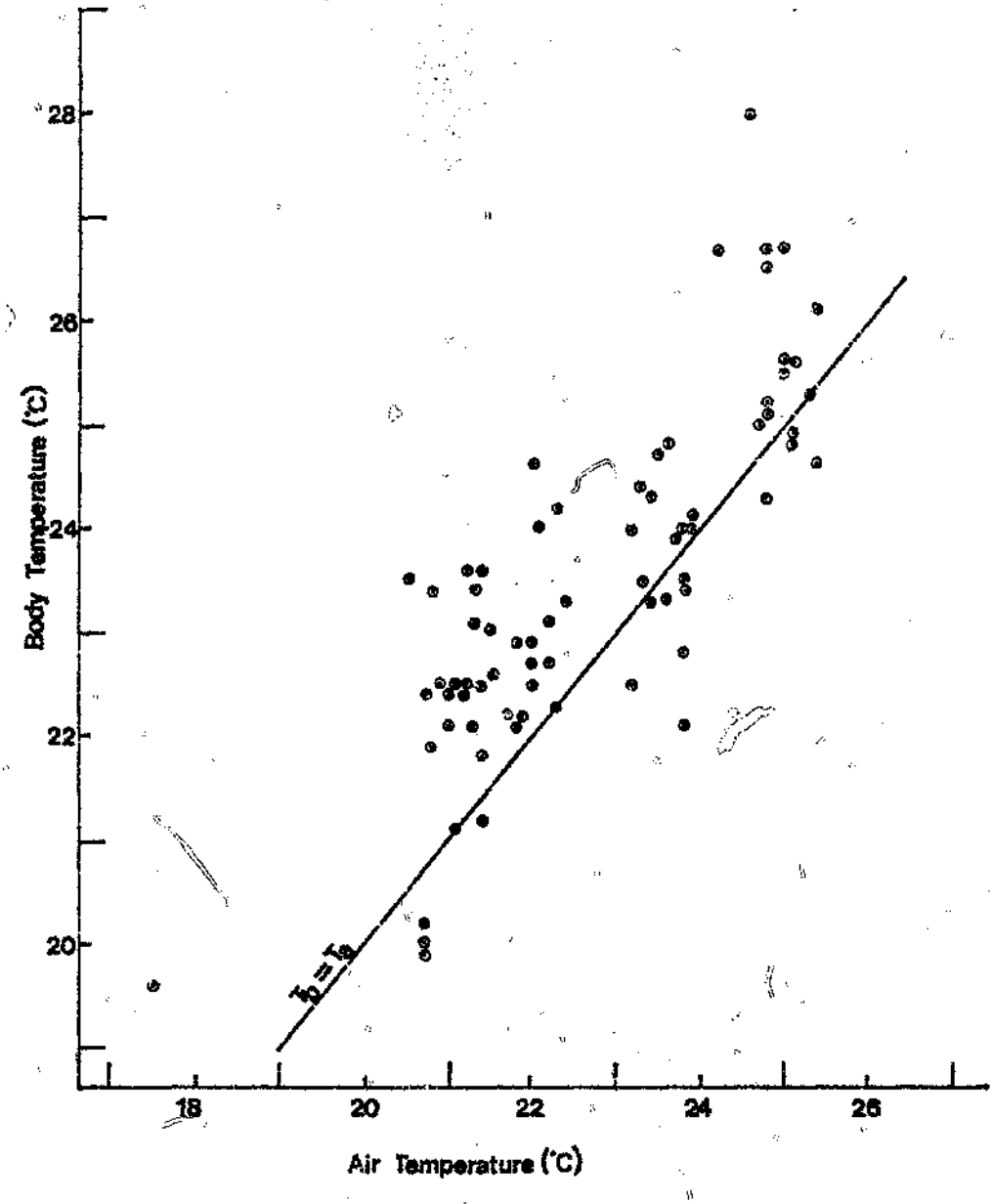

Figure 5.2 The relationship between dry-bulb air temperature and body temperature (oesophageal) for 78 male **Hyperolius marmoratus**.

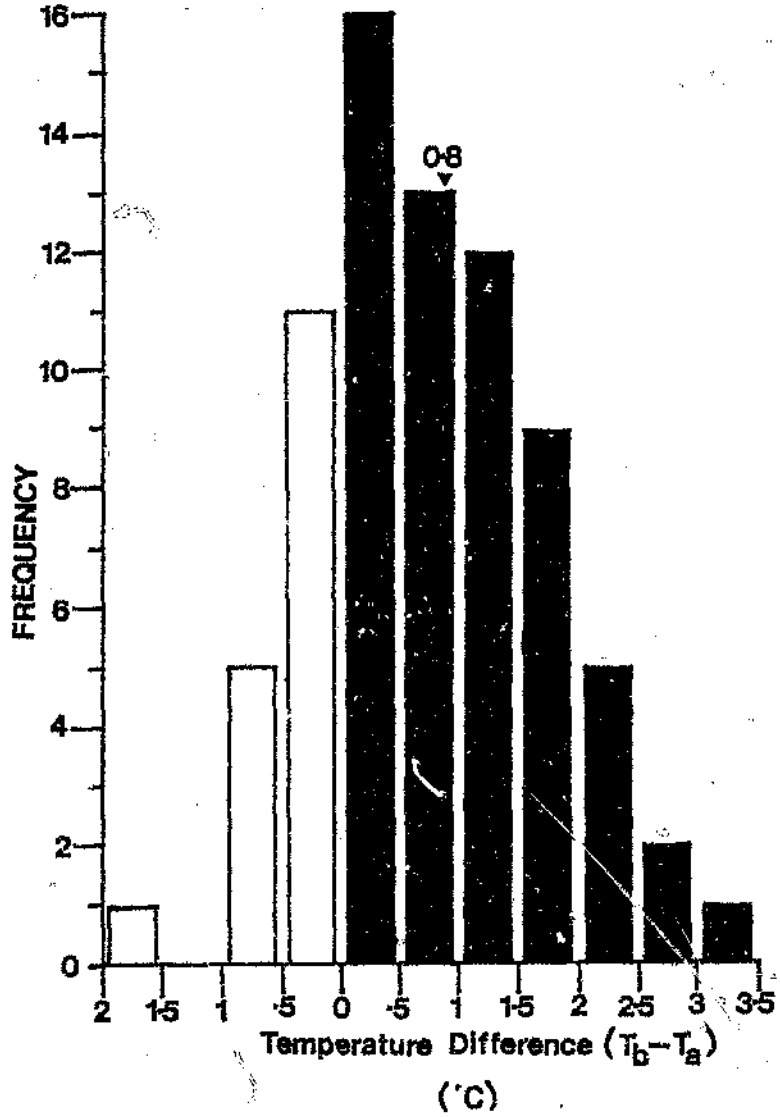

Figure 5.3 A histogram showing the distribution of the difference between body temperature and ambient temperature for 78 male **Hyperolius marmoratus**. Values greater than zero are shaded. The mean temperature elevation was 0.8 C (SD = 1.0 C).

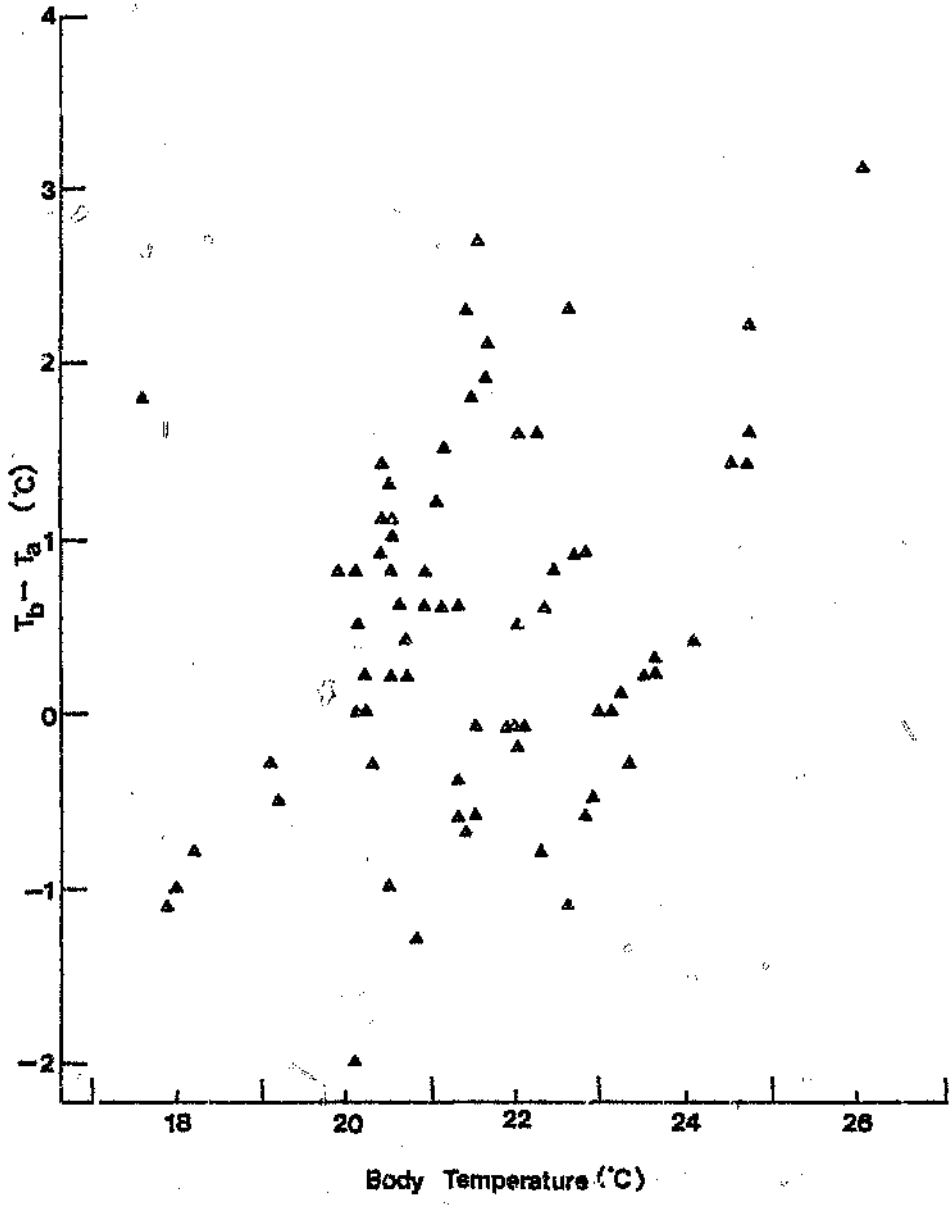

Figure 5.4 The relationship between body temperature and the magnitude of body temperature elevation (T_b - T_a). The Model I regression equation is: $T_b - T_a = -3.78 + 0.19.T_b$ [$F(1,76) = 9.14$, $P = 0.003$, $R^2 = 10.7\%$].

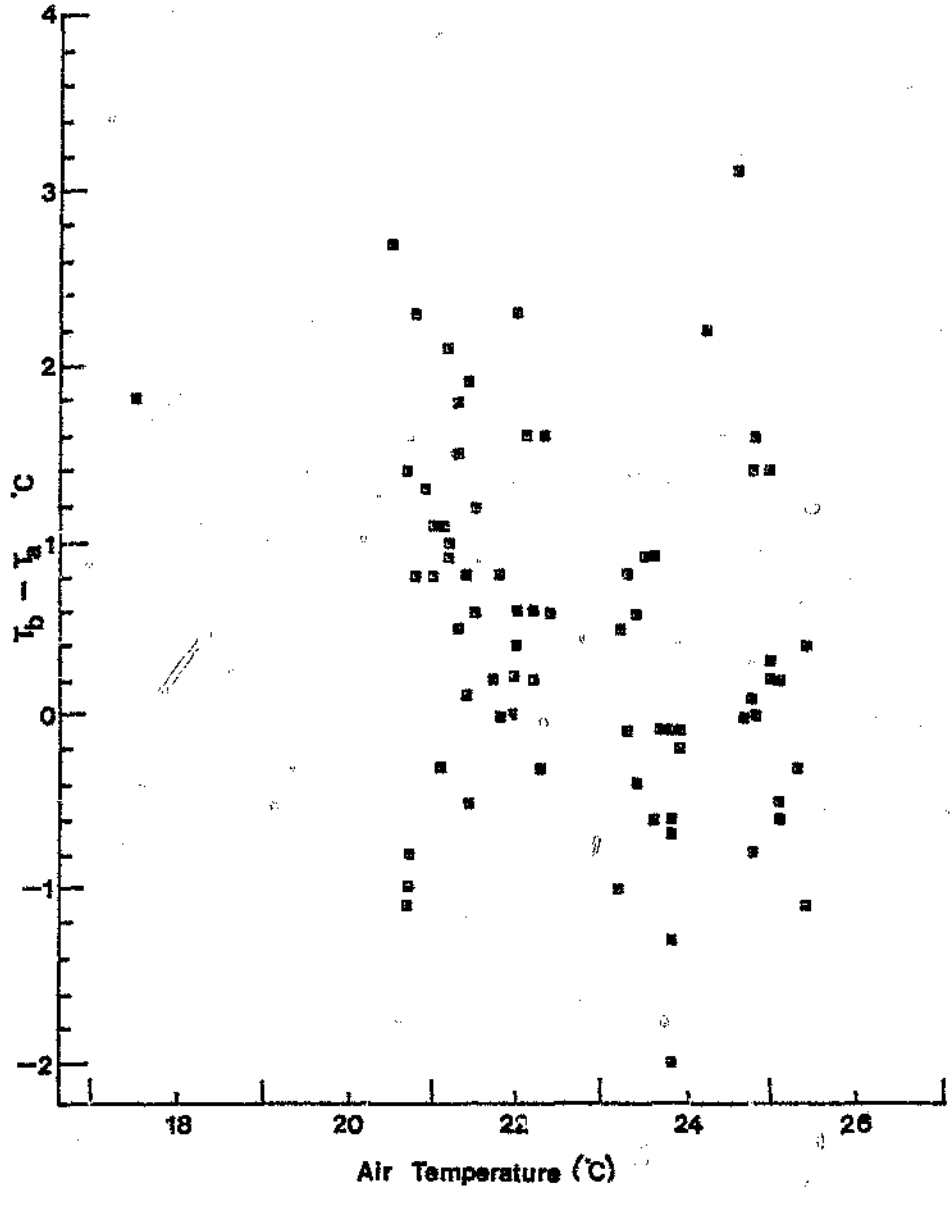

Figure 5.5 The relationship between air temperature and the magnitude of body temperature elevation ($T_b - T_a$). The Model I regression equation is: $T_b - T_a = 4.48 - 0.16.T_a$ [$F(1,76) = 5.99$, $P = 0.017$, $R^2 = 7.3\%$].

5.5 DISCUSSION

The data show that there is no significant difference between body temperature and dry-bulb air temperature in **Chiromantis xerampelina**. Dry-bulb temperature is a good predictor of body temperature. This is in agreement with results from diurnal studies of body temperature made by Shoemaker et al. (1987, 1989). Basking and low evaporative water loss do not appear to elevate nocturnal body temperature in **C. xerampelina**. I thus suggest that the elevated body temperature in **Hyperolius marmoratus** can not be attributed solely to these two factors.

The results presented here confirm that male and female **H. marmoratus** have body temperatures higher than air temperature. However, while Passmore & Malherbe (1985) found a mean difference between Tb and Ta of 3.1 C, I recorded a difference of 0.8 C. Thus, while the trend is supported, the magnitude of the difference is four-fold smaller in my study. A possible explanation for these results is that relative humidity differed between the two studies. During the study of Passmore & Malherbe (1985), rainfall was normal to heavy and relative humidity was high. During my study, extreme drought conditions occurred with almost no rainfall and relative humidity was very low. Water loss rates are inversely related to relative humidity in anurans (Campbell & Davis 1971). So a high rate of evaporative water loss by **H. marmoratus** during this study may explain the relatively low body temperatures I recorded when compared to those of Passmore & Malherbe (1985).

What is the proximate explanation for elevated body temperature in **H. marmoratus**? First, I can rule out the simple explanation that the air loses heat more rapidly than the frog in the hours of darkness. There is no correlation between (Tb - Ta) and time of night. Second, a major difference between the two species examined is that calling is sporadic in **C. xerampelina** whilst **H. marmoratus** males call continuously. Perhaps

metabolic heat is generated by calling? Passmore and Malherbe (1985) suggested that calling was not the cause of elevated body temperature. They noted that the temperature of females did not differ from that of nearby males (N = 6 females). We found the same relationship (N = 7 females). At present, a satisfactory explanation for elevated body temperature in **H. marmoratus** is unavailable.

Whatever the proximate explanation for elevated body temperatures in **H. marmoratus**, the effect on mating success is of biological significance. Passmore et al. (unpublished) have shown in a Model I regression that:

Call Rate (calls per min) = 3.84 (Ta in C) - 41.54 (R^2 = 70.7%)

As body temperature (which is positively correlated with air temperature) rises, call rate increases. Field work has shown that males with higher call rates are much more likely to mate. Two-choice phonotaxis experiments have also shown that females prefer the faster call rate when the difference between the two stimuli is 10 calls per min. There is also a non-significant preference for the faster call when the difference is 5 calls per min, but sample sizes are small and a Type II error is probable (Gerhardt 1991).

The regression shows that call rate increases by 3.84 calls per min for every 1 C change in air temperature. Thus males with body temperatures 2 C above air temperature have, on average, call rates 7.7 calls per minute faster than males whose body temperature equals air temperature. This difference is probably sufficient for females to discriminate between males. Nine of the 78 males examined (12%) had body temperatures more than 2 C above ambient. It is thus possible that sexual selection has driven the evolution of mechanisms for elevating body temperature in **H. marmoratus**.

Weygoda & Williams (1991) have shown that the mean nocturnal body temperature of **Hyla cinerea** is 0.8 C above that of a water-saturated plaster

model. They suggested that this temperature difference was of no biological significance. However, an examination of their data shows that 37.5% of the individuals examined had temperatures 1.0 - 2.9 C above that of the plaster model. Female **H. cinerea** show a preference for males with higher call rates (15% above mean, smaller differences were not tested) (Gerhardt 1987). If, as in other hylids (eg. Gayou 1984), there is a positive relationship between call rate and temperature then this temperature difference may affect patterns of female choice.

Acknowledgements - I thank S. Davies, L. Egan and M. Cooper for field assistance; and Craig Ferguson, the owners of Vienna Game Farm and Ian & Jean Garland for permission to work on their property.

CHAPTER SIX

CONCLUDING DISCUSSION

6.1 SPERM COMPETITION IN *CHIROMANTIS*

6.1.1 The evidence

Participation at nests by more than one male has been documented in all three species of **Chiromantis**. This phenomenon has been a source of controversy and two competing explanations have been advanced. First, male behaviour has been described in terms of co-operation (Coe 1967) which then led to an explanation invoking kin selection (Wilson 1975). Although kin recognition in adult frogs has not been investigated in detail, it does occur in tadpoles (but see Grafen 1990). It is possible that adult male **Chiromantis** recognize kin and co-operate in nest building if this raises their inclusive fitness. Second, male behaviour was viewed in a competitive light. In most animals, additional males associated with females during spawning or mating are there as competitors. It was thus suggested that additional males in **Chiromantis** are raising their reproductive success via sperm competition (Halliday & Verrell 1984).

I found, unsurprisingly I think, that the sperm competition hypothesis provides the best explanation for additional males. There were four main lines of evidence. First, **C. xerampelina** males have unusually large testes for frogs of their size (Chapter Three). This is consistent with a trend in birds, mammals and other frogs where testis size is related to the intensity of sperm competition. Second, additional males were shown to release sperm and possess the potential to fertilize eggs (Chapter Three) Third, there was no evidence that males co-operated in helping to build nests by churning the

foam (Chapter Two). Four, amplexus displacement attempts and jostling around the female indicated competitive interactions between males (Chapter Two).

6.1.2 Alternative mating tactics: who and why?

A continuous study throughout an entire breeding season showed that sperm competition is a dominant feature of the mating pattern in **Chiromantis xerampelina**. Over 80% of the males were seen to act as peripheral males and 57% both amplexed and acted as peripheral males (Chapter Two). There was no evidence that the tactic was size-based or age-based. I suspect that the reason for this is that female choice based on male size or morphology is very rare in prolonged breeding frogs. There is also scant evidence that large body size is advantageous in direct male-male competition in prolonged breeders. There seem to be few benefits associated with increased male body size in terms of mating success for prolonged breeders. In fish, large size is often associated with the ability to hold territories; and small size or female mimicry is associated with inconspicuousness and 'sneaking' ability. In **Chiromantis**, divergent selection pressures for two different body sizes do not exist, hence the lack of two morphs. In addition, in most frog species a large percentage of males fail to mate - even when they pursue the dominant tactic of calling to attract females. This low success rate makes it likely that an alternative mating tactic, even with moderate pay-offs, will benefit males if they can also pursue it 'opportunistically'. This trend towards a flexible response to changes in the social environment is a common feature of many frog species (Arak 1988b).

6.1.3 Consequences of sperm competition

The most obvious consequence of sperm competition in **C. xerampelina** is enlarged testis size. I suggest, however, that other more subtle effects can also be seen. Prolonged breeders are characterized by stationary males

calling loudly and persistently while females move through the chorus and choose males. Male mating success is usually associated with call rate or call intensity (Lopez & Narins 1991). The mating pattern in **C. xerampelina** does not fit this picture. Males are weak callers; they are mobile; and female choice appears to be completely absent. All of this can be explained by sperm competition. When there is a successful alternative mating tactic that is not genetically determined, it weakens selection on the dominant tactic (Halliday & Verrell 1986). For example, if there is female choice for large males, but small males sneak matings, selection for male body size will be reduced.

I suggest that weak calling is associated with reduced benefits to being in amplexus because of sneak fertilization by peripheral males. Indeed, when one considers that the average male spends more time being peripheral than in amplexus it is even possible that selection for traits associated with the peripheral male tactic is stronger. This is indicated by the fact that all males have large testis size. Given a mean of 5 males per nest and assuming equal chances of mating, on average, males will spend 80% of the time in a peripheral position and 20% in amplexus (Chapter Two).

The absence of female choice can be explained in one of two ways. First, one can view female mating preferences as mechanisms that ensure that they mate with fitter males (references in Bradbury & Andersson 1987). If peripheral males are successful, females can not choose a single male who will fertilize their eggs. There is thus little benefit associated with choosing because sperm competition circumvents female choice. If choice procedures carry costs they will actually be selected against (Pomiankowski 1988). Second, one may view female choice as a strategy to minimize the costs of locating a male which is thus directly selected for (ie. passive attraction to a male who, on average, is nearest: Arak 1988a). In **C. xerampelina**, however, the peripheral male tactic selects for male ability to locate females. A female only needs to enter the general area where males are present and males will then search for and amplex with her. The male advertisement call

is usually less intense than the ambient background noise which suggests that it plays a reduced role as a means of securing females.

Recent studies of sperm competition have focused on ways in which females influence the process (Birkhead & Hunter 1990). In **C. xerampelina** I could find little evidence for adaptive female responses to sperm competition. First, females did not appear to discriminate against peripheral males. Prior to amplexus, they did not move away from areas where unpaired males were present. On the other hand, they did not delay oviposition if peripheral males were absent. There is no indication that they prefer it if their eggs are fertilized by specific males. Second, females never tried to dislodge males from amplexus, regardless of whether the male was the first male to amplex or a former peripheral male. It might be argued, however, that this is a form of female choice where the female accepts the outcome of male-male competition. The only female response to sperm competition that I could see, the lack of female choice.

6.1.4 Fertilization rates of the two reproductive tactics

There are several lines of evidence that suggest that the fertilization rates of amplexing and peripheral males are similar. First, male behaviour when a female arrives among a group of males is unusual. The female may jump over a male or pass by him without him initiating amplexus. This can not be attributed to female choice. Females did not reject males who amplexed, even when the male did so by jumping onto the female from a distance. This suggests that there is not strong selection on males to amplex with females. Second, males often dismount when the female descends to the water despite the females being gravid. It is possible that the female gives the amplexing male a cue that causes him to dismount - although I can not see why this should occur. Even so, if amplexus is advantageous, males should be selected to remain in amplexus regardless of female cues for dismounting. Third, because of sexual size dimorphism and the position peripheral males assume

at nests, the amplexing male's cloaca is, in fact, farther from the extruded eggs than the cloaca of a peripheral male alongside the pair. Four, peripheral males have indirect access to the cue indicating that the female is ovipositing because oviposition occurs during bouts of female hind leg movements.

It would be extremely interesting to calculate fertilization rates for the two tactics. There is, I believe, the potential for a mixed Evolutionary Stable State (mESS). There do not seem to be differential costs associated with amplexus and being peripheral; males do not have to call more (expending energy) to be in amplexus; and predation risk is likely to be identical for all males at nests. Thus the fertilization rate will probably be directly related to the overall pay-off of the tactic. A possible mESS occurs in **Hyla cinerea** where satellites and callers have equal mating success (Perrill et al. 1978). However, satellites are smaller than callers and there are clearly energetic costs associated with calling (Prestwhich et al. 1989). Satellites may be pursuing a conditional tactic to reduce energetic costs (Gerhardt et al. 1987).

6.2 CONCLUSIONS

The mating behaviour of **Chiromantis xerampelina** strongly suggests that sperm competition is a regular feature of breeding. A comparative study of testis size supports this hypothesis. It is almost certain that all males are capable of pursuing the mating tactic of being a peripheral male, and engaging in sperm competition with the amplexing male. Male and female behaviour appear to have been affected by sperm competition. Male mating behaviour is characterized by reduced calling effort and increased attendance at the breeding site, while females do not discriminate between males.

The functional significance and evolution of several aspects of breeding remain obscure. Why does amplexus displacement occur if all males can fertilize eggs? If the answer is increased fertilization success by the amplexing male, then why was displacement absent in the Mkuzi population? Why do males dismount between nest sessions? Why does male behaviour prior to amplexus appear so inept - males being bypassed by females, males not amplexing? Why do males continue to call in amplexus? Why do males even bother to call? All these issues can be addressed empirically. Future studies of mating in this species will need to investigate: fertilization rates; sperm depletion; male/female localization mechanisms; and female preferences in phonotaxis. Together, these data may answer some of the questions posed above.

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APPENDIX ONE

CHORUS SIZE INFLUENCES THE ANTI-PREDATOR RESPONSE OF A NEOTROPICAL FROG

A. INTRODUCTION

Leks are the least well understood mating system because males defend neither females nor resources. Instead, males aggregate in a limited area and defend small territories (Davies 1991). Recent reviews of male behaviour on leks have focused primarily on the role of female choice in determining mating success (Balmford 1991). Males that display communally on leks, however, may also benefit in terms of reduced susceptibility to predators. When males group together there is a so-called 'dilution effect', whereby the individual risk of predation is inversely proportional to group size (Hamilton 1971). Individuals grouped for social or foraging reasons can respond adaptively to changes in predation risk that are associated with varying group size. In elk (**Cervus elaphus**), for example, individuals in larger groups are able to devote less time to vigilance behaviour and more time to other activities (Dehn 1990). To our knowledge, the effect of the number of males on a lek on anti-predator behaviour has not been directly investigated.

Male Tungara frogs (**Physalaemus pustulosus**) group together to form choruses and advertise vocally from stationary sites to attract females. There is no resource-defence and, as with many other frogs, the mating system can be considered a lek (Davies 1991). The main predator of **P. pustulosus** is the fringed-lipped bat (**Trachops cirrhosus**). These bats use the acoustic cues provide by the frog's advertisement calls to locate their prey. Male frogs take

evasive action in response to the presence of **T. cirrhosus**, or model replicas, by shutting down calling (Tuttle et al. 1982). This behaviour, however, carries a potential cost because females choose males on the basis of their calls. Males will thus be selected to resume calling as soon as possible. A male's anti-predator response should take into account the lower predation risk involved with calling in larger choruses (Ryan et al. 1981). We tested, therefore, whether the duration of non-calling evasive behaviour varies among different sized choruses because of the change in predation risk.

B. METHODS AND RESULTS

We conducted experiments in Gamboa, Panama from June to August, 1991. Male **P. pustulosus** were introduced into a plastic pool (2.2 m in diameter) containing patches of water (max depth of 3 cm) and dry areas, and natural choruses were established. Males were able to leave the pool and numbers were maintained by periodic addition of new individuals. A model of **T. cirrhosus** was made by tracing the wing outline of an individual onto plywood. The model was attached to a roller and run across the pool on a sloped, nylon, monofilament line attached to two wooden stakes (1.7 m and 0.4 m high) spaced 7.2 m apart. Ambient light levels were maintained above those of full-moon conditions by a nearby streetlight, which controlled for variation in responsiveness associated with light levels (Tuttle et al. 1982).

Trials were conducted between 2030 and 2200 hours and 74 trials were performed on 20 nights. There was at least a 10 minute interval between trials. Before each trial, we noted the number of males calling by listening to the chorus. This method limited us to choruses of five or fewer males, but had the advantage that calling was not disrupted. Shutdown Duration was defined as the time that elapsed between the termination of calling after the release of the model and its resumption with the first advertisement call.

Initial results revealed a significant difference in the shutdown durations of solitary males versus two-male choruses. We further investigated this phenomenon by measuring the shutdown duration of solitary males to the model when: a) the male was alone (SOLITARY); b) a loudspeaker placed in the pool broadcast a conspecific advertisement call every 2 s, mimicking the presence of a conspecific (BAT & CALLER); c) the loudspeaker broadcast stopped as the model passed overhead, mimicking a conspecific that had responded to a bat (BAT & NON-CALLER); and d) when the broadcast stopped without the model being released (NO BAT & NON-CALLER).

Chorus size had a significant effect on calling behaviour (Table A1.1). The shutdown duration of two-male choruses was significantly longer than that of solitary males, four-male choruses or five-male choruses (Scheffe's test, two-tailed, $P < 0.05$). The shutdown duration of three-male choruses was not significantly different from that of two-male choruses, nor from that of the other chorus combinations. In general, there was a decrease in shutdown duration as chorus size increased from two to five males. The shutdown duration of a solitary male, however, did not differ significantly from that of a five-male chorus.

In **P. pustulosus** the individual risk of predation decreases as chorus size increases (Ryan et al. 1981). Our study shows that the anti-predator response of males is influenced by chorus size over the size range examined. As chorus size increased the anti-predator behaviour of terminating calling weakened, and calling resumed sooner. Male frogs interact vocally and adjust calling behaviour in relation to the presence of conspecifics (Wells 1988). This may provide the proximate mechanism for males to assess chorus size, at least in small choruses. The loudspeaker experiments investigated potential factors causing solitary males to have a significantly shorter period of shutdown than two-male choruses. Our results showed that the treatments had a significant effect on shutdown duration (ANOVA, $F(3, 36) = 4.17$, $P = 0.012$) (Table A1.1). Furthermore, the shutdown duration for the treatment BAT &

TABLE A1.1

The effect of chorus size and experimental treatment on shutdown duration. For the chorus size experiment, ANOVA $F(4, 81) = 6.62$, $P < 0.0001$. For the loudspeaker experiment, ANOVA, $F(3, 36) = 4.17$, $P = 0.012$.

Number of males in chorus	Shutdown duration (x ± SE)
One	11.1 ± 2.8s (N = 16 choruses)
Two	83.3s ± 23.8s (N = 18 choruses)
Three	38.5s ± 8.6s (N = 15 choruses)
Four	16.6s ± 4.4s (N = 15 choruses)
Five	17.5s ± 6.2s (N = 12 choruses)

Loudspeaker Treatment	Shutdown duration (x ± SE)
SOLITARY	5.0 ± 2.2 (N = 10 males)
BAT & CALLER	10.9 ± 6.2 (N = 10 males)
NO BAT & NON-CALLER	15.4 ± 3.9 (N = 10 males)
BAT & NON-CALLER	39.2 ± 12.6 (N = 10 males)

NON-CALLER was significantly greater than that for the other three treatments (ANOVA, $F(1, 38) = 11.79$, $P < 0.001$). Shutdown duration for BAT & NON-CALLER was significantly longer than that for NO-BAT & NON-CALLER (Student's t-test, One-tailed, $P = 0.04$).

C. DISCUSSION

The shutdown duration of solitary males was surprisingly short, given that their risk of predation is greatest. The loudspeaker experiment suggests that the vocalizations of conspecific males provide additional information as to the likelihood that a predator is present. Shutdown duration was longest in the treatment which mimicked a second male alerted to a predator's presence (BAT & NON-CALLER = 39.2 s). When calls were continuously broadcast, or when the male was alone and the model was passed overhead, he ceased calling for a significantly shorter period (5.0 s and 10.9 s respectively). There appears to be a synergistic interaction between cues indicating a potential predator (eg. those derived from the model passing overhead), and the termination of calling by a conspecific (eg. switching the loudspeaker off).

Green (1990) noted that male **P. pustulosus** are attracted to conspecific calls. This behaviour leads to grouping of males and may be related both to benefits derived from the dilution effect and from the increased information provided by a conspecific's response to a potential predator. We suggest that future studies of species that lek, or display communally so that males are in vocal or visual contact, should investigate interactions between males not only in relation to mate attraction, but also in terms of vigilance and other anti-predator behaviours. In many lekking species males possess elaborate ornaments and engage in energetic displays. Interactions between males that reduce predation risks have the potential to partially offset these display costs.

This study was conducted in collaboration with Dr. P.R.Y. Backwell. We thank A.S. Rand, M.J. Ryan, the Smithsonian Tropical Research Institute, E. Stockwell, N.I. Passmore and D. Maitland for assistance.

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APPENDIX TWO

THE ADVERTISEMENT CALL: SUMMARY STATISTICS

A. INTRODUCTION

The advertisement calls of frogs provide a reliable method of distinguishing between species (Sullivan 1989). The mate recognition system of most frog species is based almost entirely on the advertisement call (Ryan 1990). Additional cues that ensure successful fertilization may occur latter in the chain of co-adapted responses between the sexes, but the initial assortment of individuals is based on the mating call. Females are preferentially attracted to the calls of conspecifics, leading to positive assortative mating and the occurrence of isolated gene pools that are the fundamental indicator of biological species (Mayr 1982). Species identity is coded in a variety of ways. In amplitude modulated calls, the rate of amplitude modulation (pulse rate) and the dominant frequency of the call are usually key elements (eg. Blair 1972). Finer scale details, such as pulse structure and the shape of the sound envelope are rarely important. With frequency modulated calls, the direction of the sweep, start and stop frequencies, and call duration may all be important (eg. Ryan 1983).

Although calls vary in a consistent manner between species, intra-specific variation also occurs. This is important, because it many allow for mate choice within a species, leading to evolution of calls through sexual selection by female choice (Ryan 1991). For selection through female choice to occur, there must be variation in the preferred trait (inter-male). In addition, if there is intra-individual consistency in call properties, these may allow females to reliably distinguish between males. Intra-individual consistency may also indicate a heritable basis to the call property, which creates the potential for evolutionary change.

Gerhardt (1991) has recently suggested that: directional selection due to female choice is stronger on variable call properties, which do not coded species identity (eg. call rate, call duration); variation at the population level may be maintained in spite of this directional selection; stabilizing selection tends to occur for relatively invariant call properties (eg. pulse rate).

B. METHODS AND RESULTS

Methods

Calls were recorded on a TCD-5M tape recorder using a Sony EMC-16T microphone and TDK-D90 audio tapes. Recordings were made at air temperatures between 22.5 and 24.0 C. Air temperature was measured using a Bat-12 Bailey Instruments digital thermometer. Two call types were recorded, 'fast' and 'slow' (see Chapter Two). Call parameters were analyzed on a Kay DSP Sona-graph Model 5500 (Kay Electronics Company, New Jersey) within the frequency range 0-8KHz. The upper and lower limits of the emphasized frequencies were measured, along with call duration and the number of pulses per call. Pulse repetition rate was also calculated. The first five 'fast' calls of 15 males were measured, and individual coefficients of variation (CV) were calculated for various call parameters for each male. Fewer 'slow' calls were available, and coefficients of variation were only calculated for 7 individuals for whom 5 calls each were analyzed. These coefficients of variation provide information on the intra-individual consistency of call parameters.

Population means, standard deviations and coefficients of variation were calculated using a single call of each type (the first measured) from each of 12 males. A Wilcoxon matched-pairs ranked-sign test was employed to test for differences between 'fast' and 'slow' calls. Coefficients of variation were also calculated for various call parameters indicating the inter-individual, or population level, variation in the trait.

Table A2.1. (A) Comparison between the two calls, based on 13 males (1 call of each type per male); (B) Within-male variability of acoustic properties of advertisement calls (based on 5 calls per male; N = 15 males for 'fast' call, 7 males for 'slow' call)

A. Inter-male

Call Property	FAST CALL	SLOW CALL	P-value *
	x ± SD (Coefficient of variation)		
Call duration (ms)	68.0 ± 13.2 (19.4)	298.2 ± 124.1 (41.6)	0.0016
Pulse Rate (pulses/s)	55.7 ± 5.4 (13.2)	20.5 ± 2.9 (28.7)	0.0016
Lower emphasized frequency (Hz)	718.5 ± 95.0 (19.0)	776.9 ± 223.3 (11.4)	0.78
Upper emphasized frequency (Hz)	2884.6 ± 548.0 (9.7)	2886.2 ± 328.1 (14.1)	0.53

* Wilcoxon matched-pairs ranked-sign test

B. Intra-male

Call Property	FAST CALL	SLOW CALL
	Mean Coefficient of variation (%) *	
Call duration (ms)	23.4 (12.5 - 45.1)	28.6 (18.5 - 40.1)
Pulse Rate (pulses/s)	6.2 (2.5 - 12.1)	10.5 (4.9 - 17.3)
Lower emphasized frequency (Hz)	6.3 (1.4 - 14.6)	10.2 (1.2 - 34.4)
Upper emphasized frequency (Hz)	11.0 (3.4 - 17.6)	7.9 (4.4 - 11.4)

* range in parenthesis

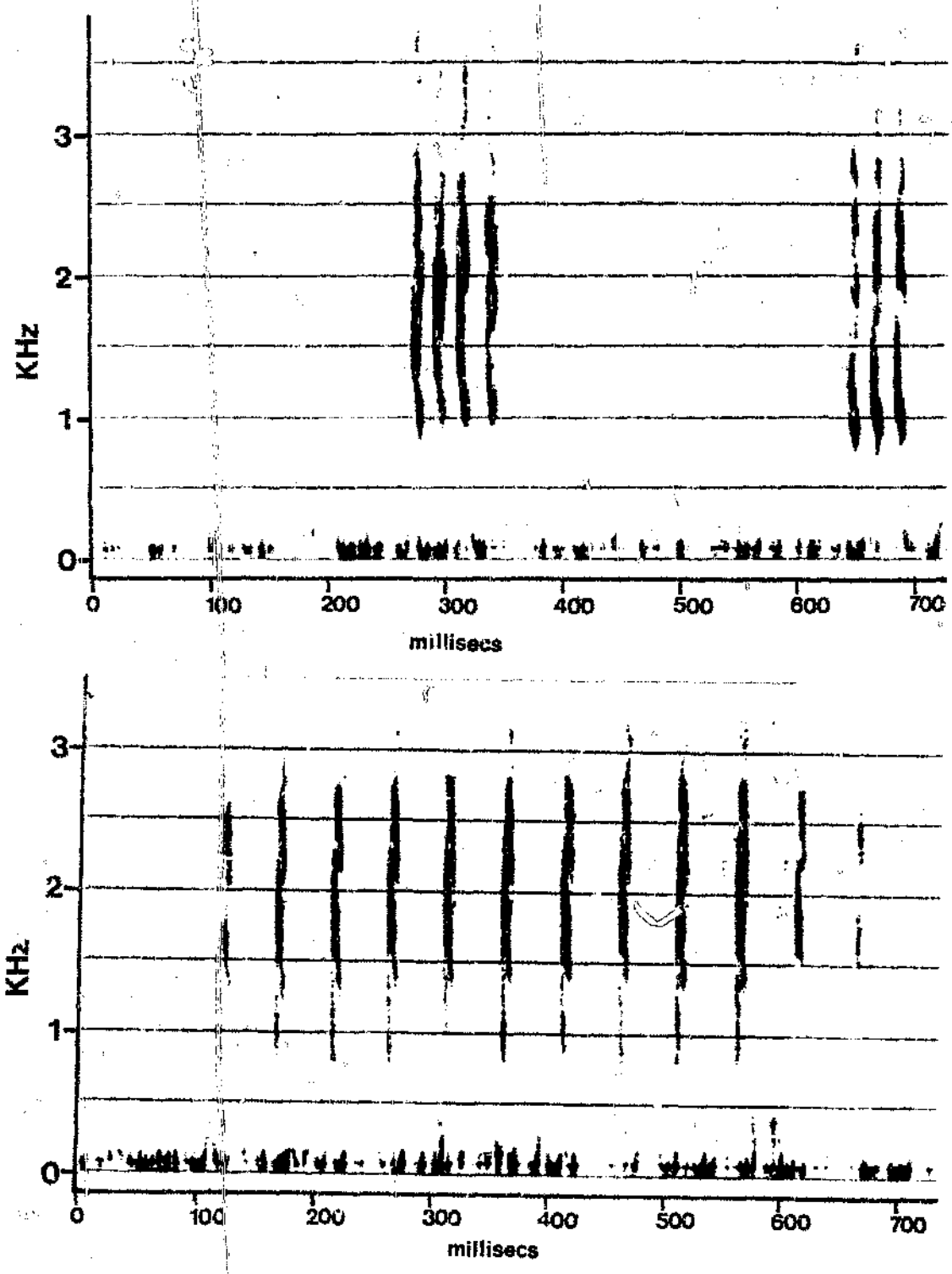

Figure A2.1 Sonagrams of male **Chiromantis xerampelina** advertisement calls.
A. Two 'Fast' calls. The first call has four pulses, and the second three pulses.
B. A 'Slow' call of 13 pulses, the first pulse is barely visible.
Both calls are from a single individual and were made at a temperature of 23.1 C. SonAgrams were made on a Kay DSP Sona-graph Model 5500 (Kay Electronics Company, New Jersey) in the frequency range of 0-4 KHz.

Results

The advertisement call of **Chiromantis xerampelina** is pulsatile, with the emphasized frequencies falling in the range 540-4420 Hz (N = 15 males). Initial analysis suggested that two distinct calls occur, 'fast' and 'slow'. Results of intra-male and inter-male variation in call parameters are presented in Table A2.1. 'Fast' and 'slow' calls differed significantly in terms of call duration (68 vs 298 milliseconds) and pulse rate (55.7 vs 20.5 pulses per second), but not in terms of emphasized frequency. The biological significance of these difference between the calls is unknown. There was significant variation between males for both call types, as indicated by the relatively high coefficients of variation (9.7 - 41.6%). However, variation in emphasized frequency should be viewed with caution. Because **C. xerampelina** has a very soft call, calls did not stand out clearly from background noise and it was thus difficult to obtain accurate measures of frequency parameters.

Pulse structure was not analyzed, it should, however, be noted that there was also variation in this parameter. Specifically, pulse duration varied, with some pulse at least twice as long as the average pulse. Inspection of the sonagrams (Figure A2.1) also indicates that, although the pulses were not divided into clear harmonics (frequency bands), there were two or more strongly emphasized frequencies within the total frequency range of the pulse (eg. in Figure A2.1 B, at 1.5KHz and 2.3KHz). These strongly emphasized frequencies also showed a degree of frequency modulation, but this was only clear when longer pulses were inspected (figure not presented).

A fairly large degree of intra-male variation was also found for the analyzed call parameters. Call duration was especially variable (23.4 and 28.6 for 'fast' and 'slow' calls respectively). Other call parameters showed less variability, but still more than expected for 'static' traits [sensu Gerhardt (1991), where 'static' was defined as less than 4%].

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C. DISCUSSION

The calls of **Chiromantis xerampelina** are unusual in that there is a relatively high degree of variability, both intra-male and inter-male, in call properties that are normally static, such as pulse rate and emphasized or dominant frequency. Gerhardt (1991) defined static traits for three species of hylids as those with coefficients of variation (CV) less than 4%. Here, I found that the smallest inter-male CV was 9.7% (upper emphasized frequency), and the smallest intra-male CV was 6.2% (pulse rate). The high degree of inter-male variability suggests that there is the potential for female choice based on call properties. However, the high degree of intra-male variability suggests that differences between males are unlikely to be reliable indicators of male phenotypic or genotypic characteristics.

The functional significance of the two call types is unclear. There usage does not appear to be related to the social situation, and it is unlikely that they are related to functional partitioning into 'female attraction' and 'male-male communication' as found in several other species (eg. Backwell 1988, Littlejohn & Harrison 1985).

The high variability in the calls of **C. xerampelina** may reflect the reduced role of calling in mate attraction. Females do not appear to select males intra-specifically on the basis of call parameters, hence there is unlikely to be directional selection which tends to erode variability. The variability in call duration is also consistent with a general trend in frogs that 'call duration is... less variable in species with unpulsed or very rapidly pulsed calls than in species with distinctly pulsatile calls' (Gerhardt 1991, p626).