# THE EVOLUTIONARY ECOLOGY AND SEXUAL SELECTION OF A MADAGASCAN POISON FROG (*MANTELLA LAEVIGATA*)

BY

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This dissertation is dedicated to the people of Madagascar,
for allowing me to live among them and observe their frogs.

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# Introduction

Natural selection is extremely powerful, and arguably the most interesting force in the Universe. It is also one of the most difficult to investigate, because we can view only a snapshot, and therefore must work backwards to understand how it functions, and by what rules. We could not do that but for one saving grace: in a general sense, natural selection repeats itself with some frequency. The resulting analogous patterns in nature allow us to evaluate which components of a system are integral to its evolution, and which are incidental. Thus, the investigation of components of reproductive success, or ecological limitation, only make sense in light of those parameters that are common between the current study and others like it.

Such scientific investigations carry a special burden, however. Historical events are necessarily individual in nature, and thus do not meet the criteria necessary for rigorous statistical interpretation. As such, investigators of evolutionary patterns of convergence need to be especially careful not to over-interpret their conclusions, as no degree of precision can be placed on their level of certainty. This dissertation focuses on the previously unstudied side of an apparently extraordinary convergence.

Species in the family of neotropical poison frogs, Dendrobatidae, are known for the lipophilic alkaloids in their skin, attendant aposematic coloration, and a diverse array of complex behavior. They are small, diurnal, myrmecophagous, and spatially clumped, with terrestrial egg deposition and the use of phytotelmata for tadpole development. Many species have elaborate, stereotyped courtship, territoriality, and parental care (e.g., see Wells 1980, Weygoldt 1987, Summers 1990). In Madagascar, members of the endemic ranoid genus *Mantella* are known to have cutaneous lipophilic alkaloids identical to those found in the dendrobatids (Daly et al. 1984, Garraffo et al. 1993, Daly et al. 1996). Furthermore, members of *Mantella* species are also small, diurnal, myrmecophagous, and spatially clumped, and oviposit in phytotelmata (Blommers-Schlösser 1979, Glaw and Vences 1994). Little else was known of their social system or behavior until now.

When Boulenger first described *Mantella* in 1882, he placed the genus in Dendrobatidae, based largely on a shared lack of teeth (Boulenger 1882). Several other taxa are known only from South America and Madagascar (e.g. iguanians, Frost and Etheridge 1989; boas, Kluge 1991), which is biogeographically consistent with current reconstructions of Gondwana (Scotese and Golonka 1992). Since Boulenger, the hypothesis of relationship between Dendrobatidae and *Mantella* has occasionally recurred (e.g. Zimmermann 1995), but there is little phylogenetic evidence to support it (e.g. Hay et al. 1995).

Given that Dendrobatidae and *Mantella* appear to be historically distinct, but convergent in many ways, and that the dendrobatids reflect social complexity not often observed in anurans, I proceeded with an investigation of the social system, ecology, and sexual selection of *M. laevigata*. Darwin (1871) first recognized the phenomenon of sexual selection (which increases reproductive success, rather than survivorship), and the difficulties inherent in trying to assess its effects. Bateman (1948) found that, in *Drosophila*, there is higher variance in the reproductive success of males than of females. Trivers (1972) later suggested that sexual selection is delimited by the relative parental investment of each sex in offspring. In species with parental investment, the sex of the parent who invests more (usually females) is limiting for the other sex. Emlen & Oring (1977) hypothesized that the ability of a portion of a population to control access of others to potential mates determines the degree of variance in mating success. Alexander & Borgia (1979) further defined the difference in reproductive effort between the sexes: males spend more time and energy in mating effort, whereas females expend more energy in parental effort.

In light of this background theory, and bearing in mind what was known from the dendrobatids, my research revolved around questions of how much, if any, post-mating parental investment was occurring in *M. laevigata*; how both males and females assessed potential mates; and how, if at all, resources were controlled (chapters two, five and six). To investigate these questions in a species whose social system and behavior were unknown, it was imperative to broaden the scope of my research to include the population ecology of not only *M. laevigata*, but of any members of its community with which it competed or had shared interests (chapter four). Furthermore, the issue of resource control, first promoted by Emlen and Oring (1977), required an understanding of what resource, or resources, were limiting for the populations I was studying (chapter three).

## Relationships Among Chapters

I wrote chapters 2-6 as independent papers to be published in journals, and as such, there is some redundancy between these chapters, such as in the description of the study site. All of the research presented was a coherent and internally coordinated enterprise. The questions I asked grew from observations in the field, from theory, and from one another, such that there is necessarily reliance on results from earlier chapters in later ones (and, to a lesser degree, vice versa).

Chapter 1 (Anuran Diversity: Reproductive Modes and Parental Care) is a review of the literature on anuran reproductive modes and parental care. This is the only chapter that does not include original research. It is included to orient the reader to the diversity of life history strategies in anurans. I focused on strategies employed by anurans, such as *Mantella laevigata*, that breed in phytotelmata (the small wells of water found within or upon plants). I proposed a new system for classifying types of anuran parental care, and reviewed fully the evidence for type 6: maternal feeding of tadpoles. At this time, several species of dendrobatids exhibit this type of parental care, as do two species of hylids, one rhacophorid, and, with this dissertation, one ranid, *M. laevigata*.

Chapter 2 (Social and Reproductive Behavior in *Mantella laevigata*, with Comparisons to the Dendrobatids) is in press in *Animal Behaviour* (manuscript #A8794). It represents the observational (non-experimental) results from this research, those 925 hours of focal observation that allowed me to put together a coherent picture of the social system and reproductive behavior of *M. laevigata*.

The questions asked and experiments undertaken in the ensuing chapters were often a result of observations reported in chapter 2.

Chapter 3 (Reproductive Limitation by Oviposition Site in a Treehole Breeding Madagascan Poison Frog (*Mantella laevigata*)) is in submission at *Evolutionary Ecology*. Based on observations during focal watches, I hypothesized that oviposition sites were limiting for this species. In this chapter I present the experiment that I conducted to test this hypothesis, and review the risks associated with breeding in phytotelmata.

Chapter 4 (*Not in My Phytotelm*: Of Neighbors, Architecture, and Water Quality: Correlates of Well Use by *Mantella laevigata*) presents a more inclusive picture of the phytotelm-dwelling community. Based on long-term monitoring of 53 individual phytotelmata, I investigated possible correlates of *M. laevigata* reproductive success such as water chemistry, water holding potential, and other inhabitants (including several other species of anurans, and insect larvae). The role of particular predators and competitors, reviewed in general terms in chapter 3, is quantified and discussed.

Chapter 5 (Female Mate Choice for Oviposition Site Quality in *Mantella laevigata*) is in submission at *Oecologia*. Based on observations during focal watches (chapter 2), and the results of limitation experiments (chapter 3), I hypothesized that females are choosing their mates based solely on their territories (“good resources”), rather than on their individual quality (“good genes”), or ability to call for long periods of time (“good current condition”). In this chapter I present the experiment I conducted to test this hypothesis, and discuss the significance of resource-based mate choice, which is common in birds but little known in anurans.

Chapter 6 (Mechanisms of Male Reproductive Success in *Mantella laevigata*) represents an investigation of those parameters that affect male reproductive success in this system with strong female choice. Using focal observations and male choice experiments to evaluate these parameters, I assessed the occurrence and relative importance of multiple male strategies, male-male competition (including prior resident advantage), male parental care, male mate choice, and male-female conflict in this species.

## Ode to Amphibians

In 1758, Linneaus had this to say about amphibians:

> "These foul and loathsome animals are abhorrent because of their cold body, pale color, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom; and so their Creator has not exerted his powers to make many of them."

After several years conducting research on said beasts, during which time I have grown quite fond of them, I would like to take this opportunity to disabuse the reader of any lingering doubts he or she may have as to the true nature of amphibians. With regard to the subject of the present volume, however, I must agree with Linneaus on two counts. *Mantella laevigata*, one of eleven currently recognized species of *Mantella*, the Madagascan poison frogs, does indeed carry with it a terrible venom, though not in such abundance as to worry the human observer, unless he intends the little frogs as a meal. And, as I hope will become evident over the course of these six chapters, *M. laevigata* must indeed have a calculating eye—and ear, and brain, for that matter—to undertake the constant assessments and reassessments necessary to exist and thrive in the complex social world it has created for itself. For that, we should not disdain the creatures, but rather, find occasion to study them further, for they are indeed captivating.

## Literature Cited

Alexander, R. D., and G. Borgia. 1979. On the origin and basis of the male-female phenomenon. Pages 417-440 *in* M. S. Blum and N. A. Blum, editors. Sexual Selection and Reproductive Competition in Insects. Academic Press, New York.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity **2**:349-368.

Blommers-Schlösser, R. M. A. 1979. Biosystematics of the Malagasy frogs. I. Mantellinae (Ranidae). Beaufortia **352**:1-77.

Boulenger, G. A. 1882. Catalogue of the Batrachia Salientia s. Ecaudata in the collection of the British Museum, 2nd edition. British Museum, London.

Daly, J. W., N. R. Andriamaharavo, M. Andriantsiferana, and C. W. Myers. 1996. Madagascan poison frogs (*Mantella*) and their skin alkaloids. American Museum Novitates **3177**:1-34.

Daly, J. W., R. J. Highet, and C. W. Myers. 1984. Occurrence of skin alkaloids in non-Dendrobatid frogs From Brazil (Bufonidae), Australia (Myobatrachidae) and Madagascar (Mantellinae). Toxicon **22**:905-919.

Darwin, C. 1871. The descent of man, and selection in relation to sex. Princeton University Press, Princeton.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science **197**:215-223.

Frost, D. R., and R. Etheridge. 1989. A phylogenetic analysis and taxonomy of Iguanian lizards (Reptilia: Squamata). University of Kansas Museum of Natural History, Miscellaneous publications **81**:1-65.

Garraffo, H. M., J. Caceres, J. W. Daly, T. F. Spande, N. R. Andriamaharavo, and M. Andriantsiferana. 1993. Alkaloids in Madagascan frogs (*Mantella*) - pumiliotoxins, indolizidines, quinolizidines, and pyrrolizidines. Journal of Natural Products **56**:1016-1038.

Glaw, F., and M. Vences. 1994. A Fieldguide to the Amphibians and Reptiles of Madagascar, 2 edition. Zoologisches Forschungsinstitut und Museum Alexander Koenig. Moos Druck, Leverkusen and FARBO, Köln, Bonn.

Hay, J. M., I. Ruvinsky, S. B. Hedges, and L. R. Maxson. 1995. Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12s and 16s ribosomal RNA genes. Molecular Biology and Evolution **12**:928-937.

Kluge, A. G. 1991. Boine snake phylogeny and research cycles. Miscellaneous publications of the Museum of Zoology, University of Michigan **178**:1-58.

Scotese, C. R., and J. Golonka. 1992. Paleomap Paleogeographic Atlas. Paleomap Project Department of Geology, University of Texas at Arlington.

Summers, K. 1990. Parental investment and sexual selection in dart-poison frogs (genus *Dendrobates*). Ph.D. University of Michigan, Ann Arbor, MI.

Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136-179 *in* B. Campbell, editor. Sexual Selection and the descent of man, 1871-1971. Aldine, Chicago.

Wells, K. D. 1980. Behavioral ecology and social organization of a dendrobatid frog (*Colostethus inguinalis*). Behavioral Ecology and Sociobiology **6**:199-209.

Weygoldt, P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). Zeitschrift für Zoölogische Systematik und Evolutions forschung **25**:51-67.

Zimmermann, H. 1995. Les Dendrobatidae et *Mantella* ont ils des ancetres communs? Pages 30 *in* International Symposium Biogeography of Madagascar, Paris, France.

# Chapter 1

## Anuran Diversity: Reproductive Modes and Parental Care

Explanations for the diversity of anuran behavior can be found in their history, their current anatomical and physiological characters, and the opportunities afforded by outmaneuvering those constraints. In this chapter, I review what makes amphibians, and anurans in particular, distinct from other vertebrates. I then survey reproductive modes in anurans, giving some examples to demonstrate the diversity of their life histories, without reviewing all that is known. I make particular note of reproductive modes of phytotelm-breeding anurans. This is in order to place my study organism, the phytotelm-breeding *Mantella laevigata*, in a broader context of anuran reproduction. I briefly review parental care in anurans, and propose a new functional organization of the types of anuran parental care. I conduct a complete review of the type exhibited by *M. laevigata* (see chapter 2), maternal feeding of tadpoles. Again, this serves to give the reader a sense of the diversity of behavior in anurans, and to place *M. laevigata* in its broader evolutionary and ecological context.

## Amphibians: an ecological summary

Amphibians are so named for the two distinct periods of their lives: the aquatic larval stage, and the terrestrial adult stage. Not all amphibians have these two stages, however, some being fully aquatic, others entirely lacking aquatic stages in their life histories. More precisely, amphibians can be (incompletely) diagnosed as quadrupedal vertebrates with no more than one sacral vertebra (Duellman and Trueb 1986).

Lissamphibia, the taxon that includes extant amphibians, have glandular skin that lacks the epidermal structures that characterize other groups of tetrapods, such as scales, feathers, or hair. Lissamphibia are diagnosed by several morphological characters, two of which are the presence of a *papilla amphibiorum* in the inner ear, which allows them to sense acoustic signals of less than 1,000 Hz; and a reliance on cutaneous respiration through their moist skin. Furthermore, lissamphibians can be distinguished from other classes of vertebrates by karyological characteristics, including a tendency toward genome hypertrophy, and large interspecific variability in genome size, even among closely related species (Duellman and Trueb 1986).

Lissamphibia is comprised of three major groups of extant species: Gymnophiona (caecilians), Caudata (salamanders and newts), and Anura (frogs and toads). The number of recognized lissamphibian species is constantly growing, but the internal make-up of the group remains largely the same: approximately 87% of extant lissamphibian species are anurans, and anuran species currently number more than 4,000 (Duellman and Trueb 1986, Stebbins and Cohen 1995).

Anurans are the tailless amphibians. They are also diagnosed by having elongated hind limbs that are modified for jumping, as well as postsacral vertebrae that are fused into a single, rodlike element, the coccyx (Duellman and Trueb 1986). Not geographically restricted to the extent that the Gymnophiona and Caudata are, anurans are, nevertheless, more prevalent in the tropics than elsewhere. Most are also nocturnal.

All amphibian eggs lack both shells and the protective membranes of amniote eggs, and so require moist microhabitats in which to develop. Furthermore, almost all anurans have external fertilization, and are oviparous. External fertilization offers individuals a high certainty of paternity, opening the door for the evolution of paternal care. Exceptions to the rule of external fertilization include the phylogenetically basal tailed-frog, *Ascaphus truei*, whose "tail," actually an extension of the cloaca, acts as an intromittent organ during copulation (Metter 1964). All of the members of the toad genus *Nectophrynoides* have internal fertilization (Noble 1926), two species being viviparous (Xavier 1977, Wake 1980), several ovoviviparous (Grandison 1978), and one oviparous (Wake 1980). *Eleutherodactylus jasperi* is also ovoviviparous (Drewry and Jones 1976), the young emerging as froglets from the cloaca. *Mertensophryne micranotis* (Grandison 1980) and *Eleutherodactylus coquí* (Townsend et al. 1981) both display the unlikely combination of internal fertilization and oviparity, and there is some evidence for the same in *Eleutherodactylus angelicus* (Hayes 1985).

During, and in many cases before, oviposition, the male anuran grasps the female in an embrace known as amplexus. In cases of extended amplexus, which can last months in some species, this may serve to restrict the female from mating with other males. In all cases, amplexus is understood to insure that the sperm and eggs will actually contact one another.

Temporal patterns of anuran reproduction vary widely, but can be roughly divided into two broad types: explosive breeding and prolonged breeding (Wells 1977). In many species, the length of the breeding period is limited by climate, or the seasonal availability of breeding sites, such as ephemeral pools. Temperate species are more likely to breed explosively—an entire population breeding over a span of a few days or weeks. By contrast, many tropical anurans breed in every month of the year, although most demonstrate some seasonality as well. Some tropical species restrict their breeding activity to either the wet or the dry season, but this does not tend to limit their breeding season to the same degree that cold limits breeding in the temperate zone (Wells 1977).

Male anurans court females, almost universally, with an advertisement call. Vocalizations are the initial attractor to potential mates in most anuran species, as they can serve to attract females from a broad area. Because of the presence of the *papilla amphibiorum*, anurans can hear and respond to a wider range of vocalizations than humans can detect. Females of most anuran species are mute, or have very weak calls.

The spatial and temporal distribution of females, as determined in part by breeding pattern, determines how males interact with each other. In species with explosive breeding, males tend to form dense aggregations and engage in scramble competition, attempting amplexus with any nearby individual, male or female. In species with prolonged breeding, males often call from stationary positions, attracting females to them, and maintaining intermale spacing (Wells 1977). These male calling sites often represent territories that contain oviposition sites or courtship areas. It is in such prolonged breeders that long-term male territoriality often evolves, and with it, the likelihood that females will choose mates not solely based on male calls, or genetic quality, but also on the resources the male defends.

## Reproductive Modes in Anurans

The diversity of life history strategies found in anurans is remarkable. Restricted, in most cases, by external fertilization, and by the need to find moist habitats for their eggs, anurans have nevertheless evolved myriad ways to thrive. Indeed, anuran reproductive modes are so diverse that there is still no single, universally cited source that encompasses all recognized modes. Furthermore, it appears that trends away from the primitive mode of eggs and tadpoles in ponds do not reflect one or two evolutions of increasing specialization, but many independently derived reproductive modes in distinct phylogenetic lineages (Duellman and Trueb 1986:27).

Part of the problem is definitional. Crump (1974) defined reproductive mode simply as the combination of deposition site and type of development. Reproductive mode is defined by Salthe and Duellman (1973), and used by Duellman and Trueb (1986:21) as a "combination of ovipositional and developmental factors, including oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatching, and type of parental care, if any." Brown and Alcala (1983) explicitly add larval nourishment to this list. There is room for interpretation in all of these definitions.

The layperson who imagines a frog during its mating season most likely conceives of an explosively breeding species. An entire population convenes about a pond, or perhaps a slowly moving stream or river. During amplexus, large masses or strings of eggs are laid in the water, and afterwards both male and female disperse, never to encounter their offspring again. Those eggs that do not get eaten develop and hatch in the water. Tadpoles survive by eating muck or small aquatic insects, until they finally metamorphose, and only then come on land. This reproductive mode—aquatic eggs deposited in relatively large, permanent bodies of water, with feeding tadpoles developing in the same water—is in fact only one of many reproductive modes now recognized in anurans. It is, however, the most common and phylogenetically widespread (Duellman and Trueb 1986:23).

Jameson (1957) was the first to systematically describe the diversity of anuran reproductive modes, and Salthe and Mecham (1974) formalized his classifications. Jameson's system includes two broad categories: aquatic and terrestrial development. He assigned aquatic development four subcategories, including 1) aquatic eggs without nests, 2) aquatic eggs in constructed aquatic nests, 3) aquatic eggs in terrestrial nests, from which tadpoles hatch and find their way to water, and 4) eggs or young tadpoles carried by a parent, before being deposited in water to complete development. The layperson's Platonic ideal of frog reproduction most closely matches the first subcategory, aquatic eggs without nests. But even in the 1950's, several examples of the more complex reproductive modes were known. The embryos of some species of *Leptodactylus* were known to develop entirely in aquatic nests (Mulaik 1937). *Phyllomedusa* had been observed ovipositing in nests made of leaves folded by the mating pair; when the eggs hatch, rain is required to move the tadpoles to water (Lutz 1947). And some species of *Alytes* and *Dendrobates* had also been observed mating and ovipositing on land, the males returning after the eggs hatched to carry their tadpoles to water (Jameson 1957).

In Jameson's classification, terrestrial development is assigned two subcategories, both of which often involve direct development, wherein eggs hatch directly into froglets, bypassing the larval stage. These two categories are 1) direct development of very large ova deposited in nests on moss or in burrows and 2) eggs or tadpoles carried by a parent before hatching into tadpoles or froglets (Jameson 1957, Salthe and Mecham 1974). Terrestrial oviposition may have evolved in response to pond-drying, competition, or predation in large bodies of water (see Magnusson and Hero 1991 for review).

Direct development in anurans can occur in the oviducts, in the case of some internally fertilized species (e.g. *Eleutherodactylus jasperi*, Townsend et al. 1981), or within eggs laid on land, as in many leptodactylids, including the majority of (neotropical) *Eleutherodactylus* species (Townsend and Stewart 1985) and several Australian species (e.g. *Myobatrachus gouldii,* Roberts 1981). Examples of Jameson's second subcategory of terrestrial development, in which eggs or tadpoles are carried by a parent, are numerous, and I list only a few here. The *Alytes obstetricans* male carries fertilized eggs entwined in his hindlimbs, releasing them into water when they are ready to hatch (Boulenger 1897). *Rhinoderma darwinii* males carry non-feeding tadpoles through metamorphosis in their vocal sacs, but *Rhinoderma rufum* males only transport tadpoles this way from the nest to water (Salthe and Duellman 1973, Formas et al. 1975). Female marsupial frogs, including those in the hylid genera *Gastrotheca* and *Flectonotus*, have special pouches on their backs in which they brood their young, which emerge either as tadpoles, or as fully formed froglets (Duellman and Maness 1980). And in gastric brooding frogs, *Rheobatrachus silus*, the female swallows fertilized eggs and broods them in her stomach for the duration of their development (Corben et al. 1974). Finally, the young froglets emerge from the passive mother's mouth (Tyler and Carter 1981). Clearly, given the diversity of strategies represented within this single mode, such a broad classification of modes is not particularly informative.

In a review of amphibian reproduction that focused on "live-bearing modes," Wake (1982) proposed a broad definition for ovoviviparity that subsumed many of the species described in the previous paragraph. She defines ovoviviparity as the retention of developing embryos without additional nutrition beyond the yolk supply, and does not specify that embryonic retention need be either maternal or original. With this definition, ovoviviparous species include those in which females retain embryos in their oviducts, stomachs or dorsal skins, or in which males retain embryos on their backs, legs, or vocal sacs (Wake 1982). Carrying of the young is thus interpreted as altering the stage at which offspring are laid or born, and reproductive mode and parental care are more tangled than before.

Most who have reviewed anuran reproductive modes have not been so creative, but this has still not led to consensus. Crump (1974) proposed 10 reproductive modes to describe the anurans of the upper Amazon basin in eastern Ecuador. Salthe and Duellman (1973) listed nine to represent all of Anura, but they excluded viviparity from consideration. Lamotte and Lescure (1977) broke anuran reproduction into six primary categories, and 18 specific modes. Brown and Alcala (1983), in their review of reproductive modes in Philippine anurans, identified the same two general modes used by Jameson (1957) and Salthe and Mecham (1974), but they recognized 18 specific modes. Duellman and Trueb (1986:26) proposed three general modes (eggs aquatic, eggs terrestrial or arboreal, and eggs retained in oviducts), and divided these into 29 specific modes. Jørgensen (1992) approached the problem qualitatively, and declined to fully separate reproductive mode from reproductive pattern, finding that climatic conditions, latitude, altitude, seasonality, the length of the breeding season and courtship are all inextricably tied to reproductive mode.

There is incomplete overlap between all of these systems, and none is considered the authority on the subject. What should be clear is that anuran reproductive modes are extremely diverse and numerous, and because they appear to have evolved independently several times, attempts to confine them neatly into a relatively small number of definitional boxes will always fall short.

## Reproductive Modes in Phytotelm-Breeding Anurans

For the present study, I am specifically interested in anurans that breed in phytotelmata, the small pools of water found within or upon plants. More than 60 species of anurans, representing at least seven families, breed in phytotelmata (see chapter 4), and the particular ways in which they accomplish this task are, not surprisingly, quite diverse. The Madagascan ranoid *Mantidactylus pulcher* appears to be an obligate pandanus-breeder, but little else is known of its reproduction (Blommers-Schlösser 1979). The Peruvian microhylid *Syncope antenori* lays its eggs directly in the water of bromeliad axils, and the non-feeding tadpoles develop in the same water (Krügel and Richter 1995). The Philippine bufonid *Pelophryne brevipes* also lays its eggs directly in the water of leaf axils, and has non-feeding tadpoles (Brown and Alcala 1983). The Thai rhacophorid *Theloderma stellatum* breeds in treeholes, laying its eggs above the water line, and the tadpoles are able to eat a wide array of food items (Wassersug et al. 1981). The Costa Rican hylid *Anotheca spinosa* breeds in bromeliads, depositing its eggs above the water line, and the tadpoles primarily eat frog eggs, but also ingest insects (Taylor 1954, Jungfer 1996).

Lamotte and Lescure (1977) first distinguished between phytotelm-breeding anurans that deposit their eggs in, as opposed to above, the water. Brown and Alcala (1983) identified three modes encompassing phytotelm-breeding, defining the first (I(6)) as a "small number of relatively large, nonpigmented eggs attached to wood above water in tree holes. Larvae fall into water where they develop and feed." The remaining two modes both have small to moderate numbers of eggs deposited in water-filled leaf axil or tree holes (one mode has feeding larvae; in the other, the larvae do not feed). Duellman and Trueb (1986) also distinguished between phytotelm-breeding frogs that deposit their eggs in the water (aquatic), versus above phytotelmata (arboreal). In some cases, these distinctions may be critical, as "arboreal" eggs may drown if deposited in water, "aquatic" ones dry out. In reality, of course, these lines are rarely so clear. In *Mantella laevigata*, for instance, a single egg is usually laid above the water line (see chapter 2). But eggs that are covered in water after heavy rains can develop and hatch, and mating pairs occasionally oviposit at or below the water line (*pers. obs.*).

It is commonly presumed that the primitive reproductive mode for anurans is by means of large numbers of relatively small aquatic eggs deposited into lentic water, in which the tadpoles also develop (Duellman and Trueb 1986:27, Jørgensen 1992). The move away from this mode to any of those associated with phytotelm-breeding enhances the possibility that one or both parents will exhibit parental care. The first requirement of parental care is met in phytotelm-breeders: the offspring can be found, as they exist in a confined space that does not move over time. Furthermore, the relatively confined space of phytotelmata limits the mass of reproductive investment at any moment in time: either the number of eggs, the size of individual eggs, or both, is restricted by physical limitations. Reduced numbers of offspring are expected to be correlated with an increase in parental investment, as parents have a given amount of energy they can apply to reproductive effort. If less energy is being shunted to the production of gametes, more can be directed to post-zygotic care (Trivers 1972). Finally, in the confined space of the phytotelm, tadpoles are less likely to encounter predators, but they are also less likely to encounter food. Tadpole species dwelling in phytotelmata are therefore predicted to disproportionately display one of two patterns among the many possible: 1) taking in no food until after metamorphosis, or 2) receiving nourishment from a parent, one of the most complex forms of parental care known in anurans.

## Evolution of Parental Care in Anurans

The origin and evolution of parental care has received much attention in the last 30 years. Trivers (1972) provided the framework for current thinking, and introduced the term "parental investment," which he defined as "any investment by the parent in an individual offspring that increases the offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring." Parental care is usually considered a subset of parental investment, and as such, assumes a cost to the parent as well. Parental care includes all nongametic investments in offspring, and is usually identified as such only after fertilization—that is, only after the care-giver is actually a parent (e.g. Wittenberger 1981).

In anurans, parental care is phylogenetically widespread, but relatively rare. Parental care is known in the great majority of currently recognized families, and those few that have no parental care tend to have very few species (Crump 1995). In 1995, approximately 6% (206/3,348) of anuran species were known to exhibit some form of parental care (Crump 1995). Since then, the number of both recognized species and those with parental care has grown, the latter faster than the former, and it is probable that McDiarmid's (1978) estimate that 10% of anuran species have parental care will ultimately turn out to be accurate.

Anuran parental care is most common in species living in the wet tropics, with extended breeding seasons, and terrestrial modes of reproduction. More than 90% of anuran species that provide parental care oviposit out of water

(Crump 1995). Terrestrial reproduction may reduce the risk of predation, but increases risks associated with desiccation and starvation, perhaps increasing the need for parental care. It certainly increases the feasibility, as young that are immobile out of water are less likely to get lost on land. Additional correlates of parental care in amphibians are smaller body size, increased egg size and decreased clutch size, relative to closely related species that do not give care (Salthe and Mecham 1974, Nussbaum and Schultz 1989, Crump 1995).

What advantages does the evolution of parental care offer anurans? Salthe and Mecham (1974:399) proposed three general explanations, which include 1) reducing predation on early life stages by guarding or active defense, 2) decreasing the developmental abnormalities that are more likely in large eggs by frequent manipulation, and 3) providing a more suitable microhabitat for terrestrial eggs, either by protecting them from desiccation or by transporting them to water at a later stage of development. In the most comprehensive and detailed review of amphibian parental care to date, Crump (1995) added to this list particular advantages of egg attendance, which include protection from pathogens (such as fungi), aeration of aquatic eggs, and assistance to hatchlings in escaping from the nest.

The evolution of parental care in anurans is far more complex than this brief review can encompass, but two more considerations are worth noting. First, the sex of the care-giving parent is highly variable across anurans, but rarely varies within a species, and even those few exceptions may not be viable. In *Dendrobates pumilio*, both females and males have been observed carrying newly hatched tadpoles to bromeliads on their backs, but tadpoles carried by males do not get fed by their mothers (Weygoldt 1987). Hypotheses explaining the perceived relationship between male parental care and external fertilization include “certainty of paternity,” which suggests that males are more likely to care for offspring they are “sure” are theirs (Alexander 1974), which is more likely when fertilization is immediate and external (Trivers 1972, Ridley 1978). The relationship has been tested in amphibians, however, and has ambiguous support at best (Gross and Shine 1981, Beck 1998).

Second, parental care exists on a continuum of necessity for the offspring, from obligate to facultative. In one of the first long-term investigations of parental care in any anuran, Kluge (1981) found that male egg attendance in the gladiator frogs *Hyla rosenbergi*, which serves to protect against other intruding males, is facultative, and correlated with population density. In other species, egg attendance may not be parental care at all, but incidental, given where the eggs are laid relative to defended territories (e.g. *Pseudophryne*, Woodruff 1977). In several species of dendrobatids, however, egg attendance is obligate, as it precedes transport of tadpoles to water, without which the offspring die.

## Types of Anuran Parental Care

Parental care, like reproductive mode, is not easily classified, largely because each “type” is in fact a conglomeration of multiple instances of independently derived behaviors. Those who have reviewed anuran parental care before have identified from five to twelve types (Salthe and Mecham 1974, Lamotte and Lescure 1977, McDiarmid 1978, Wells 1981, Duellman and Trueb 1986, Stebbins and Cohen 1995, Crump 1995). In an attempt to organize what is known, I have divided anuran parental care into six functional types. I have not split into separate categories similar behavior when it is performed on eggs versus tadpoles, by males versus females, or in aquatic versus terrestrial situations. Nor have I separated the many ways and places that parents brood their young on their bodies (e.g. gastric vs. pouch brooding), except for true viviparity. I have, however, split carrying into two types, discriminating between short-term transport and long-term brooding. These six types are attendance, nest building, transport, brooding, viviparity, and feeding. Many species exhibit more than one type. I will define and discuss each briefly, then provide a more extensive review of type 6: maternal feeding of tadpoles.

### **Type 1**: *Egg and/or larval Attendance*

The most common form of parental care known in anurans (and the only type known in Gymnophiona and Caudata), attendance refers to a parent remaining with the clutch at a fixed location. Parents may protect their young from predators by active defense (e.g. *Anodonthyla boulengeri*, Blommers-Schlösser 1975), from desiccation by emptying their bladders on terrestrial eggs (e.g. *Dendrobates auratus*, Wells 1978), from cannibalism by older conspecifics (e.g. *Eleutherodactylus coquí*, Townsend et al. 1984), or from several other risks, as mentioned above.

### **Type 2**: *Nest Building*

Because nests are usually built before fertilization occurs, technically this is not a form of parental care. But nests are clearly built with the sole intention of protecting offspring, and thus qualify as investment in future offspring.

Furthermore, nest maintenance is often performed after the offspring are in it. Anuran nests function to protect offspring from extreme fluctuations in water level, which bring risks of flooding or desiccation, and to separate offspring from larger bodies of water that may contain predators. Nests may be built by excavating soil (e.g. *Hyla rosenbergi*, Kluge 1981), from foam produced by the parents during amplexus (e.g. *Leptodactylus pentadactylus*, Heyer 1977), or by folding leaves over the eggs during mating, and sealing them shut with oviducal secretions (several species of *Afrixalus*, Wager 1965).

**Type 3**: *Tadpole Transport*

Anurans may carry their young for one of two purposes: to transport them to another microhabitat, usually from a terrestrial to an aquatic location (type 3), or to brood them, long-term, on their bodies (type 4). Tadpole transport is particularly widespread among the dendrobatids, although it is found in seven families (Crump 1995). In dendrobatids, it represents an additional phytotelm-related reproductive mode not mentioned above. This mode, Duellman and Trueb's (1986) #14, consists of terrestrial eggs that hatch into feeding tadpoles, at which point they are carried to a phytotelm by a parent. Several of the dendrobatid species then provision their young with trophic eggs (type 6). Tadpoles may be transported singly (e.g. *Dendrobates granuliferus*, Wijngaarden and Bolaños 1992), or en masse (e.g. *Colostethus inguinalis*, Wells 1980), and are usually attached by the mouthparts to the parent's back. The carrying parent may be female or male, territorial or not; for reviews of parental care in dendrobatids, see Weygoldt (1987), Crump (1995), and Summers et al. (1999).

**Type 4**: *Egg and/or Larval Brooding*

Long-term brooding of young on the body of one of the parents is well-known and diverse in anurans, as discussed extensively above under reproductive modes. This type overlaps perfectly with Wake's (1982) broad definition of ovoviviparity, and includes brooding in the stomach, vocal sacs, or pouch, or on the legs or back.

**Type 5**: *Viviparity*

In viviparous species, the young develop in the mother's oviducts and receive nutrition from her from outside of the yolk. As noted above, viviparity is extremely rare in anurans, known only in two species of toads. As in therian mammals, viviparity in anurans offers the longest, and closest, parental protection possible.

**Type 6**: *Tadpole Feeding*

In a few species with tadpoles that live in restricted aquatic habitats, such as phytotelmata, maternal feeding of tadpoles has evolved. The mother returns to her young and oviposits nutritional eggs for her developing tadpoles to eat. The final section of this chapter reviews this type of parental care in depth.

## Parental care in phytotelm-breeding anurans: tadpole feeding

Anuran eggs or tadpoles that develop in phytotelmata may receive one of three types of parental care. Adults may attend the developing eggs and/or tadpoles in the phytotelm, actively defending the clutch (e.g. adult male

*Plethodontohyla notostica*, which stay with the clutch through metamorphosis and "bark" at intruders, *pers. obs.*). Adults may transport tadpoles that hatched from terrestrial eggs to phytotelmata (e.g. several *Dendrobates* species, Weygoldt 1987). And females may return to phytotelmata in which their tadpoles are developing, and feed those tadpoles nutritive eggs. It is this maternal feeding that is the focus of this section.

Many species of tadpoles eat conspecific eggs (see Crump 1992 and Alford 1999 for reviews of cannibalism in anurans). In the restricted spaces of phytotelmata, where increasing tadpole density can be detrimental to existing individuals, cannibalistic oophagy is probably relatively common. Several phytotelm-dwelling species are known to eat conspecific eggs (e.g. *Philautus* sp., Wassersug 1981, and several *Hyla* species, Dunn 1926; see Hoff et al. 1999 for a recent review). Some species once thought to be mere egg cannibals are now known to be eating nutritive eggs deposited by their mothers for that purpose (e.g. *Anotheca spinosa*, Jungfer 1996). It is likely that, with more research on phytotelm-breeding anurans, additional species will prove to be receiving maternal care.

Obligate maternal provisioning of tadpoles probably evolved each time from populations in which tadpoles were already opportunistically cannibalizing eggs. Behavior intermediate between maternal provisioning and opportunistic cannibalistic oophagy has been described in the hylid *Osteocephalus oophagus*. In this species, mating pairs oviposit in phytotelmata, after which the female returns every 5 to 7 days, usually in amplexus with the same male, and oviposits into the same phytotelm. Existing larvae consume the newly laid eggs, and larvae starve if not provided with eggs. Those eggs not eaten hatch (Jungfer and

Schiesari 1995, Jungfer and Weygoldt 1999). Because the parents are apparently not behaving differently than they would if they had no tadpoles, they are not providing care, even though if they change their behavior, their offspring will die.

Few species are actually known to engage in maternal feeding of tadpoles. Species from only four families have been observed feeding their young: Dendrobatidae, Hylidae, Rhacophoridae, and Ranidae.

### Dendrobatidae

The neotropical family Dendrobatidae is known for the aposematic coloration, and associated skin toxins, of members of all its genera except the basal *Colostethus*. Parental care, including male and female egg attendance and larval transport, are common throughout the family, but maternal feeding has only been observed in species of the type genus, *Dendrobates* (Weygoldt 1987). The first observation of maternal feeding in any anuran was of captive *Dendrobates pumilio* (Weygoldt 1980). In 1993, the first field observation of maternal provisioning, in the same species, was made, and confirmed that feeding is obligate in this species (Brust 1993). Since then, several additional species have been observed exhibiting maternal feeding. Phylogenetic relationships within the dendrobatids are not well understood, but two recent analyses (Summers et al. 1999, Clough and Summers 2000) suggest that this behavior exists in two lineages within *Dendrobates*.

Biparental care occurs in members of the clade of Amazonian species, which has sometimes been identified as the *D. ventrimaculatus*, or *D. minutus*, group (Weygoldt 1987, Clough and Summers 2000). This behavior occurs in at least six species, although several of those were observed solely in captivity (see Weygoldt 1987). The reproductive pattern in this clade involves deposition of 2-4 eggs on bromeliad leaves above the surface of the water. The male attends the eggs, often attracting the female back to the oviposition site. When the eggs hatch, the male attracts the female again, then transports the tadpoles, singly, to different bromeliad axils. After this second deposition, the pair court again, and oviposit eggs into the axil, which the tadpole eats. The male continues to attract the female back to these axils throughout the development of the tadpoles inside, and she deposits nutritive eggs every few days (Weygoldt 1987, but see Clough and Summers 2000, and references therein, for alternative interpretations). In *D. vanzolinii*, the male and female appear to be pair-bonded (Caldwell 1997), but the female does not accompany the male to the tadpole deposition site. After he has deposited the tadpole, and every time thereafter that she deposits unfertilized eggs for her young, the male courts her, guiding her to the tadpole before she oviposits (Caldwell and de Oliveira 1999).

Female parental care is the rule in the *D. histrionicus* (or *D. pumilio*) group, which includes at least six species. Female only parental care probably evolved only once in *Dendrobates*, from biparental care (Summers et al. 1999). A few species in this clade (e.g. *D. pumilio*, Weygoldt 1987) show male egg attendance as well, but this is thought to be plesiomorphic (Weygoldt 1987, Clough and Summers 2000). The reproductive pattern in this clade involves terrestrial egg deposition of a small number of eggs, which the female attends. After hatching, the female transports tadpoles, singly, on her back, to different phytotelmata. She then provisions the tadpoles with unfertilized eggs until metamorphosis (Silverstone 1973, Weygoldt 1980, H. and E. Zimmermann 1981, Wijngaarden and Bolaños 1992, Jungfer 1985). Tadpoles do a tail-vibrating “dance” that can be seen by visiting frogs, and swim against the cloaca of the female, presumably to stimulate her oviposition of nutritive eggs (Weygoldt 1980). Maternal feeding may be obligate in all members of this group.

## Hylidae

Two neotropical hylid species are known to feed their young, one of which represents a unique, facultative variation of the behavior. *Osteopilus brunneus* oviposits relatively large clutches into phytotelmata, after which the mother returns and supplies eggs to her offspring every few days. When her existing offspring are young, she deposits fertilized eggs, some of which do not get eaten, and themselves develop. As the young develop, and are more likely to eat all that is fed to them, the mother begins depositing unfertilized eggs. Tadpoles that are not provisioned die. This behavior may serve to avoid the trade-off of offspring quality vs. quantity that is usually unavoidable in trophic egg production (Thompson 1996).

The only other hylid known to engage in maternal feeding is *Anotheca spinosa*. 48-311 eggs are laid per clutch. During amplexus, eggs are laid above the water in a phytotelm. Five to nine days after the first oviposition, the mother returns to the oviposition site and deposits unfertilized eggs on the surface of the water for her tadpoles, and continues this behavior every few days. If a male is present during her return visits, they engage in regular oviposition behavior. Tadpoles immediately eat any eggs to which they have access, and appear to communicate with single females that arrive to feed them (Jungfer 1996).

**Rhacophoridae**

*Chirixalus eiffingeri*, a species known from Taiwan and nearby islands, is the only rhacophorid known to engage in maternal feeding of tadpoles. Clutches of approximately 30 eggs are laid above the water line in treeholes or bamboo stumps. Mothers return to these phytotelmata and deposit unfertilized eggs for tadpoles. Tadpoles crowd the female and prod her cloaca during feeding (Ueda 1986). Successful tadpoles consume more than 80 eggs during their development (Kam et al. 1996). Tadpoles not fed eggs do not grow, and eventually die (Kam et al. 1996, 1997). Adverse effects of high density on tadpoles are the result of trophic egg limitation, rather than crowding (Kam et al. 1998).

**Ranidae**

The only ranid species known to exhibit maternal feeding of tadpoles is the Madagascan endemic *Mantella laevigata*, the subject of this dissertation. With a mean clutch size of 1.29 (range: 1-3), eggs are precious in this species. During amplexus, eggs are laid above the water line in bamboo stumps or treeholes. Hatching tadpoles drop into the water below, and eat conspecific eggs. Because mating pairs oviposit in the same place that oophagous tadpoles develop, and the fertilized eggs they produce are eaten by opportunistic tadpoles, maternal feeding is facultative. Single females sometimes return to phytotelmata and deposit unfertilized eggs at or below the water line, which tadpoles immediately eat. The placement of fertilized eggs above the water line in this species may not be due to physiological constraints, but an adaptation to avoid cannibalism by preexisting tadpoles (see chapter 2 for further discussion).

## Literature Cited

Alexander, R. D. 1974. The evolution of social behavior. Annual Review of Ecology and Systematics **4**:325-383.

Alford, R. A. 1999. Ecology: resource use, competition, and predation. Pages 240-278 *in* R. W. McDiarmid and R. Altig, editors. Tadpoles: The Biology of Anuran Larvae. The University of Chicago Press, Chicago and London.

Beck, C. W. 1998. Mode of fertilization and parental care in anurans. Animal Behaviour **55**:439-449.

Blommers-Schlösser, R. M. A. 1975. Observations on the larval development of some Malagasy frogs, with notes on their ecology and biology (Anura: Dyscophinae, Scaphiophryninae and Cophylinae). Beaufortia **24**:7-26.

Blommers-Schlösser, R. M. A. 1979. Biosystematics of the Malagasy frogs. I. Mantellinae (Ranidae). Beaufortia **352**:1-77.

Boulenger, G. A. 1897. The tailless batrachians of Europe. Ray Society, London.

Brown, W. C., and A. C. Alcala. 1983. Modes of reproduction of Philippine anurans. Pages 416-428 *in* A. G. J. Rhodin and K. Miyata, editors. Advances in herpetology and evolutionary biology: essays in honor of Ernest E. Williams. Museum of Comparative Zoology, Harvard University, Cambridge.

Brust, D. G. 1993. Maternal brood care by *Dendrobates pumilio* - a frog that feeds its young. Journal of Herpetology **27**:96-98.

Caldwell, J. P. 1997. Pair bonding in spotted poison frogs. Nature **385**:211-211.

Caldwell, J. P., and V. R. L. de Oliveira. 1999. Determinants of biparental care in the spotted poison frog, *Dendrobates vanzolinii* (Anura : Dendrobatidae). Copeia:565-575.

Clough, M., and K. Summers. 2000. Phylogenetic systematics and biogeography of the poison frogs: evidence from mitochondrial DNA sequences. Biological Journal of the Linnean Society **70**:515-540.

Corben, C. J., G. J. Ingram, and M. J. Tyler. 1974. Gastric brooding: a unique form of parental care in an Australian frog. Science **186**:946-947.

Crump, M. L. 1974. Reproductive strategies in a tropical anuran community. Miscellaneous Publications, The University of Kansas, Museum of Natural History **61**:1-67.

Crump, M. L. 1992. Cannibalism in amphibians. Pages 256-276 *in* M. A. Elgar and B. J. Crespi, editors. Cannibalism: ecology and evolution among diverse taxa. Oxford University Press, Oxford.

Crump, M. L. 1995. Parental care. Pages 518-567 *in* H. Heatwole and B. K. Sullivan, editors. Amphibian Biology. Surrey Beatty & Sons PTY Limited, Chipping Norton, Australia.

Drewry, G. E., and K. L. Jones. 1976. A new ovoviviparous frog, *Eleutherodactylus jasperi* (Amphibia, Anura, Leptodactylidae), from Puerto Rico. Journal of Herpetology **10**:161-165.

Duellman, W. E., and S. J. Maness. 1980. The reproductive behavior of some hylid marsupial frogs. Journal of Herpetology **14**:213-222.

Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. Johns Hopkins University Press, Baltimore, MD.

Dunn, E. R. 1926. The Frogs of Jamaica. Proceedings of the Boston Society of Natural History **38**:111-130.

Formas, J. R., E. Pugin, and B. Jorguera. 1975. La identidad del batracio chileno *Heminectes rufus* Philippi, 1902. Physis **34**:147-157.

Grandison, A. G. C. 1978. The occurrence of *Nectophrynoides* (Anura Bufonidae) in Ethiopia. A new concept of the genus with a description of a new species. Monit. Zool. Italiano (N. S.) Suppl. **11**:119-172.

Grandison, A. G. C. 1980. Aspects of breeding morphology in *Mertensophryne micranotis* (Anura: Bufonidae): secondary sexual characters, eggs and tadpoles. Bulletin of the British Museum of Natural History **39**:299-304.

Gross, M. R., and R. Shine. 1981. Parental care and mode of fertilization in ectothermic vertebrates. Evolution **35**:775-793.

Hayes, M. P. 1985. Nest structure and attendance in the stream-dwelling frog, *Eleutherodactylus angelicus*. Journal of Herpetology **19**:168-169.

Heyer, W. R., and A. S. Rand. 1977. Foam nest construction in the leptodactylid frogs *Leptodactylus pentadactylus* and *Physalaemus pustulosus* (Amphibia, Anura, Leptodactylidae). Journal of Herpetology **11**:225-228.

Hoff, K. v., A. R. Blaustein, R. W. McDiarmid, and R. Altig. 1999. Behavior: interactions and their consequences. Pages 215-239 *in* R. W. McDiarmid and R. Altig, editors. Tadpoles: The Biology of Anuran Larvae. The University of Chicago Press, Chicago and London.

Jameson, D. L. 1957. Life history and phylogeny. Systematic Zoology **6**:75-80.

Jørgensen, C. B. 1992. Growth and reproduction. Pages 439-466 *in* M. E. Feder and W. W. Burggren, editors. Environmental Physiology of the Amphibians. University of Chicago Press, Chicago and London.

Jungfer, K.-H. 1985. Beitrag zur Kenntnis von Dendrobates speciosus O. Schmidt, 1857 (Salientia: Dendrobatidae). Salamandra **21**:396-404.

Jungfer, K.-H., and L. C. Schiesari. 1995. Description of a central Amazonian and Guianan tree frog, genus *Osteocephalus* (Anura, Hylidae), with oophagous tadpoles. Alytes **13**:1-13.

Jungfer, K. H. 1996. Reproduction and parental care of the coronated treefrog, *Anotheca spinosa* (Steindachner, 1864) (Anura: Hylidae). Herpetologica **52**:25-32.

Jungfer, K. H., and P. Weygoldt. 1999. Biparental care in the tadpole-feeding Amazonian treefrog *Osteocephalus oophagus*. Amphibia-Reptilia **20**:235-249.

Kam, Y.-C., C.-F. Lin, Y.-S. Lin, and Y.-F. Tsal. 1998. Density effects of oophagous tadpoles of *Chirixalus eiffingeri* (Anura: Rhacophoridae): Importance of maternal brood care. Herpetologica **54**:425-433.

Kam, Y. C., Y. H. Chen, Z. S. Chuang, and T. S. Huang. 1997. Growth and development of oophagous tadpoles in relation to brood care of an arboreal breeder, *Chirixalus eiffingeri* (Rhacophoridae). Zoological Studies **36**:186-193.

Kam, Y. C., Z. S. Chuang, and C. F. Yen. 1996. Reproduction, oviposition-site selection, and tadpole oophagy of an arboreal nester, *Chirixalus eiffingeri* (Rhacophoridae), from Taiwan. Journal of Herpetology **30**:52-59.

Kluge, A. 1981. The life history, social organization, and parental behavior of *Hyla rosenbergi* Boulenger, a nest-building gladiator frog. Miscellaneous Publications, Museum of Zoology, University of Michigan **160**:1-170.

Krügel, P., and S. Richter. 1995. *Syncope antenori*--a bromeliad breeding frog with free-swimming, nonfeeding tadpoles (Anura, Microhylidae). Copeia **1995**:955-963.

Lamotte, M., and J. Lescure. 1977. Tendances adaptatives a l'affranchissement du milieu aquatique chex les amphibiens anoures. La Terre et La Vie **31**:225-311.

Lutz, B. 1947. Trends toward non-aquatic and direct development in frogs. Copeia **4**:242-252.

Magnusson, W. E., and J. M. Hero. 1991. Predation and the evolution of complex oviposition behavior in Amazon rainforest frogs. Oecologia **86**:310-318.

McDiarmid, R. W. 1978. Evolution of parental care in frogs. Pages 127-148 *in* G. M. Burghardt and M. Bekoff, editors. The Development of Behavior. Garland STPM Press, New York.

Metter, D. E. 1964. On breeding and sperm retention in *Ascaphus*. Copeia **1964**:710-711.

Mulaik, S. 1937. Notes on *Leptodactylus labialis* (Cope). Copeia **1**:72-73.

Noble, G. K. 1926. An analysis of the remarkable cases of distribution among the Amphibia, with descriptions of new genera. American Museum Novitates **212**:1-24.

Nussbaum, R. A., and D. L. Schultz. 1989. Coevolution of parental care and egg size. American Naturalist **133**:591-603.

Ridley, M. 1978. Paternal care. Animal Behaviour **26**:904-932.

Roberts, J. D. 1981. Terrestrial breeding in the Australian leptodactylid frog *Myobatrachus gouldii* (Gray). Australian Wildlife Research **8**:451-462.

Salthe, S. N., and W. E. Duellman. 1973. Quantitative constraints associated with reproductive mode in anurans. Pages Chapter 6 *in* J. L. Vial, editor. Evolutionary Biology of Anurans: Contemporary Research on Major Problems. University of Missouri Press.

Salthe, S. N., and J. S. Mecham. 1974. Reproductive and courtship patterns. Pages 309-522 *in* B. Lofts, editor. Physiology of the Amphibia. Academic Press, New York.

Silverstone, P. A. 1973. Observations on the behavior and ecology of a Colombian poison-arrow frog, the kõkoé-pá (*Dendrobates histrionicus* berthold). Herpetologica **29**:295-301.

Stebbins, R. C., and N. W. Cohen. 1995. A natural history of amphibians. Princeton University Press, Princeton.

Summers, K., L. A. Weigt, P. Boag, and E. Bermingham. 1999. The evolution of female parental care in poison frogs of the genus *Dendrobates*: Evidence from mitochondrial DNA sequences. Herpetologica **55**:254-270.

Taylor, E. H. 1954. Frog-egg eating tadpoles of *Anotheca coronata* (Stejneger). (Salientia, Hylidae). The University of Kansas Science Bulletin **34**:589-595.

Thompson, R. L. 1996. Larval habitat, ecology, and parental investment of *Osteopilus brunneus* (Hylidae). Pages 259-265 *in* R. Powell and R. W. Henderson, editors. Contributions to West Indian Herpetology: a tribute to Albert Schwartz. Society for the Study of Amphibians and Reptiles, Ithaca.

Townsend, D. S., and M. M. Stewart. 1985. Direct development in *Eleutherodactylus coqui* (Anura: Leptodactylidae): a staging table. Copeia **1985**:423-436.

Townsend, D. S., M. M. Stewart, and F. H. Pough. 1984. Male parental care and its adaptive significance in a neotropical frog. Animal Behaviour **32**:421-431.

Townsend, D. S., M. M. Stewart, F. H. Pough, and P. F. Brussard. 1981. Internal fertilization in an oviparous frog. Science **212**:469-471.

Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136-179 *in* B. Campbell, editor. Sexual Selection and the descent of man, 1871-1971. Aldine, Chicago.

Tyler, M. J., and D. B. Carter. 1981. Oral birth of the young of the gastric brooding frog *Rheobatrachus silus*. Animal Behaviour **29**:280-282.

Ueda, H. 1986. Reproduction of *Chirixalus eiffingeri* (Boettger). Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University **8**:109-116.

Wager, V. A. 1965. Frogs of South Africa: their fascinating life stories. Delta Books, Craighall.

Wake, M. H. 1980. The reproductive biology of *Nectophrynoides malcomi* (Amphibia: Bufonidae), with comments on the evolution of reproductive modes in the genus *Nectophrynoides*. Copeia **1980**:193-209.

Wake, M. H. 1982. Diversity within a framework of constraints. Amphibian reproductive modes. *in* D. Mossakowski and G. Roth, editors. Environmental adaptation and evolution: a theoretical and empirical approach. Gustav-Fischer-Verlag, Stuttgart and New York.

Wassersug, R. J., K. J. Frogner, and R. F. Inger. 1981. Adaptations for life in tree holes by rhacophorid tadpoles from Thailand. Journal of Herpetology **51**:41-52.

Wells, K. D. 1977. The social behaviour of anuran amphibians. Animal Behaviour **25**:666-693.

Wells, K. D. 1978. Courtship and parental behavior in a Panamanian poison-arrow frog (*Dendrobates auratus*). Herpetologica **34**:148-155.

Wells, K. D. 1980. Evidence for growth of tadpoles during parental transport in *Colostethus inguinalis*. Journal of Herpetology **14**:428-430.

Wells, K. D. 1981. Parental behavior of male and female frogs. Pages 184-197 *in* R. D. Alexander and W. D. Tinkle, editors. Natural Selection and Social Behavior. Chiron Press, New York.

Weygoldt, P. 1980. Complex brood care and reproductive behavior in captive poison-arrow frogs, *Dendrobates pumilio* O. Schmidt. Behavioral Ecology and Sociobiology **7**:329-332.

Weygoldt, P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). Zeitschrift für Zoölogische Systematik und Evolutions forschung **25**:51-67.

Wijngaarden, R. V., and F. Bolaños. 1992. Parental care in *Dendrobates granuliferus* (Anura, Dendrobatidae), with a description of the tadpole. Journal of Herpetology **26**:102-105.

Wittenberger, J. F. 1981. Animal Social Behavior. Dixbury Press, Boston.

Woodruff, D. S. 1977. Male postmating brooding behavior in three Australian Pseudophryne (Anura: Leptodactylidae). Herpetologica **33**:296-303.

Xavier, F. 1977. An exceptional reproductive strategy in Anura: *Nectophrynoides occidentalis* Angel (Bufonidae), an example of adaptation to terrestrial life by viviparity. Pages 545-552 *in* M. K. Hect, P. C. Goody, and B. M. Hect, editors. Major Patterns in Vertebrate Evolution.

Zimmermann, H., and E. Zimmermann. 1981. Sozialverhalten, fortpflanzungsverhalten und zucht dur färberfrösche *Dendrobates histrionicus* und *D. lehmanni* sowie einiger anderer Dendrobatiden. Z. Kölner Zoo **24**:83-9

# Chapter 2

# Social and Reproductive Behavior in the Madagascan Poison Frog, *Mantella laevigata*, with Comparisons to the Dendrobatids

## Abstract

I present the first behavioral study of natural populations of a Madagascan poison frog. Focal watches of marked individuals were conducted for 925 hours, in five populations, across two seasons. Like the New World dendrobatids, these diurnal anurans eat ants and are aposematically colored. Data are presented that provide additional instances of convergence with the dendrobatids, including 1) extended male-male fights over defended resources necessary for reproductive success of both sexes, 2) stereotyped, highly tactile courtships in which the female may reject initial oviposition sites, and 3) complex maternal care. Females return to water-filled phytotelmata, or wells, and lay trophic eggs for their tadpoles. *Mantella laevigata* has the minimum possible clutch size in anurans—usually one—suggesting a high degree of parental investment. Males defend wells, which attract females who oviposit in the wells. Fertilized eggs may hatch and metamorphose, or may be eaten by a tadpole already in that well, of which the territorial male is probably the father. Unfertilized eggs serve as food for tadpoles. Oviposition-site scouting behavior of both sexes, and the dependency of tadpole presence on the position of eggs laid, provide evidence of context-dependent, and assessment, behavior. Females

leave courtships most often only after visiting potential oviposition sites, while males usually leave to engage other males in aggression, suggesting that territory maintenance may be the most important component of male reproductive success.  Two other species of frogs often prevent *M. laevigata* from using defended oviposition sites, and larval crane-flies predate the eggs of all frog species using water-filled wells.

## Introduction

The poison frogs of Madagascar, genus *Mantella*, have long been of interest to students of anuran behavior, largely because there are many similarities between species of *Mantella* and the toxic members of the neotropical family Dendrobatidae, the poison-dart frogs.  Many species of dendrobatids, especially those in the genera *Dendrobates* (*sensu lato*) and *Phyllobates*, are known for their territoriality, stereotyped courtship sequences, and complex parental care.  Until now, nothing was known of the social system of any species of *Mantella*.  This study marks the first behavioral research conducted on *Mantella* in the field.

When Boulenger identified *Mantella* in 1882, he placed the genus in Dendrobatidae.  Although he later formalized this taxonomic placement on the basis of their shared lack of teeth (Boulenger 1914), it is clear that he felt that the two groups simply shared a gestalt (Boulenger 1882).  Though Boulenger did not know it, the aposematic coloration in both groups arises, in part, from identical classes of lipophilic alkaloids in the skin, including  pumiliotoxins and decahydroquinolines (Daly et al. 1996). Among anurans, these classes of toxins are otherwise known only in two other (unrelated) frog genera [*Melanophryniscus* (Bufonidae) and *Pseudophryne* (Myobatrachidae), Daly et al. 1984]. Other similarities between *Mantella* and the dendrobatids include small size (most species have a snout-vent length of 20-30 mm), diurnality, terrestrial and/or arboreal habit, and a diet consisting primarily of ants and mites. Captive dendrobatids lose their toxicity (Daly et al. 1980; Daly et al. 1992), as do captive *Mantella* (Daly et al. 1997), suggesting that the building blocks for the alkaloids are dietary. A high percentage of ants in the diet of dendrobatids is correlated with toxicity (Caldwell 1996), and some ants (genus *Solenopsis*) in the dendrobatid diet produce some of the simpler alkaloids themselves (Spande et al. 1998; Jones et al. 1999).

*Mantella laevigata*, commonly known as the climbing *Mantella*, has more characters in common with the aposematic dendrobatids than do the other ten species of *Mantella*. It is not fully terrestrial, and has expanded toe-pads. It also breeds in water-filled tree holes or broken bamboo (phytotelmata), much like the preferred bromeliads of some dendrobatids. Amplexus is neither reliably axillary nor inguinal in *M. laevigata*, which are the two most common mating positions in anurans; *M. laevigata* adopts a variety of positions during mating amplexus (*pers. obs.*). In several dendrobatids, amplexus is also unlike that of most anurans, being absent (Crump 1972; Limerick 1980), or cephalic, a highly derived form in which the male grasps the female by the head (Myers et al. 1978; Wells 1980). Finally, the larval morphology of *M. laevigata* resembles that of the oophagous dendrobatid tadpoles, having reduced labial denticles and a large, keratinized horny beak (Glaw & Vences 1994).

Several other taxa are known from both South America and Madagascar, but not Africa (e.g., side-neck turtles, Underwood 1976; iguanians, Frost & Etheridge 1989; boas, Kluge 1991). These examples are consistent with reconstructions of Gondwana that suggest that Africa and South America partially broke from the rest of the Southern landmass by the late Jurassic, and that by the early Cretaceous, Africa and South America were rifting, leaving a more recent and direct land connection between South America and Madagascar than between Africa and Madagascar (Scotese & Golonka 1992). These observations might lead to a hypothesis of sister-group relationship between the dendrobatids and *Mantella*, but phylogenetic analyses, though not complete with regard to the taxa investigated, provide evidence against such a historical hypothesis (e.g. Hay et al. 1995). Thus, it seems that all similar specializations between the dendrobatids and *Mantella* are independently evolved.

While a dendrobatid-*Mantella* relationship would support the hypothesis of Gondwana reconstruction outlined above, it provides little insight into more general causal chains that may have led to what we observe today. Complex convergences, involving distinct processes, can help identify environmental factors that might lead to similar results in the different organisms. That is, the known existence of several convergences may suggest that others are present.

Based on the known similarities described above, my goal was to discover the social system and natural history of *M. laevigata*, with the broad hypothesis that some or all of the behaviors of aposematic dendrobatids would be present in *M. laevigata.* I predicted that male *M. laevigata* would be territorial, defending calling and/or oviposition sites from other males, as several dendrobatids do (e.g., *D. granuliferus*, Crump 1972; *D. pumilio*, Bunnell 1973; *E. femoralis*, Roithmair 1992). I predicted that *M. laevigata* would have a stereotyped courtship sequence similar to that of some dendrobatids, in which a male attracts a female using advertisement calls, changes to a softer courtship call, and through repeated tactile interaction with the female leads her to one or more potential oviposition sites until she accepts one, or abandons the courtship (see review by Wells 1977; also *Colostethus stepheni* and *C. marchesianus*, Juncá 1998). I also predicted that *M. laevigata* would have complex maternal care of the type found in the oophagous dendrobatids, in which mothers return to individual tadpoles repeatedly to feed them trophic (unfertilized) eggs (see reviews by Weygoldt 1987; Crump 1996). This behavior has also been observed in three unrelated species of anurans, two hylids (*Osteopilus brunneus*, Lannoo et al. 1986; and *Anotheca spinosa*, Jungfer 1996; Thompson 1996), and one rhacophorid (*Chirixalus eiffingeri*, Ueda 1986; Kam et al. 1996).

In my 1999 field season, I had already failed to falsify these broadly defined "hypotheses of convergence," based on the behavior I had observed in *M. laevigata* in 1997. I then focused on quantifying what variables affect the outcomes of territorial encounters and courtships, and what and how often other organisms in the phytotelmata (well) community interact with *M. laevigata*. With these data it is possible to construct a coherent picture of the selective pressures affecting reproductive success for these frogs.

## Methods

*Mantella laevigata* was observed during the Malagasy summers of 1997 and 1999 (January through May, and January through April, respectively). The research was conducted on the 510 hectare island of Nosy Mangabe, which lies at the northern end of the Bay of Antongil, five kilometers south of the town of Maroantsetra, Toamasina Province, in northeastern Madagascar (15° 30′ S, 49° 46′ E). Nosy Mangabe is a "Special Reserve" in the Masoala National Park consisting mainly of 100 – 400 year old secondary forest. There are two summits, the highest being 332 m, and most of the island is inaccessible by trail. *M. laevigata* are found throughout the island, but are densest in coastal bamboo stands. The bamboo on Nosy Mangabe--*Bambusa vulgaris*--was introduced, but in Marojezy, the other region in which *M. laevigata* is found, the frogs are found in association with native bamboo species, including *Ochlandra capitata*.

The goals of the 1997 and 1999 seasons were distinct. Prior to my arrival in 1997, no researchers had observed these frogs in the wild for more than a few hours. As such, my knowledge of the system was based entirely on what I learned anew every day. I therefore made observations in 1997 on an *ad libitum* basis, in which I changed "focal" animals any time a more interesting behavior was observed elsewhere, in order to maximize knowledge about all aspects of the social system of these frogs. In 1999, the intent was to quantify behaviors identified during 1997. The descriptions of behaviors throughout this paper are based on two seasons of observations, while the quantifiable data—e.g., analysis of what percentages of behaviors ended in particular ways—are based entirely on 1999 data.

I conducted *ad libitum* sampling with continuous recording (Martin & Bateson 1993) on populations of marked frogs in coastal bamboo stands in 1997, while in 1999, I used focal watches with continuous recording (Altmann 1974). Three of the four populations watched in 1997 were in three adjacent bamboo stands, encompassing a total area of approximately 1500 $m^2$, though the area taken up by bamboo amounts to only 147 $m^2$ (stands 4, 5, and 6; Fig. 1). Some females traveled among these stands, but males were never observed to do so. The fourth population (bamboo stand 3) is 2.8 km away, and no movement of frogs was recorded between that and the other focal populations. The two populations watched in 1999 were in stands 650 meters apart (stands 2 and 4; Fig. 1), and no movement of animals was observed between these populations.

In 1997, I and an assistant (JM) observed marked individuals for 509 hours. Animals were caught by hand, and by using small aquarium dipnets. All animals were toe-clipped, in addition to being given a mark which should have enabled us to identify the animals from a distance, including tying small beaded waistbands onto them, and stitching beads directly into their dorsums. None of the marks given in 1997 was reliable for more than two weeks. In 1999, I and a second assistant (GF) conducted 416 hours of focal watches on individuals tattooed on their dorsums with a portable battery-operated tattoo machine (Dermo-Marker Tattoo Machine model 401). These tattoos required re-inking every four-six weeks, but the animals returned to their territories and resumed calling and territorial defense (if male) immediately upon being returned from tattooing, so the marking technique did not appear to interfere with behavior. In both 1997 and 1999, I recorded mass and snout-vent length (SVL) for all marked animals (N=218 in 1997, N=83 in 1999; total N=301).

We conducted focal watches in 1999 in half hour intervals. We watched any given animal for as much as two hours per day, but attempts were made to diversify the animals watched. Dominant, territorial males were the easiest to find, so they were watched more often than other individuals. Individuals who disappeared under the leaf litter or up bamboo during a focal watch were searched for until the next fifteen minute interval, at which point a new focal animal was found.

The most common behavior observed and recorded was courtship. Courtship bouts are defined as uninterrupted courtship segments, during which neither participant engages with a third individual, and those courting are within 1 meter of each other. When courtships are interrupted, they may resume later. If the same individuals began courting again within fifteen minutes of a courtship bout being interrupted, the ensuing courtship was coded as another bout in the same courtship. The sum total of bouts within one courtship is referred to as a complete courtship.

At the beginning of every day of focal watching, we examined the contents of all water-filled wells in the vicinity of the population with a mini-maglite, and *M. laevigata* eggs and tadpoles, heterospecific frogs and broods, and egg predators were censused. These observations were repeated after any frog activity was seen around a given well.

During the 1997 season, 87.8% of the focal watch hours were conducted in the morning, primarily between 5:30 and 10:00 am, when the frogs are most active. In 1999, 95.3% of focal watches were conducted in the morning. During

416 hours of focal watches in 1999, 274.5 hours (70.0%) were spent watching adult males, 101 hours (24.3%) were spent watching adult females, and the remaining hours spent watching juveniles or adults of unknown sex.

## Statistics

Fisher's Exact Test was used to analyze the difference in oviposition location between courtships and incidents of maternal care. Individual females were not clustered in the analysis presented (which would control for the effect of individuals tending to make similar choices repeatedly), but other analyses (including contingency tables using Rao and Scott correction for clustered data) revealed similar significance levels when controlled for non-independence between individuals. Chi Square goodness of fit tests were used to analyze male discrimination of wells, in which independent observations of the contents of wells in the population were used to calculate "expected" values, and the contents of wells to which males took females are the "observed" values. Again, alternate analyses, in which the same males in multiple courtships were treated as non-independent events, yielded similar results (calculated a 95% confidence interval for the ratio, using clustering). Logistic regressions with cluster analyses were used to address the significance of much of the data in which the same animal was engaged in multiple incidences of the measured behavior, including the analysis of female discrimination of wells, with repeated, individual females clustered as non-independent variables. The results presented, therefore, are as conservative as is possible with the existing data.

All of the data described in the preceding paragraph were also analyzed using resampling techniques, which make no *a priori* assumptions regarding the distribution of the data. The p-values obtained from resampling statistics were all as or more significant than those generated using more traditional tests.

## Results

## Descriptions of Three Behaviors (from 1997 & 1999 observations)

### Territorial Behavior

During 925 hours of observations, 215 fights were witnessed between males, in 367 bouts. Fight bouts between males last from ten seconds to more than 1.5 hours. Fights take place primarily over territories, the best of which include water-filled wells in which mating takes place, and eggs and tadpoles develop. Wells are the limiting resource for these frogs (see chapter three). Males defend these wells, and an area not less than 500 cm diameter around them, with advertisement calls and fights.

Males use two distinct forms of territorial defense, and an additional category of male does not defend territories at all. Among the territorial males, some defend resources, other appear not to. I define males in possession of territories with wells in them “resource-defense territorial,” or RD-territorial. I define males in possession of territories without wells as “no-resource territorial", or NR-territorial. As wells are known to be limiting, and other parameters such as food are not, territories without wells in them are defined as without resource, and therefore of low quality. Both NR-territorial and RD-territorial males scout in other males' territories, usually without advertisement calls, when they are not defending their own territory. Finally, there are "aterritorial" males that have not been seen defending any area, but that spend time in territorial males' territories and try to attract females therein. These "aterritorial" males may in fact be NR-territorial, with defended territories outside the range of my observations. When either NR-territorial or aterritorial males are in territories with wells, I refer to them as non-residents, and the territory-holder as the resident.

Both NR-territorial and aterritorial males sometimes successfully lead females into matings in wells in territories not their own. Often these non-resident males are seen by the resident (territory-holder) and attacked, at which point they respond in one of two ways. Non-resident males may fight back, often repeatedly (several bouts); or they may be docile, not call, and submit to being amplexed by the male resident. Resident males do not amplex, or try to court, non-responsive animals of either sex for more than five minutes, and leave the non-resident after a short time.

RD-territorial males can usually be found in a given territory, not larger than 2 meters square, which contains at least one water-filled well—usually broken bamboo. RD-territorial males call frequently from their territories. NR-territorial males have a high rate of site fidelity as well, but the sites they call from do not have wells in them. These males call and attract females to these low-quality territories, after which they lead females into other males' (high-quality) territories. Territory borders, and even interiors, are repeatedly fought over by the same males. Fights include male-male amplexus, belly-to-belly wrestling, tumbling, leaping on to one another, and chasing. Aterritorial males have home ranges that they do not defend against other males.

### Courtship Behavior

During 925 hours of observations, 348 courtships were witnessed in 521 bouts. Courtships involve stereotyped sequences that begin when the male gives an advertisement call, often from a raised perch, such as a palm axil or the well he defends, and a female responds by approaching. When a male sees a female approaching, he moves towards her and makes initial contact by putting his throat either on the top of her head or on her dorsum (behavior hereafter referred to as "chinning"). The male often faces the female during chinning, and less often has his body perpendicular to hers (see Fig. 2). His vocalizations change at this point from the louder, two-note advertisement call to the softer, often single-note courtship call. The male chins the female for up to five minutes before beginning to lead her to a potential oviposition site. He hops not more than 10 centimeters in front of the female, emitting courtship calls, sporadically returning to chin her again. During some courtships, the male alternates chinning of the female with non-reproductive axillary amplexus. For up to 30 minutes, the male leads the female to a potential oviposition site, then climbs to the top of it, and chins her again before allowing her to enter. She then enters, goes into the water, and the male follows, emitting continuous courtship vocalizations. If she accepts the well by remaining inside, the male amplexes her on the inside of the well, and a single egg is laid (see clutch size below). This is the first time that amplexus occurs during the courtship. If she rejects the well, she hops out while the male calls from inside the well. In most cases, he then follows and catches her, chins her again, and begins the repeated chinning, leading and calling while leading her to another potential oviposition site.

Only two pairs of *M. laevigata* were observed in reproductive amplexus without first engaging in the stereotyped courtship sequence. These two exceptions occurred when the female entered the well and began ovipositing at the water line, at which point the male followed her in and amplexed her, and an egg was laid. In both cases, the egg was quickly eaten by the resident tadpole, suggesting attempted maternal care (see below) rather than true courtship.

Some would-be courtships turn into fights, when silent aterritorial or NR-territorial males enter a calling RD-territorial male's territory, and are chinned by the resident male. It is difficult for humans, and perhaps frogs, to distinguish between the sexes without behavioral cues. This is true despite statistically significant sexual dimorphism for both SVL and mass in this species (N=44 females, 70 males; Range, Mean ± SD: female SVL (mm): 25.0 – 31.0, 28.06 ± 0.18; male SVL (mm) 24.0 – 30.0, 26.79 ± 0.14; female mass (g): 1.15 – 2.05, 1.72 ± 0.03; male mass (g): 1.10 – 1.90, 1.53 ± 0.02. Independent Samples T-test, t=5.54 for mass, t=5.50 for SVL, $p<0.0001$ for both variables).

Females were often observed in scouting behavior, in which they investigate several wells in succession, climb them, look in, and go into the water. While scouting, females ignore the advertisement calls of nearby males, and retreat when males approach them. Males using all of the three territorial categories were also seen scouting potential oviposition sites, never calling while doing so.

### Maternal Care

During 925 hours of observations, 10 incidences of maternal care of tadpoles were witnessed. Females were observed climbing wells alone, ignoring the advertisement calls of nearby males, backing into these wells, and depositing single eggs at or below the water line. In all ten cases, a resident tadpole was observed eating the egg within 30 minutes of deposition. In five of these cases, the females who returned alone had previously been seen in mating amplexus with males in those same wells. I do not, however, have more direct evidence that the tadpoles in these wells belonged to these females, as viable wells often have eggs from several ovipositions and from different mating pairs, at the same time.

Furthermore, two of the successful "courtships" observed appear to have been attempted maternal care in which a territorial male followed the female into the well and amplexed her while she was already positioned at the water line, which is otherwise never seen in mating pairs. These eggs were also promptly eaten by tadpoles. On five occasions, an egg deposited by a single or amplexed female was completely gone within five minutes of the female or mating pair depositing the egg and leaving the well. This suggests that both maternal care and oophagy generally may be more common than these data suggest, as the evidence is quickly erased, rendering the behavior invisible to the researcher. Tadpoles were never observed eating anything but conspecific eggs, and wells with tadpoles in them were not free of mosquito or other small arthropod larvae or detritus.

## Quantification of Observed Behaviors, from 1999 Data

Resident territorial males fought non-residents in 82.4% of total fights observed (total n=131), of which the residents won 88.9%. Of the remaining 17.6% of fights (n=23), 9 maintained territory boundaries between two resident males, and 14 were fought between non-resident males when the resident was absent. Individual males were predictably winners or losers in successive fights. Eleven males were observed to participate in five or more fight bouts (range: 7-45), of which five won less than 23% of fights (range: 0 - 22.2%), while the other six won more than 69% of fights (range: 69.2 - 85.7%). In addition, an initiator's advantage was apparent, as only 17 of 131 complete fights (12.9%) were won by non-initiators, 10 of which were won by residents who were attacked by non-residents.

Apparently, territory is the primary reason for fighting. Ninety-six of 131 fight bouts were fought over territory, in which no female was within 1 meter of either male participant; 31 fight bouts were fought over territory while a female was within 1 meter of the males; and 4 fights appeared to be entirely over the theft of a female from a courting male by another male.

Courtships ended for a variety of reasons (Table 2.1). These include females leaving after being inside the well a male had taken her to, males leaving to engage in territorial activities, interference of other species, and success. Of 160 courtship bouts witnessed in 1999, 128 were "terminal," 32 "non-terminal." Terminal courtship bouts are those that are not resumed within 15 minutes of interruption. Males leave non-terminal courtship bouts for territorial activities more often than they leave terminal courtship bouts for territorial activities.

Females, however, are equally likely to leave terminal and non-terminal bouts after being led inside a well by a male (Table 2.2).

In all instances in which a competitor species interrupted a courtship, the interruption terminated that courtship. The only heterospecific frog species seen to directly interfere with the courtships of *M. laevigata* was *Plethodontohyla notostica*. However, two other microhylids—*Anodonthyla boulengeri* and *Platypelis grandis*—also used wells, and prevented *M. laevigata* from using them.

Females oviposited in wells at different heights relative to the water line when they were engaged in courtship, versus when they were performing maternal care by depositing single eggs for tadpoles. During courtships, females oviposited above the water line 18 of 21 times, while during instances of maternal care, they deposited at or below the water line 4 out of 5 times (Fisher's Exact Test, $N=26$, $P \leq 0.01$). These data include only those ovipositions for which there was no ambiguity about the number or placement of resultant eggs. Of those same 21 non-ambiguous courtships observed in 1999 , mean clutch size was 1.29 ± 0.12, with a range of 1-3.

A larval crane fly (*Limonia renaudi* Alexander, Tipulidae) was observed eating the eggs of *Mantella laevigata*, as well as those of one of the other two species of frogs that breed in bamboo wells on Nosy Mangabe, *Plethodontohyla notostica*. The tipulid larvae attach their mouth parts to the gelatinous capsule, and suck out the ovum. Embryos in later stages of development stop moving during egg predation, and are then eaten.

Tadpoles were never observed to engage in cannibalism. Up to four tadpoles of different developmental stages were observed coexisting in wells, and smaller tadpoles did not disappear from wells with larger tadpoles in them more often than they disappeared from wells in which they were the only inhabitants.

Males discriminate between wells on the basis of tipulid and heterospecific frog presence (Table 2.3). Wells to which males take females during courtships do not differ significantly from average viable wells with regard to tadpole presence. Wells to which males lead females tend to have more conspecific eggs in them than do random wells, but this is non-significant (Table 2.3). Females are not left with the option to discriminate between wells on the basis of heterospecific frog presence, as males never take them to these wells. A female rejected the one tipulid-filled well that a male took her to. Furthermore, there is a significant tendency for courted females to reject wells with tadpoles in them. As with wells that males choose, wells that females accept have a non-significant tendency to contain more eggs than the average (Table 2.4.)

Most heterospecific frog species are avoided by *M. laevigata* when choosing oviposition sites, including the microhylids *Anodonthyla boulengeri*, *Plethodontohyla notostica*, and *Platypelis grandis*. *Platypelis grandis* is a large, short-term resident that presents an obstacle for less than 24 hours. In contrast, the first two species move in and produce clutches, which the fathers stay with until metamorphosis, effectively removing those wells from use by *M. laevigata* for a month or more. In one instance, I observed a preexisting *M. laevigata* tadpole survive ten days in the same well with a *Plethodontohyla notostica* father and clutch. For over one month after the *Plethodontohyla* father abandoned the clutch, it decayed in the well while the *M. laevigata* tadpole was still developing in that well. The *M. laevigata* tadpole survived to metamorphosis. In several other cases, however, when *Plethodontohyla notostica* mating pairs moved in, and fathers stayed for over a month with their developing brood, previously existing *M. laevigata* eggs or tadpoles were never seen again in those wells. The other paternal-care giving microhylid that usurps wells from *M. laevigata*, *Anodonthyla boulengeri*, is more common in forest than in bamboo stands, where the watched populations of *M. laevigata* live. Thus, no direct competition between *A. boulengeri* and *M. laevigata* for wells was seen, though *A. boulengeri* was observed occupying wells that *M. laevigata* previously had occupied. *A. boulengeri* and *M. laevigata* were both observed to move into artificial wells that were established in forest plots, suggesting possible competition for wells between these two species as well (see chapter three).

Not all heterospecific frog species are discriminated against by *M. laevigata* when choosing oviposition sites. *Platypelis cowani*, a small (25 mm SVL) microhylid species was often found in wells, then difficult to locate minutes later, when no frogs had been observed to leave. As such, no data were taken on *P. cowani* presence in wells due to suspected inaccuracies. However, in one directly observed *M. laevigata* courtship, there were two *P. cowani* easily seen inside the well in which the *M. laevigata* mating pair oviposited an egg.

## Discussion

*Mantella laevigata* reveals a surprising number of behavioral convergences with the aposematic dendrobatids. The extended, repeated fights between males over territory, the stereotyped and highly tactile courtship sequences, and the maternal care that is given by females to tadpoles in the form of unfertilized eggs all resemble behaviors of some dendrobatids. Convergent morphology or behavior may provide information about the evolution of adaptations by revealing universal rules or causal chains. For instance: toxicity is a prerequisite for aposematic coloration; aposematic coloration leads to diurnality and clumping of individuals due to lower predation pressure; clumping allows for greater complexity of social interactions, which includes males trying to control females through controlling the resources they need access to (Emlen & Oring 1977; Heying 1997; Vences et al. 1998). Perhaps of more biological significance, however, are the differences between the dendrobatids and *Mantella* that emerge on closer inspection, and the specifics of the *M. laevigata* system.

Maternal care in *M. laevigata* is probably facultative, rather than obligate, given the small number of occurrences observed during this study. The evidence of maternal care may often disappear quickly, as hungry tadpoles eat trophic eggs within minutes of deposition. Most tadpoles were never seen receiving maternal care, which suggests that many tadpoles obtain nutrition from non-maternal sources. Whereas in most parental-care-giving dendrobatids, clutches are laid in one place, allowed to hatch, and the tadpoles are then transported, usually singly, to bromeliads or other wells (reviews in Weygoldt 1987; and Crump 1995; but see Summers 1999 for an exception); in *M. laevigata* the eggs and tadpoles develop in the same space. Thus, courted females lay eggs while amplexed by males in the same phytotelmata used by lone females for maternal care when ovipositing for their already hatched offspring. These spaces are limiting for *M. laevigata* (chapter three), and males often take females to wells that already have developing eggs or tadpoles in them during courtships. If a male has fathered tadpoles currently developing in a well he actively defends, then the male's potential gain from convincing a female to oviposit in that well is increased. The egg the female lays during courtship may result in another offspring of his, or may go to feed his already existing offspring. Whether pre-zygotic or post-zygotic investment, his effort is parental in nature. This should be considered reproductive parasitism on the part of the male (Weygoldt 1987; Summers 1999).

The "icebox hypothesis," which postulates that some offspring provide indirect parental investment by providing food for other offspring, is predicted to be in effect in such a system, where future conditions are unpredictable (Mock & Parker 1997). If heavy rains bring the water level in a well up to the level of an egg laid during courtship, that egg will be eaten if the well contains a tadpole. In such a case, however, that egg, left uneaten, would likely die, due to insufficient oxygen (Seymour & Bradford 1995). Females, then, might be expected to return to wells already containing their offspring for future courtships, as egg predation in such a well still furthers the female's reproductive success. It is not clear that females are doing this, but males do lead females to defended wells during courtships that already contain the male's tadpoles. Higher certainty of paternity than maternity in individual wells might have led to this manifestation of the icebox hypothesis, in which oviposition-site defending males are more eager than non-territorial females to have eggs laid in particular viable sites.

In response to males evolving reproductively parasitic behavior to further their reproductive success, females are expected to evolve detection mechanisms by which to avoid such parasitism during courtships. In *M. laevigata*, the evidence is strong that females actively discriminate against wells with tadpoles during courtships (Table 2.4). Tadpoles, in turn, are likely to evolve mechanisms by which to hide from females. The scouting behavior of females serves to give them multiple interactions with possible oviposition sites, such that when a female is taken to a well by a male during courtship, she already has some information about the quality and inhabitants of that well.

When courted females do oviposit into wells with tadpoles in them, the resultant egg (or occasionally eggs) is usually laid above the water line. Tadpoles are unable to reach eggs above the water line, except in rare cases where their forelimbs are well-developed but they have not yet dispersed from the well; thus the oviposition location is another mechanism by which females can attempt to protect their offspring from becoming food for another. Because wells are limiting, however, it is rare that *M. laevigata* tadpoles exist in wells without conspecific eggs on the walls of that well. When other courting pairs, or individuals of either sex who are scouting wells, move around in them, eggs are often dislodged into the water below. Thus, unlike the dendrobatid species with obligately oophagous tadpoles (e. g., *Dendrobates pumilio*, Brust 1993; and the other four species in the *D. pumilio* group, Crump 1995; and *Dendrobates vanzolinii*, Caldwell & de Oliveira 1999), tadpoles of *M. laevigata* have sources of conspecific eggs other than from their mothers. However, other species of dendrobatids have also been observed to cannibalize conspecific, fertilized eggs (e.g., *Dendrobates ventrimaculatus*, Summers & Amos 1997; Summers 1999). All cases of maternal care observed in *M. laevigata* involved non-courted females and were directed at tadpoles in wells in which eggs had not been seen for at least three days, suggesting that females monitor the wells in which their tadpoles live.  There is no evidence in *M. laevigata* that tadpoles cannibalize other tadpoles.

In amphibians, small clutch size, large egg size, and terrestrial deposition of eggs are often correlated with a high degree of parental care in individual offspring (Crump 1995).  *M. laevigata* usually exhibits the minimum possible clutch size, its ova are large at approximately 3-3.5 mm in diameter, and, though not having terrestrial oviposition *per se*, females oviposit on the sides of wells above the water line, which is presumably a defense against egg predation, like terrestrial oviposition. Given these predictors of parental care, facultative maternal care probably does not describe the extent of care in offspring in *M. laevigata*.  Males may provide parental care that is not immediately identifiable as such.  Males defend wells actively, often leaving individual courtships to chase other males away from their territories. Fights between *M. laevigata* males serve, most often, to maintain the territorial status quo.  Initiators win fights most of the time, and initiators are usually resident males. (This is true across taxa in which it has been studied, e.g., Tasmanian hens, Putland & Goldizen 1998.)

Although non-resident males have been seen successfully mating in resident males' wells, this is the exception.  A male “confident” of his paternity--which I cannot assess without molecular data--stands to increase his reproductive success through courtship regardless of the future of the egg that is laid. Reproductive parasitism by a male to feed a pre-existing offspring is clearly parental investment on behalf of that offspring.  Male defense of wells when tadpoles are inside could also be considered parental investment.  If a male *M. laevigata* drives away individuals of one of the two paternal-care giving microhylid frog species from  a well with his offspring, he has probably saved his tadpole's life. Individuals of both *Plethodontohyla notostica* and *Anodonthyla boulengeri* fill a well with their clutch and father, and remain there for more than a month, making it functionally impossible for *M. laevigata* to co-exist in that space. This anuran phytotelm community is ecologically quite similar to a neotropical phytotelm community which includes females of *Dendrobates castaneoticus* (which deposit a single tadpole into each phytotelm) and *Bufo castaneoticus* (which oviposit 61-387 eggs into each phytotome, Caldwell 1993). Other competitors for well space include land crabs, which have, on occasion, been observed to move into wells and eat *M. laevigata* eggs. I predict that success of crabs moving into wells is diminished by the presence of an actively defending male, as only crabs already on the inside of a well interacted with adult *M. laevigata*.

Courtships are the most direct route to reproductive success that these frogs have, but courtships end frequently without an egg being laid (Tables 2.1, 2.2). This raises the question: what is more important to long-term reproductive success than the continuation of the present courtship? Females most frequently leave courtships only after investigating the well into which the male expects her to oviposit, suggesting that some measure of well quality that she is gauging is a better predictor of offspring success than the particular male with which she is in courtship. Sexual selection experiments support this hypothesis (see chapter five).

When males leave courtships, however, more explanation is required. Males rarely leave to court other females, even though the quality of the tadpole in the well a male defends must be critical to the father's reproductive success. Thus, males do not appear to exhibit mate choice in this species, although some parameters of the social system fit predictions of male mate choice: males provide benefits to both females and offspring by defending oviposition sites, but the courtship process is long enough to limit, though not preclude, multiple matings by males (Krupa 1995). Furthermore, the resource provided by the male in this system cannot be used simultaneously by a large number of offspring, as tadpoles will eat younger, conspecific eggs if they can. "Mate" choice for oviposition sites rather than individuals is consistent with experimental evidence that these sites are limiting for *M. laevigata* (see chapter three).

Males determine the quality of wells before they begin courting females, which is consistent with focal observations of males scouting the area and investigating various wells. Thus, before females have a chance to reject wells, males have already surveyed wells, and do not take potential mates to wells containing crane-fly larvae or members of other frog species (Table 2.3). This is not due to different habitat requirements for the various species, as the same wells discriminated against by *M. laevigata* when they contain other frogs or tipulid larvae are used for reproduction when they are free of competitors and predators. In the first reported system in which frogs assess oviposition sites for egg density—*Hyla pseudopuma*, a tropical hylid with known egg cannibalism by tadpoles--females and males are both choosy with regard to the phytotelmata in which they oviposit (Crump 1991). Findings were similar in three dendrobatid systems. Summers observed male *Dendrobates auratus* exploring oviposition sites in succession before taking tadpoles to them (Summers 1989). Caldwell hypothesized that adult *D. castaneoticus* carrying tadpoles detect predatory insect larvae in phytotelmata, and discriminate against wells containing them (Caldwell 1993). Finally, Summers demonstrated that *D. ventrimaculatus* males do discriminate against pools containing large tadpoles when courting females and when depositing tadpoles (Summers 1999).

Though the evidence is strong that *M. laevigata* discriminate against wells with crane-fly larvae in them (Table 2.3), *M. laevigata* males cannot prevent adult crane-flies from ovipositing in wells that already contain frog eggs. Because clutch size is minimal in *M. laevigata*, the effect of a single voracious crane-fly larva on the mating effort of *M. laevigata* will potentially be much greater than that on a clutch of *Plethodontohyla notostica* eggs (n > 40 in all clutches observed). Minimal clutch size may, however, be due in part to a selective response to tipulid presence, such that females scatter their eggs across multiple wells in order to reduce the likelihood of all of their offspring being eaten in a single predation event.

I predict that inter-clutch kin selection for accelerated hatching will occur when an egg inhabiting the same well is attacked by a crane-fly larva. This follows from Warkentin's work on red-eyed tree frogs, in which she found that eggs within a clutch that is being attacked by an egg-eating snake escape snake predation by hatching early and dropping into the water below (Warkentin 1995). Due to selection pressure to detect conspecific eggs being eaten, I hypothesize that the destruction of *M. laevigata* eggs by crane-fly larvae accelerates the hatching of conspecific eggs in the same well, regardless of relationship. Although other *M. laevigata* eggs in the same well are usually not full siblings, they are often, if not always, half-sibs, the parent in common being the male defending the site. The prediction of such adaptive plasticity in *M. laevigata* eggs differs from Warkentin's system primarily in that the advantage to early hatching would go to half-siblings of the predated egg, rather than members of the same clutch.

Though *M. laevigata* and *P. notostica* eggs are both at risk from crane-fly larvae, tadpoles do not appear to be, as they were frequently seen co-existing in wells with crane-fly larvae. In such cases, the crane-fly larvae were often positioned above the water line, suggesting a possible reverse predation by tadpoles on the crane-flies, though this has not been tested.

Several aspects of the reproductive behavior of *M. laevigata* are clearly context-dependent. Examples include males scouting for oviposition sites, and not taking females to previously favored wells which are now changed for the worse, due to new inhabitants or low water level; females both scouting for wells alone, and discriminating between wells at the time of courtship, depending on the current contents of the well; and females ovipositing at different positions relative to the water line when they are in courtship, versus providing maternal care. The assessment required for all of these choices points to a sophisticated set of standards and rules on which choices are based.

To summarize, males defend water-filled wells, which are used as oviposition sites by females. Extremely low clutch size suggests that rare instances of direct maternal provisioning of tadpoles are not the only form of parental care in this species. Males win, reproductively, whenever they court a female that oviposits in a well in which one of the male's offspring already exists. A father’s reproductive success is aided whenever his tadpole eats an egg, be it from the tadpole's mother, the result of a courtship involving the father, or the result of a courtship involving neither mother nor father. Males do not take females to wells that contain frog species larger than themselves, nor do they expose courted females to the predatory crane-fly larvae that eat frog eggs. Females avoid courtships in wells that already have tadpoles in them, but tadpoles are probably selected to hide from disturbance, so as to increase their chances of being provisioned. The previously unknown social system and well community of *Mantella laevigata* is as complex and evolutionarily fascinating as that of the better-studied dart-poison frogs.

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## Literature Cited

Altmann, J. 1974. Observational study of behavior: sampling methods. *Behavior: An International Journal of Comparative Ethology* **49**:227-267.

Boulenger, E. G. 1914. Reptiles and Batrachians. J. M. Dent & Sons, Ltd., London.

Boulenger, G. A. 1882. Catalogue of the Batrachia Salientia s. Ecaudata in the Collection of the British Museum, 2nd edition. British Museum, London.

Brust, D. G. 1993. Maternal brood care by *Dendrobates pumilio* - a frog that feeds its young. Journal of Herpetology **27**:96-98.

Bunnell, P. 1973. Vocalizations in the territorial behavior of the frog *Dendrobates pumilio*. Copeia **1973**:277-284.

Caldwell, J. P. 1993. Brazil nut fruit capsules as phytotelmata - interactions among anuran and insect larvae. Canadian Journal of Zoology-Revue Canadienne De Zoologie **71**:1193-1201.

Caldwell, J. P. 1996. The evolution of myrmecophagy and its correlates in poison frogs (family Dendrobatidae). Journal of Zoology **240**:75-101.

Caldwell, J. P., and V. R. L. de Oliveira. 1999. Determinants of biparental care in the spotted poison frog, *Dendrobates vanzolinii* (Anura : Dendrobatidae). Copeia:565-575.

Crump, M. L. 1972. Territoriality and mating behavior in *Dendrobates granuliferus* (Anura: Denrobatidae). Herpetologica **28**:195-198.

Crump, M. L. 1991. Choice of oviposition site and egg load assessment by a treefrog. Herpetologica **47**:308-315.

Crump, M. L. 1995. Parental care. Pages 518-567 *in* H. Heatwole and B. K. Sullivan, editors. Amphibian Biology.

Crump, M. L. 1996. Parental care among the Amphibia. Advances in the Study of Behavior **25**:109-144.

Daly, J. W., N. R. Andriamaharavo, M. Andriantsiferana, and C. W. Myers. 1996. Madagascan poison frogs (*Mantella*) and their skin alkaloids. American Museum Novitates **3177**:1-34.

Daly, J. W., H. M. Garraffo, G. S. E. Hall, and J. F. Cover. 1997. Absence of skin alkaloids in captive-raised Madagascan mantelline frogs (*Mantella*) and sequestration of dietary alkaloids. Toxicon **35**:1131-1135.

Daly, J. W., R. J. Highet, and C. W. Myers. 1984. Occurrence of skin alkaloids in non-Dendrobatid frogs From Brazil (Bufonidae), Australia (Myobatrachidae) and Madagascar (Mantellinae). Toxicon **22**:905-919.

Daly, J. W., C. W. Myers, J. E. Warnick, and E. X. Albuquerque. 1980. Levels of Batrachotoxin and lack of sensitivity to Its action in poison-dart frogs (*Phyllobates*). Science **208**:1383-1385.

Daly, J. W., S. I. Secunda, H. M. Garraffo, T. F. Spande, A. Wisnieski, C. Nishihira, and J. F. Cover. 1992. Variability in alkaloid profiles in neotropical poison frogs (Dendrobatidae): genetic versus environmental determinants. Toxicon **30**:887-898.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science **197**:215-223.

Frost, D. R., and R. Etheridge. 1989. A phylogenetic analysis and taxonomy of Iguanian lizards (Reptilia: Squamata). University of Kansas Museum of Natural History, Miscellaneous publications **81**:1-65.

Glaw, F., and M. Vences. 1994. A Fieldguide to the Amphibians and Reptiles of Madagascar, 2 edition. Zoologisches Forschungsinstitut und Museum Alexander Koenig. Moos Druck, Leverkusen and FARBO, Köln, Bonn.

Hay, J. M., I. Ruvinsky, S. B. Hedges, and L. R. Maxson. 1995. Phylogenetic relationships of amphibian families inferred from DNA sequences of mitochondrial 12s and 16s ribosomal RNA genes. Molecular Biology and Evolution **12**:928-937.

Heying, H. 1997. Behaviour and sexual selection in *Mantella*, with comparisons to dendrobatids. *in* Third World Congress of Herpetology, Prague, Czech Republic.

Jones, T. H., J. S. T. Gorman, R. R. Snelling, J. H. C. Delabie, M. S. Blum, H. M. Garraffo, P. Jain, J. W. Daly, and T. F. Spande. 1999. Further alkaloids common to ants and frogs: Decahydroquinolines and a quinolizidine. Journal of Chemical Ecology **25**:1179-1193.

Juncá, F. A. 1998. Reproductive biology of *Colostethus stepheni* and *Colostethus marchesianus* (Dendrobatidae), with the description of a new anuran mating behavior. Herpetologica **54**:377-387.

Jungfer, K. H. 1996. Reproduction and parental care of the coronated treefrog, *Anotheca spinosa* (Steindachner, 1864) (Anura: Hylidae). Herpetologica **52**:25-32.

Kam, Y. C., Z. S. Chuang, and C. F. Yen. 1996. Reproduction, oviposition-site selection, and tadpole oophagy of an arboreal nester, *Chirixalus eiffingeri* (Rhacophoridae), from Taiwan. Journal of Herpetology **30**:52-59.

Kluge, A. G. 1991. Boine snake phylogeny and research cycles. Miscellaneous publications of the Museum of Zoology, University of Michigan **178**:1-58.

Krupa, J. J. 1995. How likely is male mate choice among anurans? Behaviour **132**:643-664.

Lannoo, M. J., D. S. Townsend, and R. J. Wassersug. 1986. Larval life in the leaves: arboreal tadpole types, with special attention to the morphology, ecology, and behavior of the oophagous *Osteopilus brunneus* (Hylidae) larva. Fieldiana **1381**:1-31.

Limerick, S. 1980. Courtship behavior and oviposition of the poison-arrow Frog *Dendrobates pumilio*. Herpetologica **36**:69-71.

Martin, P., and P. Bateson. 1993. Measuring behavior: an introductory guide, 2 edition. Cambridge University Press, Cambridge.

Mock, D. W., and G. A. Parker. 1997. The Evolution of Sibling Rivalry. Oxford University Press, Oxford.

Myers, C. W., J. W. Daly, and B. Malkin. 1978. A dangerously toxic new frog (*Phyllobates*) used by Embera Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. Bulletin of the American Museum of Natural History **161**:307-366.

Putland, D. A., and A. W. Goldizen. 1998. Territorial behaviour in the Tasmanian native hen: group and individual performance. Animal Behaviour **56**:1455-1463.

Roithmair, M. E. 1992. Territoriality and male mating success in the dart-poison frog, *Epipedobates femoralis* (Dendrobatidae, Anura). Ethology **92**:331-343.

Scotese, C. R., and J. Golonka. 1992. Paleomap Paleogeographic Atlas. Paleomap Project Department of Geology, University of Texas at Arlington.

Seymour, R. S., and D. F. Bradford. 1995. Respiration of amphibian eggs. Physiological Zoology **68**:1-25.

Spande, T. F., P. Jain, H. M. Garraffo, L. K. Pannell, H. J. C. Yeh, J. W. Daly, S. Fukumoto, K. Imamura, T. Tokuyama, J. A. Torres, R. R. Snelling, and T. H. Jones. 1998. Occurrence and significane of decahydroquinolines from dendrobatid poison frog and a myrmicine ant: Use of 1H- and 13-CNMR in their conformational analysis. Journal of Natural Products **65**:5-21.

Summers, K. 1989. Sexual selection and the cost of polygyny in the green poison-dart frog, *Dendrobates auratus*. American Zoologist **29**:2A.

Summers, K. 1999. The effects of cannibalism on Amazonian poison frog egg and tadpole deposition and survivorship in *Heliconia* axil pools. Oecologia **119**:557-564.

Summers, K., and W. Amos. 1997. Behavioral, ecological, and molecular genetic analyses of reproductive strategies in the Amazonian dart-poison frog, *Dendrobates ventrimaculatus*. Behavioral Ecology **8**:260-267.

Thompson, R. L. 1996. Larval habitat, ecology, and parental investment of *Osteopilus brunneus* (Hylidae). Pages 259-265 *in* R. Powell and R. W. Henderson, editors. Contributions to West Indian Herpetology: a tribute to Albert Schwartz. Society for the Study of Amphibians and Reptiles, Ithaca.

Ueda, H. 1986. Reproduction of *Chirixalus eiffingeri* (Boettger). Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University **8**:109-116.

Underwood, G. 1976. A systematic analysis of boid snakes. Pages 151-175 *in* A. d. A. Bellairs and C. B. Cox, editors. Morphology and Biology of Reptiles.

Vences, M., F. Glaw, and W. Bohme. 1998. Evolutionary correlates of microphagy in alkaloid-containing frogs (Amphibia : Anura). Zoologischer Anzeiger **236**:217-230.

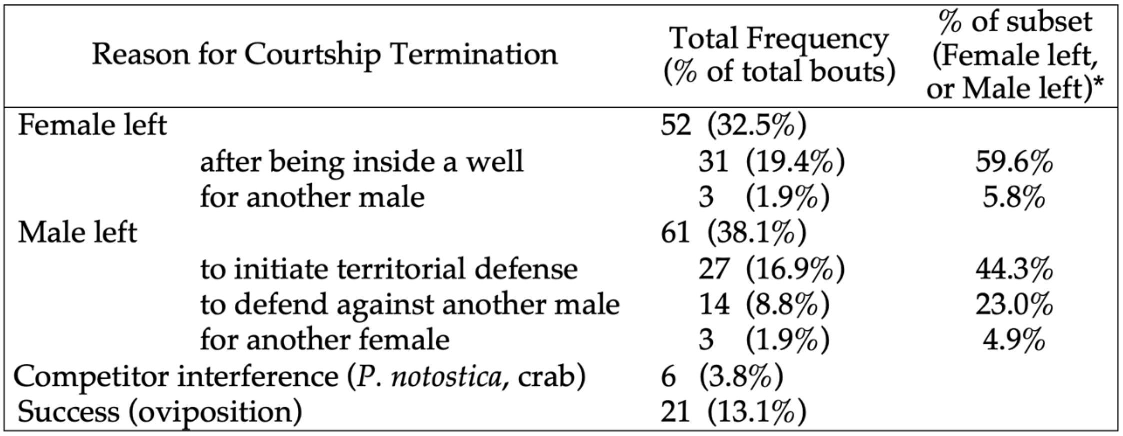
Warkentin, K. M. 1995. Adaptive plasticity in hatching age: a response to predation risk trade-offs. Proceedings of the National Academy of Sciences of the United States of America **92**:3507-3510.

Wells, K. D. 1977. The courtship of frogs. *in* D. H. Taylor and S. I. Guttman, editors. The Reproductive Biology of Amphibians. Plenum Publishing Corporation.

Wells, K. D. 1980. Behavioral ecology and social organization of a dendrobatid frog (*Colostethus inguinalis*). Behavioral Ecology and Sociobiology **6**:199-209.

Weygoldt, P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). Zeitschrift für Zoölogische Systematik und Evolutions forschung **25**:51-67.

## Table 2.1: How Courtship Bouts End



| Reason for Courtship Termination | | Total Frequency (% of total bouts) | % of subset (Female left, or Male left)* |
|---|---|---|---|
| Female left | | 52 (32.5%) | |
| | after being inside a well | 31 (19.4%) | 59.6% |
| | for another male | 3 (1.9%) | 5.8% |
| Male left | | 61 (38.1%) | |
| | to initiate territorial defense | 27 (16.9%) | 44.3% |
| | to defend against another male | 14 (8.8%) | 23.0% |
| | for another female | 3 (1.9%) | 4.9% |
| Competitor interference (*P. notostica*, crab) | | 6 (3.8%) | |
| Success (oviposition) | | 21 (13.1%) | |

Courtship bouts (N=160) end in a variety of ways, including females or males abandoning the courtship for any of several reasons. Frequency does not total 100% because it was sometimes impossible to gauge why or how a courtship ended.

*The final column is a measure of the conditions under which males and females left courtships (e.g., Of those females who left courtships, 59.6% (31/52) left only after being inside a well, while only 5.8% (3/52) left for another male).

**Table 2.2: Differences in Courtship Termination between Terminal and Non-Terminal Bouts**

| | Termination Reason | Non-Terminal Bouts (%) | Terminal Bouts (%) | Difference (p value) |
|---|---|---|---|---|
| A | Female leaves after being inside well | 7/32 (21.9) | 24/128 (18.75) | 0.65 |
| B | Male leaves to initiate territorial defense | 10/32 (31.3) | 17/128 (13.3) | 0.02 |
| C | Male leaves to defend himself against another male | 6/32 (18.8) | 8/128 (6.3) | < 0.0001 |
| D | Male leaves to engage in territorial activity (B + C) | 16/32 (50.0) | 25/128 (19.6) | 0.001 |

P-values generated using Logistic Regression with clustering of females (A) or males (B-D).

## Table 2.3: Male Discrimination of Wells in Bamboo Stand 4

| Type of well | Number of wells containing other organisms | | | |
|---|---|---|---|---|
| | Eggs | Tadpoles | Tipulids | Other frog species* |
| Average Well (N=238) | 71<br>(29.8%) | 90<br>(37.8%) | 43<br>(29.8%) | 71<br>(29.8%) |
| Wells Males take Females to (N=31) | 13<br>(41.9%) | 15<br>(48.4%) | 1<br>(3.2%) | 0<br>(0%) |
| Difference (Chi-Square Goodness of fit p-value) | p=0.11 | p=0.27 | p=0.02 | p=0.0004 |

*Other frog species include only *Plethodontohyla notostica* and *Anodonthyla boulengeri*

"Average Wells" include all data from wells in stand 4, which were surveyed every three days independent of focal watches. Each well was included in the survey only if, at some point during the 3.5 months of survey, evidence of *M. laevigata* reproductive activity was observed in that well (eggs, tadpoles, or courting adults directly observed).

## Table 2.4: Female Discrimination of Wells during Courtships

| Type of well | Egg Occupied | Tadpole Occupied |
|---|---|---|
| Wells Males take Females to (N=31) | 13 (41.9%) | 15 (48.4%) |
| Wells Females Accept (N=18) | 9 (50.0%) | 6 (33.3%) |
| Difference (Logistic regression 2-tailed p-values) | p=0.12 | p=0.02 |

Females reject wells which are already tadpole-occupied for oviposition. They do not discriminate against egg presence in wells; rather, there is a non-significant trend for females to favor wells with eggs in them.

**Figure 2.1: Map of Bamboo Stands containing Focal Populations on Nosy Mangabe**

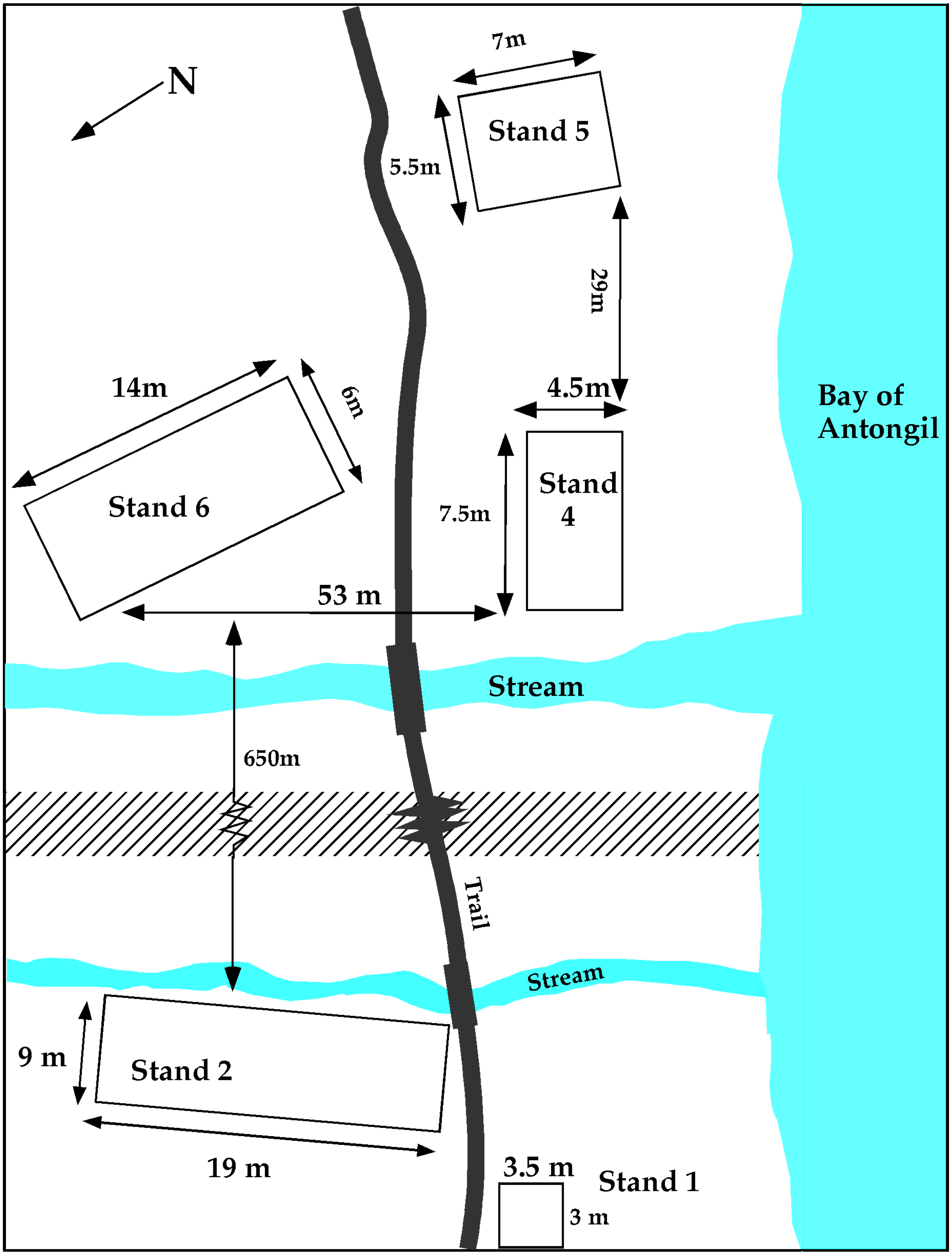


**Figure 2.2: Chinning Behavior of Male *Mantella laevigata* during Courtship**

During both initiation and continuation of courtship, the male repeatedly makes contact with the female by resting his throat on either the top of her head, or her back, while softly emitting a courtship call. The relative position of the courting animals is variable, but usually the male is facing the female, or perpendicular to her.

**Figure 2.2: Chinning Behavior of Male *Mantella laevigata* during Courtship**

# Chapter 3

# Reproductive Limitation by Oviposition Site in a Treehole Breeding Madagascan Poison Frog (*Mantella laevigata*)

## Abstract

Oviposition sites are a critical parameter of reproductive success in any species that does not move its young immediately after laying. Based on my previous observations, I hypothesized that oviposition sites—water-filled treeholes—are limiting for *M. laevigata*. I subjected *M. laevigata*, a Madagascan poison frog, to experimental manipulation to test this hypothesis.

In most systems, natural selection should adjust the effort required to procure necessary resources such that multiple resources ultimately co-limit population size. Experimental results failed to falsify the hypothesis that oviposition sites are limiting for *M. laevigata*, which suggests that, in this system, there are not multiple limiting factors. When a resource provides a unique benefit not otherwise replicable in the environment of the organism, and when critical resources aren't readily interchangeable, multiple limitation is not expected to occur. In the *M. laevigata* system, the number of viable oviposition sites cannot be increased by diverting energy from other resources. In light of the importance of oviposition sites, benefits of high quality treeholes are discussed, including protection from predation and physical mutilation from fast moving water, and the ability to keep track of offspring. Risks associated with low quality treeholes include desiccation, predation, cannibalism, and

competition. The importance of being choosy about oviposition site quality is discussed.

## Introduction

Limiting elements are defined as those aspects of the environment which, if increased, would result in an increase in population. The limiting element for a population can act in multiple ways, but its effect is always the same. The mechanism may be that adding more of a limiting agent causes existing individuals to become larger, more fecund, or at lower risk of predation, resulting in population growth in the next generation from increased offspring production, while the death rate remains stable. Alternately, the mechanism may be that the limiting agent causes increased immigration and reduced emigration from an area, such that population growth is a result of increased numbers in the current generation, and future generations are scaled larger as well. In systems with either of these mechanisms, the limiting agent could conceivably be nutrients, water, space, shelter from weather or predators, or oviposition sites.

Von Liebig (1855) was the first to formulate an ecological "law of the minimum," which hypothesizes that, for plants, growth is limited by a single resource at any one time. A plant is expected to increase growth in response to the addition of its single limiting resource, until it becomes limited by another resource.

In 1941, however, Hutchinson observed that, though it may be a simple matter to demonstrate a single limiting resource in the lab, in nature, many factors often contribute simultaneously to an organism's success. In particular, he noted that competition between species for resources may alter an individual species' "limit of tolerance" (Hutchinson 1941). Following from these early observations, optimal foraging theory suggests that, in nature, organisms should minimize effort spent foraging for non-limiting resources, and maximize time spent searching for and acquiring a limiting resource. Taken to its logical conclusion, optimal foraging theory thus predicts that morphology, physiology and behavior will be altered over evolutionary time such that no resource is taken up in excess, and thus all resources will ultimately simultaneously limit growth (Chapin et al. 1987, Gleeson and Tilman 1992).

The hypothesis of multiple limitation can be refined with judicious use of economic theory. All resources are not equally costly to obtain, and resources have not only an absolute cost, but also costs relative to other resources—their exchange ratios (Bloom et al. 1985). The extent to which a resource is limiting will depend on its exchange ratio with other resources. The exchange ratio between two resources depends on their supply, the demand for them, and the type of reserve being expended to acquire them (Bloom et al. 1985). Organisms that are selected to equalize exchange ratios are predicted, as a result, to have multiple limiting factors.

These economic-based predictions—that organisms should adjust allocation so that all resources equally limit growth—are not borne out by the data. There is evidence, however, that plants manipulate resource usage, and thus change the degree to which individual resources limit their primary productivity (Bloom et al. 1985). No single limiting factor was suggested when an attempt to distinguish between pollen and resource limitation in a plant failed (Campbell and Halama 1993). Multiple resources were observed to limit different parts of a biennial herb (Meekins and McCarthy 2000). In grasshoppers, both nitrogen and carbohydrate are limiting (Joern and Behmer 1997). Habitat quality appears to mediate the effects of nitrogen limitation in cotton rats (Schetter et al. 1998). And multiple limitations on breeding densities have been found in several species of birds (see Newton 1994 for a review). Thus, multiple resources may limit productivity, even when all resources do not.

Changing environmental conditions make availability of resources unpredictable, which will restrict the ability of organisms to adjust allocation of resources such that they all limit growth equally. But natural selection can be expected to expand usage capabilities for, or decrease reliance on, the single limiting factor, to that of the next limiting one. Limitation by a single resource implies excess capacity for acquiring other, non-limiting resources, which would be currency better spent in acquiring the limiting resource (Chapin et al. 1987). However, the finite nature of most resources will lead to a situation of diminishing returns, in which increases in effort yield smaller and smaller increases in availability. So, some systems will retain a single limiting factor, and in such cases, intense effort is likely to be exerted in competition over available resources rather than in seeking new sources (see discussion).

I studied resource limitation in *Mantella laevigata*, one of 11 species of Madagascan poison frog (Mantellinae, Ranidae). Adults of this species prey opportunistically on mites, ants, and other small terrestrial and flying insects. Males call regularly from defended territories, which include oviposition sites (water-filled phytotelmata: wells in bamboo or tree holes). Females approach only males issuing advertisement calls; after attracting a female, the male leads her to possible oviposition sites (Heying, in press, and chapter two). Females assess oviposition sites both before and during courtships, and most often reject courtships only after investigating the well to which the male has led her. Males defend their territories against other males, who often attempt to sneak matings in oviposition sites not their own. Males often abandon courtships in order to engage in territorial defense (chapter two). Females choose mates based on the quality of oviposition sites they call from, and not based on individual male calls, or the length of male calls (chapter five). Females provide facultative maternal care to their tadpoles by feeding them trophic eggs; tadpoles get most of their nutrition by cannibalizing fertilized eggs (Heying, in press, and chapter two).

The observations that females leave courtships after assessing oviposition sites; that males leave courtships to defend their oviposition site-containing territories; and that females choose mates based solely on oviposition site quality, all suggest that high-quality oviposition sites may be limiting in the environment of *M. laevigata*. This hypothesis prompted the current research.

Organisms with multiple limiting resources are not expected to respond to experiments in which single resources are increased in the short term. The experiment described here, in which oviposition sites were added to a population of *M. laevigata*, is therefore a test both of whether oviposition sites are limiting for *M. laevigata*, and of whether oviposition sites are the *only* limiting factor for *M. laevigata*, as it is impossible to demonstrate the first, without also demonstrating the latter.

## Methods

In each of two years (1997 and 1999), I conducted an experiment to test whether oviposition sites ('wells") were limiting for *Mantella laevigata*, by adding artificial wells to plots that were monitored by visual survey before and after addition of those wells. This experimental design followed from Donnelly's (1989) work on the dart-poison frog *Dendrobates pumilio*, which also use wells (bromeliads) as discrete reproductive resources, and forage opportunistically, decreasing the likelihood that food is limiting.

Research was conducted on the 510 hectare island of Nosy Mangabe, which lies at the Northern end of the Bay of Antongil, five km South of the town of Maroantsetra, Toamasina province, in northeastern Madagascar (15° 30′ S, 49° 46′ E). Nosy Mangabe is a "Special Reserve" in the Masoala National Park, consisting mainly of 100 – 400 year old secondary forest.

From surveys of the island of Nosy Mangabe, six areas were found (2 in 1997, 4 in 1999) in which *M. laevigata* were occasionally seen, very few natural treeholes were found, and there were no bamboo stands within 400 m (see Figure 3.1). The highest density of *M. laevigata* is found in and around bamboo stands, so it was desirable to eradicate the effect of bamboo from this experiment. Marked individuals from focal populations in bamboo stands were never observed to move more than 100 m (Heying, unpublished data).

For each of these unique areas, random compass directions were chosen, and three plots were laid, in one large transect, with the starting corner 2 meters off the nearest trail, moving in the compass direction chosen. Each transect was

10 m wide by 35 m long, and included three parallel plots which were 10 m long by 5 m wide, each 10 m apart from the next (see Figure 3.2 for one example).

Three days after establishing the plots, I began conducting visual surveys. Observers conducted visual scans of 15 minutes, with established starting points within plots, counting every *M. laevigata* adult and juvenile observed. We surveyed the plots this way approximately every three days. Observers were rotated through plots, to control for observer effect.

After eight (1997) or six (1999) surveys, during which time each observer had surveyed every plot multiple times, 1 plot in each area was randomly chosen to receive artificial wells which were filled with water, and maintained ("filled well treatment"). Another plot in each area was randomly chosen to receive wells which were not filled with water, nor maintained, except to insure that they remained upright and attached to trees ("unfilled well treatment"). The third plot in each area had no wells added to it, and served as a control plot.

Artificial wells consisted of small, brightly colored plastic cups, purchased in the nearby town of Maroantsetra. Seven artificial wells were placed in each treatment plot. In each treatment plot, well 1 had a 100 mm diameter, and a 575 ml capacity (filled wells were maintained at 475 ml); well 2 had a 90 mm diameter, and a 400 ml capacity (filled wells were maintained at 345 ml); and wells 3-7 had a 55 mm diameter, and a 125 ml capacity (filled wells were maintained at 95 ml). Wells 1, 3, and 5 were placed at 0.5 m above the ground; wells 2 and 4 at 1 m above the ground, and wells 6 and 7 at 1.5 m above the ground.

Three days after artificial wells were added to the treatment plots, visual surveys resumed, and continued for 14 (1997) or 18 (1999) more surveys, approximately every three days, as before. Data were kept on how many adult and juvenile *M. laevigata* were seen during each survey, who conducted each survey; weather conditions; and time of day (before 11 a.m., between 11 a.m. and 1 p.m., or after 1 p.m.).

Experimental set-up and design in 1997 and 1999 were identical, with the following exceptions. In 1997 there were only 2 experimental areas, in 1999 there were 4. In 1997, there were two observers (the author, and Jessica Metcalf), in 1999, there were 3 (the author, Bret Weinstein, and Glenn Fox). In 1997, eight surveys were conducted, over the course of 23 days, before wells were added to treatment plots. 14 more surveys were conducted following well addition, over a span of 38 days. In 1999, 6 surveys were conducted, over the course of 16 days, before wells were added to treatment plots. 16 more surveys were conducted following well addition, over a span of 46 days. Research was conducted during the rainy season, February through April, in both years.

This experimental design allowed me to address the following questions with my data. These include:

- Does the presence of wells affect population density in *M. laevigata*?
- Do maintained wells (filled with water and cleared of detritus after each survey) attract significantly more *M. laevigata* than do wells that are neither filled with water, nor cleared of detritus?
- Can the difference in population density after the addition of wells be attributed to seasonality? Is there an effect on population density in the

"control" plots over time, which remained without artificial wells for the entire experiment?

- Is there an "area" effect (e.g. do frogs show up in higher numbers in some areas, each of which contains three plots, one of each category, than they do in others?)
- Is there an "observer" effect?
- Is there a "weather" effect?
- Is there a "time of day" effect?
- Is there a "year" effect?

## Results

The presence of artificial wells is correlated with an increase in population density in *M. laevigata*. Both filled well plots and unfilled well plots are significantly different, with respect to *M. laevigata* population density, from control plots (Figure 3.3). When treatments are lumped (plots with filled/maintained wells with plots with unfilled/unmaintained wells), and time is held constant, *M. laevigata* population density in plots with added wells is significantly higher than in control plots (Figure 3.4). Unfilled plots are not significantly different from filled plots with respect to population density before wells were added. After wells were added, non-filled wells actually attracted more frogs than filled wells, although these plots did have a higher baseline (Figure 3.5). There was a non-significant *decrease* in population density in the control plots after wells were added to adjacent plots within an area (Figure 3.3).

The six distinct areas, in each of which three plots were laid (2 in 1997, 4 in 1999), were significantly different from each other with respect to frog density (Figure 3.6). Weather also affected the numbers of frogs seen, with cooler, wetter weather generally being correlated with higher numbers of observed frogs (Figure 3.7). This result is even more clear when the weather data is collapsed into two categories: hot and/or dry, and wet and/or cool (Mann-Whitney U, $U=18608.0$, $p<0.0021$). Time of day had no significant effect on observed population density (Figure 3.8), nor did observer (Kruskal-Wallis, $H=2.985$, $p=0.2952$). Year did have a significant effect on population density of *M. laevigata*, with significantly more individuals observed per survey in 1997 than in 1999 (Mann-Whitney U, $U=15893.5$, $p=0.0027$). The experimental design controlled for all of these potential correlates of observed population density, so none affect the basic question of whether wells limit *M. laevigata* density.

## Discussion

Wells are limiting for *M. laevigata*. Only two previous studies have demonstrated population limitation by resources in anurans (Stewart and Pough 1983, Donnelly 1989). My two experimental treatments (unfilled wells added versus filled wells added) did not differ, probably because, during the rainy season, “unfilled” wells quickly fill and remain filled naturally with rainwater. The added security of having a well that is maintained at a constant level, and cleaned of excess detritus, is an evolutionary novelty, and one that *M. laevigata* should not be expected to respond to. Indeed, the addition of rainwater, and regular disturbance by humans, might be a deterrent, making the “filled” wells less desirable than the “unfilled” ones in times of water excess, although all wells, of both treatment types, were visited, and manually disturbed, after each survey, to control for experimenter influence. During a time of drought, when the “unfilled” wells were in fact empty of water some of the time, I would predict a difference between these two treatments. Furthermore, the “control” plots actually showed a non-significant trend towards a decrease in population density after the wells were added in the treatment plots. This suggests that the mechanism of limitation in this system is to reduce immigration when resources (wells) are in short supply. Furthermore, the populations from which animals are immigrating from and emigrating to are very local. As such, a local bounty—such as the addition of 14 artificial wells within a single area—apparently causes emigration out of the plot without additional wells.

I failed to falsify my hypothesis that wells are limiting for *M. laevigata*. This begs the question, in light of earlier arguments regarding multiple limiting resources: what about this system has caused natural selection to allow a single limiting factor?

Multiple limitation occurs when effort devoted to acquiring one (non-limited) resource is shunted towards effort to acquire a different (limited) resource. When extra, limited resource can be obtained by exchanging effort in this way, natural selection will tend to equalize availability of multiple resources. For instance, if there were an infinite number of oviposition sites (wells) in the environment of *M. laevigata*, the frogs would need only find those wells in order to utilize them. By converting the non-limiting resource of food into energy used to discover additional (limiting) wells, the frogs could, theoretically, ultimately exist in a system of multiple limitation. But wells are not infinite, and all wells in the unmanipulated system are already being used. No matter how much food energy is put into searching for new oviposition sites, there will be no more; other resources cannot be converted into wells.

## Why breed in treeholes?

Anuran treehole (well) breeders utilize a distinct niche during their larval stage. In the absence of direct development or extreme behavioral adjustment on the part of the parents (found in the gastric brooding frog, or when one of the parents carries the clutch on its back), which is known from very few anurans, these species must go through both an egg and tadpole stage in which they rely on a single, wet environment. That environment is the result of oviposition site choice by one or both parents.

On Nosy Mangabe and the Masoala peninsula, where *M. laevigata* are known, small ponds of surface water do not exist. The fresh water in these forests is entirely in rivers; small, fast moving streams which flood regularly during the rainy season; and treeholes. In a system that currently utilizes treeholes, the adaptive valley between this state and the nearest possible one, that of utilizing small, fast moving, often-flooded streams, is vast and deep. Moving between these two adaptive peaks would require a set of modifications in the offspring that would be distinctly suboptimal in these two, divergent habitats (Wright 1932).

*M. laevigata* has a clutch size of one, which is apparently adaptive in a treehole breeding environment in which parents can keep track of their offspring

(Heying, in press, and chapter two). However, this minimal clutch size would almost certainly condemn most or all clutches to either physical mutilation in the fast moving environment of a stream or river, or to predation, especially by fish or dragonfly larvae, known predators of amphibian eggs and larvae in other systems (e.g. Resetarits and Wilbur 1989, Kats and Sih 1992, Hopey and Petranka 1994, Holomuzki 1995).

Minimal clutch size is possible in *M. laevigata*, in part, because of the reduced number of predators in temporary treeholes. Freedom from predation is a clear advantage to breeding in restricted water bodies. Furthermore, treeholes provide an easily defensible territory for males, which allows them to increase their certainty of paternity by excluding other males. Continuing defense of and courtship in wells already containing tadpoles constitutes, at the very least, passive paternal investment by the male territory holder. Males dissuade competitor species from displacing tadpoles. And eggs resulting from later courtships between the father/territory holder and an unrelated female often go to feed an existing tadpole (Heying, in press, and chapter two). Females gain whenever males invest in offspring. As in the coquí frog (Stewart and Rand 1991), defense of retreat sites offers advantages to both sexes in *M. laevigata.*

## Why be choosy about oviposition site quality?

Resetarits, Jr. (1996) argued that oviposition site choice must be under equally strong selection as egg and clutch size, in order to generate locally adapted life history phenotypes and optimize parental fitness. If oviposition site quality is variable, and correlated with offspring success, both sexes are expected to be choosy about this parameter. In *M. laevigata*, females are choosing oviposition site and *not* male quality or current condition (chapter five), suggesting a particularly important role for oviposition site quality in reproductive success in this species.

Oviposition sites act as patches, each of which contain various degrees of resource and risk (Resetarits Jr. 1996). The resources available to *M. laevigata* in high-quality treeholes in the Masoala include deep, and therefore reliable, water, which is likely to attract future courtships, providing food for tadpoles in the form of fertilized eggs; and territorial defense by the resident male against intruding competitors (Heying, in press, and chapter two). The risks of low-quality treeholes include desiccation, predation, cannibalism, and competition from heterospecifics.

Desiccation of eggs or larvae is a real danger for amphibians. Semlitsch and Gibbons (1990) found that pond drying is inversely correlated with larval success in salamanders. In treehole breeders, the amount of water in the well and the ambient humidity are both known to affect survivorship (damselflies, Fincke 1994; rhacophorid frogs, Kam et al. 1998). Several studies have revealed oviposition site preference for a wetter, or potentially wetter, environment in amphibians (treefrogs: Crump 1991; salamanders: Figiel and Semlitsch 1995;

ranid frogs: Spieler and Linsenmair 1997). *M. laevigata* do not oviposit in dry or shallow wells (see chapter four).

Predation is reduced in treeholes, but it is generally a prominent source of mortality in anuran eggs and tadpoles. Adults choose oviposition sites that reduce the risk of predation in several species, including from bullfrogs predated by leeches (Howard 1978); treefrogs predated by salamanders and fish (Resetarits Jr. and Wilbur 1989); from fish on wood frogs (Hopey and Petranka 1994); American toad larvae predated by wood frog tadpoles (Petranka et al. 1994); pickerel frogs and American toads predated by fish (Holomuzki 1995); and red-eyed tree frogs predated by shrimp (Warkentin 1999). In treeholes, the risk of predation from salamanders, fish and shrimp is essentially zero. Insect larvae remain a threat, however. In Brazil nut fruit capsules, which are analogous to treeholes, poison frog tadpoles are susceptible to predation by both mosquito and damselfly larvae (Caldwell 1993). In bamboo wells, chironimid and tipulid larvae prey on the eggs of well-breeding rhacophorids (Kam et al. 1998). And crane-fly larvae (*Limonia renaudi* Alexander, Tipulidae) prey on the eggs of at least three anuran well-breeders in the Masoala, including *M. laevigata*. Adult female *M. laevigata* reject oviposition sites that contain predatory crane-fly larvae (Heying, in press, and chapter two).

Cannibalism, a subset of predation, is also a threat to anuran larvae. It is, therefore, a parameter that females choosing oviposition sites should take into account. Crump (1991) demonstrated experimentally that female treefrogs prefer to oviposit in artificial pools lacking conspecific tadpoles, which are known to be cannibalistic. Similarly, African ranid frogs avoid ovipositing in pools where there are cannibalistic conspecifics (Spieler and Linsenmair 1997), as do leptodactylid frogs (Halloy and Fiaño 2000). In *M. laevigata*, females reject oviposition sites that already contain conspecific tadpoles, though they do not reject sites with conspecific eggs (Heying, in press, and chapter two).

Competition from other species is less well known in anuran larvae. Male gobies experience competition for nest sites from invertebrates and larger fish (Breitburg 1987, Kroon et al. 2000), and female salmon compete with breeding space with other, related species (Essington et al. 2000). Damselflies compete for treeholes with other species of odonates (Fincke 1992). In anurans, adults of one species of treefrog avoid ovipositing in ponds that already contain the competitive larvae of another treefrog (Resetarits Jr. and Wilbur 1989). In *M. laevigata*, it is the males, rather than the females, who discriminate against oviposition sites containing competing species of microhylid frogs and their clutches (Heying, in press, and chapter two).

Given the prevalence of risk from desiccation, predation, cannibalism, and competition for anuran larvae generally, oviposition site choice must be critical to reproductive success. In other taxa, oviposition site preference by females has been correlated with increased parental reproductive success. Choosy damselflies show increased offspring survivorship (Siva-Jothy et al. 1995), and choosy pied flycatchers gain an increase in clutch size (Siikamäki 1995). In *M. laevigata*, where oviposition sites are limiting, and both sexes discriminate among these oviposition sites, it is likely that parental reproductive success is enhanced by this parental behavior.

For the same reasons that many resources or factors may be simultaneously limiting, females may select multiple aspects of males/resources when choosing mates. That which is most variable in the population at the time of choice is likely to be that which is most actively chosen by females (as in sparrows, Reid and Weatherhead 1990). If natural selection is continually readjusting the need for and availability of limiting factors, then we should expect populations to evolve in response to those changes as well. Females will choose mates differently if food is limiting and males control access to the food, than if food is widely available, and males control no resources that females require. Thus, it is important to conduct choice experiments at the same time (during the same season) as limitation experiments, in order to attempt to "match up" what's limiting with what females are choosing.

## Conclusions

In any stable population, some parameter is limiting population growth. Given the propensity for natural selection to act on parameters that limit survivorship, growth, and reproduction, individuals are expected to be limited by multiple factors under most conditions. In some systems, however, multiple limitation does not occur. *M. laevigata* presents one such system. The single limiting factor for *M. laevigata* is treehole oviposition sites, a resource so unique in the environment of *M. laevigata* that abandoning treeholes as oviposition sites would result in catastrophic reproductive failure. Given the current reliance on treeholes for reproduction, *M. laevigata* is expected to shunt effort spent acquiring other resources to finding and/or acquiring oviposition sites. Given a finite number of oviposition sites, however, additional effort acquired through food energy will, ultimately, fail to result in the discovery of additional oviposition sites. If there is still excess food in the environment, which is likely for the opportunistically foraging *M. laevigata*, natural selection might convert those resources into increased competition for oviposition sites. Though competition for oviposition sites is not a resource, per se, but a behavior that can lead to the acquisition of limiting resources, this prediction points to a possible role for non-limiting factors. Non-limiting food could, with increased uptake, result in increased levels of competition for oviposition sites, altering the nature of territorial disputes and, ultimately, the social system.

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## Literature Cited

Bloom, A. J., F. S. Chapin, and H. A. Mooney. 1985. Resource limitation in plants—an economic analogy. Annual Review of Ecology and Systematics **16**:363-392.

Breitburg, D. L. 1987. Interspecific competition and the abundance of nest sites: factors affecting sexual selection. Ecology **68**:1844-1855.

Caldwell, J. P. 1993. Brazil nut fruit capsules as phytotelmata - interactions among anuran and insect larvae. Canadian Journal of Zoology-Revue Canadienne De Zoologie **71**:1193-1201.

Campbell, D. R., and K. J. Halama. 1993. Resource and pollen limitations to lifetime seed production in a natural plant population. Ecology **74**:1043-1051.

Chapin, F. S., A. J. Bloom, C. B. Field, and R. H. Waring. 1987. Plant responses to multiple environmental factors. Bioscience **37**:49-57.

Crump, M. L. 1991. Choice of oviposition site and egg load assessment by a treefrog. Herpetologica **47**:308-315.

Donnelly, M. A. 1989. Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. Ecological Monographs **59**:207-221.

Essington, T. E., T. P. Quinn, and V. E. Ewert. 2000. Intra- and inter-specific competition and the reproductive success of sympatric Pacific salmon. Canadian Journal of Fisheries and Aquatic Sciences **57**:205-213.

Figiel, C. R., and R. D. Semlitsch. 1995. Experimental determination of oviposition site selection in the marbled salamander, *Ambystoma opacum*. Journal of Herpetology **29**:452-454.

Fincke, O. M. 1992. Interspecific competition for tree holes: consequences for mating systems and coexistence in Neotropical damselflies. American Naturalist **139**:80-101.

Fincke, O. M. 1994. Population regulation of a tropical damselfly in the larval stage by food limitation, cannibalism, intraguild predation and habitat drying. Oecologia **100**:118-127.

Gleeson, S. K., and D. Tilman. 1992. Plant allocation and the multiple limitation hypothesis. American Naturalist **139**:1322-1343.

Halloy, M., and J. M. Fiaño. 2000. Oviposition site selection in *Pleurodema borellii* (Anura: Leptodactylidae) may be influenced by tadpole presence. Copeia:606-609.

Heying, H. E. in press. Social and reproductive behaviour in the Madagascan poison frog, *Mantella laevigata*, with comparisons to the dendrobatids. Animal Behaviour.

Holomuzki, J. R. 1995. Oviposition sites and fish-deterrent mechanisms of two stream anurans. Copeia:607-613.

Hopey, M. E., and J. W. Petranka. 1994. Restriction of wood frogs to fish-free habitats: how important is adult choice? Copeia:1023-1025.

Howard, R. D. 1978. The influence of male-defended oviposition sites in early embryo mortality in bullfrogs. Ecology **59**:789-798.

Hutchinson, G. E. 1941. Ecological aspects of succession in natural populations. American Naturalist **75**:406-418.

Joern, A., and S. T. Behmer. 1997. Importance of dietary nitrogen and carbohydrates to survival, growth, and reproduction in adults of the grasshopper *Ageneotettix deorum* (Orthoptera: Acrididae). Oecologia **112**:201-208.

Kam, Y. C., C. F. Yen, and C. L. Hsu. 1998. Water balance, growth, development, and survival of arboreal frog eggs (*Chirixalus eiffingeri*, Rhacophoridae): Importance of egg distribution in bamboo stumps. Physiological Zoology **71**:534-540.

Kats, L. B., and A. Sih. 1992. Oviposition site selection and avoidance of fish by streamside salamanders (*Ambystoma barbouri*). Copeia:468-473.

Kroon, F. J., M. de Graaf, and N. R. Liley. 2000. Social organisation and competition for refuges and nest sites in Coryphopterus nicholsii (Gobiidae), a temperate protogynous reef fish. Environmental Biology of Fishes **57**:401-411.

Meekins, J. F., and B. C. McCarthy. 2000. Responses of the biennial forest herb *Alliaria petiolata* to variation in population density, nutrient addition and light availability. Journal of Ecology **88**:447-463.

Newton, I. 1994. Experiments on the limitation of bird breeding densities: a review. Ibis **136**:397-411.

Petranka, J. W., M. E. Hopey, B. T. Jennings, S. D. Baird, and S. J. Boone. 1994. Breeding habitat segregation of wood frogs and American toads: the role of interspecific tadpole predation and adult choice. Copeia:691-697.

Reid, M. L., and P. J. Weatherhead. 1990. Mate choice criteria of Ipswich sparrows: the importance of variability. Animal Behaviour **40**:538-544.

Resetarits Jr., W. J. 1996. Oviposition site choice and life history evolution. American Zoologist **36**:205-215.

Resetarits Jr., W. J., and H. M. Wilbur. 1989. Choice of oviposition site by *Hyla chrysosocelis*: role of predators and competitors. Ecology **70**:220-228.

Schetter, T. A., R. L. Lochmiller, D. M. Leslie, D. M. Engle, and M. E. Payton. 1998. Examination of the nitrogen limitation hypothesis in non-cyclic populations of cotton rats (*Sigmodon hispidus*). Journal of Animal Ecology **67**:705-721.

Semlitsch, R. D., and J. W. Gibbons. 1990. Effects of egg size on success of larval salamanders in complex aquatic environments. Ecology **71**:1789-1795.

Siikamäki, P. 1995. Habitat quality and reproductive traits in the Pied Flycatcher--an experiment. Ecology **76**:308-312.

Siva-Jothy, M. T., D. W. Gibbons, and D. Pain. 1995. Female oviposition site preference and egg hatching success in the damselfly *Calopteryx splendens Xanthostoma*. Behavioral Ecology and Sociobiology **37**:39-44.

Spieler, M., and K. E. Linsenmair. 1997. Choice of optimal oviposition sites by *Hoplobatrachus occipitalis* (Anura: Ranidae) in an unpredictable and patchy environment. Oecologia **109**:184-199.

Stewart, M. M., and F. H. Pough. 1983. Population density of tropical forest frogs: relation to retreat sites. Science **221**:570-572.

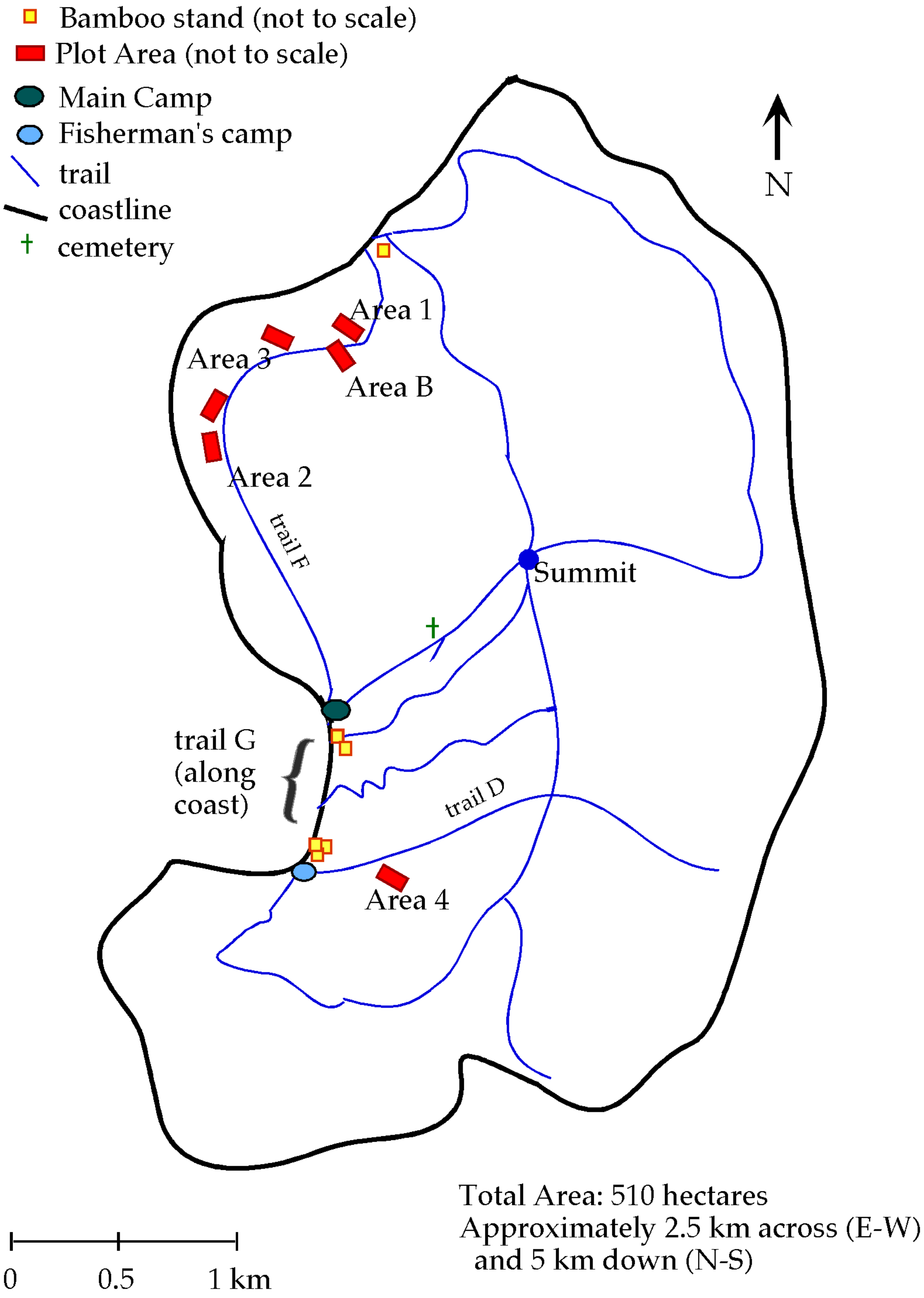
Stewart, M. M., and A. S. Rand. 1991. Vocalizations and the defense of retreat sites by male and female frogs, *Eleutherodactylus coqui*. Copeia **1991**:1013-1024.

Von Liebig, J. 1855. Die Grundsatze der Agriculturchemie. Braunschweig, Viewig.

Warkentin, K. M. 1999. The development of behavioral defenses: a mechanistic analysis of vulnerability in red-eyed tree frog hatchlings. Behavioral Ecology **10**:251-262.

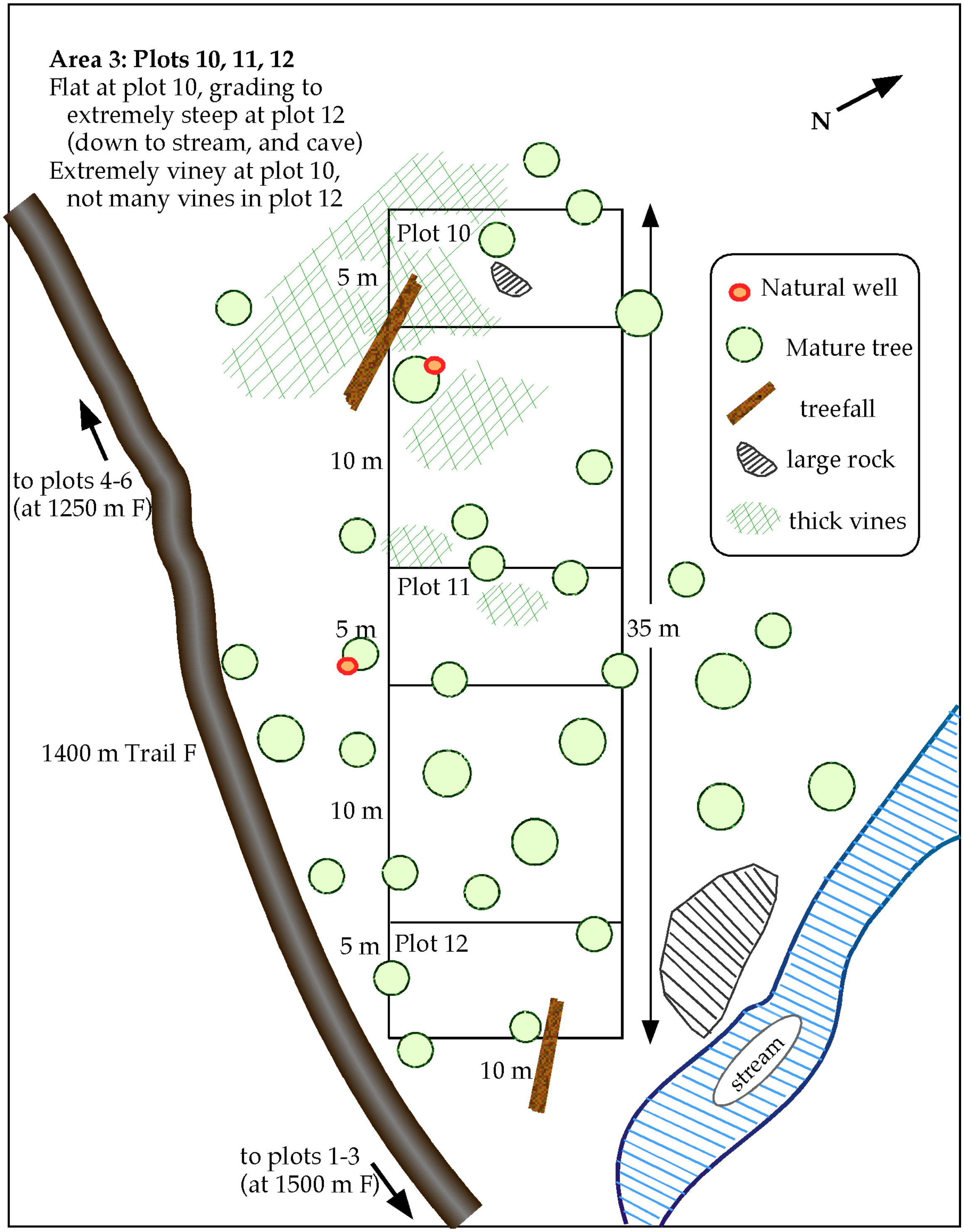
Wright, S. 1932. The roles of mutation, inbreeding, crossbreeding and selection in evolution. Proceedings of the Sixth International Congress of Genetics **1**:356-366.

**Figure 3.1: Plot Areas on Nosy Mangabe**



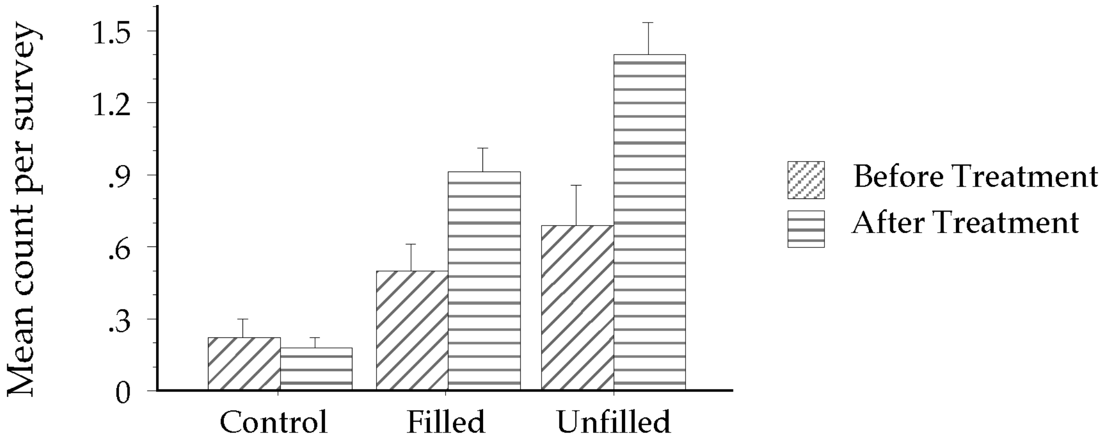
Total Area: 510 hectares
Approximately 2.5 km across (E-W)
and 5 km down (N-S)

## Figure 3.2: Physical Environment of Area 3, with parallel plots



## Figure 3.3: Effect of the Addition of Wells on Population Density of *M. laevigata*

(Before and after addition of wells to treatment plots)



The addition of wells significantly affected population density in *M. laevigata* in plots to which unfilled wells were added ("Unfilled").

Mann-Whitney U, U=1258.0, tied-p=0.002

The addition of wells also significantly affected population density in *M. laevigata* in plots to which filled wells were added ("Filled").

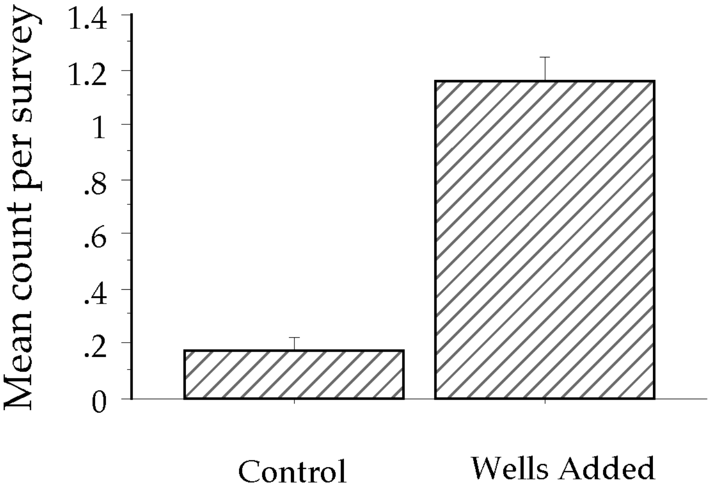
Mann-Whitney U, U=1473.0, tied-p=0.040

Plots to which wells were never added ("Control") did not differ significantly between the same time periods, although there was an unexpected, non-significant trend for frogs to leave these plots, probably as a result of movement into neighboring treatment plots.

Mann-Whitney U, U=1764.0, p=0.406

**Figure 3.4: Effect of the Addition of Wells on Population Density of *M. laevigata***

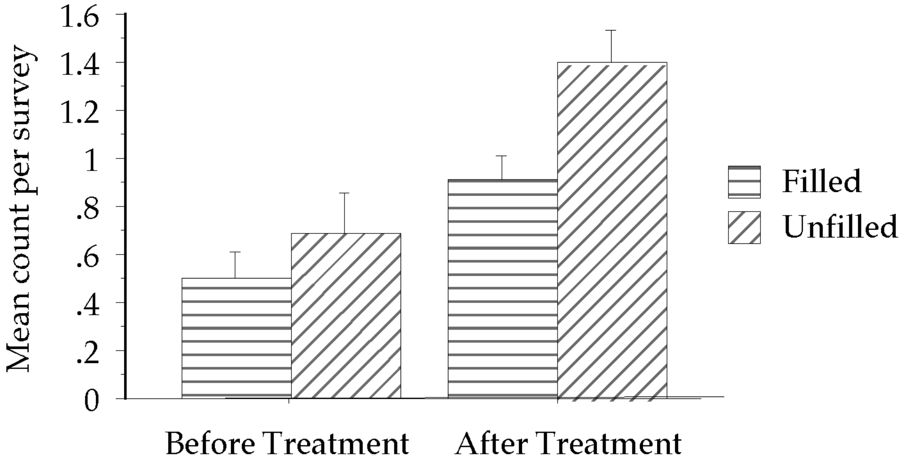
(During concurrent surveys, after wells were added)



Data from surveys 7-24 only: after wells were added to "Treatment" plots. During the identical timeperiod, all plots with wells in them attracted significantly more *M. l aevigata* than did control plots.

Mann-Whitney U, U=5121.5, $p<0.0001$

## Figure 3.5: Effect of Unfilled versus Filled Wells on Population Density of *M. laevigata*



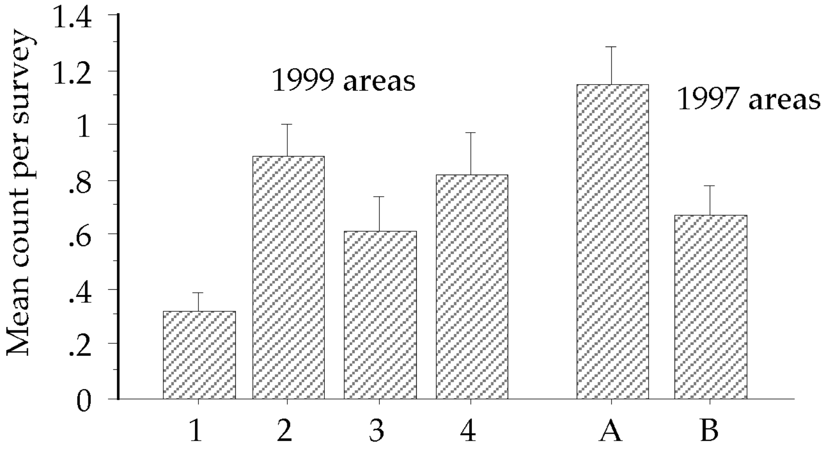
Before the addition of wells, plots which would later receive unfilled and filled wells were not statistically different wth respect to frog density.

Mann-Whitney U, U=606.0, p=0.594

Contrary to expectations, plots with added unfilled wells attracted significantly more M. laevigata than did plots with filled wells.

Mann-Whitney U, U=4311.0, p=0.008

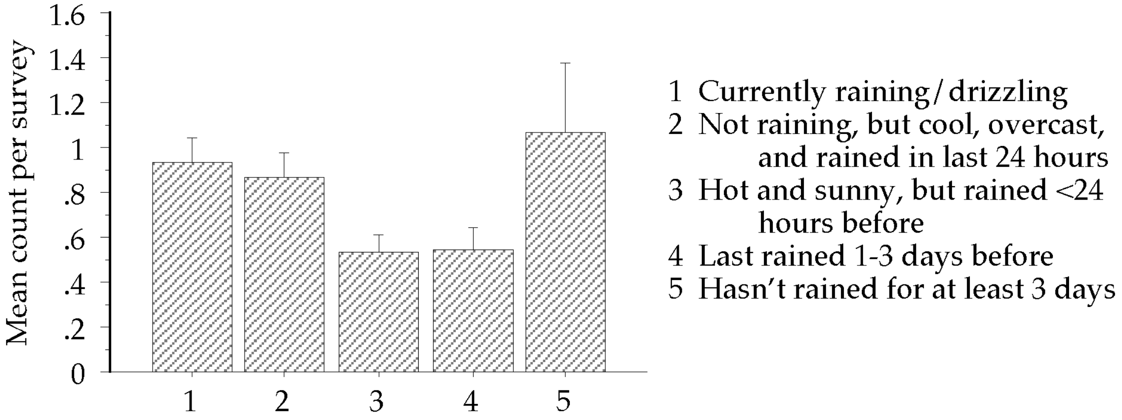
## Figure 3.6: Area Effect on Population Density of *M. laevigata*



Area has a significant effect on population density.

Kruskal-Wallis, H=27.112, p<0.0001

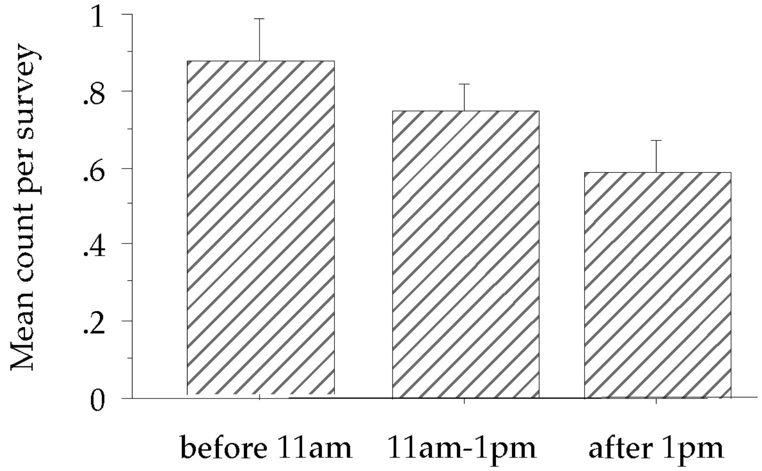
## Figure 3.7: Weather Effect on (observed) Population Density of *M. laevigata*



Weather significantly affects the apparent population density (the observers' ability to find frogs).

Kruskal-Wallis, H=11.145, p=0.0079

**Figure 3.8: Time of Day Effect on Population Density of *M. laevigata***



There was no effect of time of day on observed population density.

Kruskal-Wallis, H=2.562, p=0.2039

# Chapter 4

## *Not in My Phytotelm*
## Of neighbors, architecture, and water quality: Correlates of well use by *Mantella laevigata*

## Abstract

Phytotelmata, the small wells of water found within or upon plants, can provide relatively safe refuges for a variety of organisms. I monitored 53 wells every three days for ten weeks, collecting data on *M. laevigata* eggs and tadpoles, water level, pH, conductivity, dissolved oxygen, and the presence or absence of several other species in the phytotelm assemblage, including larval crane-flies and mosquitoes, adult crabs, and adults and clutches of five other frog species. *Mantella* eggs and tadpoles were more likely to be found in wells that were acidic (pH < 4.4), short, and full of water. Wells that produced the most *Mantella* metamorphs had the lowest pH, and contained the fewest crane-fly larvae and members of other frog species. The presence of the microhylid frog *Plethodontohyla notostica* is predictive of a reduction in new *Mantella* eggs, but also of a reduction in the loss of preexisting *Mantella* eggs. The paternal care offered by *P. notostica* to its clutch may benefit preexisting *Mantella* eggs as well, but the male's "bark" at intruders serves to dissuade *Mantella* from ovipositing once a well is inhabited by a *P. notostica*. Finally, the presence of (cannibalistic) *Mantella*

tadpoles predicts the loss of *Mantella* eggs from a well. After a metamorph leaves a well, however, there is an immediate positive change in the number of eggs in that well, suggesting that adults of both sexes are constantly assessing the status of viable oviposition sites. I delineate the food web of the phytotelmata of Nosy Mangabe, and describe the relative importance of each of the factors, abiotic and biotic, contributing to the success of *M. laevigata*.

## Introduction

Phytotelmata, the small pools of water found within or upon plants, can provide relatively safe refuges for a variety of organisms. First formally described by Varga in 1928, they are more common in the tropics than in the temperate zone (Thienemann 1954). Phytotelmata (sometimes referred to as phytotomes) may contain liquid derived from plant secretions, or from rainwater, and may be formed out of leaf axils, flowers, stems, or from the fallen parts of plants (Kitching 1971).

Phytotelmata are found in at least 29 plant families (Fish 1983). These include bamboo (Corbet 1964, Berry 1972, Kurihara 1983, Louton et al. 1996, Sota and Mogi 1996), bromeliads (see review in Frank 1983), pitcher plants (see review in Beaver 1983, also Ratsirarson and Silander 1996), wild ginger (Thienemann 1954), *Pandanus* (Kitching 1971), traveler's palms (Blommers-Schlösser 1975), Brazil nut fruit capsules (Caldwell 1993), and many species of large canopy trees that have formed shallow cavities in their surface, either through natural growth or through rot (Kitching 1971, Wassersug et al. 1981). Studies of the fauna of phytotelmata are so prevalent as to have warranted a published bibliography more than 20 years ago (Fish and Beaver 1978), and several papers since then contain tables and appendices summarizing the fauna (e.g. Frank and Lounibos 1983). More than 1,500 animal species are documented to use phytotelmata (Fish 1983), and probably many more than that. Invertebrate fauna using phytotelmata include several species of nematodes and annelids (Sota and Mogi 1996), rotifers (Laessle 1961, Kitching 1971), mites (Kitching 1971, Ratsirarson and Silander 1996), copepods (Laessle 1961, Kitching and Orr 1996), and crabs (Laessle 1961). Several orders of arthropod larvae are also known to live and develop in phytotelmata. Most prevalent among these are the Diptera, especially mosquitoes (which are known from all phytotelm studies to date), and crane-flies (Fish 1983, Louton et al. 1996, Kam et al. 1998); Odonata (see review in Corbet 1983, also Fincke 1998), and Coleoptera (Laessle 1961, Kitching 1971). Many studies of particular types of phytotelmata find representatives of all of these arthropods (e.g. Laessle 1961, Sota and Mogi 1996).

Vertebrate inhabitants of phytotelmata are more restricted in number and diversity. Temporary residents include lizards (e.g. lacertids, Corbet 1961; gekkos, Ratsirarson and Silander 1996) and adult frogs that do not breed in phytotelmata (e.g. *Heterixalus tricolor*, Hyperoliidae, Ratsirarson and Silander 1996; *Afrixalus fulvovittatus*, Polypedatidae, Corbet 1964; *Eleutherodactylus jamaicensis*, Leptodactylidae, Laessle 1961). Longer-term vertebrate residents are restricted to anuran eggs and tadpoles, and, in some species, an attending parent (e.g. *Plethodontohyla notostica*, Microhylidae, Blommers-Schlösser 1975).

Frogs across several families are known to breed in phytotelmata, including bufonids (Brown and Alcala 1983), dendrobatids (Silverstone 1975), hylids (Dunn 1926), leptodactylids (Peixoto 1981), microhylids (Blommers-Schlösser 1975), ranids (Blommers-Schlösser 1979), and rhacophorids (Wassersug et al. 1981; also see Lannoo et al. 1986 for review of well-breeding anurans). Among the ranids, phytotelm breeders are limited to several Malagasy species of Mantellinae (Blommers-Schlösser 1979), and the Philippine tree frog (Gibbons and Guinea 1983). Within the mantellines, *Mantella laevigata* is the only member of its genus to breed in phytotelmata.

In all well-breeding anuran species, tadpoles, and usually eggs, necessarily interact with other members in the restricted space of the phytotelm. It is, therefore, of interest to study habitation patterns of phytotelm-dwelling fauna, in order to infer both positive and negative interactions between species. There has been extensive work on food webs in phytotelmata (e.g. Beaver 1983, Kitching 1983, Louton et al. 1996, Kitching and Orr 1996), but work focusing specifically on the effect of co-inhabitants on larval anurans has been limited, and restricted to the Neotropics (e.g. Caldwell 1993).

In addition to the many animal inhabitants a single phytotelm might have, phytotelm structure is altered by abiotic and plant parameters. Water chemistry variables in phytotelmata, including pH, dissolved oxygen, and conductivity, have been well-studied (Laessle 1961, Yanoviak 1999b, see Baltzley et al. 1999 for additional references), but largely in reference to insect larvae, particularly mosquito, inhabitants. Water chemistry parameters have been studied in non-phytotelm breeding anuran species (e.g. Fegraus and Marsh 2000), and are known to affect larval anuran success in some of these (e.g. Fioramonti et al. 1997). Furthermore, plant parameters including height of the phytotelm above the ground, and its volume, are often correlated with faunal abundances (Kam et al. 1996, Yanoviak 1999a, Eterovick 1999).

The present study aimed to discern the correlates of *Mantella laevigata* reproductive activity in the phytotelmata in which they breed. Phytotelmata are limiting (chapter 3), and both females and males rely on well quality for their reproductive success (chapters 5 and 6). Active selection of oviposition sites is expected in such a system, so the variation in potential sites, as well as the parameters that vary with evidence of *M. laevigata* reproductive activity, can provide insight into both interactions among abiotic and biotic factors, and what factors may be important to *M. laevigata* reproductive success.

Three water chemistry parameters were predicted to vary with evidence of *M. laevigata* reproductive activity: eggs, but especially tadpoles, were predicted to be in wells with more dissolved oxygen (although they may use it up, making data interpretation difficult); tadpoles were expected to produce organic material, and so be found in wells with higher conductivity; and pH was predicted to vary with both egg and tadpole presence (and possibly used as a form of niche partitioning), but in which direction was not clear.

*M. laevigata* eggs and tadpoles were expected to be more prevalent in higher wells, where the risk from terrestrial predators is less; in deeper wells (measured both as maximum capacity and current volume), where the risk of desiccation is lower; and in narrower wells, which reduce the number of potential predators/competitors than can enter the wells.

Two species of well-breeding microhylid frog also compete for space with *M. laevigata*. Several more species of frogs, as well as land crabs, use wells as temporary retreats. Larval crane-flies predate frog eggs in phytotelmata. All of these interactions within the phytotelm-dwelling species assemblage were predicted to negatively affect *M. laevigata* egg and tadpole presence in wells, due to competition or predation. It was therefore predicted that tipulids, crabs, and heterospecific anurans would all be negatively correlated with egg and tadpole presence in wells.

## Methods

Research was conducted on the 510 hectare island of Nosy Mangabe, which lies at the Northern end of the Bay of Antongil, five km South of the town of Maroantsetra, Toamasina province, in northeastern Madagascar (15° 30′ S, 49° 46′ E). Nosy Mangabe is a “Special Reserve” in the Masoala National Park, consisting mainly of 100 – 400 year old secondary forest. *M. laevigata* are found throughout the island, but are densest in stands of coastal bamboo. The bamboo on Nosy Mangabe--*Bambusa vulgaris*--was introduced, but in Marojezy, the other region in which *M. laevigata* is found, the frogs are found in association with native bamboo species, including *Ochlandra capitata* (F. Andreone, *pers. comm.*, Nicoll and Langrand 1989).

From surveys of the island of Nosy Mangabe, six coastal bamboo stands were found, in which 48 bamboo water-filled wells were identified. In addition, five treehole wells in the forest were identified, and marked. A total of 53 wells were marked and monitored approximately every three days for ten weeks, from February 8 through April 18, 1999. Some wells were discovered late, and included in the study after other wells had already been monitored. Data from wells that did not hold water for 72 hours or more were not used, as these were therefore non-viable habitats for *M. laevigata* eggs and tadpoles. In each bamboo stand, data from several such wells were discarded. Most of the 53 included wells were monitored from 21-24 times. In total, 1,156 monitoring observations were made for which data were used.

Data were collected by one of two researchers, who alternated visits to each well. Data collected on each visit included number of *M. laevigata* eggs, number of *M. laevigata* tadpoles, water level, pH, conductivity, dissolved oxygen (DO), and the presence or absence of other species in the phytotelm assemblage. These included larval tipulids (crane-flies), mosquito larvae, adult crabs, adult *Anodonthyla boulengeri* (Anura: Microhylidae), adult male *A. boulengeri* with a clutch (eggs or tadpoles), adult *Plethodontohyla notostica* (Anura: Microhylidae), adult male *P. notostica* with a clutch, *P. notostica* clutch only, adult *Platypelis grandis* (Anura: Microhylidae), adult *Mantidactylus webbi* (Anura: Ranidae), and other adult *Mantidactylus* species. In addition, date, well type (bamboo or treehole), and area (bamboo stand number (1-6) or forest, if a treehole) were recorded. Once during the study period, data on the height, diameter, and maximum depth were taken for each well.

Water level was assessed qualitatively at every visit, on a scale of zero to four, where 0 = dry, 4 = full to capacity, and 1-3 are at approximately 25, 50 and 75% capacity, respectively. Water level data reflects the current amount of water in a well; well depth reflects the maximum capacity in each well. Water chemistry data were taken with the following instruments: pH: Oakton #35615-03; conductivity: Oakton #35661-10 (range 0 – 1990 ppm); DO: Sentry / Ray Jeff.

Due to intermittent equipment failure, pH was not recorded for 705 visits, conductivity was not recorded for 287 visits, and DO was not recorded for 72 visits. Due to early and permanent equipment failure, water temperature was recorded for only 20 visits, which were not considered sufficient to be included in this analysis.

Because incidences of many of the non-*Mantella* frog species were low, I combined several variables. All *Plethodontohyla* observations (adult only, male with clutch, and clutch only) were combined into a single variable: PlethCom. Both *Anodonthyla* observations (adult only, and male with clutch) were combined into a single variable: AnodCom. And all observations of *Mantidactylus* species were combined into a single variable: ManCom.

Independent variables used in the analysis therefore included: date, well, well type, area, well height, well diameter, well depth, water level, pH, conductivity, DO, tipulids, mosquito larvae, crabs, PlethCom, AnodCom, *Platypelis grandis*, and ManCom. All combined species variables (e.g. PlethCom) will hereafter be referred to with the species name only.

Data on the dependent variables—number of *M. laevigata* eggs, and number of *M. laevigata* tadpoles—were later recoded to better assess pattern in the dataset. Binary variables, describing presence/absence of eggs and tadpoles (instead of counts), were created. Five additional variables, representing transformations of the original data, were coded from the raw data as follows. For each of these five variables, the first visit to each well is treated as missing data, and is given no value (= no observation). All five variables are binary.

1) Metamorph: observation immediately after a metamorph disperses from a well = 1; all other observations = 0 (except the first visit to every well,

which is treated as missing data). This variable was only used in select analyses. Due to small numbers of observations of metamorphs, analyses using this variable did not cluster observations by well (see Statistics, below).

2. Positive change in *Mantella* reproductive activity: more eggs than during the previous visit to a given well = 1; all other observations (fewer eggs, or no change in the number of eggs) = 0.
3. Negative change in *Mantella* eggs: fewer eggs than during the previous visit = 1; all other observations = 0.
4. Negative change in *Mantella* tadpoles: fewer tadpoles than during the previous visit = 1; all other observations = 0.
5. Negative change in *Mantella* reproductive activity: negative change in either eggs or tadpoles = 1; neither fewer eggs nor tadpoles since previous observation = 0.

## Statistics

I performed regressions on all dependent variables except Metamorph (8), against all independent variables (18), using the SAS 8.0 genmod procedure. Logistic regressions were performed when the dependent variable was binary; Poisson regressions were performed when the dependent variable was count data, and fit a Poisson distribution. Observations were clustered by well, such that different visits to the same well were not treated as independent observations. Specific generalized linear models, taking clustering into account, and using either binary or Poisson regressions, were Generalized Estimating Equations (Diggle et al. 1994). These produce Score $\chi^2$ values.

SAS code used was as follows (where "abswell" is the variable name for individual well identities): "proc genmod; class abswell; model (dependent variable) (independent variable) / dist = bin (or poisson); repeated subject = abswell / type = ind;".

Additional regressions were performed using the same models, with combinations of variables that were biologically relevant. Wells were clustered in all analyses unless otherwise noted. Conductivity data were log-transformed. No other variables were transformed. Analyses using count data as dependent variables (number of eggs, number of tadpoles) often had unrecoverable errors, whereas models using binary data rarely did.

## Results

One water chemistry parameter (pH), and one parameter of well structure (well height) differed significantly with both egg and tadpole presence, with lower pH and lower height correlated with greater *Mantella* reproductive activity (Table 4.1; Eggs x pH: Score $\chi^2$ for pH = 4.62, 1 df, P = 0.032; Eggs x Well height: Score $\chi^2$ for Height = 7.77, 1 df, P = 0.005; Tads x pH: Score $\chi^2$ for pH = 8.70, 1 df, P = 0.003; Tads x Well height: Score $\chi^2$ for Height = 4.91, 1 df, P = 0.027). In wells from which tadpoles disappeared before metamorphosis, however (negative change in the number of tadpoles), the pH was significantly lower than in other wells (mean ± SD pH from which tadpoles disappeared = 4.04 ± .46; mean ± SD pH from which tadpoles did not disappear = 4.39 ± 1.13; Score $\chi^2$ for pH = 4.08, 1 df, P = 0.043). Only 10 of 451 (2.22%) pH readings were above 7.0; while 195 of 451 (43.24%) pH readings were below 4.0.

Conductivity was almost a significant predictor of egg presence, with higher conductivity correlated with eggs (Score $\chi^2$ for Conductivity = 3.34, 1 df, P = 0.068). Tadpoles showed non-significant trends to survive better in wells of smaller diameter (Score $\chi^2$ for Diameter = 3.06, 1 df, P = 0.080), and in wells with higher water levels (Score $\chi^2$ for Water Level = 3.65, 1 df, P = 0.056); see Table 4.1. Tipulid presence showed a strong correlation with water level (Score $\chi^2$ for Water level = 7.30, 1 df, P = 0.007), but did not differ with well height (Score $\chi^2$ for Well height = 2.40, 1 df, P = 0.122). Neither dissolved oxygen nor (maximum) well depth were correlated with egg or tadpole presence/absence.

Biotic correlates of *Mantella* eggs in wells did not include tipulids, mosquito larvae, or crabs (Figure 4.1). *Mantella* egg presence was significantly correlated with presence of *Plethodontohyla* (Score $\chi^2$ for Pleth = 4.69, 1 df, P = 0.030), *Anodonthyla* (Score $\chi^2$ for Anodon = 3.86, 1 df, P = 0.049), and *Platypelis* (Score $\chi^2$ for Pgrandis = 4.76, 1 df, P = 0.029). Observations of *Mantidactylus* species were too low to perform analyses.

Biotic correlates of *Mantella* tadpoles in wells did not include tipulids, crabs, *Plethodontohyla* or *Anodonthyla* (Figure 4.2). *Mantella* tadpole presence was significantly correlated with presence of mosquito larvae (Score $\chi^2$ for Larvae =

9.26, 1 df, P = 0.002). Observations of *Platypelis grandis* and *Mantidactylus* species were too low to perform analyses.

*Plethodontohyla* presence was negatively correlated with the addition of new eggs to wells (Score $\chi^2$ for PlethCom = 6.01, 1 df, P = 0.014), but also negatively correlated with the disappearance of eggs from wells (Score $\chi^2$ for PlethCom = 5.09, 1 df, P = 0.024); see Figure 4.3. *Plethodontohyla* presence did not have an effect on *M. laevigata* tadpole disappearance (Score $\chi^2$ for PlethCom = 0.50, 1 df, P = 0.478).

Twenty three of the 53 wells monitored never contained a tadpole during the study, but did contain *Mantella* eggs. Two additional wells never contained any *Mantella* eggs or tadpoles (#211 and #402). Only three of the seven areas monitored—bamboo stands 2, 4, and 5—produced metamorphs. Of 12 metamorphs that dispersed from their natal well during this study, three emerged from a single well (#509), and 2 from another (#404). These highly successful wells had lower pH and lower height than average wells, and also never contained any tipulids or other species of frogs (see Table 4.2). The two avoided wells were of greater height and had higher pH (though still quite acid) than the average. They also had significantly higher incidences of either predators (tipulids) or competitors (*Plethodontohyla*); Table 4.2. Two of the 23 wells that attracted eggs but never produced a tadpole (#303 and #706), analyzed separately, showed average pH, slightly lower conductivity, and greater height than average wells. They both showed extremely high incidence of tipulids, which are the most direct threat to *Mantella* eggs.

The presence of tadpoles was correlated with negative changes in the number of *Mantella* eggs in wells (Figure 4.4; Score $\chi^2$ for Tadpoles = 7.00, 1 df, P = 0.008). After tadpoles metamorphosed, however, the number of eggs deposited in those same wells increased by two measures (Figure 4.5): the number of eggs in wells with recent metamorphs was higher than in those without (no clustering; logistic regression $\chi^2$ for Metamorph = 3.93, 1 df, P = 0.047), and wells with recent metamorphs were more likely to show a positive change in the number of *Mantella* eggs, than those without (no clustering; logistic regression $\chi^2$ for Metamorph = 6.62, 1 df, P = 0.010).

Several parameters differed by well type (bamboo vs. tree hole). Bamboo wells tend to be lower (Score $\chi^2$ for Well height = 4.06, 1 df, P = 0.044) and deeper (Score $\chi^2$ for Well Depth = 4.42, 1 df, P = 0.036), with higher conductivity (Score $\chi^2$ for L(conduct) = 5.94, 1 df, P = 0.015) and lower dissolved oxygen (Score $\chi^2$ for DO = 4.88, 1 df, P = 0.027). Bamboo wells also showed a non-significant tendency to be narrower than treeholes (Score $\chi^2$ for Well Diameter = 3.41, 1 df, P = 0.065). None of the faunal variables differed by well type.

## Discussion

### Better living through chemistry

Habitat preferences of amphibians have frequently been studied, but only occasionally with regard to water chemistry parameters such as pH, DO, and conductivity (Magnusson and Hero 1991, Ildos and Ancona 1994, Vos and Stumpel 1995, Stumpel and van der Voet 1998, Fegraus and Marsh 2000). Research on the ability of tadpoles to live in harsh environments has demonstrated that tadpoles can withstand high ammonia levels resulting from urea synthesis (Kam et al. 1997, Schmuck et al. 1994). In *M. laevigata*, however, the minimal clutch size, and therefore low density of conspecifics to contribute to ammonia concentrations, in combination with frequent, probably acidifying rainfall, means that tadpoles must cope not with (basic) ammonia levels, but with high acid levels.

Phytotelm pH varies widely across studies. In tropical phytotelmata, the contents are usually weakly to moderately acid (e.g. <4.0 – 7.0 in bromeliads, Laessle 1961; means 4.7 – 6.0 in bromeliads, Eterovick 1999; 4.4 – 4.7 in tree holes, Kitching and Orr 1996), though some studies show highly variant, and weakly alkaline, conditions (e. g. means 4.6 – 7.7 across phytotelm types, Fincke 1998; means 7.4 – 7.9 in tree holes, Fincke 1999). Rainfall is typically acid, especially in the tropics, and in a study that compared phytotelm acidity in two tropical forests, the wetter forest, with more frequent rainfall, contained phytotelmata with significantly lower pH (Fincke 1998).

In the rainforests of Nosy Mangabe, both eggs and tadpoles of *M. laevigata* are more likely to exist in strongly acid wells (Table 4.1). Eterovick (1999) found that well-breeding *Phyllodytes luteolus* preferentially used bromeliads with lower than average pH, and Stumpel and van der Voet (1998) also found an effect of pH in one species studied. Similarly, Kurihara (1983) found that mosquito larvae of the genus *Aedes* preferred ovipositing in acid bamboo wells (although none of these other results report pH as low as in the current study). Given strong competition from several other species of well-breeding frogs, *M. laevigata* may preferentially oviposit in strongly acidic wells as a form of niche partitioning. But my finding that tadpoles are particularly likely to disappear from even more strongly acid wells suggests a possible cost to extremely low pH. Experiments in *M. laevigata* egg and tadpole tolerance to acidity and variation in pH are called for, in addition to tolerance by their predator and competitors, to better explain this extreme result.

Dissolved oxygen is available to aquatic, respiring organisms, and all else being equal, as it decreases, those organisms should have more difficulty respiring. *Physalaemus pustulosus* tadpoles, when in treeholes rather than foam nests, occupy holes with higher than average DO (Fincke 1999), as do *Bufo viridis* (Ildos and Ancona 1994). But in *Osteopilus brunneus* tadpoles, lower DO concentrations were associated with fewer air gulps per hour, suggesting that they were not struggling to breathe (Lannoo et al. 1987). This is consistent with findings that, in hypoxic environments (at low DO concentrations), anuran larvae are actually more likely to shed oxygen into the water through their skin and buccopharyngeal surfaces, than they are to absorb it (Feder and Burggren 1984, Lannoo et al. 1987).

In addition, DO has been observed to fluctuate widely during the day within individual phytotelmata (Laessle 1961). I have no data on either oxygen use by *M. laevigata* tadpoles, or on diel variation in DO in the phytotelmata they use, but if either parameter is similar to these early studies, the result that neither *M. laevigata* egg nor tadpole presence are predicted by DO concentration should not be surprising.

Water conductivity increases with the amount of dissolved material in solution. It is therefore expected that conductivity will rise with biomass, be it living (e.g. anuran or insect larvae) or dead (e.g. leaf litter). Conductivity is known to be negatively correlated with the presence of the adults of one pond-breeding anuran species, but not with its reproductive stages (*Rana temporaria*, Stumpel and van der Voet 1998). As conductivity rises, pH decreases (Kam et al. 1996); the almost significant relationship between tadpole presence and conductivity in the current study may therefore simply reflect the fact that the pH in wells with tadpoles is even lower than in those with eggs. It is impossible to tell, with these data, which parameter is driving which, and why either parameter might be important for *Mantella* adults to assess. The conductivity, or amount of biomass, in a potential oviposition site, although a possible predictor of other inhabitants of that site, may be a less direct, and therefore less accurate, route to assessing the risks of a site. In *M. laevigata*, adults may use visual and tactile surveys to assess the presence of predators or competitors, rather than relying on an indirect water chemistry measure that is probably less accurate.

## Nobody likes being high and dry

Habitat drying is a problem for any organism dependent for its survival on temporary pools (Fincke 1994, Aspbury and Juliano 1998). Water depth plays an important role in oviposition site choice for several species of anurans (e.g. *Rana sylvatica*, Seale 1982; *Hyla pseudopuma*, Crump 1991; *Bufo bufo*, Ildos and Ancona 1994; *Rana temporaria*, Stumpel and van der Voet 1998), at least two of which breed in phytotelmata (*Chirixalus eiffingeri*, Kam et al. 1996; *Phyllodytes luteolus*, Eterovick 1999). In *Hoplobatrachus occipitalis*, there is evidence that females are assessing not only current water depth, but also water holding capacity, of shallow, temporary pools (Spieler and Linsenmair 1997). Such a parameter might have better revealed oviposition site preference by *M. laevigata* as well.

In *M. laevigata*, high water level predicted both tadpole and tipulid presence in wells, but was not correlated with egg presence (Table 4.1). Water level may be a tricky variable for adult *Mantella* to discriminate, for several reasons. Eggs are laid above the water line in wells (see chapter 2), so do not require submersion in water to develop and hatch. (Eggs do require positive water uptake as they develop, but probably obtain water through capillary action from the wall of the well, as in *Chirixalus eiffingeri*, Kam et al. 1998). Tipulids, which prey on eggs, are found more often in wells with more water, so you might expect courting adults to prefer wells with lower water levels, to reduce the risk of tipulid predation on their eggs. Once the eggs hatch, however, the tadpoles do require water. The risk of desiccation is great, and though all observations were made during the rainy season, there were several periods of three days or longer when there was no precipitation, and previously wet wells began to dry up, or did in fact dry completely. When assessing viable habitat for eggs, adults are faced with conflicting concerns: less water means less risk of predation from tipulids, but greater risk of desiccation for the tadpoles once they hatch. Perhaps it is precisely this conflict that explains the lack of significant difference in water level between wells that do and do not contain *Mantella* eggs.

Lower wells attract more *M. laevigata* reproductive activity than do higher wells. This went against predictions, and runs counter to evidence from other phytotelm-breeding anurans (*Chirixalus eiffingeri*, Kam et al. 1996; *Phyllodytes luteolus*, Eterovick 1999), although abundance of some insect larvae have been observed to decrease with height of the phytotelm (Yanoviak 1999a). Although I refer to two completely unused wells as "avoided" (Table 4.2), a better descriptor might be "unfound." Apparent preference for lower wells may simply be an artifact of which wells are found first by these mostly terrestrial frogs.

Although bamboo and tree hole wells differed with regard to several parameters, there were no differences in egg or tadpole presence by well type. *M. laevigata* are more common in bamboo stands than in the forest, but this is likely due to the fact that bamboo, being clonal, is spatially clumped, whereas tree holes are dispersed throughout the forest.

## “A beast so sagacious, and very voracious”

The differences between individual wells are stark when analyzed on a case by case basis (Table 4.2). Highly successful wells have all the parameters expected by the other analyses presented: low height, low pH, and a complete absence of tipulids and other frog species. Avoided wells, too, look as expected: they are higher, less acidic, and have much higher incidences of tipulids and/or *P. notostica* than do average wells. The two (of 23) wells analyzed that received eggs, but never contained tadpoles, however, provide new data. None of the measured parameters look different than average wells, except for one: they were full of tipulids. While I failed to discern any negative correlation between *M. laevigata* eggs and tipulids in overall analyses (Figure 4.1), individual well analyses tell a different story. I repeatedly observed ravenous tipulids eating *M. laevigata* (and other frog) eggs, and also obtained anecdotal data that “unsuccessful” wells containing tipulids attract courtships and eggs within 24 hours of removal of those tipulids. (Although odonate larvae are widely known to prey on anuran larvae (e.g. Heyer et al. 1975, Semlitsch 1990, Caldwell 1993), only one other study has observed tipulid larvae preying on the eggs of frogs (*Chirixalus eiffingeri*, Kam et al. 1998).) These observations suggest that the analysis presented in Figure 4.1 is less representative of the truth than that portrayed by the individual well analysis of Table 4.2. I propose that there is a predator/prey ratchet in place, whereby tipulid adults look for frog egg containing wells in which to oviposit, *M. laevigata* adults look for wells lacking tipulid larvae in which to oviposit, and while both sides have individual successes, they also both experience failures. Other species of anurans actively avoid oviposition sites which pose high risk of predation to their eggs and larvae (e.g. *Rana catesbeiana*, Howard 1978; *Hyla chryososcelis*, Resetarits and Wilbur 1989; *Bufo americanus*, Petranka et al. 1994), and there is no evidence to suggest that *M. laevigata* are less vigilant in this regard.

### *Mi casa es su casa?*

*Mantella laevigata* eggs were unlikely to be found in the same well with *Anodonthyla boulengeri*, *Plethodontohyla notostica*, or *Platypelis grandis* frogs (Figure 4.1). All three of these microhylid species breed in phytotelmata, with the father attending the clutch (Blommers-Schlösser 1975), but I never observed *P. grandis* doing so on Nosy Mangabe. Rather, this large (>45 mm SVL) species moved into bamboo wells and filled them with its body, but typically only stayed for 24-48 hours. *A. boulengeri* and *P. notostica*, on the other hand, frequently bred in phytotelmata, and gave apparently obligate paternal care, in which the father remained with the clutch throughout its development, leaving only when the metamorphs began to disperse. Whether it was the massive body of *P. grandis*, or the 50+ eggs or tadpoles and father of *A. boulengeri* or *P. notostica*, all three of these microhylid frogs precluded *M. laevigata* from sharing wells with them.

*Anodonthyla boulengeri* and *P. grandis* were relatively rare in this survey (see Table 4.2), and other frogs occasionally observed in phytotelmata, members of the genus *Mantidactylus*, were so rare that the sample sizes precluded their inclusion in any analyses. *P. notostica*, however, had a substantial niche overlap with *M. laevigata*. *P. notostica* presence prevents *M. laevigata* eggs from being oviposited. However, *P. notostica* presence also appears to provide some protection from whatever threatens *M. laevigata* eggs, as eggs disappear less often when sharing wells with *P. notostica* fathers and their broods (Figure 4.3). Two possible mechanisms for *P. notostica* protection of *M. laevigata* eggs include, first, that the attendant father of *P. notostica* clutches dissuade egg predators of *M. laevigata* eggs inadvertently while dissuading predators of their own eggs. Second, there may be predator satiation of tipulids in a well with a *P. notostica* clutch. *P. notostica* have clutches of >50 eggs; *M. laevigata* have a clutch size of 1; an *M. laevigata* egg among a *P. notostica* clutch simply has a much lower probability of being predated by a hungry tipulid, all other things being equal. These results do beg the question: if presence of *P. notostica* reduces the loss of *M. laevigata* eggs, why are fewer *M. laevigata* eggs laid when *P. notostica* inhabit wells? Given this selective advantage, courting *M. laevigata* should seek out wells with *P. notostica* in them. In practice, though, they probably don't have a choice. The most obvious defense that *P. notostica* fathers give their clutches is the emission of a sharp "bark" when anyone tries to enter their well—be it a conspecific, an *M. laevigata*, or a human. *M. laevigata* are routinely turned back when they approach the openings of wells containing *P. notostica* fathers with their broods.

*Mantella laevigata* adults may be dissuaded from entering wells already containing *P. notostica*, but if their offspring precede those of *P. notostica* in a well, they appear to fare well. Not only do *M. laevigata* eggs disappear less often in the presence of *P. notostica*, but their tadpoles do not appear to suffer any ill effects from co-existence. In other species of co-existing anuran larvae, one species of tadpole may predate the other (e.g. Heyer et al. 1975, Petranka et al. 1994), but no interspecific tadpole predation appears to be occurring in this system.

### Love your mother, eat your brother

When *M. laevigata* tadpoles are present in wells, *M. laevigata* eggs are more likely to disappear (Figure 4.4). This follows from the finding that tadpoles eat conspecific eggs, receiving facultative maternal care in the form of trophic eggs, and otherwise eating any fertilized eggs available to them (chapter 2). Adult females, then, should be expected to gauge tadpole presence, and avoid ovipositing in wells with tadpoles. But wells with successful tadpoles are clearly viable in every other respect—aspects of well architecture, water chemistry, and co-inhabitants are appropriate for a tadpole to survive and grow. As such, as soon as a tadpole metamorphoses and disperses from its natal well, females begin laying eggs in that well in greater numbers than would otherwise be predicted, as gauged by two, related measures (Figure 4.5). This is indirect evidence that part of what females are gauging when they investigate wells prior to and during courtships is tadpole presence, and that they not only avoid ovipositing in wells with tadpoles in them, but preferentially return to those wells once the tadpoles have metamorphosed.

Tadpoles that eat eggs are obligately oophagous in several other anuran species (e.g. all members of the *Dendrobates histrionicus* group, Weygoldt 1987; *Chirixalus eiffingeri*, Ueda 1986; *Osteocephalus oophagus*, Jungfer and Schiesari 1995), although several other species have tadpoles that eat conspecific eggs in addition to other material (e.g. members of the *Dendrobates minutus* group, Weygoldt 1987; *Anotheca spinosa*, Taylor 1954). The diets of most of these tadpoles could be predicted by their mouth morphology, as obligately oophagous species tend to have reduced denticle rows, and often a keratinized horny beak that they use to break through the outer capsule of the egg (Duellman and Trueb 1986). Tadpoles of *M. laevigata* have reduced denticle rows and enlarged beaks (Glaw and Vences 1994), and I never observed them eating anything but conspecific eggs. But the current study demonstrates that mosquito larvae are widespread throughout all wells monitored, but significantly reduced in wells containing *M. laevigata* tadpoles (Figure 4.2). This result suggests that *M. laevigata* tadpoles, despite their mouth morphology, may be feeding on mosquito larvae in addition to conspecific eggs. A gut content analysis would be required to determine this for certain.

### Be it ever so humble, there's no place like home

Phytotelmata are used as oviposition sites by many species of tropical anurans. Several hypotheses have been proposed to explain the repeated evolution of arboreal oviposition with free-living aquatic larvae from wholly aquatic development. These range from predator avoidance, advantage in competition, side-stepping the physical dangers associated with life in swift water, and avoiding unfavorable water quality such as low DO or pH (see Magnusson and Hero 1991 for review). In the phytotelmata that *M. laevigata* use, I have presented evidence of unavoidable egg predation by crane-flies

(Tipulidae) in wells, frequent interaction with heterospecific frog competitors, and extremely acid and hypoxic water conditions. In this landscape of rivers, fast moving streams, and phytotelmata (see chapter 3), the "avoidance of risk from swift water" hypothesis seems a plausible first approximation when asking why *M. laevigata* breed in phytotelmata.

Given that they do breed in phytotelmata, *M. laevigata* must assess a large array of risks and threats, as well as beneficial attributes, when choosing potential oviposition sites (Figure 4.6). Contributing positively to egg abundance is adult abundance, well acidity, well abundance, and low well height. Cannibalistic conspecific tadpoles, predatory tipulid larvae, and the presence of other species of frogs, with or without their broods, negatively affect *M. laevigata* egg success (with the possible exception of *P. notostica*, which seems to simultaneously have a positive and negative effect on *M. laevigata* egg presence). *M. laevigata* tadpole populations are positively affected by egg abundance, low pH, low well height and narrow diameter, perhaps by mosquito larvae (which may serve as food), and by water level. Tadpoles are unlikely to exist in extremely acid conditions, or with *P. grandis*, the largest frog competitor in the system.

*M. laevigata* and the rest of the species assemblage in Nosy Mangabe phytotelmata are remarkable in several ways. First, although tadpoles do disappear from wells, there are apparently no tadpole predators inhabiting these wells. None of the tadpoles I observed were predatory on other tadpoles (the microhylid tadpoles apparently do not feed at all, Blommers-Schlösser 1975), and there are no insect larvae that do so either. The usual suspects as top predator in phytotelmata, dragonfly and damselfly larvae (e.g. Caldwell 1993, Fincke 1999), are missing from the phytotelmata on Nosy Mangabe. The only larval arthropod recognized as a predator of larval anurans in these wells, the crane-fly, is a threat only to eggs, not tadpoles.

Second, although phytotelm oviposition sites are an essential resource for all anuran members of the assemblage that use phytotelmata, as well as for tipulids, only *M. laevigata* appear to defend wells between reproductive events. When they are defending a clutch inside a well, fathers of *P. notostica* bark at intruders, but they leave when, or before, their clutch metamorphoses, and I have no evidence that they preferentially return to the same well for future matings.

Third, the mean acidity of these phytotelmata is higher than in other studies of phytotelm water quality, and the acidity of wells containing *M. laevigata* eggs and tadpoles is even higher. Acid tolerance is not expected in aquatic organisms, but it may, in this case, be an adaptation to avoid competition from, or predation by, other members of the assemblage who do not share the tolerance. Possible acid tolerance is limited, though, as *M. laevigata* tadpoles tend to disappear at the lowest pH readings. As desiccation is a threat in these temporary, shallow pools, tadpoles rely on frequent rainfall to stay alive, but the rain also acidifies their environment, which may ultimately kill them.

Finally, there seems to be on-going, condition-dependent assessment of changing well conditions by adult *M. laevigata*. Females don't oviposit fertilized eggs in wells with cannibalistic tadpoles in them, but as soon as these tadpoles metamorphose, those wells become prime real estate for *M. laevigata* courtships. Furthermore, it appears, anecdotally, that *M. laevigata* oviposit in wells soon after *P. notostica* and *A. boulengeri* clutches leave. These phytotelmata are highly complex, variable microhabitats with an ever-changing array of fauna inhabiting them. *M. laevigata*, constantly assessing these changing conditions, and meeting with frequent (reproductive) success, are in integral part of this ecological system.

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## Literature Cited

Aspbury, A. S., and S. A. Juliano. 1998. Negative effects of habitat drying and prior exploitation on the detritus resource in an ephemeral aquatic habitat. Oecologia **115**:137-148.

Baltzley, M. J., C. J. Paradise, and W. A. Dunson. 1999. Interactive effects of density and water sodium concentration on growth of insect larvae inhabiting treeholes. Journal of Freshwater Ecology **14**:113-124.

Beaver, R. A. 1983. The communities living in *Nepenthes* pitcher plants: fauna and food webs. Pages 129-160 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Medford, New Jersey.

Berry, P. Y. 1972. Undescribed and little known tadpoles from West Malaysia. Herpetologica **28**:338-346.

Blommers-Schlösser, R. M. A. 1975. Observations on the larval development of some Malagasy frogs, with notes on their ecology and biology (Anura: Dyscophinae, Scaphiophryninae and Cophylinae). Beaufortia **24**:7-26.

Blommers-Schlösser, R. M. A. 1979. Biosystematics of the Malagasy frogs. I. Mantellinae (Ranidae). Beaufortia **352**:1-77.

Brown, W. C., and A. C. Alcala. 1983. Modes of reproduction of Philippine anurans. Pages 416-428 *in* A. G. J. Rhodin and K. Miyata, editors. Advances in herpetology and evolutionary biology: essays in honor of Ernest E. Williams. Museum of Comparative Zoology, Harvard University, Cambridge.

Caldwell, J. P. 1993. Brazil nut fruit capsules as phytotelmata - interactions among anuran and insect larvae. Canadian Journal of Zoology-Revue Canadienne De Zoologie **71**:1193-1201.

Corbet, P. S. 1961. Entomological studies from a high tower in Mpanga Forest, Uganda. IV. Mosquito breeding at different levels in and above the forest. Trans. R. Ent. Soc. Lond. **113**:275-283.

Corbet, P. S. 1964. Observations on mosquitoes ovipositing in small containers in Zika forest, Uganda. Journal of Animal Ecology **33**.

Corbet, P. S. 1983. Odonata in phytotelmata. Pages 29-54 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Inc., Medford, New Jersey.

Crump, M. L. 1991. Choice of oviposition site and egg load assessment by a treefrog. Herpetologica **47**:308-315.

Diggle, P. J., K.-Y. Liang, and S. L. Zeger. 1994. Analysis of Longitudinal Data. Clarendon Press, Oxford.

Duellman, W. E., and L. Trueb. 1986. Biology of Amphibians. Johns Hopkins University Press, Baltimore, MD.

Dunn, E. R. 1926. The Frogs of Jamaica. Proceedings of the Boston Society of Natural History **38**:111-130.

Eterovick, P. C. 1999. Use and sharing of calling and retreat sites by *Phyllodytes luteolus* in a modified environment. Journal of Herpetology **33**:17-22.

Feder, M. E., and W. W. Burggren. 1984. Cutaneous gas exchange in vertebrates: Design, patterns, control and implications. Biological Reviews **60**:1-45.

Fegraus, E. H., and D. M. Marsh. 2000. Are newer ponds better? Pond chemistry, oviposition site selection, and tadpole performance in the tungara frog, *Physalaemus pustulosus*. Journal of Herpetology **34**:455-459.

Fincke, O. M. 1994. Population regulation of a tropical damselfly in the larval stage by food limitation, cannibalism, intraguild predation and habitat drying. Oecologia **100**:118-127.

Fincke, O. M. 1998. The population ecology of *Megaloprepus coerulatus* and its effect on species assemblages in water-filled tree holes. Pages 391-416 *in* J. P. D. a. I. F. G. McLean, editor. Insect Populations. Kluwer Academic Publishers, Dordrecht.

Fincke, O. M. 1999. Organization of predator assemblages in Neotropical tree holes: effects of abiotic factors and priority. Ecological Entomology **24**:13-23.

Fioramonti, E., R. D. Semlitsch, H. U. Reyer, and K. Fent. 1997. Effects of triphenyltin and pH on the growth and development of *Rana lessonae* and *Rana esculenta* tadpoles. Environmental Toxicology and Chemistry **16**:1940-1947.

Fish, D. 1983. Phytotelmata: flora and fauna. Pages 1-28 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Medford, New Jersey.

Fish, D., and R. A. Beaver. 1978. A bibliography of the aquatic fauna inhabiting bromeliads (Bromeliaceae) and Pitcher Plants (Nepenthaceae and Sarraceniaceae). *in* Proceedings of the Florida Anti-Mosquito Association 49th Meeting, April 2-5, 1978.

Frank, J. H. 1983. Bromeliad phytotelmata and their biota, especially mosquitoes. Pages 101-128 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Medford, N. J.

Frank, J. H., and L. P. Lounibos, editors. 1983. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus, Medford, N. J.

Gibbons, J. R. H., and M. L. Guinea. 1983. Observations on development of the Fijian tree frog, *Platymantis vitiensis*. Herpetofauna **14**:83-86.

Glaw, F., and M. Vences. 1994. A Fieldguide to the Amphibians and Reptiles of Madagascar, 2 edition. Zoologisches Forschungsinstitut und Museum Alexander Koenig. Moos Druck, Leverkusen and FARBO, Köln, Bonn.

Heyer, W. R., R. W. McDiarmid, and D. L. Weigmann. 1975. Tadpoles, predation and pond habitats in the tropics. Biotropica **7**:100-111.

Howard, R. D. 1978. The influence of male-defended oviposition sites in early embryo mortality in bullfrogs. Ecology **59**:789-798.

Ildos, A. S., and N. Ancona. 1994. Analysis of habitat preferences in a farmland area (Po plain, northern Italy). Amphibia-Reptilia **15**:307-316.

Inger, R. F. 1966. The systematics and zoogeography of the amphibia of Borneo. Fieldiana Zool. **52**:1-402.

Jungfer, K.-H., and L. C. Schiesari. 1995. Description of a central Amazonian and Guianan tree frog, genus *Osteocephalus* (Anura, Hylidae), with oophagous tadpoles. Alytes **13**:1-13.

Kam, Y. C., Y. H. Chen, Z. S. Chuang, and T. S. Huang. 1997. Growth and development of oophagous tadpoles in relation to brood care of an arboreal breeder, *Chirixalus eiffingeri* (Rhacophoridae). Zoological Studies **36**:186-193.

Kam, Y. C., Z. S. Chuang, and C. F. Yen. 1996. Reproduction, oviposition-site selection, and tadpole oophagy of an arboreal nester, *Chirixalus eiffingeri* (Rhacophoridae), from Taiwan. Journal of Herpetology **30**:52-59.

Kam, Y. C., C. F. Yen, and C. L. Hsu. 1998. Water balance, growth, development, and survival of arboreal frog eggs (*Chirixalus eiffingeri*, Rhacophoridae): Importance of egg distribution in bamboo stumps. Physiological Zoology **71**:534-540.

Kitching, R. L. 1971. An ecological study of water-filled tree-holes and their position in the woodland ecosystem. Journal of Animal Ecology **40**:281-302.

Kitching, R. L. 1983. Community structure in water-filled treeholes in Europe and Australia--comparisons and speculations. Pages 205-222 *in* J. H. Frank and L. P. Lounibos, editors. Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Medford, N. J.

Kitching, R. L., and A. G. Orr. 1996. The foodweb from water-filled treeholes in Kuala Belalong, Brunei. Raffles Bulletin of Zoology **44**:405-413.

Kurihara, Y. 1983. The succession of aquatic dipterous larvae inhabiting bamboo phytotelmata. Pages 55-77 *in* J. H. Frank and L. P. Lounibos, editors.

Phytotelmata: Terrestrial plants as hosts for aquatic insect communities. Plexus Publishing, Medford, New Jersey.

Laessle, A. M. 1961. A micro-limnological study of Jamaican bromeliads. Ecology **42**:499-517.

Lannoo, M. J., D. S. Townsend, and R. J. Wassersug. 1986. Larval life in the leaves: arboreal tadpole types, with special attention to the morphology, ecology, and behavior of the oophagous *Osteopilus brunneus* (Hylidae) larva. Fieldiana **1381**:1-31.

Louton, J., J. Gelhaus, and R. Bouchard. 1996. The aquatic macrofauna of water-filled bamboo (Poaceae: Bambusoideae: *Guadua*) internodes in a Peruvian lowland tropical forest. Biotropica **28**:228-242.

Magnusson, W. E., and J. M. Hero. 1991. Predation and the evolution of complex oviposition behavior in Amazon rain forest frogs. Oecologia **86**:310-318.

Nicoll, M. E., and O. Langrand. 1989. Madagascar: Revue de la Conservation et des Aires Protégées. WWF, Gland, Switzerland.

Peixoto, O. L. 1981. Notas sobre a girino de *Crossodactylodes pintoi* Cochran (Amphibia, Anura, Leptodactylidae). Revista Brasileira de Biologia **41**:339-341.

Petranka, J. W., M. E. Hopey, B. T. Jennings, S. D. Baird, and S. J. Boone. 1994. Breeding habitat segregation of wood frogs and American toads: the role of interspecific tadpole predation and adult choice. Copeia:691-697.

Ratsirarson, J., and J. A. Silander. 1996. Structure and dynamics in *Nepenthes madagascariensis* pitcher plant micro-communities. Biotropica **28**:218-227.

Resetarits Jr., W. J., and H. M. Wilbur. 1989. Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. Ecology **70**:220-228.

Schmuck, R., W. Geise, and K. E. Linsenmair. 1994. Life cycle strategies and physiological adjustments of reedfrog tadpoles (Amphibia, Anura, Hyperoliidae) in relation to environmental conditions. Copeia:996-1007.

Seale, D. B. 1982. Physical factors influencing oviposition by the woodfrog, *Rana sylvatica*, in Pennsylvania. Copeia:627-635.

Semlitsch, R. D. 1990. Effects of body size, sibship, and tail injury on the susceptibility of tadpoles to dragonfly predation. Canadian Journal of Zoology-Revue Canadienne De Zoologie **68**:1027-1030.

Silverstone, P. A. 1975. A revision of the poison-arrow frogs of the genus *Dendrobates* Wagler. Natural History Museum of Los Angeles County Science Bulletin **27**:1-53.

Sota, T., and M. Mogi. 1996. Species richness and altitudinal variation in the aquatic metazoan community in bamboo phytotelmata from North Sulawesi. Researches On Population Ecology **38**:275-281.

Spieler, M., and K. E. Linsenmair. 1997. Choice of optimal oviposition sites by *Hoplobatrachus occipitalis* (Anura: Ranidae) in an unpredictable and patchy environment. Oecologia **109**:184-199.

Stumpel, A. H. P., and H. van der Voet. 1998. Characterizing the suitability of new ponds for amphibians. Amphibia-Reptilia **19**:125-142.

Taylor, E. H. 1954. Frog-egg eating tadpoles of *Anotheca coronata* (Stejneger). (Salientia, Hylidae). The University of Kansas Science Bulletin **34**:589-595.

Thienemann, A. 1954. Chironomus. Leben, Verbreitung und wirtschaftliche Bedeutung der Chironomiden. Binnengawässer **20**.

Ueda, H. 1986. Reproduction of *Chirixalus eiffingeri* (Boettger). Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University **8**:109-116.

Varga, L. 1928. Ein interessanter Biotop des Biocönose von Wasserorganismen. Biologisches Zentralblatt **48**:143-162.

Vos, C. C., and A. H. P. Stumpel. 1995. Comparison of habitat-isolation parameters in relation to fragmented distribution patterns in the tree frog (*Hyla arborea*). Landscape Ecology **11**:203-214.

Wassersug, R. J., K. J. Frogner, and R. F. Inger. 1981. Adaptations for life in tree holes by rhacophorid tadpoles from Thailand. Journal of Herpetology **51**:41-52.

Weygoldt, P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). Zeitschrift für Zoölogische Systematik und Evolutions forschung **25**:51-67.

Yanoviak, S. P. 1999a. Distribution and abundance of *Microvelia cavicola* Polhemus (Heteroptera : Veliidae) on Barro Colorado Island, Panama. Journal of the New York Entomological Society **107**:38-45.

Yanoviak, S. P. 1999b. Effects of leaf litter species on macroinvertebrate community properties and mosquito yield in Neotropical tree hole microcosms. Oecologia **120**:147-155.

**Table 4.1: Well structure and water chemistry correlates of *Mantella laevigata* reproductive activity**

| | Eggs Present | | Eggs Absent | Tadpoles Present | | Tadpoles Absent |
|---|---|---|---|---|---|---|
| pH | 4.15 ± 1.08 | < | 4.53 ± 1.13 | 3.85 ± 0.49 | << | 4.47 ± 1.18 |
| Conductivity, ppm | 2.08 ± 0.41 | ≤ | 2.16 ± 0.38 | 2.22 ± 0.37 | ns | 2.11 ± 0.40 |
| Dissolved $O_2$, ppm | 2.85 ± 1.08 | ns | 2.82 ± 1.04 | 2.97 ± 1.02 | ns | 2.79 ± 1.07 |
| Well Height at rim, cm | 61.00 ± 31.40 | << | 74.82 ± 33.70 | 54.37 ± 29.45 | < | 73.43 ± 33.66 |
| Well Diameter, cm | 5.72 ± 2.15 | ns | 5.59 ± 2.26 | 5.00 ± 1.46 | ≤ | 5.82 ± 2.36 |
| Well Depth, cm | 9.22 ± 6.60 | ns | 8.95 ± 6.51 | 9.56 ± 5.80 | ns | 8.92 ± 6.74 |
| Water Level (0-4, 0=dry, 4=full) | 2.96 ± 1.02 | ns | 2.87 ± 1.21 | 3.20 ± 0.87 | ≥ | 2.82 ± 1.19 |

Values are means ± SD
<< and >> indicate a significant difference, at $P < 0.01$
< and > indicate a significant difference, at $P=0.05$, between wells containing eggs (or tadpoles) and those without.
≤ and ≥ indicate a non-significant trend, at $0.05 < P < 0.09$
ns are non-significant differences, where $P > 0.09$

## Table 4.2: Well parameters of six individual wells used by *Mantella laevigata*

Wells: 2 reproductively successful (S); 2 avoided (A); and 2 used but unsuccessful wells (no tads)

Well Codes:

- S: Successful, productive well (509 produced 3 metamorphs; 404 produced 2 metamorphs)
- A: Avoided well (no *Mantella* eggs or tadpoles for the duration of the study)
- no tads: no tadpoles observed, but frequent eggs (303 contained eggs in 78.3% of observations; 613 contained eggs in 55.6% of observations; across all wells, eggs were observed in 40.4% of total observations.)

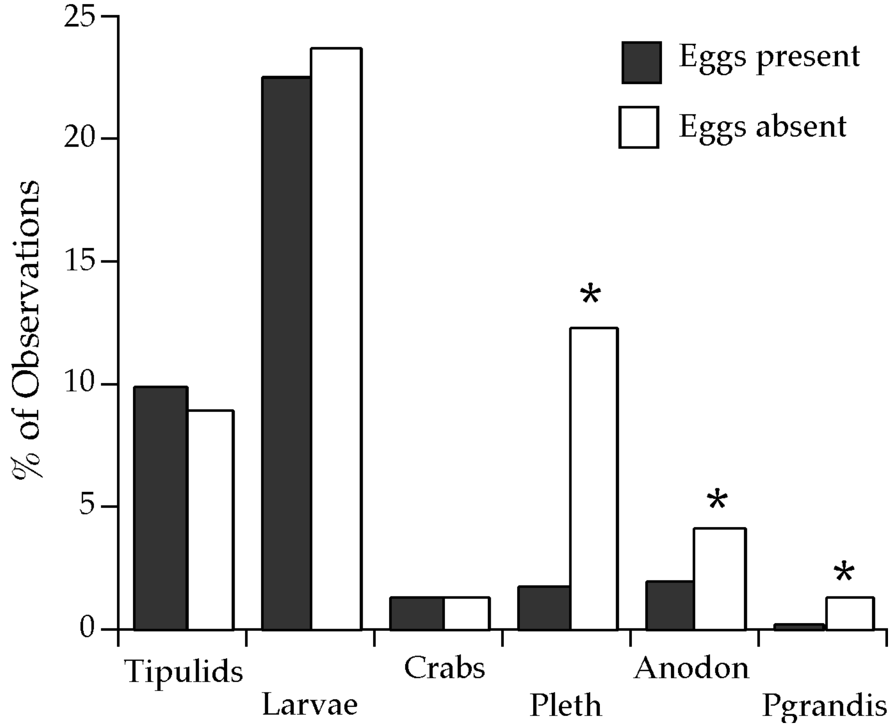
Data are presented as Means ± S.D. Conductivity and DO are in ppm. Well height, depth, and diameter are in cm. Tipulids, *Plethodontohyla* (Pleth), and *Anodonthyla* (Anodon) data are numbers of time present / number of observations. *Platypelis grandis* was too infrequently observed in the present study (All wells: 10/1156, 0.86%) to provide informative numbers for individuals wells.

**Table 4.2: Well parameters of six individual wells used by *Mantella laevigata***

| Well | | pH | Conduc. | DO | Height | Depth | Diam. | Water Level | Tipulids | *Pleth.* | *Anodon.* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S | 509 | 3.69 ± 0.16 | 93.33 ± 20.24 | 3.72 ± 0.56 | 60 | 18 | 5 | 3.59 ± 0.67 | 0/23 (0%) | 0/23 (0%) | 0/23 (0%) |
| | 404 | 3.56 ± 0.42 | 619.55 ± 340.16 | 2.11 ± 0.54 | 30 | 12.5 | 4 | 3.46 ± 0.66 | 0/24 (0%) | 0/24 (0%) | 1/24 (4.2%) |
| A | 211 | 4.64 ± 1.03 | 85.00 ± 91.92 | 2.78 ± 1.34 | 107 | 24.5 | 2.5 | 3.41 ± 0.85 | 0/22 (0%) | 8/22 (36.4%) | 1/22 (4.5%) |
| | 402 | 4.73 ± 0.52 | 245.91 ± 109.80 | 2.48 ± 0.97 | 115 | 12 | 4.5 | 3.38 ± 0.82 | 8/24 (33.3%) | 20/24 (83.3%) | 0/24 (0%) |
| no tads | 303 | 3.63 ± 1.69 | 164.19 ± 101.11 | 3.26 ± 0.89 | 96 | 27 | 7 | 3.04 ± 1.07 | 17/23 (73.9%) | 0/23 (0%) | 0/23 (0%) |
| | 706 | 4.43 ± 0.84 | 42.62 ± 19.68 | 3.21 ± 0.61 | 106 | 5 | 3 | 3.29 ± 0.56 | 5/21 (23.8%) | 1/21 (4.8%) | 0/21 (0%) |
| All (N=53) | | 4.36 ± 1.12 | 207.37 ± 245. 63 | 2.83 ± 1.06 | 69.24 ± 33.70 | 9.06 ± 6.55 | 5.64 ± 2.22 | 2.91 ± 1.14 | 106/1136 (9.3%) | 93/1156 (8.0%) | 37/1156 (3.2%) |

## **Figure 4.1:** ***M. laevigata*** **Egg Presence: Effect of the Coexistence of Other Species**

N=1156 for all variables except tipulids, for which N=1136.

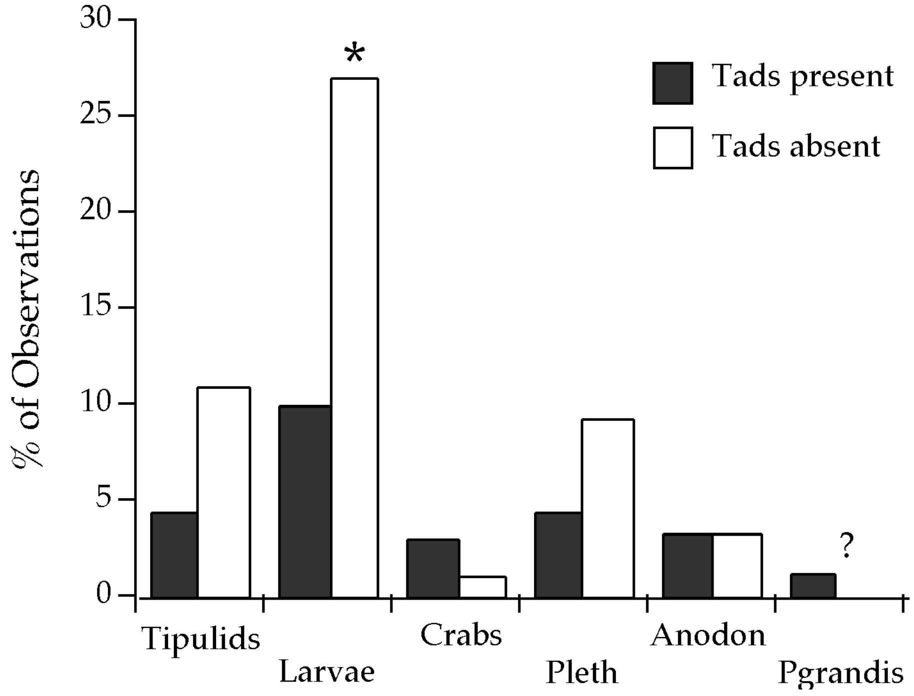


Occurrence of tipulids, mosquito larvae, and crabs were not correlated with *Mantella* egg presence.

Three other anuran species (*Plethodontohyla notostica*, *Anodonthyla boulengeri*, and *Platypelis grandis*) were significantly negatively related to presence of *Mantella* eggs (see text for statistical details).

## Figure 4.2: *M. laevigata* Tadpole Presence: Effect of the Coexistence of Other Species

N=1156 for all variables except tipulids, for which N=1136.

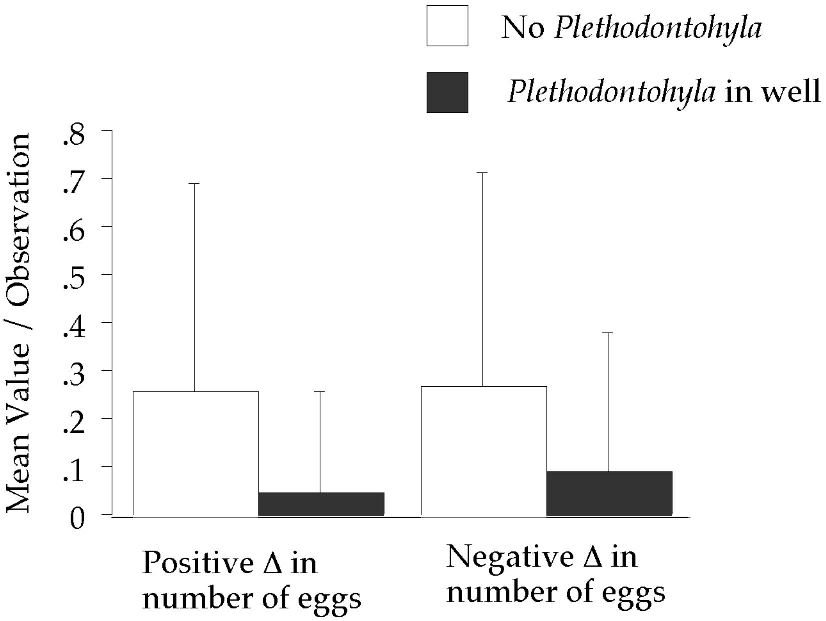


Occurrence of tipulids, crabs, *Plethodontohyla notostica*, and *Anodonthyla boulengeri* were not correlated with *Mantella* egg presence.

Mosquito larvae were negatively correlated with tadpole presence (see text for statistical details).

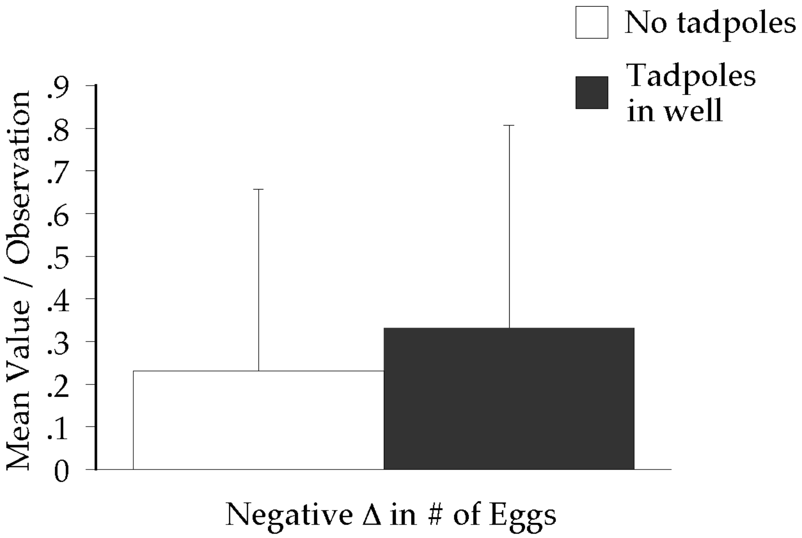
There were no observations of wells in which both *Platypelis grandis* and *Mantella* tadpoles co-existed.

## Figure 4.3: Effect of *Plethodontohyla notostica* on *M. laevigata* Oviposition



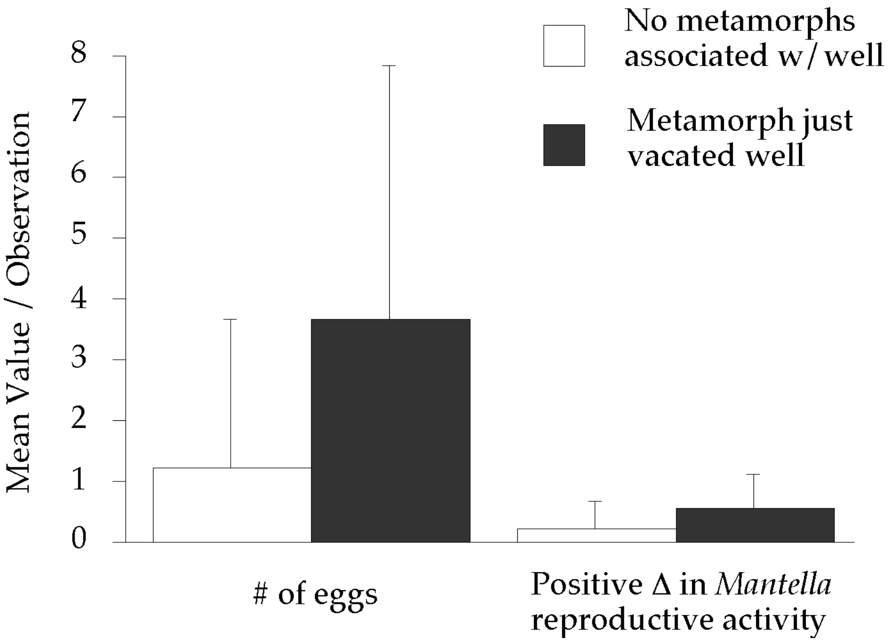
*Plethodontohyla notostica* presence in a well is negatively correlated with both positive changes in the number of *Mantella* eggs, and with negative changes in the number of *Mantella* eggs. Both pairs of Means (± 1 SD) are significantly different (see text for statistical details).

## Figure 4.4: Effect of Tadpole Presence on Egg Loss



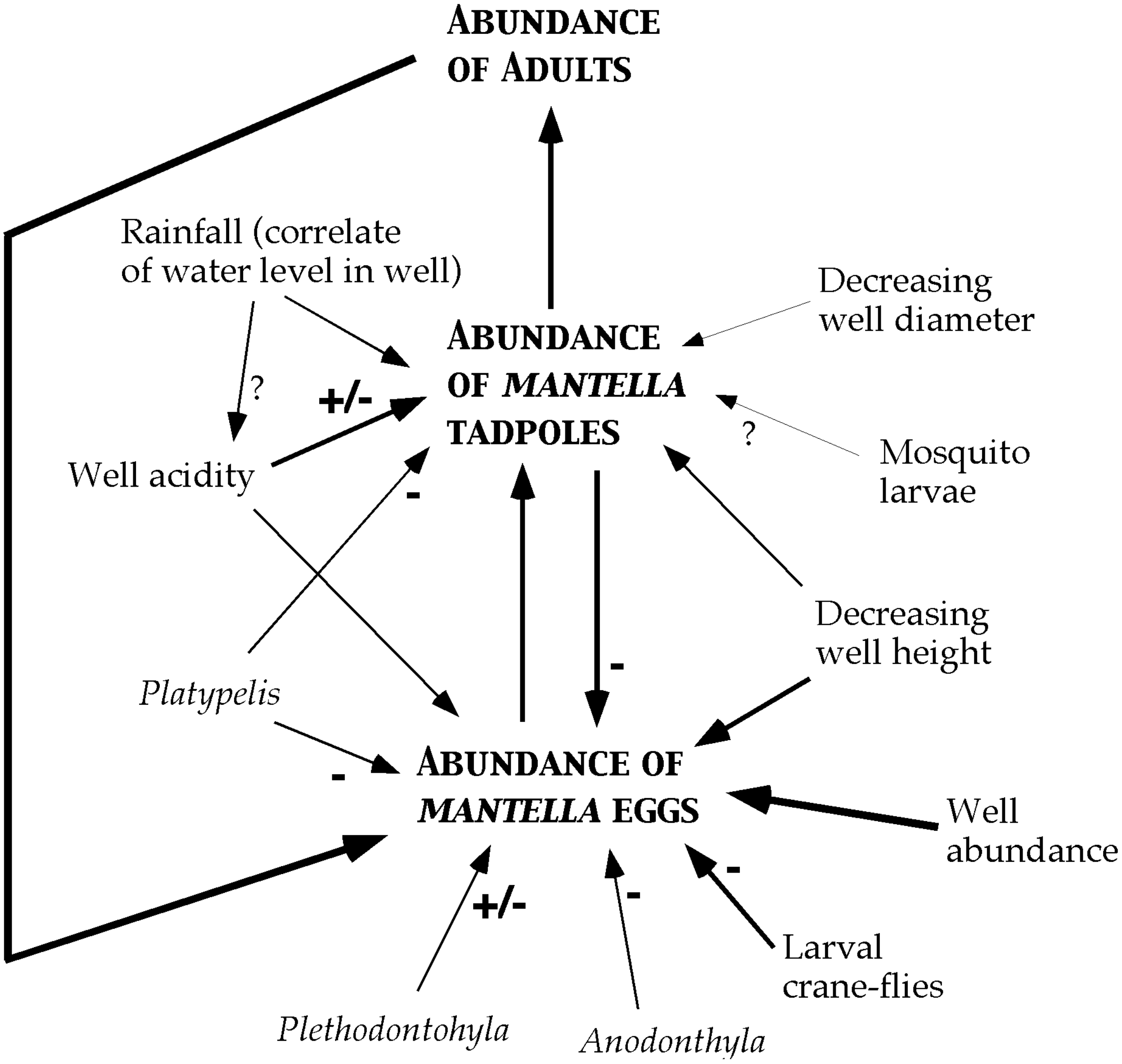
Eggs are more likely to disappear from wells between observations when those wells have tadpoles in them. Means (± 1 SD) are significantly different.

## Figure 4.5: Relationship between Metamorph Dispersal and *M. laevigata* Oviposition



Dispersal of metamorphs from their natal well produces an increase in *Mantella* eggs in that well, as measured both by the absolute number of eggs across all wells, and by change in number of eggs since the previous observation in that well. Both pairs of Means (± 1 SD) are significantly different.

## Figure 4.6: Biotic and Abiotic Factors affecting *M. laevigata* Abundance in Wells



Biotic and abiotic factors affecting *Mantella laevigata* abundance in wells.
Relationships are positive unless indicated by a negative sign.
Arrow thickness indicates the relative qualitative importance of each factor.

# Chapter 5

# Female Mate Choice for Oviposition Site Quality in a Madagascan Poison Frog

## Abstract

Mechanisms of female choice in *Mantella laevigata* were investigated in a playback experiment in the field. Females were allowed to choose between calls of individual males ("good genes"), of varying length calls ("good current condition"), in different quality territories ("good resources"). Observations of females scouting for oviposition sites before courtships, males leaving courtships to engage in territorial defense of oviposition sites, and the presence of biparental care in this species, all suggested the "good resources hypothesis." Experimental results failed to falsify this hypothesis, and did not provide support for either of the other hypotheses tested. Whereas female choice of resources such as territory is common in birds, in which parental care is prevalent, it is less common in anurans, in which parental care is known for less than 10% of species. *M. laevigata* is similar to many pair-bonded bird species in that the oviposition site for few, sessile young (eggs) incapable of self-defense is critical to the survival of offspring. Both parents are expected to be choosy with regard to oviposition site in such a system, though the parameters of particular importance to each sex will be different. Further, species which utilize restricted, transient oviposition sites which vary in quality, such as phytotomes, will likely converge on aspects of

their mating systems. In these regards, *M. laevigata* fits a model more common to birds than to anurans.

## Introduction

Darwin first observed that male displays were likely to differentially attract females to mate with them (1871). Since then, models of mate choice have evolved in many directions, and the diversity of behaviors referred to as male displays expanded on, no longer restricted to obvious signals such as bright plumage in birds or complex calls in anurans. Among these, honest advertisement models (derived from Zahavi 1975; see Borgia 1987, Kirkpatrick 1987, Pomiankowski 1988, Kirkpatrick & Ryan 1991, Maynard Smith 1991, and Hill 1994 for recent reviews) are unique in addressing the issue of exactly what females are cueing on, as opposed to how the mate choice first evolved.

Honest advertisement models may be broken down into three broad categories, on the basis of what is being advertised (Table 5.1). The corollary of this is: what are females seeking from mates. First, females may choose males with traits for which there is additive genetic variance for fitness in the population. Such traits are therefore heritable, and this is termed the "good genes" model (Hamilton and Zuk 1982, Andersson 1986). In anurans, females discriminating on the basis of good genes are likely to do so by assessing advertisement calls of males (see review in Ryan 1991). Anuran examples of female choice for aspects of male song—such as call rate and dominant frequency—abound (e.g. Ryan 1980, Lopez and Narins 1991, Passmore et al.

1992, Cherry 1993, Gerhardt et al. 1996, Giacoma et al. 1997, Wollerman 1998, ), and there is growing evidence that call parameters may be honest indicators of male quality (e.g. Welch et al. 1998).

Second, females may choose an (honestly advertising) male based on the quality of the resources he is promising, such that the benefits the female accrues are direct. In anurans, these resources may have immediate effects on reproductive success, such as with high-quality oviposition sites that males defend (Wells 1977, Howard 1978a and 1978b, Greer and Wells 1980, Kluge 1981, Seale 1982, Crump 1991, Petranka et al. 1994, Spieler and Linsenmair 1997, Halloy and Fiaño 2000) or paternal care which goes into effect immediately after oviposition (e.g. midwife toads, Verrell and Brown 1993), or the resources may be delayed, as with the majority of paternal care (Hoelzer 1989, Summers 1989, Summers 1992a, Bourne 1998). Females may also choose oviposition sites, and the attendant male, even in species without strong male site fidelity (e.g. Marsh et al. 2000).

Third, females may want to gauge the current condition of males, which may predict their ability to procure resources, and thus potentially correlate with genetic quality, or defense of their young in paternal-care giving species. The duration of an anuran male's advertisement call may be an indicator of his current condition, as calling is energetically expensive (Taigen and Wells 1985, Prestwich 1995), and only a male with current resources to spare can afford to call for extended periods of time (Grafe 1997). Call duration has been linked to genetic quality in grey treefrogs (Welch et al. 1998), and females prefer long calls in both that species (Klump and Gerhardt 1987) and in midwife toads (Márquez and Bosch 1997). Furthermore, in species with territorial defense of food, rather than oviposition sites, the current condition of a male may indicate that resources are available in his territory, and therefore be used as a proxy for territory quality by females (Arvidsson and Neergaard 1991). Neither the “good resources” nor the "good current condition" models require heritability of the traits being chosen, though there may be heritable correlates. More likely they represent female choice for proximate gains in offspring, or maternal, survival.

All three of these subsets of the honest advertisement model have potential overlap with one another. Howard (1978a, 1978b) found that, in bullfrogs, females choose high-quality oviposition sites, but that the ability to procure and defend such sites is correlated with age in males, which is, in turn, correlated with size. Furthermore, in systems where males choose calling sites, and females choose oviposition sites by calling males, the parallels, or differences, between male and female choices can provide information about intersexual differences in strategies to maximize reproductive success (Resetarits and Wilbur 1991).

The relative abundance of anuran studies demonstrating female choice for male size or parameters of male vocalizations suggests that female choice for territory or other resource quality is rare in frogs. This may, in part, be an artifact of the difficulty of testing female choice for resource quality and other direct benefits. Additionally, the literature is skewed towards examples of anuran mate choice in choruses, which often occur in explosively-breeding populations, in large part because most species of anurans are chorus breeders (e.g. Bertram et al. 1996). But the prevalence of external fertilization in anurans, in combination with restricted mating conditions, such as is often found in tropical species that breed on land or in isolated water bodies, together increase certainty of both paternity and maternity. Both of these factors probably lead to higher rates of parental care (Wells 1981, Gross and Shine 1981). Female choice for resource quality will be more prevalent in such systems, when males offer resources, such as oviposition sites or paternal care, which can be discriminated by females.

*Mantella laevigata*, one of 11 species of Madagascan poison frog (Mantellinae, Ranidae), is a good system in which to investigate models of female choice. Males call regularly from defended territories, which include oviposition sites (water-filled phytotelmata: wells in bamboo or tree holes). Advertisement calls consist of varying length string of repeated two-note elements. Females provide maternal care by provisioning tadpoles with trophic eggs. Male defense of oviposition sites which contain their young might be considered a form of paternal care as well. Females in courtships may reject an oviposition site to which a male leads her, without rejecting the male, if the male is able to attract the female back and lead her to a new oviposition site (chapter two). Females approach only males issuing advertisement calls; silent males do not have the opportunity to engage in courtships (Heying, unpub. data). A male may call from an oviposition site directly, or from the territory he defends, which contains from zero to five oviposition sites. Tadpoles cannibalize eggs, eating either unfertilized eggs supplied by their mothers, or fertilized, conspecific eggs laid during courtships (chapter two).

I conducted a choice test in the field in which non-captive females were given choices between particular males, in particular territories, calling for unique periods of time. Differential female response to calls from particular males would be interpreted as a failure to falsify the "good genes" model for this system. Differential female response to calls from particular territories would be interpreted as a failure to falsify the "good resources" model for this system. And differential female response to calls of different lengths would be interpreted as a failure to falsify the "good current condition" model for this system. My experimental design allowed for the observation of simultaneous choice for multiple characters, as has been described in some birds (Buchanan and Catchpole 1997) and frogs (Howard 1978a, 1978b).

I hypothesized that females follow a "good resources" model in *Mantella laevigata*. This hypothesis is based on previous observations that non-territorial males may successfully sneak matings in other male's territories, and that courting males leave courtships most often to engage in territorial defense (Figure 5.1), suggesting that a male's long term reproductive success is better served by leaving courtships to defend his territory against other males, than by remaining in all potentially successful courtships and allowing other males to use the wells he defends (chapter two). Furthermore, females scout for, and investigate, potential oviposition sites before they are receptive to courtship. These observations suggest that the quality of oviposition sites is patchy, with varying levels of risk between them; it is in such an environment that oviposition site choice is likely to evolve (Resetarits, Jr. 1996). Furthermore, my observations imply that females are not gauging male quality, as displayed by their ability to capture and defend territories, but rather that females are gauging potential oviposition sites, which are defended by males, as a predominant factor in their mate choice.

## Methods

### Study Site

All research was conducted on the 510 hectare island of Nosy Mangabe, which lies at the Northern end of the Bay of Antongil, five km South of the town of Maroantsetra, Toamasina province, in northeastern Madagascar (15º 30′ S, 49º 46′ E). Nosy Mangabe is a “Special Reserve” in the Masoala National Park, consisting mainly of 100 – 400 year old secondary forest. *M. laevigata* are found throughout the island, but are densest in coastal bamboo stands (*Bambusa vulgaris)*.

### Acoustic Recording, Digitalization, and Playback

Advertisement calls of six males from a marked and daily focal-watched population were recorded. The males recorded ranged from those who actively defended territories containing multiple oviposition sites, to those who sporadically defended territories with no oviposition sites, but snuck in to other males' territories while attempting to attract females.

Call recordings were made with a Sennheiser K6 electret condenser microphone system, using the ME66 shot-gun microphone, and a Sony Professional Walkman, model WM-D6C, using Radio Shack HD, High Bias

Advanced Cobalt Ferric cassettes. Recordings were made of each of the six frogs on multiple days, from February 17 - March 18, 1999. Calls were digitized on a Macintosh PowerBook 3400 using Adobe Premiere 4.2.1 software, then re-recorded onto new, identical audio tapes. In order to control for variation in call frequency within each male, five minutes of constant call were edited, then copied twice and recorded back-to-back, such that each 15 minute recording contained three identical five minute recordings. As such, playbacks of five and fifteen minutes did not differ in the quality of the calls, only the duration. Playbacks were conducted using the Sony Professional Walkman and a single, internally amplified Sony SRS-A33 speaker.

Due to theft, the equipment used to record and playback the digitized frog calls was different on the final day of the experiment. Playback for three of the six frogs (D4, K2, and M4) were already completed, so only tapes for frogs A1, A3, and N5 were re-recorded, using a Yamaha Natural Sound Stereo Double Cassette Deck,, model KX-W392. Record levels were 7.5 for A1 and A3, and 9.5 for N5. This equipment belongs to the radio station in the nearby town of Maroantsetra, and its history is not precisely known. Playbacks on the final day of the experiment were conducted with a Panasonic stereo cassette player, model RQ-SW10; a Radio Shack Mini Amplifier, cat. no. 277-1008C; and a non-amplified Sony SRS-A33 speaker, the matching speaker of the internally amplified one used on the previous eleven days.

## Mate-Choice Experiments

In an undisturbed population of *M. laevigata* (bamboo stand 6), ten territories were chosen. I located water-filled wells, which are used as oviposition sites, defended by resource-defense territorial males (chapters two and six), and are limiting for the population (chapter 3). I went repeatedly to these potential oviposition sites, looked for evidence of reproductive activity at these sites, and waited ten minutes to record if any adult *M. laevigata* of either sex came to those sites. If adult *M. laevigata* arrived within 0.5 meters of the site a majority of the time that I visited it, and there was evidence of reproductive activity in the well more than 50% of the time, I coded it as a "high quality territory." This assessment follows Ens et al. (1992), who suggest that territory quality should be correlated with habitat features (water-filled wells), and the "critical resource" provided for the organisms be known. This assessment also follows Weatherhead and Boak's (1986) work, which used nonrandom variation in occupation of territories as a proxy for territory quality.

For each of five such high quality territories, a low quality territory was delineated 2.5 meters away, in a randomly chosen compass direction from the high quality territory. None of the low quality territories contained obvious oviposition sites. Males are known to defend territories without oviposition sites—these "non-resource defense territorial" males intercept females and sneak courtships in other males' territories (Heying, in press, also chapter 2). Females are not territorial, and individual females travel distances much greater than 2.5 meters most days (Heying, unpub. data). Thus, environmental variables were controlled for by using spatially linked pairs of territories ('areas"), while maintaining all territories within the range of known *M. laevigata* populations. This method of choosing territories yielded high quality territories containing viable oviposition sites (water-filled phytotelmata), and low quality territories with either no, or non-viable (i.e. dry) oviposition sites. Because females travel longer distances every day than that between paired territories, females are not expected to be clumped, or limited, to only one type of territory.

Tapes of each of the six males, in lengths of 5 and 15 minutes, were played-back in each of the ten territories. For each of twelve days, ten unique combinations of territory, male, and call length were pulled out of a hat, and playbacks were performed in the order in which they were pulled. Playbacks were performed over the course of 23 days, from March 19 through April 10, 1999. *M. laevigata* are reproductive throughout the year (Heying, unpub. data), but are most active during the rainy season, which usually lasts from late November through May.

Before playbacks, I moved the equipment into position and sat down one meter from the speaker, then waited for one minute before beginning the tape. During playbacks, I monitored female approaches to the territory/speaker, and counted as an approach any individual that came within 0.5 meters of the speaker. Individuals that left the 0.5 meter radius and returned during the same playback were counted only once.

Statistical analysis was performed using SAS software, and the proc genmod routine therein. The number of female approaches during a given playback was modeled using a Poisson regression. The models included territory quality, individual males, call length, and area as predictors. A likelihood ratio Chi Square test was performed to assess the significance of each predictor. An alpha level of 0.05 was used throughout.

## Results

In total, 53 female approaches to playback tapes were observed, over the course of 1200 minutes of actual playback time.

Females differentially approached high-quality territories over low-quality ones (likelihood ratio $\chi^2$=76.33 with 1 D.F., $p<0.0001$). Females did not discriminate based on individual male's calls (likelihood ratio $\chi^2$=4.52 with 5 D.F., $p=0.4766$), or call length (likelihood ratio $\chi^2$=1.95 with 1 D.F., $p=0.1622$); see Figure 5.2. There was a non-statistically significant, combined effect of territory quality and call length ($p=0.0975$), while no other combination of variables produced an effect.

The five areas, each of which consisted of a pair of territories—one high and one low quality—were compared (Figure 5.3). Statistically significant differences were found between areas (likelihood ratio $\chi^2$=20.34 with 4 D.F., $p=0.0004$). The experiment was designed with areas to avoid potential confounds from environmental variation. This result suggests that including an area control is important in such tests.

## Territory Quality

Females approached playback tapes in all 5 of the high-quality territories, but in only 2 of 5 of the low-quality territories (Figure 5.3). 51 approaches were to playbacks in high-quality territories; 2 were in low-quality territories.

## Male Quality

Females approached playbacks of all 6 males. A1's playbacks were approached 9 times; A3's, 10 times; D4's, 10 times; K2's, 7 times; M4's, 9 times; and N5's, 8 times.

## Call Duration

Females approached both short and long calls. To control for the length of time available to females to approach playbacks, while testing for the effect of call duration on female receptivity, the number of female approaches to long calls (15 minutes) was divided by three, and this number was compared, statistically, to the number of female approaches to short calls (5 minutes). If call duration were a factor in female choice of mates, we would expect to see more females approaching longer calls, even after this correction. In fact, with corrected data, 12 approaches were made to long calls, 17 to short ones. This is consistent with the observation that most female approaches were made in the early, rather than late, parts of playbacks of both lengths.

## Discussion

The bird literature is filled with examples of female choice for territory quality rather than, or in addition to, male quality. Both pied flycatchers (Alatolo et al. 1986) and field sparrows (Best 1977) choose mates based on the quality of their territory rather than the quality of aspects of the male, such as song parameters, plumage brightness, size, or age. Female house wrens prefer already mated males, but when that is controlled for, they choose high-quality nest sites (Johnson and Searcy 1993). Great reed warbler females choose high-quality territories, despite the fact that territory quality appears not to have an effect on female reproductive success (Bensch and Hasselquist 1991a). Territory size, plumage, and male size are all factors affecting female choice in Darwin's finches (Price 1984). In all of these examples, either the breeding pair or one of the two parents are providing parental care for the young, and the success of that care is presumably dependent on the quality of the nest site. It is rare that, in a species with paternal care, female birds choose characters that are negatively correlated with the male's likelihood to give parental care (though female blackcaps choose high territory quality over paternal care, Hoi-Leitner et al. 1995).

By contrast, the anuran literature contains few examples of females choosing mates based on territory or other resources rather than male quality. Birds are more likely than anurans to provide parental care. Perhaps parental care, and its correlate, having offspring that can be found once laid, are more important predictors of territory-quality based mate-choice than is an organism's broad phylogenetic classification.

In anurans, the most prevalent cue to mate-choosing females is male vocalizations. Any anuran system in which vocalization cues are insufficient for females to choose the best mate will likely include non-vocal male signals, however. Examples include that of *Dendrobates pumilio* in the Bocas del Toro archipelago of Panama, in which rapid evolution of divergent color morphs has apparently led to assortative mating for like color morph by female choice (Summers et al. 1999). In another diurnal frog, *Hylodes asper*, noisy habitat has decreased reliance on acoustic cues and increased female choice for visual cues (Haddad and Giaretta 1999). Finally, in any anuran system with paternal care, it is expected that females will choose mates, at least in part, on the basis of the quality of the promised care (Summers 1989, 1992).

In *Mantella laevigata*, the degree of paternal investment is somewhat ambiguous, but the importance of high-quality oviposition sites is not, as any egg laid into a well that does not hold water, or that contains the egg predating larval crane-fly *Limonia renaudi Alexander* (Tipulidae), will not survive (chapter three). In this system, females rely more on cues of resource quality than of male quality. To what degree high quality males are able to monopolize territories—by securing, calling from, and obtaining mates exclusively in high-quality ones—is an open question. If no males were ever observed calling and successfully mating from territories defended by other males, it could be assumed that the quality of the male and the territory he is calling from are highly correlated. In such a case, high-quality oviposition sites/territories could be considered a simple proxy for male quality. In *M. laevigata*, however, sneaky males have been observed in successful courtships with females in territories not their own

(Heying, unpub. data). Thus, it cannot be assumed that females are simply using territory quality as a proxy for male quality.

In organisms with parental care and mobility-restricted young, territory quality at the place of egg laying is critical to the offspring's survival, except in those cases where offspring are moved from the oviposition site by attendant parents (as in several dendrobatids; see reviews in Weygoldt 1987, and Crump 1995). In these species, therefore, we expect to see evidence that territory quality is gauged by both sexes, even if both sexes are not providing care for their offspring. In birds this has been well documented. In the Savi's warbler, early arriving males choose territories which correlate with later breeding success, even though females do not appear to be choosy with regard to any aspect of their mates, individual male or territory (Aebischer et al. 1996). Early arrival to the nesting area, with coincident acquisition of the best territory, is the best predictor of breeding success for both sexes in this species. In Eurasian treecreepers, the success of breeding pairs is reduced in territories with wood ants (Aho et al. 1999). In oystercatchers, territory quality is a better predictor of offspring success than parental effort (Ens et al. 1992, Kersten 1996). In frogs, however, few examples are known in which both sexes assess territory quality in the same way. One elegant experiment revealed that both female and male treefrogs discriminate against sites containing the same species of predators and competitors. Males, however, were not as choosy about their calling sites, failing to discriminate against one potential predator species that females did discriminate against when choosing oviposition sites (Resetarits Jr. and Wilbur 1989, 1991). Adults of both sexes were also observed to be selective about oviposition sites in American toads (Petranka et al. 1994) and wood frogs (Hopey and Petranka 1994).

In *Mantella laevigata*, in which both sexes disperse from their natal well, both males and females scout for oviposition sites before engaging in courtships, and their assessment of high-quality sites changes with the condition of those sites. Wells that are active (multiple courtships observed per week) one month may receive no courtships the following month, depending on the other inhabitants and condition of those wells (Heying, unpub. data). Males discriminate against the presence of heterospecific frogs attending clutches, and predatory tipulid larvae, and are significantly less likely to take females to such wells. Courted females are never taken to wells with heterospecific frogs and attendant clutches in them, and discriminate against the few wells they are led to which contain tipulid larvae (chapter two).

The parallel, in systems such as *M. laevigata* with biparental care, between female choice for territory quality, and male-male competition for high quality territories, is predicted by a shared selected goal in these species for territories that can successfully harbor offspring. Male-male competition over male-selected territories/oviposition sites, and female choice, are working in tandem, towards the same goal. This is also observed in song sparrows, in which each sex appears to independently assess territory quality (Weatherhead and Boak 1986). Bisexual territory choice will have the effect of bringing together mates who, broadly, have similar standards for territory quality. Thus, intersexual choice may look like simple convergence of behavior, which results in bringing individuals to the same microhabitat without any interaction between them. I would argue that this is a kind of mate choice, albeit for a subset of individuals who have made the same resource decisions, rather than for a single individual.

Even when territory quality is being actively chosen by both sexes, the mating strategies of the two sexes under non-monogamous conditions can look quite different from one another. Males are often described as competing to convince females of their value, in order to maximize their number of mates, while females try to assess those males who have competed or displayed themselves, in order to maximize the number or quality of their offspring. In green frogs, both males and females discriminate between territories based on the suitability of the vegetation as oviposition sites, but only males discriminate against territories without cover from predators (Wells 1977). In this non-parental care giving species, the male's future survivorship is of no reproductive interest to the female.

Despite the overall similarity of goals in *M. laevigata*, as described above for avoidance of competitors and predators, the sexes do differ with regard to choice of oviposition site in at least one important parameter. Males take females to wells that are not significantly different from average viable wells with regard to conspecific tadpole presence. In contrast, females discriminate against wells that already have conspecific, cannibalistic tadpoles in them (chapters 2 and 4). This observation fits with the prediction that, though both sexes want their offspring to survive, there are different routes to maximizing reproductive success in each sex. Males, with high certainty of paternity of the eggs and tadpoles in the wells they defend, gain reproductively whenever a new egg is laid and fertilized by them in one of their wells. By contrast, females in courtship at a well with a tadpole not their own may suffer a cost from ovipositing in such wells.

Sexual selection should favor females that choose mates on the basis of characteristics that 1) affect their reproductive success, 2) can be assessed prior to mating, and 3) vary among breeding situations (Searcy 1979). Under an honest advertisement model, such characteristics might include male vocalizations, other characteristics of male phenotype, such as size or color, or resources that males defend which are likely to increase the reproductive success of the choosing female. In birds, females often choose some combination of resource and male characteristic, or choose solely based on resources. In anurans, females are far more likely to choose on the basis of male characteristics. The anuran pattern will converge on the avian pattern only when resources are being defended by males, or provided via parental care. When males control access to oviposition sites, but non-territorial males can secure matings within defended sites, and females effectively choose the male who is at the right place at the right in time, the model turns from one of resource-defense polygyny, to one of multiple clutch polygamy (Emlen and Oring 1977), as individuals of both sexes can simultaneously brood several clutches at one time.

I propose that anurans that breed in restricted, transient water bodies are a particularly likely group to display the evolution of mate choice for territory quality for three broad reasons. First, the near universality of external fertilization in anurans means that the mating site is equivalent to the oviposition site, and is therefore where the offspring will develop (except in rare cases in which offspring are transported). Second, derived breeding systems in which the young are traceable, such as in well-breeders, allow parents to identify their offspring on the basis of location. Third, well-breeding, probably an adaptation to prevent against predation, generates new risks, such as desiccation of eggs. Females are under pressure to find a place on land which has a reliable water-source, and it is often these oviposition sites, rather than food, that are limiting for such populations. In *M. laevigata*, oviposition sites do limit population size (see chapter 3).

## Conclusions

Several factors in the social system and ecology of *M. laevigata* have likely facilitated female choice for resources rather than for the genes or current condition of males. The transient, restricted water bodies used as oviposition sites are patchily distributed, of varying qualities, critical to reproductive success, and defended by males. Sexual size dimorphism is not great enough for males to control access to multiple females by coercion. Eggs are large, and clutch size is usually one, suggesting high parental investment in each offspring. Biparental care exists, though the parents do not have a pair-bond. Maternal care begins after eggs hatch into tadpoles. Ongoing male defense of oviposition sites may be considered a form of paternal care, but males also attract females to wells with tadpoles in them, and successful matings frequently end up as food for the male’s preexisting tadpole(s). All of these factors contribute to a system in which high-quality oviposition sites are key to the reproductive success of both sexes.

Not explained by these parameters is why females do not also choose males based on calls, or call duration.

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## Literature Cited

Aebischer, A., N. Perrin, M. Krieg, J. Studer, and D. R. Meyer. 1996. The role of territory choice, mate choice and arrival date on breeding success in the Savi's warbler *Locustella luscinioides*. Journal of Avian Biology **27**:143-152.

Aho, T., M. Kuitunen, J. Suhonen, A. Jantti, and T. Hakkari. 1999. Reproductive success of Eurasian Treecreepers, *Certhia familiaris*, lower in territories with wood ants. Ecology **80**:998-1007.

Alatalo, R. V., A. Lundberg, and C. Glynn. 1986. Female pied flycatchers choose territory quality and not male characteristics. Nature **323**:152-153.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. Evolution:4.

Arvidsson, B. L., and R. Neergaard. 1991. Mate choice in the willow warbler - a field experiment. Behavioral Ecology and Sociobiology **29**:225-229.

Bensch, S., and D. Hasselquist. 1991. Territory infidelity in the polygynous great reed warbler *Acrocephalus arundinaceus*: the effect of variation in territory attractiveness. Journal of Animal Ecology **60**:857-871.

Bertram, S., M. Berrill, and E. Nol. 1996. Male mating success and variation in chorus attendance within and among breeding seasons in the gray treefrog (*Hyla versicolor*). Copeia:729-734.

Best, L. B. 1977. Territory quality and mating success in field sparrow (*Spizella pusilla*). Condor **79**:192-204.

Borgia, G. 1987. A critical review of sexual selection models. Pages 55-66 *in* J. W. Bradbury and M. B. Andersson, editors. Sexual Selection: Testing the Alternatives. Wiley & Sons, New York.

Bourne, G. R. 1998. Amphisexual parental behavior of a terrestrial breeding frog *Eleutherodactylus johnstonei* in Guyana. Behavioral Ecology **9**:1-7.

Buchanan, K. L., and C. K. Catchpole. 1997. Female choice in the sedge warbler, *Acrocephalus schoenobaenus*: Multiple cues from song and territory quality. Proceedings of the Royal Society of London Series B-Biological Sciences **264**:521-526.

Cherry, M. I. 1993. Sexual selection in the raucous toad, *Bufo rangeri*. Animal Behaviour **45**:359-373.

Crump, M. L. 1991. Choice of oviposition site and egg load assessment by a treefrog. Herpetologica **47**:308-315.

Crump, M. L. 1995. Parental care. Pages 518-567 *in* H. Heatwole and B. K. Sullivan, editors. Amphibian Biology.

Darwin, C. 1871. The descent of man, and selection in relation to sex. Princeton University Press, Princeton.

Ens, B. J., M. Kersten, A. Brenninkmeijer, and J. B. Hulscher. 1992. Territory quality, parental effort and reproductive success of oystercatchers (*Haematopus ostralegus*). Journal of Animal Ecology **61**:703-715.

Gerhardt, H. C., M. L. Dyson, and S. D. Tanner. 1996. Dynamic properties of the advertisement calls of gray tree frogs: Patterns of variability and female choice. Behavioral Ecology **7**:7-18.

Giacoma, C., C. Zugolaro, and L. Beani. 1997. The advertisement calls of the green toad (*Bufo viridis*): Variability and role in mate choice. Herpetologica **53**:454-464.

Grafe, U. 1997. Use of metabolic substrates in the gray treefrog Hyla versicolor: Implications for calling behavior. Copeia:356-362.

Greer, B. J., and K. D. Wells. 1980. Territorial and reproductive behavior of the tropical American frog *Centrolenella fleischmanni*. Herpetologica **36**:318-326.

Gross, M. R., and R. Shine. 1981. Parental care and mode of fertilization in ectothermic vertebrates. Evolution **35**:775-793.

Haddad, C. F. B., and A. A. Giaretta. 1999. Visual and acoustic communication in the Brazilian torrent frog, *Hylodes asper* (Anura : Leptodactylidae). Herpetologica **55**:324-333.

Halloy, M., and J. M. Fiaño. 2000. Oviposition site selection in *Pleurodema borellii* (Anura: Leptodactylidae) may be influenced by tadpole presence. Copeia **2000** :606-609.

Hamilton, W. D., and M. Zuk. 1982. Heritable true fitness and bright birds: a role for parasites? Science **218**:384-387.

Hill, G. E. 1994. Trait elaboration via adaptive mate choice: sexual conflict in the evolution of signals of male quality. Ethology Ecology & Evolution **6**:351-370.

Hoelzer, G. A. 1989. The good parent process of sexual selection. Animal Behaviour **38**:1067-1078.

Hoi-Leitner, M., H. Nechtelberger, and H. Hoi. 1995. Song rate as a signal for nest-site quality in blackcaps (*Sylvia atricapilla*). Behavioral Ecology and Sociobiology **37**:399-405.

Hopey, M. E., and J. W. Petranka. 1994. Restriction of wood frogs to fish-free habitats: how important is adult choice? Copeia:1023-1025.

Howard, R. D. 1978. The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. Evolution **32**:850-871.

Howard, R. D. 1978. The influence of male-defended oviposition sites in early embryo mortality in bullfrogs. Ecology **59**:789-798.

Johnson, L. S., and W. A. Searcy. 1993. Nest site quality, female mate choice, and polygyny in the house wren *Troglodytes aedon*. Ethology **95**:265-277.

Kersten, M. 1996. Time and energy budgets of oystercatchers *Haematopus ostralegus* occupying territories of different quality. Ardea **84A**:291-310.

Kirkpatrick, M. 1987. Sexual selection by female choice in polygynous animals. Annual Review of Ecology and Systematics **18**:43-70.

Kirkpatrick, M., and M. J. Ryan. 1991. The evolution of mating preferences and the paradox of the lek. Nature **350**:33-38.

Kluge, A. 1981. The life history, social organization, and parental behavior of *Hyla rosenbergi* Boulenger, a nest-building gladiator frog. Miscellaneous Publications, Museum of Zoology, University of Michigan **160**:1-170.

Klump, G. M., and H. C. Gerhardt. 1987. Use of non-arbitrary acoustic criteria in mate choice by female gray tree frogs. Nature **326**:286-288.

Lopez, P. T., and P. M. Narins. 1991. Mate choice in the neotropical frog, *Eleutherodactylus coqui*. Animal Behaviour **41**:757-772.

Márquez, R., and J. Bosch. 1997. Male advertisement call and female preference in sympatric and allopatric midwife toads. Animal Behaviour **54**:1333-1345.

Marsh, D. M., Rand, A. S. & Ryan, M. J. (2000) Effects of inter-pond distance on the breeding ecology of tungara frogs. *Oecologia* **122:**505-13.

Maynard Smith, J. 1991. Theories of sexual selection. Trends in Ecology & Evolution **6**:146-151.

Passmore, N. I., P. J. Bishop, and N. Caithness. 1992. Calling behaviour influences mating success in male painted reed frogs, *Hyperolius marmoratus*. Ethology **92**:227-241.

Petranka, J. W., M. E. Hopey, B. T. Jennings, S. D. Baird, and S. J. Boone. 1994. Breeding habitat segregation of wood frogs and American toads: the role of interspecific tadpole predation and adult choice. Copeia:691-697.

Pomiankowski, A. 1988. The evolution of female mate preferences for male genetic quality. Oxford Surveys in Evolutionary Biology **5**:136-184.

Prestwich, K. N. 1994. The energetics of acoustic signaling in anurans and insects. American Zoologist **34**:625-643.

Price, T. D. 1984. Sexual selection on body size, territory and plumage variables in a population of Darwin's finches. Evolution **38**:327-341.

Resetarits Jr., W. J. 1996. Oviposition site choice and life history evolution. American Zoologist **36**:205-215.

Resetarits Jr., W. J., and H. M. Wilbur. 1989. Choice of oviposition site by *Hyla chrysosocelis*: role of predators and competitors. Ecology **70**:220-228.

Resetarits Jr., W. J., and H. M. Wilbur. 1991. Calling site choice by *Hyla chrysoscelis*: effect of predators, competitors, and oviposition sites. Ecology **72**:778-786.

Ryan, M. J. 1980. Female mate choice in a neotropical frog. Science **209**:523-525.

Ryan, M. J. 1991. Sexual selection and communication in frogs. Trends in Ecology & Evolution **6**:351-355.

Searcy, W. A. 1979. Female choice of mates: a general model for birds and its application to red-winged blackbirds (*Agalaius phoeniceus*). American Naturalist **114**:77-100.

Spieler, M., and K. E. Linsenmair. 1997. Choice of optimal oviposition sites by *Hoplobatrachus occipitalis* (Anura: Ranidae) in an unpredictable and patchy environment. Oecologia **109**:184-199.

Summers, K. 1989. Sexual selection and intra-female competition in the green poison-dart frog, *Dendrobates auratus*. Animal Behaviour **37**:797-805.

Summers, K. 1992. Mating strategies in two species of dart-poison frogs: a comparative study. Animal Behaviour **43**:907-919.

Summers, K., R. Symula, M. Clough, and T. Cronin. 1999. Visual mate choice in poison frogs. Proceedings of the Royal Society of London Series B-Biological Sciences **266**:2141-2145.

Taigen, T. L., and K. D. Wells. 1985. Energetics of vocalization by an anuran amphibian (*Hyla versicolor*). Journal of Comparative Physiology **155**:163-170.

Verrell, P. A., and L. E. Brown. 1993. Competition among females for mates in a species with male parental care, the midwife toad *Alytes obstetricans*. Ethology **93**:247-257.

Weatherhead, P. J., and K. A. Boak. 1986. Site infidelity in song sparrows. Animal Behaviour **34**:1299-1310.

Welch, A. M., R. D. Semlitsch, and H. C. Gerhardt. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. Science **280**:1928-1930.

Wells, K. D. 1977. Territoriality and male mating success in the green frog (*Rana clamitans*). Ecology **58**:750-762.

Wells, K. D. 1981. Parental behavior of male and female frogs. Pages 184-197 *in* R. D. Alexander and W. D. Tinkle, editors. Natural Selection and Social Behavior. Chiron Press, New York.

Weygoldt, P. 1987. Evolution of parental care in dart poison frogs (Amphibia: Anura: Dendrobatidae). Zeitschrift für Zoölogische Systematik und Evolutions forschung **25**:51-67.

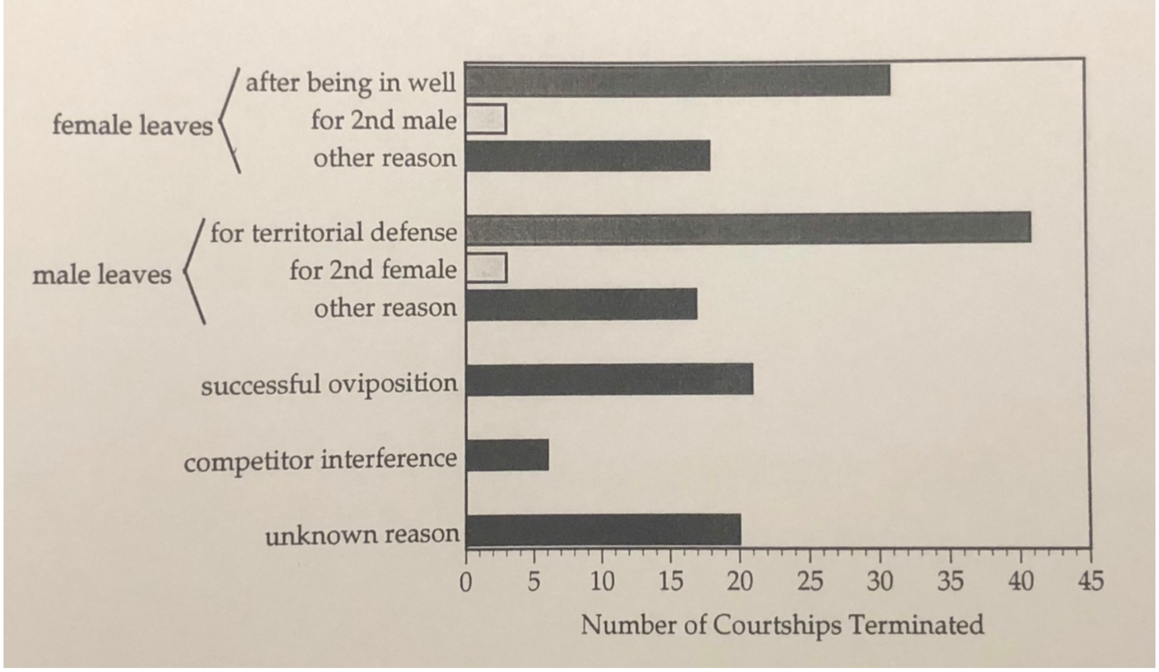
Wollerman, L. 1998. Stabilizing and directional preferences of female *Hyla ebraccata* for calls differing in static properties. Animal Behaviour **55**:1619-1630.

Zahavi, A. 1975. Mate selection - Selection for a handicap. Journal of Theoretical Biology **53**:205-214.

## Table 5.1: Benefits of Female Choice in Anurans

| Type of Honest Advertisement | Specific Cue Female Uses | Heritability and Benefit |
|---|---|---|
| Good Genes | Vocalizations | Heritable: offspring will be similarly robust |
| | Size, Color, Parasite Resistance | |
| Good Resources | Oviposition Site Quality | Not heritable: increase in offspring survivorship |
| | Immediate Paternal Care (e.g. midwife toads) | |
| | Delayed Paternal Care (egg attendance, tadpole transport) | |
| Good Current Condition | Call Duration (to assess current condition) | Indirectly heritable? |
| | Call Duration (to assess availability of food in territory, as proxy for good resources) | Not heritable: increase in maternal survivorship or current condition |
| | Age (by proxy) | Indirectly heritable? (Often correlated with size) |

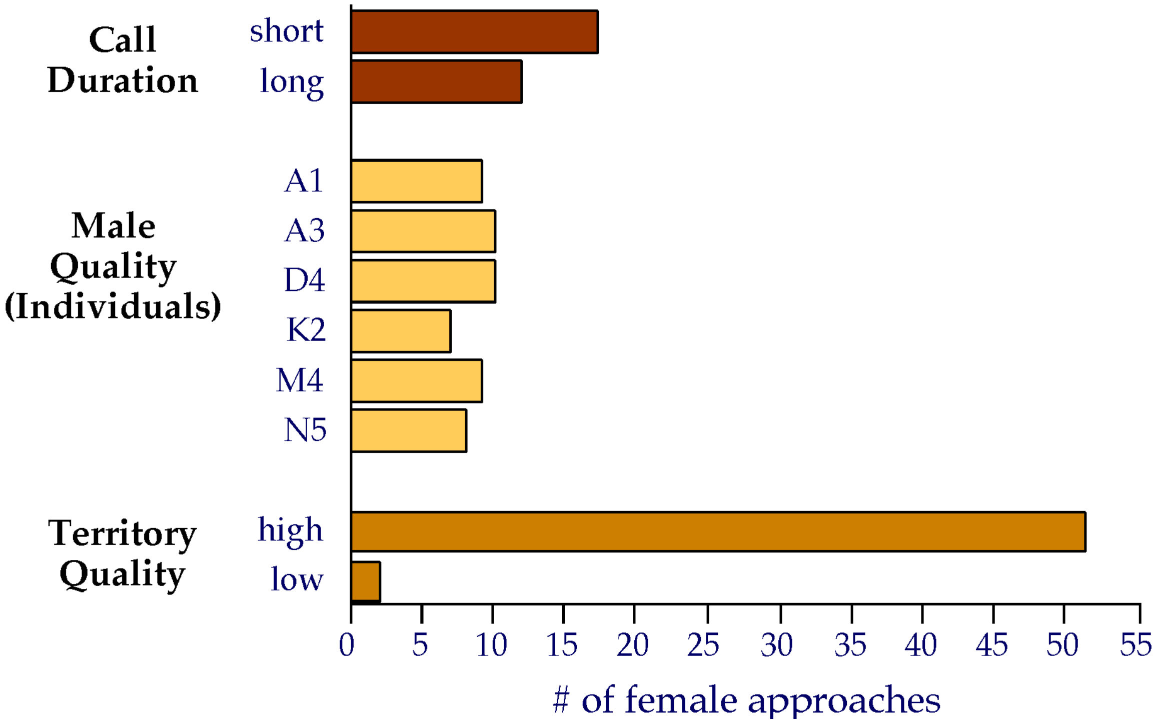
**Figure 5.1: Causes for termination of courtship bouts in *M. laevigata****



160 courtship bouts were observed during 925 hours of focal observations of marked animals. Females most often left after investigating the potential oviposition site ("after being in well"); males most often left to engage in territorial defense (chapter two).

*The original figure is corrupted and no longer exists in digital form. This is a photograph of the figure as it appears in the printed dissertation.

## Figure 5.2: Female response to experimental playbacks

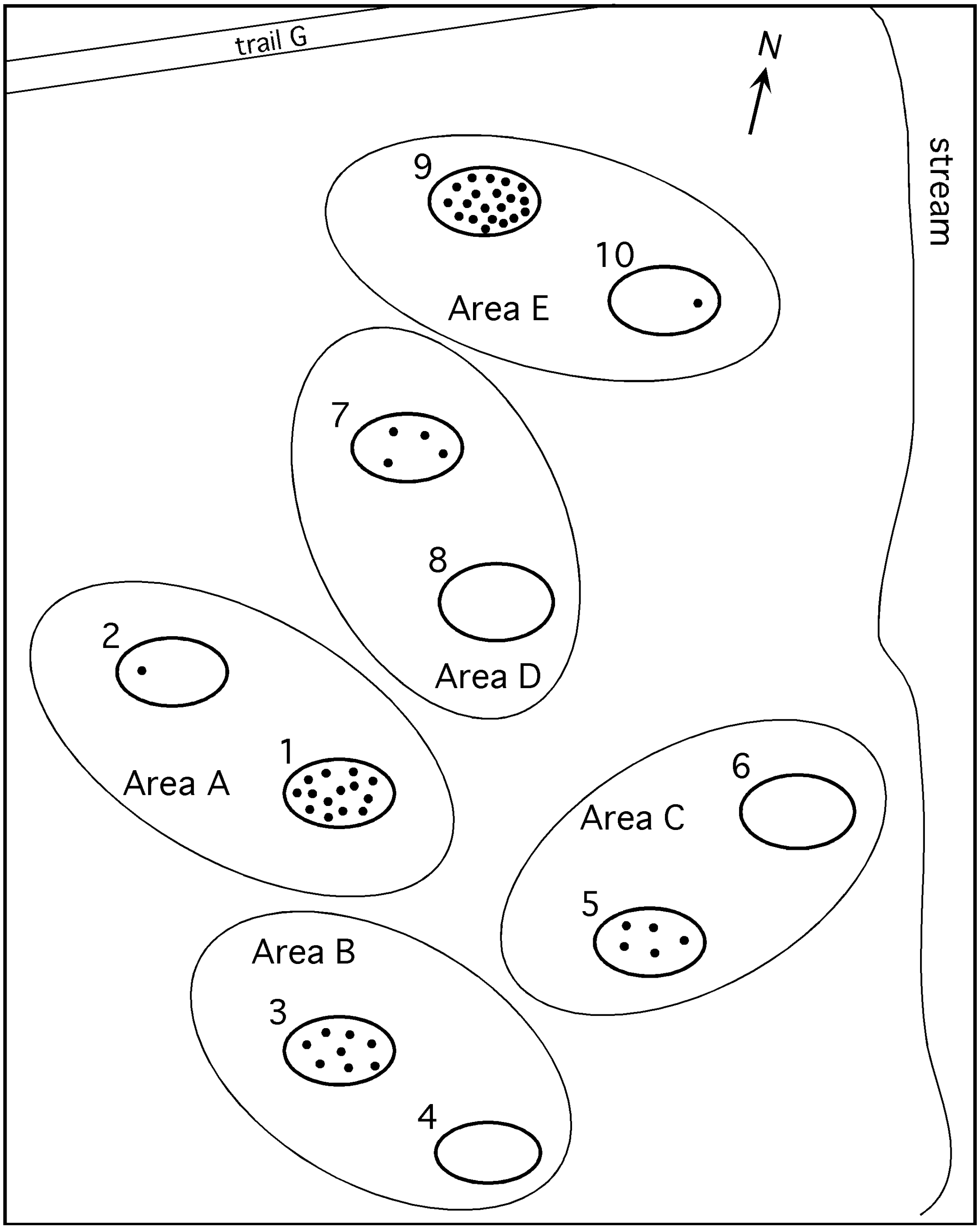


Females approached playback tapes under each of three concurrent experimental treatments: call duration (5 min. versus 15 min.), male quality (6 individual male's calls), and territory quality (high and low). Data presented for female approaches to long calls are divided by three, to prevent time confounds (see text). Of the three treatments, only "territory quality" yielded significant differences in female approaches.

## Figure 5.3: Differential female approaches to territories and areas

Female approaches, during playbacks, differed significantly between territories of high-quality (odd numbered ovals) and low-quality (even numbered ovals). Each female approach is depicted as a dot within that territory. Five areas, each containing one low- and one high-quality territory, were also significantly different from each other with regard to female approaches during playbacks.

**Figure 5.3: Differential female approaches to territories and areas**



# Chapter 6

# Mechanisms of Male Reproductive Success in *Mantella laevigata*: Territoriality, Paternal Care, and Male-Female Conflict

## Abstract

I conducted a combination of male choice experiments and focal observations to assess parameters of male reproductive success. *M. laevigata* males affect their reproductive success through direct male-male competition over defended resources, by adopting one of several mating/territorial strategies; and by providing paternal care in the form of egg attendance, or through indirect feeding, by which the male deceives a courted female into ovipositing into a well already containing one of his tadpoles. Only males that defend limiting reproductive resources are likely to procure matings. Males adopting either of two other, less successful, strategies must be sneaky to obtain mates. In order to become and remain territorial, a male must win fights. Predictors of success in territorial encounters include prior residency, and fight initiation. There is little evidence of male mate choice in this species. Male-female conflict exists when a male courts a female at a well already containing the male's tadpole. The male gains reproductive success any time an egg is deposited in such a well, the female gains only if it develops, and does not get cannibalized by the preexisting tadpole. This conflict is hypothesized to be manifested as divergent maternal and paternal contributions to hatchling size and rate of development.

## Introduction

Male reproductive success in anurans is a result of multiple parameters, including factors affecting mating success—female choice and male-male competition—and post-mating factors, such as parental care. Female choice in *M. laevigata* was discussed in chapter 5, in which I showed that females do not choose individual males, but rather the territories they defend. Male establishment of territories may be understood as the manifestation of preference for areas in which they have had rewarding (reproductively successful) interactions (Stamps and Krishnan 1999). Male-male competition may manifest in a variety of ways beyond conflicts over territorial borders, however, including the adoption of alternative mating strategies on the parts of some males, such as satellite behavior (see review in Arak 1983). And paternal care may be ambiguous in both intent and intensity, when apparently parental behavior is synonymous with territorial behavior or mating effort. The multitude of mechanisms by which males may attain reproductive success in *M. laevigata* is the subject of this paper.

Early studies of sexual selection presumed that variation in the number of mates is the only important cause of sex differences in fecundity (Bateman 1948). Bateman found that, in *Drosophila*, male reproductive success increases with the number of copulations, but female reproductive success does not increase after the second copulation (Bateman 1948). Trivers (1972) later suggested that sexual selection is delimited by the relative parental investment of each sex in offspring. In species with parental investment, the sex of the parent who invests more (usually females) is limiting for the other sex (Trivers 1972). Emlen & Oring (1977) hypothesized that the ability of part of a population to control access of others to potential mates determines the degree of variance in mating success. This may be realized as indirect control, such that the resources necessary for mate attraction or successful reproduction, rather than the mates themselves, are controlled (Emlen & Oring 1977).

In *Drosophila*, there is higher variance in male reproductive success than in female reproductive success (Bateman 1948), and this is predicted in any species where females are limiting for males (Trivers 1972). Males are unlikely to exhibit mate choice when females are limiting, but are likely to exhibit intrasexual competition. Such male-male competition is often expressed as territoriality, a critical parameter determining an individual male's reproductive success.

Territoriality is a response to competition for limited resources needed for individual survival or reproduction (Wells 1977). Male anurans usually establish and maintain their territories by calling to maintain inter-male spacing, and in some species, respond aggressively to intruders, with posturing, chasing, wrestling and jump attacks (Wells 1977). Anuran territoriality is widespread, with examples from several families (e.g. Bufonidae, Cherry 1993; Dendrobatidae, Goodman 1971; Hylidae, Kluge 1981; Leptodactylidae, Weygoldt et al. 1992; Ranidae, Howard 1978a). Only two studies have previously experimentally demonstrated resource limitation with regard to anuran territoriality, however (*Eleutherodactylus coquí*, Stewart and Pough 1983; *Dendrobates pumilio*, Donnelly 1989; see Mathis et al. 1995 for review). *M. laevigata* can now be added to this list (see chapter 3). There are three general categories of territorial behavior in anurans: defense of resources needed for survival, such as retreat sites or food; defense of oviposition sites; and defense of courtship areas (Wells 1977).

As aposematically colored, poisonous frogs, *Mantella laevigata* need specialized retreat sites less than most anurans; when they dive for cover, they simply disappear under the ubiquitous leaf litter. *M. laevigata* are opportunistic foragers on ants, mites, and small flying insects, and food is not limited. Resources necessary for their individual survival are not limited. Oviposition sites are limiting (chapter 3). Males are thus predicted to defend oviposition sites in territorial encounters. Further evidence to support this prediction is found in the nature of female choice in *M. laevigata*. Females are not choosy about the identity of their mates, only the nature of their oviposition sites (chapter 5). If males can control oviposition sites—which are both limiting, and the object of female choice—their reproductive success is likely to be high.

Alexander & Borgia (1979), defining the difference in reproductive effort between the sexes, suggested that males spend more time and energy in mating effort, whereas females expend more energy in parental effort. Indirect control of reproductive resources and territoriality constitute mating effort on the part of males. But indirect care of offspring through guarding them and the areas surrounding the oviposition sites they live in, and acquiring food for them, is parental effort. Although more energy is predicted to be spent in mating effort than in parental effort by males, parental effort is a clear mechanism by which some males can increase their reproductive success. This is particularly true in anurans, the vast majority of which have external fertilization, and therefore higher certainty of paternity than many animals. Paternal care in anurans often takes the form of egg attendance within the adult male's territory (e.g. *Chirixalus* *eiffingeri*, Ueda 1986; *Anotheca spinosa*, Jungfer 1996; *Eleutherodactylus johnstonei*, Bourne 1998). In these cases, the line between mating effort and parental effort is obscured—it may be unclear if any parental effort is either intended or actually effected, especially when there is no attendant decrease in mating effort. Such opportunistic paternal care is a short additional step from the mating effort—territoriality and advertisement calling—that was already taking place.

Finally, male-female conflict necessarily arises when females are discriminating among potential mates (or their territories). Recognition of this conflict can potentially allow males—or females—to increase their individual reproductive success. In *M. laevigata*, males and females have distinct interests when choosing where and when to mate, and a male's reproductive success will likely improve when his interests are reflected in mating choices more often than hers.

## Methods

### Study Site

All research was conducted on the 510 hectare island of Nosy Mangabe, which lies at the Northern end of the Bay of Antongil, five km South of the town of Maroantsetra, Toamasina province, in northeastern Madagascar (15° 30′ S, 49° 46′ E). Nosy Mangabe is a "Special Reserve" in the Masoala National Park, consisting mainly of 100 – 400 year old secondary forest. *M. laevigata* are found throughout the island, but are densest in coastal bamboo stands (*Bambusa vulgaris)*.

## Focal Observations

Data from focal observations were obtained using Methods described in Chapter 2: Social and reproductive behavior in *Mantella laevigata*, with comparisons to the dendrobatids. A summary of these methods follows.

*Mantella laevigat*a were observed during the Malagasy summers of 1997 and 1999 (January through May, and January through April, respectively). Behavioral descriptions throughout this paper are based on observations from both seasons; quantifiable data are based entirely on observations from 1999, due to differences in sampling methods.

*Ad libitum* sampling with continuous recording (Martin & Bateson 1993) was conducted on populations of marked frogs in coastal bamboo stands in 1997, while in 1999, focal watches with continuous recording (Altmann 1974) were used.

In 1997, I and an assistant (JM) observed marked individuals for 509 hours. All animals were toe-clipped, in addition to being given a mark which should have enabled us to identify the animals from a distance, including tying small beaded waistbands onto them, and stitching beads directly into their dorsums. None of the marks given in 1997 was reliable for more than two weeks. In 1999, I and a second assistant (GMF) conducted 416 hours of focal watches on individuals tattooed on their dorsums with a portable battery-operated tattoo machine (Dermo-Marker Tattoo Machine model 401). These tattoos required re-inking every four-six weeks, but the animals returned to their territories and resumed calling and territorial defense (if male) immediately upon being returned from tattooing, so the marking technique did not appear to interfere with behavior.

Focal watches in 1999 were conducted in half hour intervals. Any given animal was watched for as much as two hours per day, but attempts were made to diversify the animals watched. Dominant, territorial males were the easiest to find, so they were watched more often than other individuals. Individuals who disappeared under the leaf litter or up bamboo during a focal watch were searched for until the next fifteen minute interval, at which point a new focal animal was found.

## Playback Experiment

### Acoustic Recording, Digitalization, and Playback

The data for this experiment were obtained simultaneously with those for the female choice experiment described in chapter 5. As female approaches to playback tapes were recorded, so too were male approaches. A summary of the methodology follows.

Advertisement calls of six males from a marked and daily focal-watched population (in bamboo stand 4) were recorded. The males recorded included all three identified types (chapter 2): males who actively defend territories containing resources, males who actively defend territories without resources, and males without territories.

Call recordings were made with a Sennheiser K6 electret condenser microphone system, and a Sony Professional Walkman, model WM-D6C.. Recordings were made of each of the six frogs on multiple days, from February 17 - March 18, 1999. Calls were digitized on a Macintosh PowerBook 3400 using Adobe Premiere 4.2.1 software, then re-recorded onto new, identical audio tapes. Each 15 minute recording contained three identical five minute recordings, so that playbacks of five and fifteen minutes did not differ in the quality of the calls, only the duration. Playbacks were conducted using the Sony Professional Walkman and a single, internally amplified Sony SRS-A33 speaker.

## Playback Experimental Design

In an undisturbed population of *M. laevigata* (bamboo stand 6), ten territories were chosen. I went repeatedly to potential oviposition sites and waited ten minutes to record if any adult *M. laevigata* of either sex came to those sites. If adult *M. laevigata* arrived within 0.5 meters of the site a majority of the time that I visited it, I coded it as a "high quality territory," following Weatherhead and Boak's (1986) work, which used nonrandom variation in occupation of territories as a proxy for territory quality. After this assessment was made, I observed that all high quality territories contained oviposition sites, which are limiting for *M. laevigata* (chapter 3). For each of five such high quality territories, a low quality territory was delineated 2.5 meters away, in a randomly chosen compass direction from the high quality territory. None of the low quality territories contained obvious oviposition sites. Thus, environmental variables were controlled for by using spatially linked pairs of territories (‘areas”), while maintaining all territories within the range of known *M. laevigata* populations. Although most males are territorial, even resource-defense territorial males (see chapter 2, Results) assess territories not their own, and respond to advertisement calls in nearby territories, so playbacks from within territories are likely to attract males.

Tapes of each of the six males, in lengths of 5 and 15 minutes, were played-back in each of the ten territories. For each of twelve days, ten unique combinations of territory, male, and call length were pulled out of a hat, and playbacks were performed in the order in which they were pulled. Playbacks were performed over the course of 23 days, from March 19 through April 10, 1999.

During playbacks, I monitored male approaches to the territory/speaker, and counted as an approach any individual that came within 0.5 meters of the speaker. Individuals that left the 0.5 meter radius and returned during the same playback were counted only once.

Statistical analysis was performed using SAS software, and the proc genmod routine therein. The number of female approaches during a given playback was modeled using a Poisson regression. The models included territory quality, individual males, call length, and area as predictors. A likelihood ratio Chi Square test was performed to assess the significance of each predictor. An alpha level of 0.05 was used throughout.

## Results

### Male Strategies and Territoriality: Descriptive

During 925 total hours of focal observations, males were observed displaying territorial behavior during every hour of observation. All seven parameters coded by Wells (1977) as evidence of aggressive behavior related to site-specific territoriality were observed in *M. laevigata*: site attachment, encounter calls, postural displays, residents chasing intruders, jump attacks, wrestling, and oviposition within the defended territory.

Advertisement calls occurred whenever frogs were active. Single frogs sometimes called alone for extended periods, but more often engaged in antiphonal calling, alternating with one or two other neighbors between notes. RD-territorial males often sat silent at calling perches, even when there was distant ambient call, but always called when a neighbor began, or when they saw another adult of either sex.

Two hundred and fifteen fights were witnessed between males, in 367 bouts. Fight bouts between males last from ten seconds to more than 1.5 hours. Fights take place primarily over territories, the best of which include water-filled wells in which mating takes place, and eggs and tadpoles develop. Males defend these wells, and an area not less than 500 cm diameter around them, with advertisement calls and fights.

Individual males are of three types: using one of two distinct forms of territorial defense, or not defending territories at all. Among the territorial males, some defend resources, others appear not to. I define males in possession of territories with wells in them “resource-defense territorial,” or RD-territorial. The number of viable oviposition sites in the territories of RD-territorial males ranged from 1-6. I define males in possession of territories without wells as “no-resource territorial", or NR-territorial. As wells are known to be limiting (chapter 3), and other parameters such as food are not, territories without wells in them are defined as without resource, and therefore of low-quality. Both NR-territorial and RD-territorial males scout in other males’ territories, usually without advertisement calls, when they are not defending their own territory. Finally, there are "aterritorial" males that have not been seen defending any area, but that spend time in territorial males’ territories and try to attract females therein. These "aterritorial" males may in fact be NR-territorial, with defended territories outside the range of my observations. When either NR-territorial or aterritorial males are in territories with wells, I refer to them as non-residents, and the territory-holder as the resident. I use new terms to describe the three alternative male strategies because an identical system does not appear to exist in the literature. The closest is that of bullfrogs, with three alternative strategies: territorial, parasitic, and opportunistic (Howard 1978b). The first two strategies closely resemble *M. laevigata* strategies (bullfrog territorial: *Mantella* RD-territorial; and bullfrog parasitic: *Mantella* aterritorial), but the bullfrog opportunistic strategy differs substantially from the *Mantella* NR-territorial in that *M. laevigata* males defend permanent, resource-less territories, and do not abandon them when other males approach.

Both NR-territorial and aterritorial males sometimes successfully lead females into matings in wells in territories not their own. Often these non-resident males are seen by the resident (territory-holder) and attacked, at which point they respond in one of two ways. Non-resident males may fight back, often repeatedly (several bouts); or they may be docile, not call, and submit to being amplexed by the male resident.

When courtships begin, males lower the volume of their advertisement call, and sometimes emit a single note “courtship vocalization” in addition. Both the courtship vocalization, and the advertisement call, when emitted during courtships, are softer than the usual advertisement calls.

RD-territorial males can usually be found in a given territory, not larger than 2 meters square, which contains at least one water-filled well—usually broken bamboo. RD-territorial males call frequently from their territories. NR-territorial males have a high rate of site fidelity as well, but the sites they call from and patrol do not have wells in them. These males call and attract females to these low-quality territories, after which they lead females into other males’ (high-quality) territories. Territory borders, and even interiors, are repeatedly fought over by the same males. Fights include male-male amplexus, belly-to-belly wrestling, tumbling, leaping on to one another, and chasing. Aterritorial males have home ranges that they do not defend against other males.

## Fight Outcomes: Quantitative

In 1999, 131 complete fights were observed between known males. RD-territorial males won more fights than NR-territorial males, who won more fights than aterritorial males (Table 6.1). Unlike male type, male size is not correlated with ability to win fights (Figure 6.1). Furthermore, male size is not correlated with male type (SVL: Kruskal-Wallis, H=0.152, p=0.9266; mass: Kruskal-Wallis, H=0.752, p=0.6865).

During courtship, males discriminate against oviposition sites containing tipulids (crane-fly larvae), and those containing other frog species. Males prefer oviposition sites that already contain tadpoles (Table 6.2). Females are significantly more likely to reject oviposition sites containing tadpoles (Table 6.3). Furthermore, males left courtships frequently—more often than females did—and when they did so, it was usually to engage in territorial defense (Figure 6.3).

Of 131 complete fights observed during focal watches, 108 (82.4%) of those fights were between a territory holder and a non-resident. The remaining fights were between two males maintaining the border between their adjacent territories, or between two non-resident males, when the resident, territory holder was absent (Figure 6.3). Of the 108 fights between territory holders and non-residents, 96 (88.9%) of the fights were won by the resident, territory holder (Figure 6.4). When residents lost fights, there was usually a temporary redefinition of the territory border in favor of the winner. I also observed an initiator's advantage, as only 17 of 131 complete fights (12.9%) were won by non-initiators, 10 of which were won by residents who were attacked by non-residents.

## Playback Experiment

In total, 49 male approaches to playback tapes were observed, over the course of 1200 minutes of actual playback time.

Males differentially approached high-quality territories over low-quality ones (likelihood ratio $\chi^2=56.13$ with 1 D.F., $p<0.0001$). Males also approached shorter calls more often than longer calls (likelihood ratio $\chi^2=4.75$ with 1 D.F., $p=0.0293$). Males did not discriminate based on individual male's calls (likelihood ratio $\chi^2=4.13$ with 5 D.F., $p=0.5307$); see Figure 6.5. No combination of variables (e.g. territory quality and call length) produced a significant effect.

The five areas, each of which consisted of a pair of territories—one high and one low quality—were compared (Figure 6.6). Statistically significant differences were found between areas (likelihood ratio $\chi^2=13.95$ with 4 D.F., $p=0.0074$). The experiment was designed with these "areas" to control for potential confounds from environmental variation. This result suggests that including an area control is important in such tests.

## Territory Quality

Males approached playback tapes in all 5 of the high-quality territories, but in only 2 of 5 of the low-quality territories (Figure 6.6). 45 approaches were to playbacks in high-quality territories; 4 were in low-quality territories.

## Male Quality

Males approached playbacks of all 6 males. A1's playbacks were approached 7 times; A3's, 10 times; D4's, 9 times; K2's, 7 times; M4's, 7 times; and N5's, 9 times.

## Call Duration

Males approached both short and long calls. To control for the length of time available to males to approach playbacks, while testing for the effect of call duration on male receptivity, the number of male approaches to long calls (15 minutes) was divided by three, and this number was compared, statistically, to the number of male approaches to short calls (5 minutes). If call duration were a factor in male approach to playbacks, we would expect to see more males approaching longer calls, even after this correction. In fact, with corrected data, 10.3 approaches were made to long calls, 18 to short ones, yielding a significant result in the opposite direction predicted. Qualitative observations of when, during the playbacks, males tended to approach suggest that males respond quickly to conspecific calls. Most male approaches were made in the early, rather than late, parts of playbacks of both lengths.

## Discussion

Male reproductive success in *M. laevigata* is accomplished through a variety of behaviors, including male-male competition, and indirect male parental care. Territoriality, the expression of which is plastic in *M. laevigata*, is central to both of these behaviors. Any complex social system with the potential for variable success in mate acquisition may produce multiple strategies. Behavioral tactics may vary in response to variation in resource quality, competitor quality, population size, or energetic cost of the behavior (see Robakiewicz 1992 for review). In a system where controlling female access to reproductive resources, interception of females *en route* to courtships with territorial males, paternal care, and manipulation of male-female conflict are all possible mechanisms by which males obtain reproductive opportunities, each individual male is expected to pursue and obtain reproductive success with a unique combination of these alternative mating strategies.

### Male-Male Competition and Alternate Mating Strategies

Male *M. laevigata* who successfully defend high quality territories (which include oviposition sites and calling perches) attract females. Males who don't defend high quality territories (NR-territorial and aterritorial males) have to be sneaky to attract, court, and mate with females, and are not successful very often. High quality territories, which contain oviposition sites, are limited, and limiting (chapter 3), and quality is, to a first approximation, binary: either a territory contains a viable oviposition site, or it does not. (Territory quality can change over time, however; see chapter 4). Females do not approach low quality territories, or males in them (chapter 5). It is, therefore, critical for males to accurately assess the quality of territories, and reject those that could not support eggs or tadpoles. In treefrogs, calling males are most likely to be found at oviposition sites with low risk of larval mortality (Crump 1991). Similarly, in *M. laevigata*, males fight for access to high quality territories, and ignore other males in low quality territories. Males do not discern, or do not choose to discriminate between, their competitors, or how long they call, but do discern territory quality (Figures 6.5 and 6.6).

Multiple male strategies are predicted to be common in systems with steep gradients in site or male quality (Waltz 1982). In *M. laevigata*, an extremely limiting reproductive resource is the focus of all reproductive behavior of both sexes. There is no possibility of reproduction outside of the narrowly defined treehole oviposition site. In other anuran species with alternative mating strategies, males are similarly struggling for the same, limiting resources. In American toads, which show two distinct male mating behaviors—calling from the breeding site, versus mate searching on land—both tactics are reproductively viable (Forester and Thompson 1998). In *Eleutherodactylus johnstonei*, three male mating tactics coexist (calling from territories, satellite behavior, and interfering in courtships of neighbors), but it is unclear if all the tactics are viable (Ovaska and Hunte 1992). As a result of extreme reproductive limitation in *M. laevigata*, there are successful males (RD-territorial males who defend oviposition sites), and less successful males (NR-territorial and aterritorial males, who must sneak into oviposition sites to mate). RD-territorial males tend to enjoy reproductive success; NR-territorial and aterritorial males tend not to.

Given the persistence of alternate strategies in *M. laevigata*, however, aterritorial and NR-territorial males must sometimes gain reproductive success. Males are engaged in a constant assessment of the reproductive potential around them. When high quality territories become temporarily vacant, they move in quickly. In gray treefrogs (*Hyla versicolor)*, satellite males occupy preferred calling perches that have been vacated when dominant males achieve amplexus (Fellers 1979). In *M. laevigata*, both NR-territorial and aterritorial males are often observed replacing resident, RD-territorial males at calling sites when the residents leave to actively pursue courtships. Such males attract females as easily as residents do, as females don’t distinguish between individual males. The behavior remains risky, though, as in all observed cases, the territory owner ultimately returned and regained his territory. In some cases, however, it was not until after a successful courtship had occurred, and eggs had been laid in the territory owner's well.

Tenure in the chorus, and not calling activity, is the variable influencing male mating success in several chorusing species (e.g. *Hyla versicolor*, Sullivan and Hinshaw 1992; *Hyla chrysoscelis*, Ritke and Semlitsch 1991). In a non-chorusing species such as *M. laevigata*, in which most males are territorial, and females range more widely than males do, the sneaky male strategy of spending time at the edges of areas of concentrated good territories (bamboo stands) may be effective. As females approach calling males in those high-quality territories, they get waylaid by sneaky males (utilizing either of the two non-dominant strategies), who then try to lead them to an oviposition site in someone else’s territory.

Once they have obtained courtships, NR-territorial and aterritorial males reduce the volume of their calls. Wells (1988) hypothesized that the softer calls of males in several anuran species during courtship may be advantageous by reducing the probability of detection by rivals. This hypothesis is supported in *Eleutherodactylus antillensis* (Ovaska and Caldbeck 1997), and is likely to explain the reduced volume of courtship vocalizations in *M. laevigata* as well. NR-territorial and aterritorial males need to successfully court females within other males' territories in order to enjoy reproductive success, and avoiding detection by the resident, RD-territorial male is critical for this to happen.

The most reliable route to male reproductive success is to be an RD-territorial male. The only route to becoming, and remaining, an RD-territorial male, is to win aggressive encounters, usually fights, with other males (see Table 6.1). Territorial disputes take place in high quality territories, and at the borders between them. What predicts the outcome of territorial encounters? Some parameters recognized to play a role in fight success in other anuran species include male size, residency status, age, calling effort, and initiator advantage.

## Who Wins Fights?

Larger males win fights in *Uperoleia rugosa* (Robertson 1986), and in species with alternative male strategies, males often sort by size, with smaller males tending to adopt sneaky, or non-territorial roles (e.g. Given 1988, Dunn et al. 1999). In some systems, male size is confounded by prior resident advantage. Both field and lab research has demonstrated that, in a majority of territorial animals studied, when individuals interact aggressively over space, the resident usually wins (see reviews in Huntingford and Turner 1987, Archer 1988, also Olsson and Shine 2000). Prior resident advantage is well known in frogs (e.g. *Rana virgatipes*, Given 1988; *Eleutherodactylus coquí*, Stewart and Rand 1991), and persists in several species even when the resident is smaller than the intruder (e.g. *Rana clamitans*, Wells 1978; *Hyla rosenbergi*, Kluge 1981; *Atelopus varius*, Crump 1988 ). In *M. laevigata*, there is no male size advantage (Figure 6.1), but there is a clear prior resident advantage, which trumps male size (Figure 6.3). Resident males are likely to have an advantage because they know the territory, and because they obtained it, presumably from fighting, and are therefore capable at male-male competition. More basically, though, once a territory is obtained, it is easier to retain it. This has been demonstrated in *Dendrobates pumilio* (Robakiewicz 1992, Baugh and Forester 1994), and Forester et al. (1993) demonstrated in the same species that frogs that have been in residency on a territory for longer are more aggressive in defending that territory.

In bullfrogs, male strategy is largely determined by age, and older males tend to win territorial encounters (Howard 1978b). I have no evidence to address strategy-by-age sorting in *M. laevigata*. Older males may be better able to secure high quality territories, simply because they have been around for longer, and thus able to take advantage of territory vacancies when residents die or become weak. Even if older males are in fact more likely to be RD-territorial, and thus more likely to acquire matings, and have higher reproductive success, there is still no evidence that females prefer older males.

In green frogs (*Rana clamitans)*, males distinguish between other males' calls, and respond differently depending on the type and frequency of call. Males are communicating through their calls, and territorial encounters are affected by these calls (Bee and Perrill 1996). In *M. laevigata*, there is no evidence that males discriminate other males based on their calls. There is evidence, however, of an initiator advantage, wherein the frog who starts a fight is more likely to win it.

In *M. laevigata*, there is no evidence of a size or calling effort advantage in predicting the outcomes of territorial encounters. Age may play a role, but is probably working in concert with prior resident advantage if so. Prior residency and initiator advantage are the only clear rules to predict the outcomes of fights in *M. laevigata*. RD-territorial males are, by definition, the residents at high quality territories, so they tend to win fights (which take place almost exclusively in these territories). The only parameter I detected that non-resident males could use to unseat an RD-territorial male from his territory is the initiator advantage. NR-territorial and aterritorial males are predicted to initiate fights with RD-territorial males, if they want their strategy, and therefore their likely reproductive success, to change for the better.

## Male Parental Care

In the vast majority of anurans, external fertilization is the rule. In such a system, as in monogamous and polyandrous mating systems, male confidence of paternity should approach, or even exceed, that of females (Trivers 1972). With confidence of paternity comes the possibility of paternal care.

Egg attendance and defense against predators are common forms of anuran paternal care. In several species males do not call while they are brooding their young, and therefore miss mating opportunities (e.g.

*Eleutherodactylus coquí*, Townsend et al. 1984, Townsend 1986; *Hyla faber*, Martins et al. 1998). In *M. laevigata*, only RD-territorial males could conceivably engage in paternal care, as they are the only males who continue to have access to oviposition sites after the mating is over. Males do not appear to alter their calling behavior when they have eggs or tadpoles in wells, but part of the potential for paternal care in this species is feeding the young conspecific eggs. As such, males are expected to continue to try to garner matings, such that the newly laid eggs may either serve as "lunch" for preexisting tadpoles, or develop into their own individuals (the "icebox hypothesis," Mock and Parker 1997).

Finally, male choice of oviposition site is known to have an effect on egg or larval survivorship in two species of frogs (Howard 1978a, Townsend 1989), as well as grasshoppers (Greenfield et al. 1989) and damselflies (Fincke 1992). Because females only approach males at high quality territories, male discrimination of oviposition site is clearly mating effort, but if larval survivorship is affected by this choice, it might be considered parental effort as well.

## Male-Female Conflict

In *M. laevigata*, males have an opportunity to contribute to their reproductive success in such a way that their actions may result in either mating or parental effort, but which one cannot be predicted beforehand. To do so, the male must successfully deceive the female he is courting, which puts his reproductive interest at direct odds with hers. Males show a non-significant preference to take courted females to wells already containing tadpoles (Table 6.2), although females show a significant preference for empty, viable wells (Table 6.3), and often abandon courtships after being led to unacceptable wells (Figure 6.2). Presumably, these males have high certainty of paternity in these tadpoles, as the tadpoles exist in wells in the males' territories. Males gain from successful courtships in wells already containing their tadpoles in one of two ways. If the oviposited egg develops and hatches, he was engaged in successful mating effort. If the oviposited egg is eaten by the tadpole inside, he has procured food for his offspring, and has engaged in parental effort. Because these frogs are not pair-bonded, the courted female is rarely the mother of the resident tadpole. The female, then, has an interest in avoiding wells already containing tadpoles. In pair-bonded indigo buntings, extra-pair copulations provide an additional source of reproductive success not otherwise accounted for in the clean division between mating and parental effort (Westneat 1988). In *M. laevigata*, male courtship activities move across the boundary between mating and parental effort, depending on context.

The male-female conflict over the contents of oviposition sites at the time of oviposition leads to a hypothesis of conflict over hatching size and rate of development as well. Females have no interest in their offspring remaining eggs any longer than they must; threats to their eggs come both from cannibalistic tadpoles, and from predatory crane-fly larvae. RD-territorial males, on the other hand, have occasionally conflicting interests. Feeding-age tadpoles are more likely to survive to adulthood than newly laid eggs, so the father of both has an interest in one of his offspring eating the other. This might be more easily achieved if the egg has a slower development rate, or hatches when it is larger.

In two species of *Rana*, maternal contribution affects the size of hatchlings, but the male contribution alone does not (Semlitsch and Schmiedehausen 1994). I hypothesize that maternal and paternal contributions to hatchling size and rate of development in *M. laevigata* are similarly divergent.

## Evidence for Male Choice?

Male reproductive success is clearly distinct from female reproductive success, as evidenced from male-female conflict in this species, by which the different sexes make unique choices about oviposition site parameters. Several parameters of the *M. laevigata* social system fit predictions of male mate choice: males provide benefits to both females and offspring by defending oviposition sites, but the courtship process is long enough to limit, though not preclude, multiple matings by males (Krupa 1995). Furthermore, the resource provided by the male in this system cannot be used simultaneously by a large number of offspring, as tadpoles will eat younger, conspecific eggs if they can. Despite this array of mechanisms by which males can affect their own reproductive success in *M. laevigata*, there is little evidence to support direct male mate choice of females in this species. Males reassess their situations continuously, and frequently abandon courtships to engage in territorial defense; they rarely leave one receptive female to court another (Figure 6.2).

Instead, males actively seek and defend oviposition sites. Males and females are similar in this regard, focusing primarily on the quality and/or defense of oviposition sites, rather than on the quality of their mates. “Mate” choice for oviposition sites rather than individuals is consistent with experimental evidence that these sites are limiting for *M. laevigata* (chapter 3). Males influence their reproductive success through a combination of several strategies, but not through intersexual choice.

## Summary

*Mantella laevigata* males can affect their reproductive success through direct male-male competition, by adopting one of several mating/territorial strategies; and by providing paternal care in the form of egg attendance, or through indirect feeding, by which the male deceives a courted female into ovipositing into a well already containing one of his tadpoles.

Only males that defend limiting reproductive resources are likely to procure matings and be reproductively successful. Males adopting either of two other, less successful, strategies must be sneaky to obtain mates. They usurp the calling sites of RD-territorial males when those males are elsewhere; they frequent the edges of high quality territories and intercept approaching females; and they reduce the volume of their vocalizations during courtships, perhaps to avoid detection by resident males. In order to become and remain RD-territorial, a male must win fights. Predictors of success in territorial encounters include prior residency, and initiating the fight. RD-territorial males almost always have the prior residency advantage.

Parental care is facultative, can only be performed by RD-territorial males, and does not involve much, if any, change of behavior on the part of resident males. Males choose viable oviposition sites to defend, they attend eggs and tadpoles, and generate food for their tadpoles by bringing gravid females to court at oviposition sites where the oviphagous tadpoles live. Males have an interest in deceiving courted females into ovipositing into wells that already contain the male's tadpoles, which is a source of male-female conflict. Finally, there is no evidence for male mate choice in this species, though males choose oviposition sites carefully, as do females.

## Acknowledgments

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## Literature Cited

Alexander, R. D., and G. Borgia. 1979. On the origin and basis of the male-female phenomenon. Pages 417-440 *in* M. S. Blum and N. A. Blum, editors. Sexual Selection and Reproductive Competition in Insects. Academic Press, New York.

Altmann, J. 1974. Observational study of behavior: sampling methods. Behavior: An International Journal of Comparative Ethology **49**:227-267.

Arak, A. 1983. Male-male competition and mate choice in anuran amphibians. Pages 181-210 *in* P. Bateson, editor. Mate Choice. Cambridge University Press, Cambridge.

Archer, J. 1988. The behavioural biology of aggression. Cambridge University Press, Cambridge.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity **2**:349-368.

Baugh, J. R., and D. C. Forester. 1994. Prior residence effect in the dart-poison frog, *Dendrobates pumilio*. Behaviour **131**:207-224.

Bee, M. A., and S. A. Perrill. 1996. Responses to conspecific advertisement calls in the green frog (*Rana clamitans*) and their role in male-male communication. Behaviour **133**:283-301.

Bourne, G. R. 1998. Amphisexual parental behavior of a terrestrial breeding frog *Eleutherodactylus johnstonei* in Guyana. Behavioral Ecology **9**:1-7.

Cherry, M. I. 1993. Sexual selection in the raucous toad, *Bufo rangeri*. Animal Behaviour **45**:359-373.

Crump, M. L. 1988. Aggression in harlequin frogs: male-male competition and a possible conflict of interest between the sexes. Animal Behaviour **36**:1064-1077.

Crump, M. L. 1991. Choice of oviposition site and egg load assessment by a treefrog. Herpetologica **47**:308-315.

Donnelly, M. A. 1989. Demographic effects of reproductive resource supplementation in a territorial frog, *Dendrobates pumilio*. Ecological Monographs **59**:207-221.

Dunn, D. W., C. S. Crean, C. L. Wilson, and A. S. Gilburn. 1999. Male choice, willingness to mate and body size in seaweed flies (Diptera : Coelopidae). Animal Behaviour **57**:847-853.

Emlen, S. T., and L. W. Oring. 1977. Ecology, sexual selection, and the evolution of mating systems. Science **197**:215-223.

Fellers, G. M. 1979. Mate selection in the gray treefrog, *Hyla versicolor*. Copeia:286-290.

Fincke, O. M. 1992. Consequences of larval ecology for territoriality and reproductive success of a neotropical damselfly. Ecology **73**:449-462.

Forester, D. C., J. Cover, and A. Wisnieski. 1993. The influence of time of residency on the tenacity of territorial defense by the dart-poison frog *Dendrobates pumilio*. Herpetologica **49**:94-99.

Forester, D. C., and K. J. Thompson. 1998. Gauntlet behaviour as a male sexual tactic in the American toad (Amphibia: Bufonidae). Behaviour **135**:99-119.

Given, M. F. 1988. Territoriality and aggressive interactions of male carpenter frogs, *Rana virgatipes*. Copeia **2**:411-421.

Goodman, D. E. 1971. Territorial behavior in a neotropical frog, *Dendrobates granuliferus*. Copeia **2**:365-370.

Greenfield, M. D., T. E. Shelley, and A. Gonzalez-Coloma. 1989. Territory selection in a desert grasshopper: the maximization of conversion efficiency on a chemically defended shrub. Journal of Animal Ecology **58**:761-771.

Howard, R. D. 1978a. The influence of male-defended oviposition sites in early embryo mortality in bullfrogs. Ecology **59**:789-798.

Howard, R. D. 1978b. The evolution of mating strategies in bullfrogs, *Rana catesbeiana*. Evolution **32**:850-871.

Huntingford, F. A., and Angela K. Turner. 1987. Animal Conflict. Chapman and Hall, London.

Jungfer, K. H. 1996. Reproduction and parental care of the coronated treefrog, *Anotheca spinosa* (Steindachner, 1864) (Anura: Hylidae). Herpetologica **52**:25-32.

Kluge, A. 1981. The life history, social organization, and parental behavior of *Hyla rosenbergi* Boulenger, a nest-building gladiator frog. Miscellaneous Publications, Museum of Zoology, University of Michigan **160**:1-170.

Krupa, J. J. 1995. How likely is male mate choice among anurans? Behaviour **132**:643-664.

Martin, P., and P. Bateson. 1993. Measuring behavior: an introductory guide, 2 edition. Cambridge University Press, Cambridge.

Martins, M., J. P. Pombal, and C. F. B. Haddad. 1998. Escalated aggressive behaviour and facultative parental care in the nest building gladiator frog, *Hyla faber*. Amphibia-Reptilia **19**:65-73.

Mathis, A., R. G. Jaeger, W. H. Keen, P. K. Ducey, S. C. Walls, and B. W. Buchanan. 1995. Aggression and territoriality by salamanders and a comparison with the territorial behaviour of frogs. Pages 633-676 *in* B. K. Sullivan and H. Heatwole, editors. Amphibian Biology. Surrey Beatty & Sons, Chipping Norton.

Mock, D. W., and G. A. Parker. 1997. The Evolution of Sibling Rivalry. Oxford University Press, Oxford.

Olsson, M., and R. Shine. 2000. Ownership influences the outcome of male-male contests in the scincid lizard, Niveoscincus microlepidotus. Behavioral Ecology **11**:587-590.

Ovaska, K., and W. Hunte. 1992. Male mating behavior of the frog *Eleutherodactylus johnstonei* (Leptodactylidae) in Barbados, West Indies. Herpetologica **48**:40-49.

Ovaska, K. E., and J. Caldbeck. 1997. Vocal behaviour of the frog *Eleutherodactylus antillensis* on the British Virgin Islands. Animal Behaviour **54**:181-188.

Ritke, M. E., and R. D. Semlitsch. 1991. Mating behavior and determinants of male mating success in the gray treefrog, *Hyla chrysoscelis*. Canadian Journal of Zoology **69**:246-250.

Robakiewicz, P. 1992. Behavioral and physiological correlates of territoriality in a dart-poison frog *Dendrobates pumilio*. Schmidt. Ph.D. University of Connecticut, Storrs.

Robertson, J. G. M. 1986. Male territoriality, fighting and assessment of fighting ability in the Australian frog *Uperoleia rugosa*. Animal Behaviour **34**:763-772.

Semlitsch, R. D., and S. Schmiedehausen. 1994. Parental contributions to variation in hatchling size and its relationship to growth and metamorphosis in tadpoles of *Rana lessonae* and *Rana esculenta*. Copeia:406-412.

Stamps, J. A., and V. V. Krishnan. 1999. A learning-based model of territory establishment. Quarterly Review of Biology **74**:291-318.

Stewart, M. M., and F. H. Pough. 1983. Population density of tropical forest frogs: relation to retreat sites. Science **221**:570-572.

Stewart, M. M., and A. S. Rand. 1991. Vocalizations and the defense of retreat sites by male and female frogs, *Eleutherodactylus coqui*. Copeia:1013-1024.

Sullivan, B. K., and S. H. Hinshaw. 1992. Female choice and selection on male calling behavior in the gray treefrog *Hyla versicolor*. Animal Behaviour **44**:733-744.

Townsend, D. S. 1986. The costs of male parental care and its evolution in a neotropical frog. Behavioral Ecology and Sociobiology **19**:187-195.

Townsend, D. S. 1989. The consequences of microhabitat choice for male reproductive success in a tropical frog (*Eleutherodactylus coqui*). Herpetologica **45**:451-458.

Townsend, D. S., M. M. Stewart, and F. H. Pough. 1984. Male parental care and its adaptive significance in a neotropical frog. Animal Behaviour **32**:421-431.

Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136-179 *in* B. Campbell, editor. Sexual Selection and the descent of man, 1871-1971. Aldine, Chicago.

Ueda, H. 1986. Reproduction of *Chirixalus eiffingeri* (Boettger). Scientific Report of the Laboratory for Amphibian Biology, Hiroshima University **8**:109-116.

Waltz, E. C. 1982. Alternative mating tactics and the law of diminishing returns: the satellite threshold model. Behavioral Ecology and Sociobiology **10**:75-83.

Wells, K. D. 1977. The social behaviour of anuran amphibians. Animal Behaviour **25**:666-693.

Wells, K. D. 1978. Territoriality in the green frog (*Rana clamitans*) - vocalizations and agonistic behavior. Animal Behaviour **26**:1051-1063.

Wells, K. D. 1988. The effect of social interactions on anuran vocal behavior. Pages 433-454 *in* B. Fritzsch, M. J. Ryan, W. Wilczynski, T. E. Hetherington, and W. Walkowiak, editors. The Evolution of the Amphibian Ear. John Wiley, New York.

Westneat, D. F. 1988. Male parental care and extrapair copulations in the indigo bunting. Auk **105**:149-160.

Weygoldt, P., and S. P. de Carvalho e Silva. 1992. Mating and oviposition in the hylodine frog *Crossodactylus gaudichaudii* (Anura: Leptodactylidae). Amphibia-Reptilia **13**:35-45.

## Table 6.1: Fight Results by Male Type

| Male ID | Male type | Fight Bouts | Bouts won | % won |
|---|---|---|---|---|
| D4-4* | RD-territorial | 45 | 37 | 82.2% |
| A1-4* | | 31 | 26 | 83.9% |
| A1-2 | | 26 | 22 | 84.6% |
| A3-4* | | 13 | 9 | 69.2% |
| G7-2 | | 11 | 9 | 81.8% |
| N5-4* | | 7 | 6 | 85.7% |
| Q5-4 | | 5 | 3 | 60.0% |
| F6-2 | | 4 | 4 | 100.0% |
| K2-4* | NR-territorial | 28 | 6 | 21.4% |
| C3-2 | | 18 | 4 | 22.2% |
| J1-4 | | 5 | 2 | 40.0% |
| Z7-4 | Aterritorial | 17 | 1 | 5.9% |
| M4-4* | | 15 | 3 | 20.0% |
| E6-4 | | 11 | 0 | 0.0% |

Data presented on fight results for all males observed in more than three fights in 1999.

RD-territorial males are more likely to win fights than either NR-territorial males or aterritorial males. Part of this effect is probably due to prior residence, as fights usually occur in territories with resources (oviposition sites), in which RD-territorial males are resident. Male ID codes (e.g. A1-4) reflect the tattoo on the individual's dorsum (A1), followed by the bamboo stand in which they lived (stand 4). Male ID's that are followed by an asterisk reflect the use of that individual in the playback experiment.

## Table 6.2: Male Discrimination of Wells in Bamboo Stand 4

| Type of well | Number of wells containing other organisms | | | |
|---|---|---|---|---|
| | Eggs | Tadpoles | Tipulids | Other frog species* |
| Average Well (N=238) | 71<br>(29.8%) | 90<br>(37.8%) | 43<br>(29.8%) | 71<br>(29.8%) |
| Wells Males take Females to (N=31) | 13<br>(41.9%) | 15<br>(48.4%) | 1<br>(3.2%) | 0<br>(0%) |
| Difference (Chi-Square Goodness of fit p-value) | p=0.11 | p=0.27 | p=0.02 | p=0.0004 |

*Other frog species include only *Plethodontohyla notostica* and *Anodonthyla boulengeri*

"Average Wells" include all data from wells in stand 4, which were surveyed every three days independent of focal watches. Each well was included in the survey only if, at some point during the 3.5 months of survey, evidence of *M. laevigata* reproductive activity was observed in that well (eggs, tadpoles, or courting adults directly observed).

Table 6.2 is equivalent to Table 2.3.

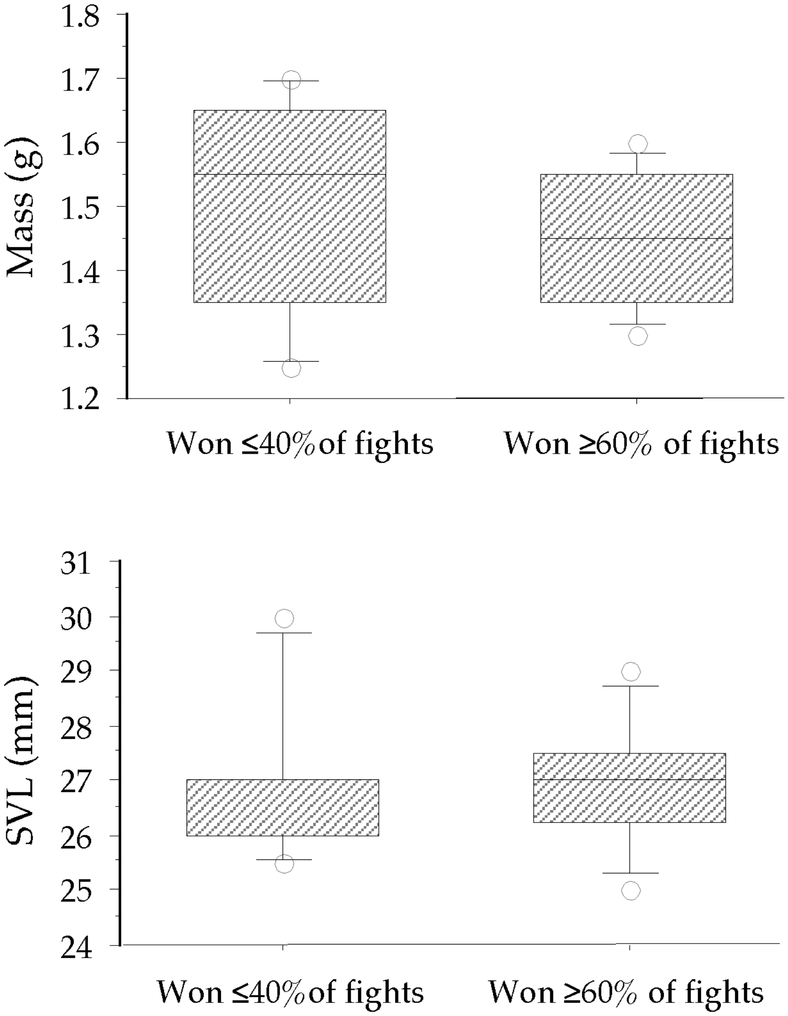
## Table 6.3: Female Discrimination of Wells during Courtships

| Type of well | Egg Occupied | Tadpole Occupied |
|---|---|---|
| Wells Males take Females to (N=31) | 13 (41.9%) | 15 (48.4%) |
| Wells Females Accept (N=18) | 9 (50.0%) | 6 (33.3%) |
| Difference (Logistic regression 2-tailed p-values) | p=0.12 | p=0.02 |

Females reject wells which are already tadpole-occupied for oviposition. They do not discriminate against egg presence in wells; rather, there is a non-significant trend for females to favor wells with eggs in them.

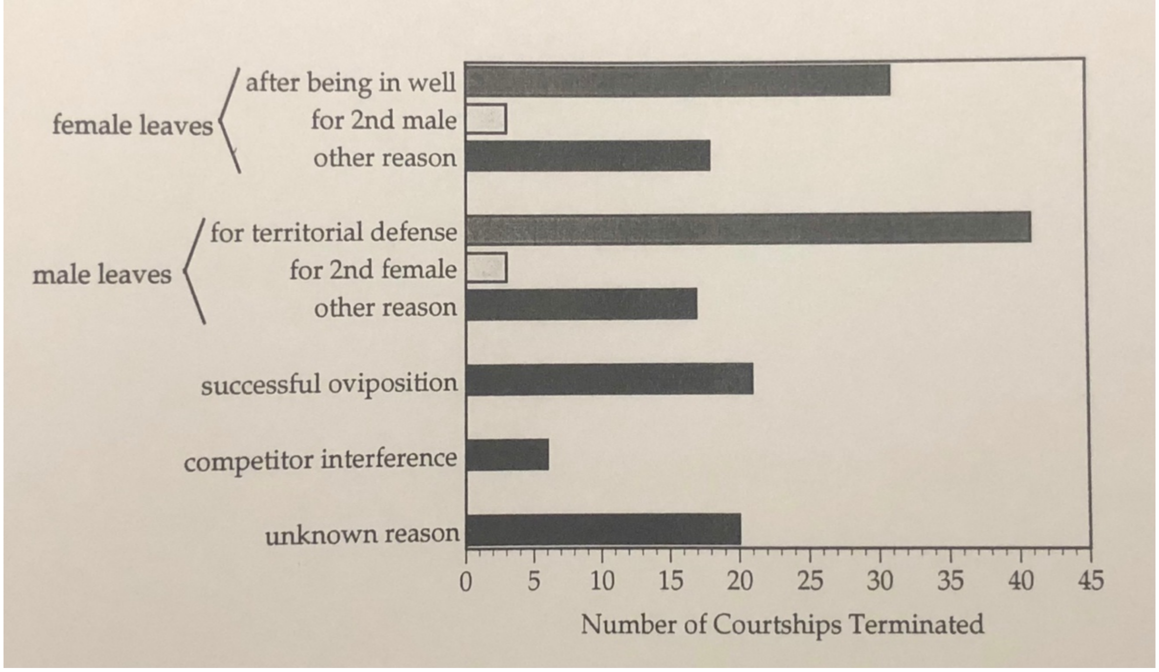
Table 6.3 is equivalent to Table 2.4.

## Figure 6.1:
## Male Size Does Not Predict Success in Territorial Encounters



Neither mass nor snout-vent length is correlated with male success in fights. All males observed in 4 or more fights in 1999 (N=14) either won 60% or more of their fights, or lost 60% or more of their fights. Male mass did not predict fight outcome (Mann-Whitney U, U=18.0, $p<0.4314$), nor did male SVL (Mann-Whitney U, U=24.0, $p>0.9999$).

**Figure 6.2: How Courtships End***



Males leave courtships more often than females do (N=160 courtship bouts recorded in 1999. Females left 52 (32.5%) of these; males left 61 (38.1%) of them.). Of male-terminated courtships, males leave most often to engage in territorial defense (41 out of 61, or 67.2%). Males were observed leaving courtships for another female only three times. There is thus little evidence for male mate choice.

These data were also presented in this form in Figure 5.1.

*The original figure is corrupted and no longer exists in digital form. This is a photograph of the figure as it appears in the printed dissertation.

**Figure 6.3:**

**Fight Descriptions for all Complete Fights Observed in 1999**

(N=131)

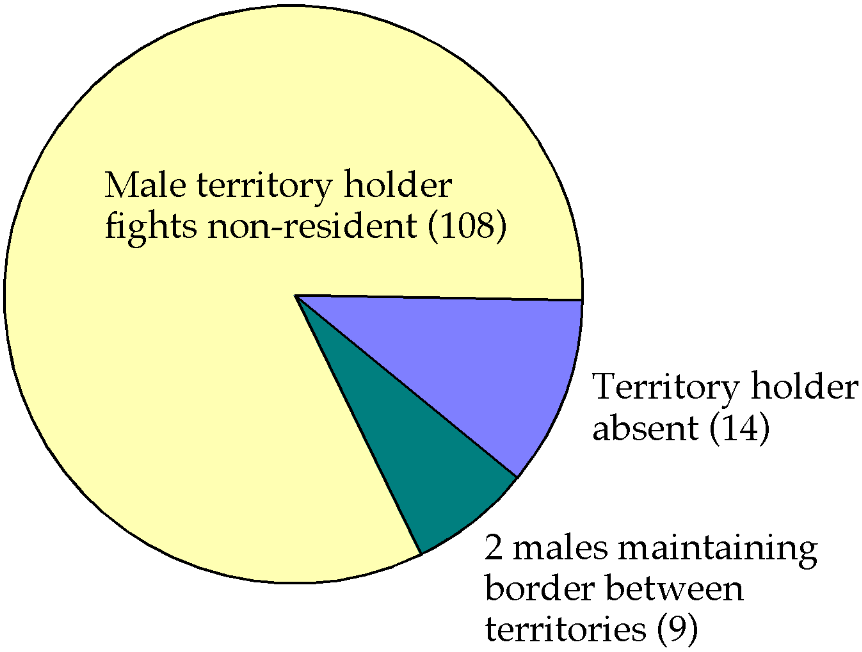


**Figure 6.4:**

**Fight Outcomes for 108 fights in which the Territorial Male Fought a Non-Resident**

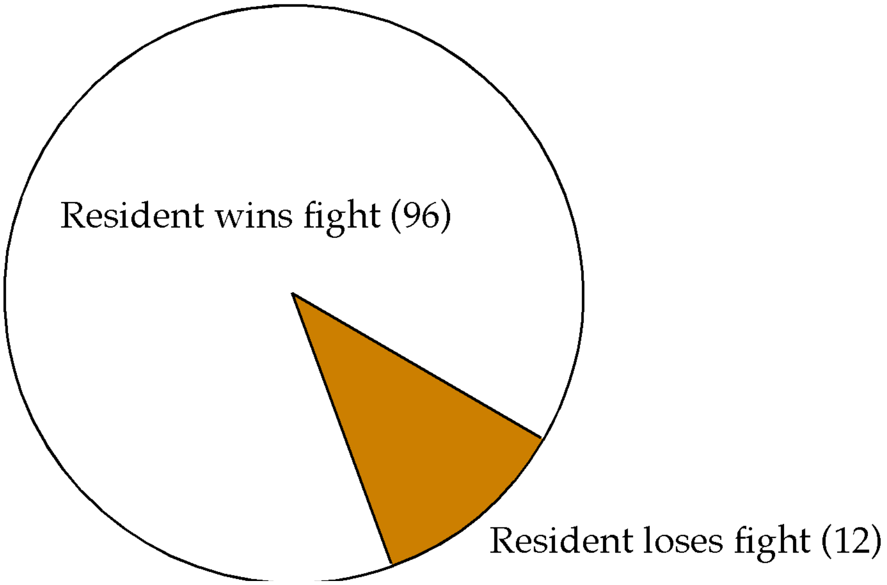
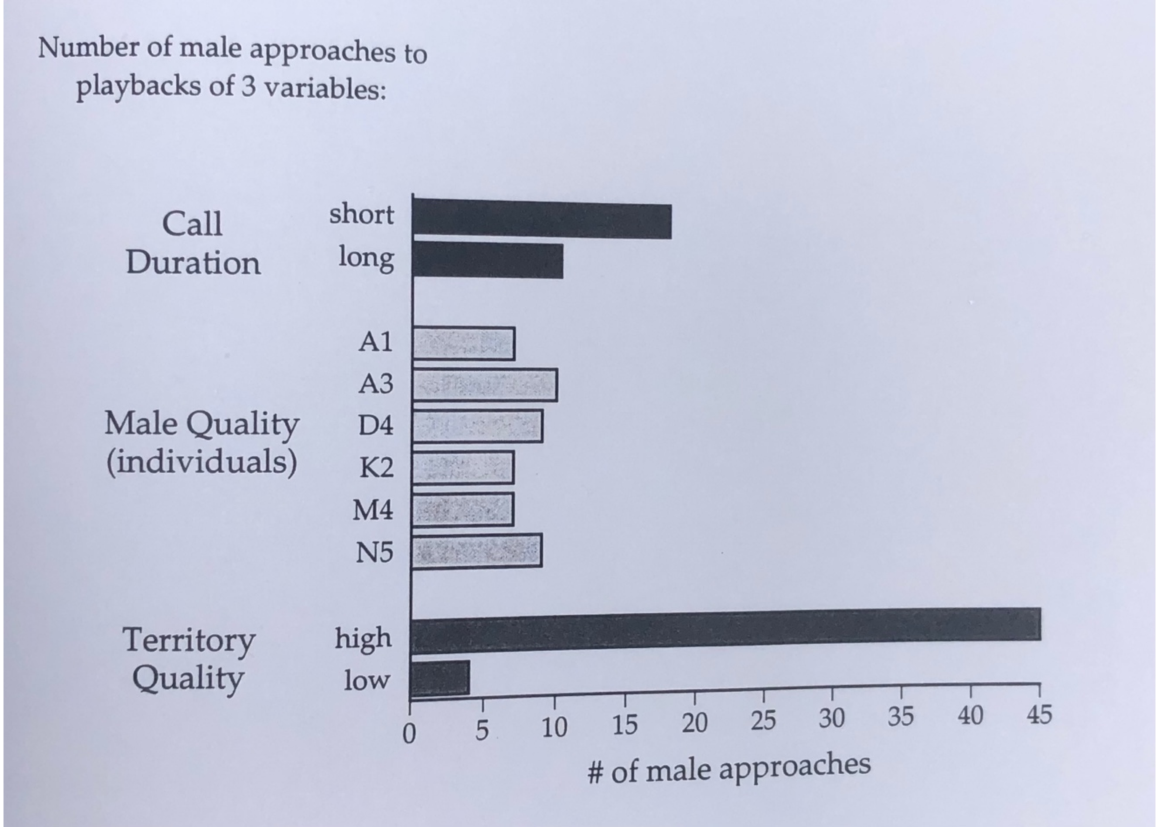


**Figure 6.5: Male response to experimental playbacks***



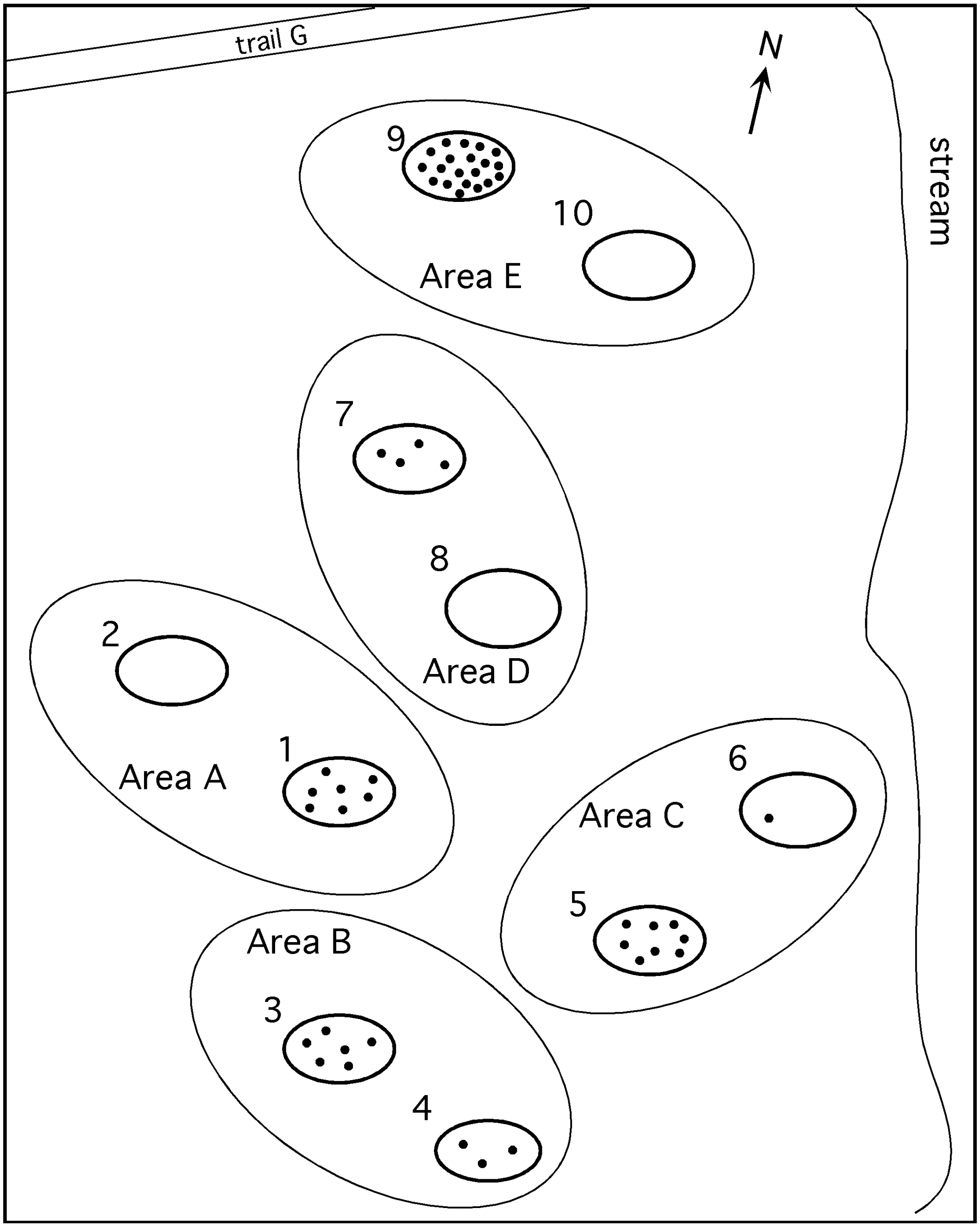
Males approached playback tapes under each of three concurrent experimental treatments: call duration (5 min. versus 15 min.), male quality (6 individual male's calls), and territory quality (high and low). Data presented for male approaches to long calls are divided by three, to prevent time confounds (see text). Of the three treatments, "territory quality" yielded significant differences in male approaches, as did "call duration," though in the opposite direction than expected.

*The original figure is corrupted and no longer exists in digital form. This is a photograph of the figure as it appears in the printed dissertation.

## Figure 6.6: Differential male approaches to territories, and areas

Male approaches, during playbacks, differed significantly between territories of high-quality (odd numbered ovals) and low-quality (even numbered ovals). Each male approach is depicted as a dot within that territory. Five areas, each containing one low- and one high-quality territory, were also significantly different from each other with regard to male approaches during playbacks.

**Figure 6.6: Differential male approaches to territories and areas**



# CONCLUSIONS

*Mantella laevigata* is a toxic Madagascan frog (Mantellinae: Ranidae: Anura) that is aposematically colored, small, terrestrial, diurnal, and breeds in rain-filled phytotelmata, the small pools of water found in plants. In the field, I undertook the first study of its social system and evolutionary ecology, and the ecology of members of the phytotelm guild with whom it shares oviposition sites.

The study of animal behavior relies on a solid understanding of an organism's requirements, be they for survival (naturally selected) or for reproduction (sexually selected); its ecology, and that of the organisms with whom it interacts; and basic life history characters such as diet and social system. Because Madagascar is remote, and its borders have been closed until recently, few people have asked these questions of any Malagasy animals besides lemurs. The background ecology and life history on which most behavioral studies are based does not exist for Malagasy anurans. When embarking on a study of the evolutionary ecology of a species about which little is known, one must discover the basics before proceeding to complex questions predicted by theory. This requires a combination of literature review to appreciate what is predicted by theory, and to understand the breadth of diversity already known; assessments to predict which species might reveal behaviors worth studying; long hours of observation, during which the researcher tries to discover pattern and meaning;

hypothesis generation based both on these observations and theory; experimental design and execution to assess those hypotheses; and a constant return to observation as a source of knowledge, which produces new hypotheses, and new questions, which, in turn, require answering before the system can be understood as a functioning whole. Such is the research cycle for discerning the evolutionary ecology of a species for which there is little prior background information. This dissertation reflects that process.

In this volume, I reviewed the literature on anuran reproductive modes and parental care, and proposed a new organization to describe the types of anuran parental care (Chapter 1). Phytotelm-breeding, which is most common in the wet tropics, is associated with a few highly specialized reproductive modes, and each of these is associated with a high degree of parental care. Parental care type 6, maternal feeding of trophic eggs to tadpoles, is currently known in only four anuran families, including several species of dendrobatids, two species of hylids, one rhacophorid and, with this dissertation, one ranid.

I described the social system and behavioral ecology of *Mantella laevigata* (henceforth *Mantella*), based on 925 hours of observation (Chapter 2). These comprised the basis for the hypotheses tested and questions posed in the remaining four chapters of the dissertation. Several complex behaviors were discovered, including extended male-male fights over defended resources necessary for the reproductive success of both sexes; stereotyped, highly tactile courtships in which the female may reject initial oviposition sites; and maternal feeding of tadpoles. Behavioral convergence with the Neotropical dendrobatids, which have identical skin toxins, is discussed. Clutch size is one, which was previously unknown in anurans. Larval crane-flies (tipulids) predate eggs, and two other treehole-breeding anurans (*Plethodontohyla notostica* and *Anodonthyla boulengeri*, both microhylids) compete for space with *Mantella*.

Oviposition sites are a critical parameter of reproductive success in any species that does not move its young immediately after laying. Observations in *Mantella* suggested that they might be limiting for this species; experiments supported this hypothesis (Chapter 3). I discuss the occurrence of multiple limiting factors, and why, in this system, they have not evolved. I also considered the particular benefits of phytotelmata as breeding sites, and concomitant risks to young, including desiccation, predation, cannibalism and competition.

Phytotelmata can provide relatively safe refuges for a variety of organisms (Chapter 4). In order to understand the correlates of use and reproductive success of *Mantella* in these limiting oviposition sites, I monitored wells for several months, collecting data on water chemistry, water holding potential, and other inhabitants. *Mantella* oviposition is positively correlated with low pH; short, wet wells; and few tipulids, conspecific tadpoles, or heterospecific frogs. *P. notostica* adults protect preexisting *Mantella* eggs, but discourage new oviposition. After *Mantella* metamorphs disperse from a well, reproductive activity increases. I delineate a foodweb of the phytotelmata of NE Madagascar.

The theoretical core of this research is sexual selection (Chapters 5 and 6). In the experiment presented in Chapter 5, females were allowed to choose between calls of individual males (“good genes”), of varying length calls (“good current condition”), in different quality territories (“good resources”). My previous observations of females scouting for oviposition sites before courtships, males leaving courtships to engage in territorial defense of oviposition sites, and biparental care, suggested the “good resources hypothesis.” This hypothesis was supported, and I discuss why this system fits a model more common to birds than anurans.

Finally, I assessed mechanisms of male reproductive success in this species with strong female choice (Chapter 6). *Mantella* males affect their reproductive success through direct male-male competition, by adopting one of several mating/territorial strategies. Only males that defend limiting reproductive resources are likely to procure matings. Males adopting either of two other, less successful, strategies must be sneaky to obtain mates. In order to become and remain territorial, a male must win fights. Predictors of success in territorial encounters include prior residency, and fight initiation. Males also provide paternal care both in the form of egg attendance, and through manipulation of male-female conflict. A male can indirectly feed his young by deceiving a courted female into ovipositing into a well that already contains his tadpole.

*Mantella laevigata* has a complex social system that is context-dependent, in which the players are constantly assessing relevant factors and basing their behavior on these reassessments. Predators, competitors, cannibals, and desiccation all put individuals at risk, but populations thrive. Natural variation in the abiotic parameters of oviposition sites results in niche partitioning among competing species. Males and females have different interests, and their behavior is accordingly divergent as well. This research reminds us that while natural selection is generally slow, with few tools at its disposal, it remains a remarkably powerful force, capable of subtle alterations of organisms in the service of their genetic fitness.