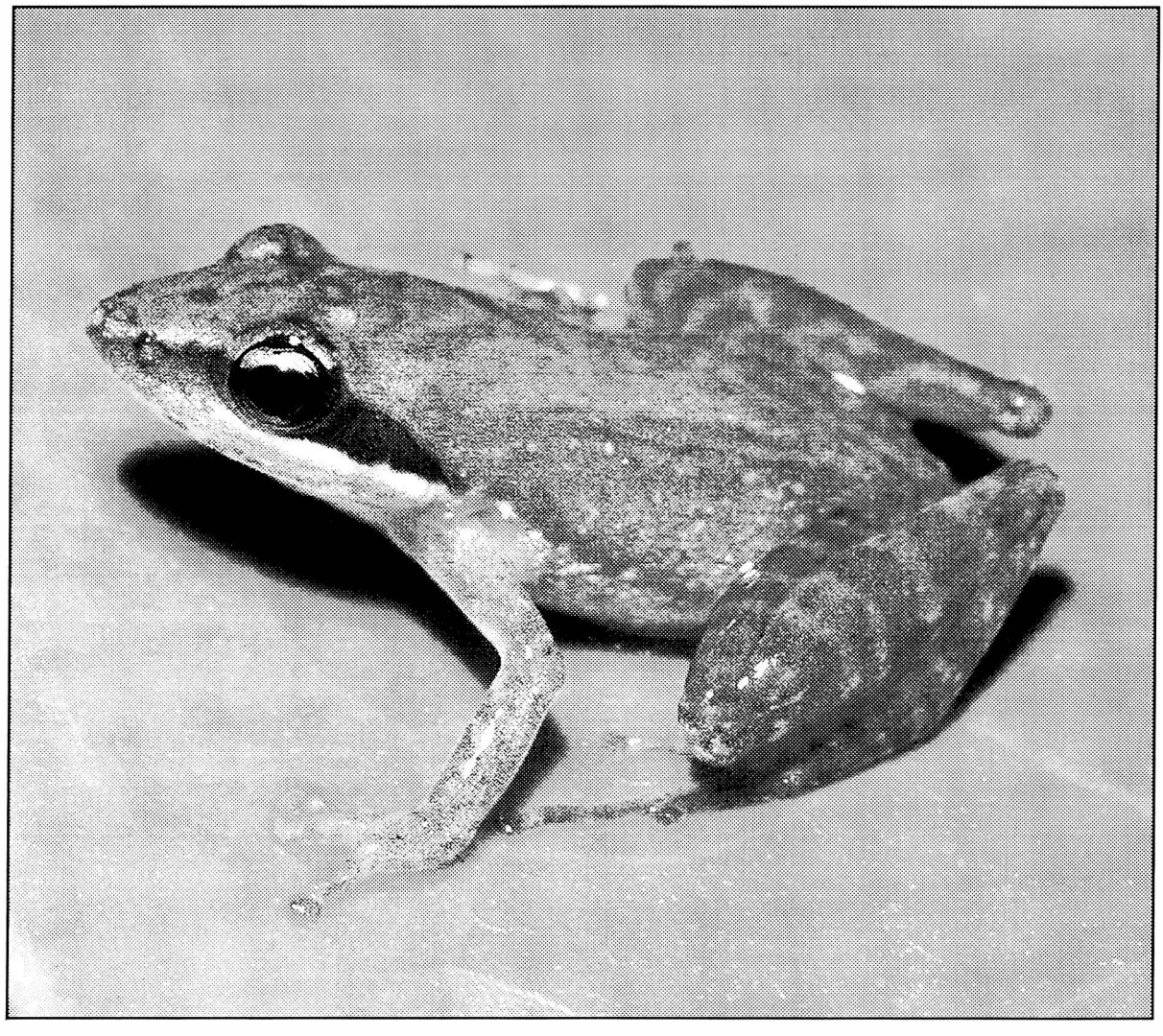
Volume 12, Number 1

January 2002

ISSN 0268-0130

THE HERPETOLOGICAL JOURNAL



Published by the
BRITISH HERPETOLOGICAL SOCIETY

Indexed in
Current Contents

The Herpetological Journal is published quarterly by the British Herpetological Society and is issued free to members. Articles are listed in *Current Awareness in Biological Sciences*, *Current Contents*, *Science Citation Index* and *Zoological Record*.

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FRONT COVER: Male *Mantidactylus sarotra* (F. Glaw & M. Vences)

HERPETOLOGICAL JOURNAL, Vol. 12, pp. 1-9 (2002)

REVIEW:

MICROSATELLITE MARKERS IN AMPHIBIAN CONSERVATION GENETICS

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Recent technical advances allow straightforward access to genetic information directly drawn from DNA. The present article highlights the suitability of high variation molecular genetic markers, such as microsatellites, for studies relevant to amphibian conservation. Molecular markers appear particularly useful for i) measuring local gene flow and migration, ii) assigning individuals to their most likely population of origin, iii) measuring effective population size through the between-generation comparison of allele frequencies, and iv) detecting past demographic bottlenecks through allele frequency distortions. We demonstrate the use of some newly developed analytical tools on newt (*Triturus* sp.) microsatellite data, discuss practical aspects of using microsatellites for amphibians, and outline potential future research directions.

Key words: amphibians, conservation, microsatellites, *Triturus cristatus*

INTRODUCTION

The introduction of enzyme electrophoretic techniques in the 1970s enabled direct access to genetic information from wild populations (Lewontin, 1991). In the European amphibian fauna, protein variants facilitated the description of several previously unrecognized species (e.g. Busack, 1986; Beerli *et al.*, 1994; Arntzen & García-París, 1995; Lanza *et al.*, 1995 - reviewed in Veith, 1996 and García-París & Jockush, 1999). However, owing to the relatively low level of genetic variation documented by protein variants, their application for evolutionary and ecological inferences was often limited to large-scale analyses, typically at the level of species and subspecies (e.g. Rafinski & Babik, 2000). Only in the last one or two decades have laboratory advancements such as the advent of routine sequencing and PCR (Polymerase Chain Reaction) technology permitted access to genetic information from across geographical ranges (Alexandrino *et al.*, 2000, 2001; Riberon *et al.*, 2001; Zeisset & Beebee, 2001). Such information can, for example, be used for the definition of 'Evolutionary Significant Units', an operational level of organization for assessing biodiversity independent from taxonomic hierarchy (Moritz, 1994; Crandall *et al.*, 2000).

One class of newly developed DNA-based markers – microsatellite loci (Goldstein & Schlötterer, 1999) – is currently receiving particular attention. Microsatellites occur in high numbers in every eukaryote genome, and consist of tandem repetitive units of DNA typically less than five basepairs in length, with a high variability due to different repeat numbers (e.g. [CA]n); for more information on specific properties of amphibian microsatellites see, for example, Neff & Gross (2001). Microsatellites are amplified with specific PCR primers and the different alleles separated along an electrophoretic gradient in routine laboratory procedures. However, the development of statistical tools for the analysis of the data has lagged behind, and some new methods, such as computer-aided Maximum Likelihood, Bayesian statistics and Markov Chain Monte Carlo procedures (for overviews see Beaumont & Bruford, 1999; Luikart & England, 1999; Sunnucks, 2000) are not yet included in the standard toolbox of many population ecologists.

Most amphibians depend on both aquatic and terrestrial habitats, and for protection and management plans their local population dynamics as well as the degree of population connectivity must be considered (Semlitsch, 2000). Furthermore, amphibians have relatively low dispersal abilities and are often philopatric, leading to distinct populations that can represent unique genetic entities despite geographic proximity (Kimberling *et al.*, 1996; Waldmann & Tocher, 1997; Driscoll, 1999; Scribner *et al.*, 2001). Amphibians therefore appear highly suitable for addressing population and conservation genetic issues, but are as yet under-represented in this research area. For example, the otherwise prominently debated global amphibian population decline is only little studied from a genetic point of view (but see Shaffer *et al.*, 2000), although there is evidence that the amount of genetic variation could have an impact on fitness-related traits (Rowe *et al.*, 1999). The aim of the present article is to demonstrate the power and utility of highly variable DNA-based markers and some recently developed analytical methods for population studies on amphibians, and the value of such studies for conservation issues.

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METAPOPULATION PROCESSES

Amphibians are well suited to address questions at the level of metapopulations (e.g. Hanski, 1998), and have in recent years become a focus for studies on the effect of landscapes and landscape alterations to wildlife (e.g. Halley *et al.*, 1996; Vos *et al.*, 2001*a*). Population size fluctuations can be addressed in a straightforward way with standard field methods, but to assess the exchange of individuals between populations at the landscape scale is difficult with fieldwork alone. We anticipate that the most ground-breaking applications of fine-tuned, DNA-based markers will be at the regional, metapopulation scale. Questions on how processes like source-sink dynamics, population connectivity and extinction-recolonization frequencies affect the maintenance of within-species genetic diversity have so far only been addressed through modelling (Whitlock & Barton, 1997; Pannell & Charlesworth, 2000; Higgins & Lynch, 2001). We now have the molecular tools at hand for obtaining empirical data on such issues, and amphibians are particularly promising study organisms in this respect.

Allozymes have already revealed some influences of human-induced landscape fragmentation on the genetic structure of European anurans of the genera *Bufo* and *Rana* (Reh & Seitz, 1990; Hitchings & Beebee, 1997; 1998), and lower genetic differentiation in natural situations relative to human-altered regions (Seppä & Laurila, 1999). Using microsatellites, Garner *et al.* (submitted) demonstrated for *R. latastei* that populations at the periphery of the distribution range are genetically depleted and should be considered as particular conservation units. Vos *et al.* (2001*b*) showed that linear barriers to dispersal such as roads had a significant impact on genetic distance measures of European moor frogs (*R. arvalis*), whereas only little fine-scale genetic differentiation was found for North American frogs (*R. sylvatica*) in a highly dynamic wetland landscape (Newman & Squire, 2001). Moreover, Rowe *et al.* (2000*a*) demonstrated the fit of a mixed 'island/island-mainland' metapopulation model to three sets of neighbouring breeding sites of remnant natterjack toad (*B. calamita*) populations in Britain. However, all four microsatellite-based studies have not yet fully explored the new analytical tools now available (e.g. Beerli & Felsenstein, 2001), and further insights into fine-scale metapopulation processes are to be expected in future studies.

Because not only the connectivity of populations but also the availability of summer habitats shapes the amphibian metapopulation structure (Pope *et al.*, 2000), detailed quantitative assessment of all utilized aquatic and terrestrial habitats is required to predict accurately the determinants of metapopulation processes. Among the most promising approaches to studies addressing this issue is perhaps the combination of data derived from microsatellites with fine-scaled landscape data – such as those obtained using GIS techniques – to identify colonization routes along environmental gradients (Arntzen *et al.*, in preparation).

INDIVIDUAL IDENTIFICATION AND ASSIGNMENT METHODS

Estimates of population structure and local migration have traditionally been obtained through the identification of individuals from phenotypic features, or some sort of tag. Alternatively, the number of alleles segregating over a panel of microsatellite loci enables the genetic recognition of individuals without physical marking ("genetic tagging", Palsböll, 1999; Taberlet & Luikart, 1999). Genetic tagging may be particularly recommendable for amphibians, as it circumvents traditional physical markings frequently claimed to be harmful, such as the removal of several toes.

Among the latest developments in the toolbox of the population geneticist are methods to classify individuals according to the most likely population of origin, based on their genotype (Rannala & Mountain, 1997; Waser & Strobeck, 1998; Dawson & Belkhir, 2001; Hansen *et al.*, 2001). Although such "assignment tests" are not new (Jamieson, 1965), they became widely applicable only with the availability of large amounts of genotypic data in combination with high computational speed and appropriate software (GENECLASS: Cornuet *et al.*, 1999; WHICHRUN: Banks & Eichert, 2000; STRUCTURE: Prichard *et al.*, 2000). Using a sufficient number of variable loci in combination with an adequate sample size the approach is surprisingly powerful, even when the reference populations are genetically rather similar (Bernatchez & Duchesne, 2000).

Assignment methods are currently far from fully explored in amphibian conservation, despite several potential applications. Illegal cases of collecting and trade could be revealed by genetically tracing the source population of involved individuals (as done for fish, Primmer *et al.*, 2000). Similarly, the source of local introductions could be inferred, an important issue when determining whether a species belongs to a regional fauna or not (Szymura, 1998; Arntzen, 2001; Zeisset & Beebee, 2001). The most promising application of assignment methods for conservation-related research on amphibians, however, lies not in tracing "alien" individuals, but in measuring between-population connectivity at a scale equal to or smaller than the migratory range of the species under study.

To provide an example of the power of assignment methods, we applied the likelihood Bayesian approach provided by GENECLASS to two French *Triturus cristatus* populations ca. 10 km apart (data from Jehle *et al.*, 2001). Thirty-five and 168 adults (48% and 29% of the estimated population census sizes, respectively) and some larval offspring (40 and 87 individuals, respectively) were assayed for eight microsatellite loci with between two and nine alleles each. The populations were differentiated at the level of Fst=0.045. We tested the null hypothesis that individual larvae would be as-

signed to their true parent population. From 74-88% of the larvae were correctly classified ($P<0.001$ for both populations, G-test for goodness of fit, $G=25.3$ and 20.1, respectively, df=1). In another analysis, without having any knowledge about potential source populations, we also estimated the probability for every adult individual that its genotype belongs to the population where it was captured. Eight adult newts (6%) did not fit the genetic profile of the population in which they were caught ($P<0.01$). Such individuals could be immigrants or their offspring, although a rigorous assessment of migration patterns would require genetic data from all regional populations, in order to assign such putative migrants to their most likely origin. It also has to be kept in mind that the results of assignment tests are inferential and, in contrast to physical capture-mark-recapture, are not based on actually recorded movements.

Measuring Effective Population Size

The size of a population is generally taken to be the total number of individuals at a certain locality, but from an evolutionary point of view, only those individuals which are successful in reproduction are important. Therefore, the census size of a population is distinguished from the "effective population size" (N_e, Wright, 1931). Current efforts for protecting and sustaining endangered and rare species often focus on the maintenance of genetic diversity (Sherwin & Moritz, 2000; Hedrick & Kalinowski, 2000; Hedrick, 2001), and it is the effective population size that determines the amount of genetic variation maintained over time. Intuitively one might expect the effective population size to be close to the adult population census size, but parameters such as reproductive failures, skewed sex ratios and substantial reproductive skews caused by specific mating systems can bias N_e up to several orders of magnitude below census size (Frankham, 1995). Because N_e is not easy to measure, comprehensive data are not yet available for many taxa, rendering the routine use of N_e in practical conservation controversial (Mace & Lande, 1991).

Measures of N_e can be obtained through demographic methods that incorporate life-history data into analytical equations (Nunney & Elam, 1994; Basset *et al.*, 2000). Unfortunately, obtaining precise life-table parameters – and particularly their variances – can be difficult. Genetic methods, however, enable calculation of N_e from one or more genetic samples without detailed life-history knowledge (Schwartz *et al.*, 1998). The "temporal method", which is based on two samples taken from one population, is particularly straightforward (Waples, 1989; Williamson & Slatkin, 1999). As N_e increases, genetic drift – and therefore the temporal change in allele frequencies – decreases. Assuming that selection, mutation, migration and population subdivision is negligible, one parameter can be estimated from the other (Fig. 1), with highly polymorphic loci such as microsatellites providing particularly precise estimates (Turner *et al.*, 2001). The main practical disadvantage of the temporal method is that at least two samples are required, ideally with several generations between sampling dates. For discussions on possible violations of assumptions of the temporal method when using microsatellites, see Jehle *et al.* (2001, and references therein).

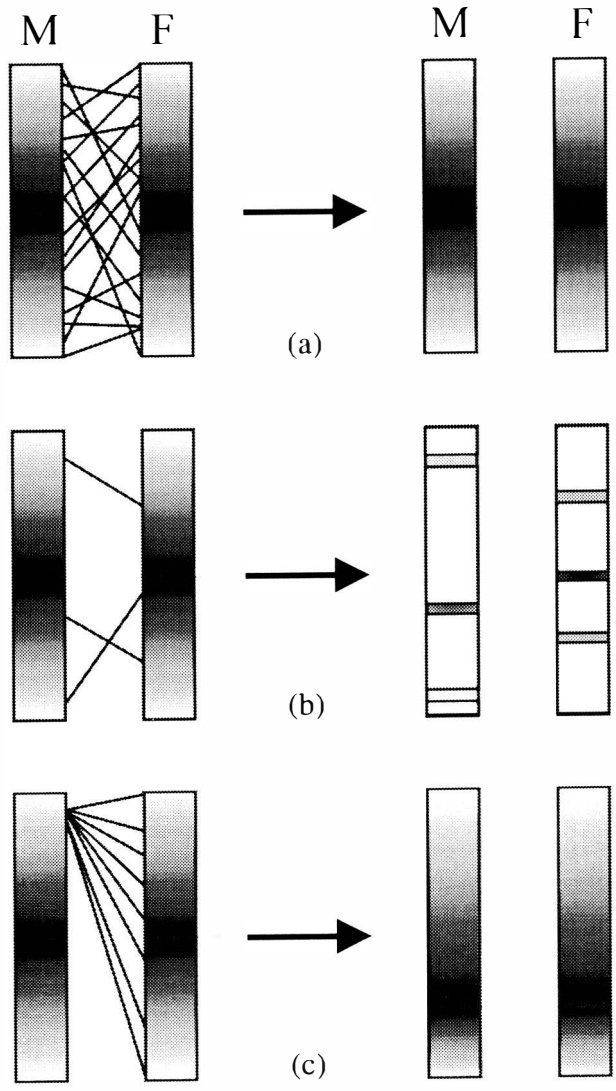

FIG. 1. Schematic and simplified representation on the effect of effective population size on between-generation changes in the spectrum of allele frequencies; (a) large numbers of breeders: the amount of genetic diversity does not change markedly between generations; (b) low number of breeders: the maintained amount of genetic diversity decreases; (c) skewed operational sex ratio: the allele frequency distribution becomes distorted. In both (b) and (c), the between-generation variance in allele frequencies is high, and the effective population size is low. M, males; F, females.

In amphibians, the methodological basis of measuring N_e ranges from counting the number of egg clutches to various demographic and genetic estimates (Merrell, 1968; Gill, 1978; Easteal, 1985; Berven & Grudzien, 1990; Driscoll, 1999; Seppä & Laurila 1999). Estimates of N_e with the temporal method were made for common toads (*Bufo bufo*, Scribner *et al.*, 1997, based on minisatellites), north American salamanders (*Ambystoma*

macrodactylum, Funk *et al.*, 1999, based on allozymes) and European newts (*Triturus cristatus* and *T. marmoratus*, Jehle *et al.*, 2001, based on microsatellites). The toad study revealed that just 1% of the adult population successfully reproduced in a particular year. In newts, N_e was 10-20% of the adult population census size. The difference in N_e between toads and newts was in accordance with knowledge of the species' reproductive modes, characterized by large and small variances in reproductive success, respectively. A potentially very accurate method for calculating the effective number of breeders in a population would be to reconstruct paternal genotypes for offspring with known mothers (Pearse *et al.*, 2001), but such data are difficult to obtain in natural amphibian populations.

Notwithstanding some methodological differences, effective population size in amphibians has generally been estimated as under a hundred individuals, whereas the minimum effective population size required to maintain genetic variation sufficient for demographically viable populations is thought to be between 500 and 5000 individuals (Franklin & Frankham, 1998; Lynch & Lande, 1998). Given that many European amphibian species are subject to increasing population isolation, these findings suggest that the long-term survival of many populations is in danger, more so than field ecological studies would reveal. Future studies could also model spatial genetic structure by combining N_e measures with an assessment of population connectivity. This would enable the assessment of the effective size of a whole metapopulation (Whitlock & Barton, 1997), a measure that could determine the rate of within-species genetic erosion on a large scale.

Detecting Past Population Bottlenecks

Genetic bottlenecks occur when populations experience severe, temporary reductions in their effective size, and can dramatically reduce the genetic diversity of populations. For example, a high degree of inbreeding depression is often interpreted as the result of a past population bottleneck (Hedrick & Kalinowski, 2000). Traditional measures of genetic diversity, such as heterozygosity and allelic diversity, can be used to infer a past bottleneck, but require a reference sample either from before the event or from another, non-bottlenecked population (Spencer *et al.*, 2000; for a simulation program on the effects of bottlenecks on genetic diversity see e.g. England & Osler, 2001). Statistical methods have recently been developed to infer the demographic history of a population from a single genetic sample. One method is based on the premise that bottlenecking gives rise to an excess of heterozygotes compared to the level of heterozygosity expected at mutation-drift equilibrium, because under bottlenecks rare alleles have a higher risk of going extinct than common alleles (Cornuet & Luikart, 1996; Luikart *et al.*, 1998; Garza & Williamson, 2001, see Fig. 2). Depending on the sample size and marker variability, this method can detect such a heterozygote excess for up to about ten generations after its occurrence (Luikart *et al.*, 1998*a*), although false bottlenecks signals can appear in the data when the populations are not fully isolated (Pope *et al.*, 2000).

The ability to trace back population bottlenecks opens the door for addressing a variety of questions. The detection of past bottlenecks could, for example, indicate the decimation of a population due to disease, or the colonization of a newly formed habitat. Bottlenecks are also expected to occur during extinctions-recolonization processes in metapopulations, which are of vital importance for the maintenance of overall genetic variation (Whitlock & Barton, 1997). Such events could now be reconstructed from genetic data, without historical knowledge of population demography. Furthermore, for studies which aim to trace population reductions over longer time scales, additional maximum likelihood and coalescent-based methods are available in the literature (Beaumont, 1999; Goldstein *et al.*, 1999).

For amphibians, the distribution of microsatellite allele frequencies has been proven to be very powerful by successfully identifying known bottlenecks in British *B.*

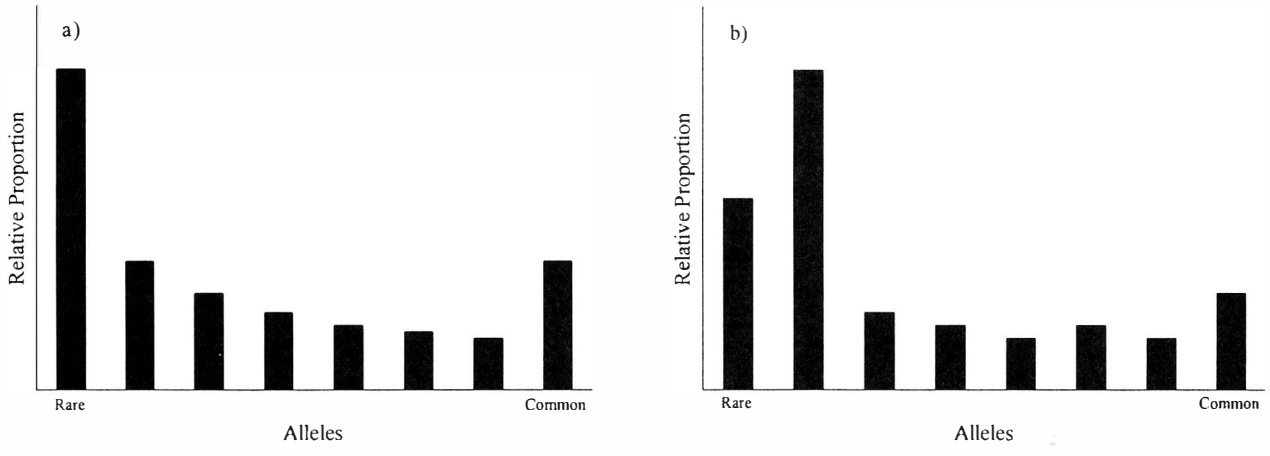

FIG. 2. Schematic representation of the consequences of a population bottleneck on gene diversity; (a) unbottlenecked population, (b) bottlenecked population. Rare alleles, under mutation-drift equilibrium more abundant than high-frequency alleles, have a higher probability of becoming extinct under bottlenecks, leading to a distortion of allelic frequencies which is detectable with hypervariable markers several generations after a bottleneck occurred. After Luikart *et al.* (1998).

calamita populations (Beebee & Rowe, 2001), but, based on allozyme data, has failed to detect significant bottlenecks associated with an introduction of the newt *T. carnifex* (Arntzen, 2001). In another example, we found evidence for one *T. cristatus* populations having experienced a genetic bottleneck, in line with the documented recent colonization of the study area (Jehle *et al.*, 2001). However, we noted that support for the case crucially depended on the microsatellite mutation mechanism assumed to operate. Microsatellites mostly mutate through the addition or deletion of one repeat unit, following a stepwise mutation model (SMM) or, alternatively, by a certain number of repeats simultaneously, following the infinite allele model (IAM, Goldstein & Schlötterer, 1999). We estimated the contributions of SMM and IAM over all loci, using a Markov chain method implemented in the software MISAT (Nielsen, 1997), as approximately 95% and 5%, respectively. Under these mixed model conditions significant bottlenecks were not found, similar to assuming the SMM alone. However, it should be mentioned that, unless the bottleneck is very severe, the statistical power of one-sample tests is lower than when an additional sample before the bottleneck occurred is available (Luikart *et al.*, 1998b). This could, for example, be achieved by extracting DNA from museum specimens (as in Beebee *et al.*, 1998).

PRACTICAL CONSIDERATIONS

Apart from their high variability, DNA-based markers offer several practical advantages over allozyme methods. Samples can be stored in concentrated ethanol, rendering the immediate freezing of samples, as usual for enzyme electrophoresis, unnecessary. Furthermore, DNA can be extracted from "old" (dried, alcohol – or even formalin – preserved) material, such as that kept in museum collections (Beebee *et al.*, 1998). For any PCR based assay, minute amounts of tissue are required, allowing non-destructive sampling (Taberlet & Luikart, 1999). Amphibian DNA samples can be obtained by removing a toe (Gonser & Collura, 1996), a procedure which at the same time can serve as a physical mark (e.g. for population size estimated with capture-mark-recapture). In urodeles, samples can be obtained by taking the tail tip (Arntzen *et al.*, 1999); larval amphibians can be non-destructively sampled by clipping the tail-fin (Rowe *et al.*, 1999), or by removing an external gill (Jehle *et al.*, 2000).

User-friendly software for performing the Maximum Likelihood, Bayesian and Monte Carlo methods is now freely available from the internet (for an overview of sources see, for example, Luikart & England, 1999), complementing the packages designed for "traditional" population genetic analyses (e.g. GENEPOP, Raymond & Rousset, 1995). However, when using microsatellite-based data it should be borne in mind that estimating population genetic parameters which depend on mutational processes can be ambiguous, as precise mutation mechanisms of microsatellites are still subject to debate and can also vary from locus to locus.

Co-dominant markers such as microsatellites are highly informative, but their biggest disadvantage is probably that they cannot be applied "off the bench". In fact, the development of the required PCR primers can be costly and time-consuming, a potentially prohibitive fact when planning a study with limited duration and finance. Within amphibians, it has been shown for ranid frogs that cross-species amplification success rates are significantly lower than for birds and mammals (Primmer & Merilä, in press). The often very large genome of urodele amphibians makes the development of successfully amplifying primers particularly difficult for them (Garner, 2002), rendering enrichment procedures highly recommended for obtaining a sufficient number of amplifiable and polymorphic loci (e. g. following Gibbs *et al.*, 1997). For European amphibians, published microsatellite PCR primers are so far available for *Bufo bufo* (Scribner *et al.*, 1994; Brede *et al.*, 2001), *B. calamita* (Rowe *et al.*, 1997; Rowe *et al.*, 2000*b*), *Hyla arborea* (Arens *et al.*, 2000), *Rana arvalis* (Vos *et al.*, 2001*b*), *R. latastei* (Garner & Tomio, 2001), *R. lessonae/R. ridibunda* (Garner *et al.*, 2000; Zeisset *et al.*, 2000), *R. temporaria* (Berlin *et al.*, 2000; Rowe & Beebee, 2001), and *Triturus cristatus/T. marmoratus* (Krupa *et al.*, 2002). Once developed, the primer systems enable the efficient derivation of genotypes, currently at a cost of about 1 Euro per datum point.

FUTURE DIRECTIONS

High-throughput microsatellite genotyping is currently facilitated by, for example, using PRC primers labelled with fluorescent dyes, in combination with semi-automated sequencing machines and associated technology such as pipetting robots. Microsatellites are in the vast majority of cases situated in non-coding regions, and their neutral character is a basic assumption underlying the above-described analytical methods. However, this also implies that they only reflect indirectly the genetic variation relevant for fitness and adaptation. The great majority of ecological and demographic characteristics relevant for population viability are based on quantitative genetic traits, which are typically determined by multiple loci with various and additive effects (Falconer & Mackay, 1996). Assuming that a large number of loci is known (in the order of >100 distributed across the whole genome), such QTLs ("Quantitative Trait Loci") could be mapped on the basis of microsatellites, an approach currently used for better-known model species. Microsatellites also play a significant role in the upcoming field of "population genomics", where numerous loci are sampled across the whole genome, with locus-specific effects of population genetic parameters being distinguishable from the general sample distribution across all loci (e. g. Black *et al.*, 2001).

Beyond microsatellites, another genetic marker widely applied for other vertebrates and directly related

to fitness, the MHC ("Major Histocompatibility Complex", e.g. Edwards & Hedrick, 1998), has, to our knowledge, not yet been applied in amphibian conservation studies. Technical advances might in the future lead to a shift from marker-based data to information directly at the sequence level, such as information derived from single-basepair substitutions ("Single Nucleotide Polymorphisms", SNPs). However, despite being promising for phylogeographic studies, due to their low mutation rate their usefulness for fine-scale population inferences might be limited. Recent technical advances also include microarrays or "DNA chips", which are microscopic plates on which short DNA strands can be bound and visualized. For example, in combination with ESTs ("Expressed Sequence Tags"), which mark those regions in the genome which are expressed under certain circumstances, microarrays now would enable to relate the activity of specific genes to environmental conditions such as ecological stress. However, these methods are as yet beyond the financial scope of most amphibian ecologists.

Genetic considerations are probably most useful when incorporated early in a species' conservation plan, when the existence of some robust populations across a species' geographical range offers the possibility of a variety of creative solutions to conservation problems (Hedrick, 2001). Many European amphibian species suffer serious declines but are not yet exposed to the imminent risk of extinction, rendering molecular studies particularly timely. However, an increased knowledge of the population genetic structure alone is not sufficient to guarantee conservation, and new scientific findings based on high variation genetic markers will only help amphibian conservation when integrated into current and future action plans.

ACKNOWLEDGEMENTS

An earlier version of this paper is included in the conference volume *Atti del Terzo Convegno Salvaguardia Anfibi, Edizioni Cogecstre.* Trevor Beebee, Trent Garner and an anonymous referee provided constructive comments. RJ is supported by a European Community Marie Curie Fellowship and FWF grant P-14799.

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Accepted 18.12.01

HERPETOLOGICAL JOURNAL, Vol. 12 , pp. 11-20 (2002)

A NEW SIBLING SPECIES OF THE ANURAN SUBGENUS *BLOMMERSIA* FROM MADAGASCAR (AMPHIBIA: MANTELLIDAE: *MANTIDACTYLUS*) AND ITS MOLECULAR PHYLOGENETIC RELATIONSHIPS

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A new species of the *Mantidactylus domerguei* species group in the subgenus *Blommersia* is described from central eastern Madagascar. *Mantidactylus sarotra* sp. n. is morphologically similar to the syntopic *M. blommersae* but differs by smaller size, vocal sac coloration, advertisement calls and habitat of calling males. A phylogenetic analysis of 565 nucleotides of the mitochondrial 16S rRNA gene of all described species of the *M. domerguei* group revealed that *M. sarotra* and *M. blommersae* are not sister species but are genetically highly differentiated (9.73% sequence divergence). *M. sarotra* was grouped with high bootstrap support as a sister species of *M. kely*. The two species share a general advertisement call structure, vocal sac coloration and small size, but differ from each other in terms of skin texture, dorsal coloration, and pulse rate of vocalizations. The high differentiation among all species of the group (4.96% divergence between the closest relatives, *M. blommersae* and *M. domerguei*) indicate that speciation of the currently recognized taxa probably occurred several million years before the present.

Key words: *Mantidactylus sarotra* sp. n., *Blommersia,* Madagascar, phylogeny, mitochondrial DNA, advertisement calls

INTRODUCTION

The speciose Malagasy genus *Mantidactylus* is a diverse assemblage of about 75 nominal species of frogs, divided into 12 subgenera. Recent molecular and morphological data indicate that it is paraphyletic relative to the well established genus *Mantella* (Richards *et al.*, 2000; pers. obs.). Subdivision of *Mantidactylus* into several genera also seems adequate considering the high morphological and biological diversity of the included species (Glaw & Vences, 1994). However, stability of such a partitioning can only be reached if the natural history and relationships of the taxa involved are satisfactorily known. One group with unresolved taxonomy in the subgenus *Blommersia* is composed of small, mainly swamp-breeding and partly arboreal frogs. It was named *Mantidactylus wittei* complex by Glaw & Vences (1994); here we define it as *Mantidactylus domerguei* species group, named after the historically first described taxon included in the group. Although rather common in eastern Madagascar, the species of the *M. domerguei* group were not discovered before the early 1970s, when Guibé (1974*a,b*, 1975) described four species: *M. blommersae, M. domerguei, M. grandisonae* and *M. wittei*. Blommers-Schlösser (1979) provided the first information on the natural history of three species (*M. blommersae, M. domerguei, M. wittei*). Glaw & Vences (1994) added information on *M. grandisonae*, described a further species (*M. kely*) and recognized two additional undescribed species (*Mantidactylus* sp. a and *M.* sp. b). During the last six years, additional information on morphological, bioacoustic and genetic differentiation of species of *Blommersia* has become available. These new data enable us to diagnose reliably one of the previously recognized new species (*M.* sp. b). In the present paper, we describe this species and assess its phylogenetic relationships to other species of the *M. domerguei* group using mitochondrial DNA sequences.

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MATERIALS AND METHODS

Specimens were collected during day and night by localization of calling males. Whenever possible, we collected specimens only after identification by vocal sac inflation during call climax, to reliably link call recordings to voucher specimens. Vocalizations were recorded using portable tape recorders with external microphones and were analysed either with the MEDAV sound analysing system Spektro 3.2 or on a PC using the software CoolEdit (Syntrillium Corp.). Vouchers were fixed in 96% ethanol and subsequently stored in 70% ethanol. Museum acronyms used are MNHN (Muséum national d'Histoire naturelle, Paris), UADBA (Université d'Antananarivo, Département de Biologie Animale), ZFMK (Zoologisches Forschungsinstitut und Museum A. Koenig, Bonn), and ZSM (Zoologische Staatssammlung, München). As no definitive catalogue numbers of UADBA specimens were available, we report here the provisional field numbers of F. Glaw and M. Vences (FG/MV) for specimens stored in this collection. The following morphological measurements were taken with dial calipers to the nearest 0.1 mm: SVL (snout-vent

length), HW (head width), HL (head length), ED (horizontal eye diameter), END (eye-nostril distance), NSD (nostril-snout tip distance), NND (nostril-nostril distance), TD (tympanum diameter), HAL (hand length), FORL (forelimb length), HIL (hindlimb length), FOL (foot length), FOTL (foot length including tarsus), FGL and FGW (length and width of femoral gland). Temporal and metric measurements are given as range, with mean ± standard deviation in parentheses. DNA was extracted from muscle tissue samples preserved in ethanol. We sequenced a fragment of the mitochondrial 16S rRNA gene comprising up to 565 nucleotides (nt). Detailed information on DNA extraction, primers, PCR and sequencing are given in Vences *et al.* (2000*b*). Alignment required the inclusion of gaps in the sequences of one or more taxa at a total of 16 nucleotide sites, mainly to account for divergent sequences of outgroups in the hypervariable loop regions. Prior to phylogenetic reconstruction, we looked at which substitution model fits our sequence data the best. We applied a hierarchical likelihood ratio test for testing the goodness-of-fit of nested substitution models using the program MODELTEST (Posada & Crandall, 1998). Phylogenetic analyses were carried out using PAUP* (Swofford, 2001). We performed heuristic searches using the Maximum Likelihood (ML) method under the substitution model proposed by MODELTEST, and with random addition sequences with 10 replications and tree bisection reconnection (TBR) branch-swapping. Additionally, we calculated Maximum Parsimony (MP) cladograms with gaps treated as a fifth character, and Neighbor-joining (NJ) trees with LogDet distances which are robust against possible variation of sequence evolution among lineages (Lockhart *et al.*, 1994).

The analyses were repeated after exclusion of two hypervariable regions (spanning over a total of 62 nucleotides) and of the only two additional sites with gaps in one or more taxa in the alignment. Two-thousand bootstrap replicates were run in all analyses. The robustness of nodes was tested by Kishino-Hasegawa tests (Kishino & Hasegawa, 1989) as implemented in PAUP* (RELL bootstrap, 1000 replicates, one-tailed test).

Sequences were submitted to Genbank (see appendix for accession numbers). The following specimens were used for genetic analysis: *Boophis tephraeomystax*, ZFMK 66685 (Cap Est), Genbank accession AF215333; *Mantella laevigata*, ZFMK 65637 (no locality data), AF215280; *Mantidactylus blommersae*, UADBA-FG/MV 2000.65 (Andasibe), AF317688; *Mantidactylus depressiceps*, ZFMK 60131 (Andasibe), AF215326; *Mantidactylus domerguei*, ZSM 370/2000 (Manjakatompo), AF317689; *Mantidactylus grandisonae*, ZFMK 66669 (Ambatobe), AF215315; *Mantidactylus kely*, ZSM 363/2000 (Ambatolampy), AF317690; *Mantidactylus liber*, ZSM 491/2000 (Montagne d'Ambre), AF317686; *Mantidactylus sarotra*, ZSM 354/2000 (Mandraka), AF317687; *Mantidactylus wittei*, UADBA-FG/MV 2000.123 (Ambanja), AF317691.

RESULTS

A New Species of *Mantidactylus*

Field data gathered during 1995, 1996 and 2000 supported the observations of Glaw & Vences (1994) regarding the syntopic occurrence of two forms which morphologically corresponded to the description of *Mantidactylus blommersae* (Guibé, 1975). Morphological examination (Table 1) revealed no differences between them except size. Re-examination of the type series of *M. blommersae* (Table 1) corroborated that holotype and paratypes agree in size and morphology with the larger form which had already been defined as *M. blommersae* by Glaw & Vences (1994). The female holotype and paratypes were not conspicuously larger than the male paratypes, as usual in many *Mantidactylus* (Blommers-Schlösser, 1979). The smaller form corresponds to an undescribed form, designated *Mantidactylus* sp. b in Glaw & Vences (1994).

Males of *M. blommersae* generally called from the underside of leaves 10-20 cm above swamps, while specimens of the undescribed species (Figs. 1,2) called from positions near or on the ground, very well hidden in deep vegetation, and generally not directly from structures above the water surface. Vocalizations of the two were consistently different: *M. blommersae* emit-

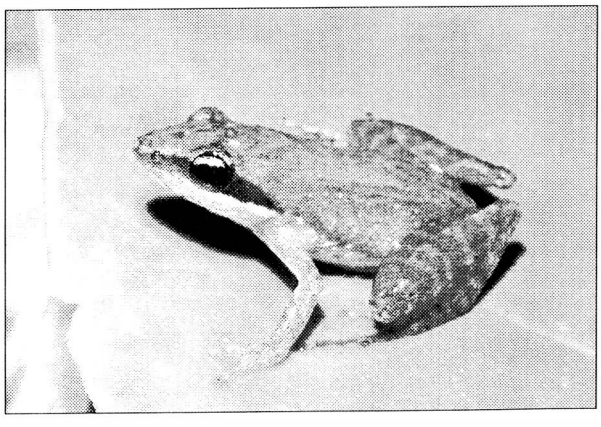

FIG. 1. Dorsolateral view of *Mantidactylus sarotra* (male holotype) in life.

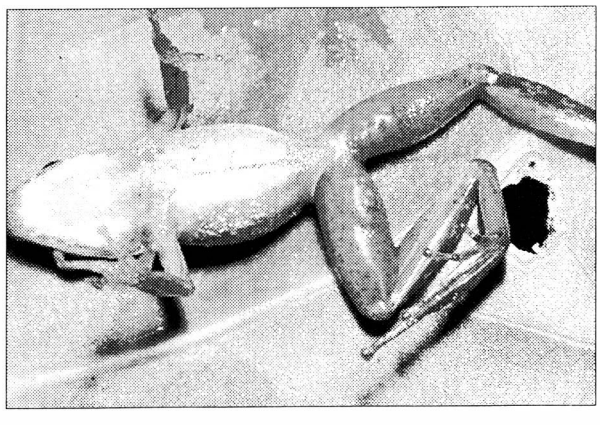

FIG. 2. Ventral view of *Mantidactylus sarotra* (male holotype) in life.

TABLE 1. Morphometric measurments (all in mm) of specimens of *Mantidactylus sarotra, M. blommersae* and *M. kely*. For abbreviations of measured variables, see Materials and Methods; further abbreviations used: M (male), F (female), HT (holotype), PT (paratype), RHL (relative hindlimb length: point reached by tibiotarsal articulation when the hindlimb is adpressed along the body: 0, anterior eye margin; 1, between eye and nostril; 2, nostril; 3, snout tip).

Catalogue number	Status	Sex	Locality	SVL	HW	HL	TD	ED	END	NSD	NSD	FORL	HAL	HIL	FOTL	FOL	FGL	FGW	RHL
M. sarotra																			
ZFMK 62887	PT	M	Mandraka	15.2	4.7	6.0	1.1	2.0	1.3	1.2	1.7	10.0	4.4	27.7	12.6	7.8	3.0	1.3	2
ZSM 351/2000	HT	M	Mandraka	16.8	5.0	6.6	1.1	2.0	1.6	1.5	1.9	12.5	4.9	30.5	13.9	8.7	3.0	1.4	3
ZSM 354/2000	PT	M	Mandraka	15.7	4.8	6.0	1.2	1.9	1.4	1.1	1.9	10.6	4.6	28.6	13.4	8.7	2.4	1.3	2
M. kely																			
ZFMK 57444	HT	M	Manjakatompo	15.3	5.0	6.0	0.8	2.3	1.5	1.2	1.9	10.3	4.0	24.7	12.4	8.1	2.8	1.3	1
ZSM 363/2000	-	M	Ambatolampy	14.2	4.0	5.6	0.9	1.7	1.1	1.0	1.6	8.8	3.5	22.9	11.0	7.2	2.9	1.0	0
ZSM 364/2000	-	M	Manjakatompo	15.9	4.7	6.0	0.9	2.3	1.4	1.2	1.7	10.4	4.0	27.0	12.8	8.3	2.8	1.4	1
M. blommersae																			
MNHN 1975.05	HT	F	25 km S Moramanga	20.1	6.0	7.6	1.3	2.3	1.8	1.4	2.4	12.5	5.1	34.0	15.7	9.9	-	-	1
MNHN 1975.06	PT	F	Ranomafana	19.6	5.6	7.4	1.4	2.2	1.9	1.3	2.3	12.0	4.9	30.3	14.4	9.5	-	-	0
MNHN 1975.07	PT	M	Andasibe	21.3	6.7	8.2	1.2	2.4	1.9	1.6	2.4	14.1	6.4	36.9	16.7	11.2	3.2	1.5	1
MNHN 1975.08	PT	M	Andasibe	20.2	6.1	7.3	1.2	2.5	1.8	1.5	2.3	14.0	6.2	34.9	17.2	11.2	3.0	1.3	1
MNHN 1975.09	PT	M	Andasibe	18.4	6.0	7.0	1.6	2.2	1.5	1.4	2.2	11.8	5.4	31.2	14.9	9.8	3.9	1.4	2
MNHN 1975.10	PT	M	Andasibe	18.8	6.0	7.6	1.3	2.2	1.8	1.3	2.3	14.3	6.5	34.1	15.8	10.6	3.3	1.2	2
MNHN 1975.11	PT	F	Andasibe	20.4	6.3	8.1	1.4	2.2	1.6	1.5	2.1	11.5	5.0	32.4	15.1	9.7	-	-	0
MNHN 1975.12	PT	F	Andasibe	19.3	5.6	7.6	1.4	2.1	1.9	1.5	2.3	11.7	4.8	28.7	13.0	8.3	-	-	0
MNHN 1975.13	PT	M	Andasibe	19.0	5.6	6.9	1.3	2.2	1.5	1.3	2.0	12.3	5.4	31.7	14.8	9.4	2.8	1.4	2
ZFMK 59819	-	F	Andasibe	19.3	5.3	6.9	1.1	2.1	1.6	1.5	2.1	12.2	4.8	31.1	14.4	9.1	-	-	0
ZFMK 59877	-	M	Andasibe	19.2	5.8	7.3	1.2	2.4	1.7	1.5	2.2	13.0	5.8	31.8	15.2	9.6	3.9	1.7	1
ZFMK 59878	-	M	Andasibe	19.4	6.0	7.1	1.3	2.3	1.6	1.4	2.4	13.2	5.6	33.6	15.2	9.8	2.6	1.4	2
ZFMK 62226	-	M	Andasibe	21.0	6.2	7.5	1.3	2.2	1.7	1.5	2.1	13.3	5.9	32.6	15.4	10.2	4.2	1.4	0
ZFMK 62227	-	M	Andasibe	20.0	5.8	7.2	1.4	2.3	1.6	1.5	2.0	13.0	5.5	33.3	15.8	10.2	3.8	1.5	0
ZFMK 62228	-	M	Andasibe	19.5	6.1	7.5	1.5	2.3	1.7	1.5	2.0	12.3	5.7	33.2	15.2	10.0	3.5	1.5	1
ZFMK 62229	-	M	Andasibe	20.0	6.0	7.5	1.2	2.0	1.6	1.4	2.2	12.5	5.2	31.8	14.6	9.1	3.5	1.6	0
ZFMK 62230	-	M	Andasibe	18.6	5.7	7.8	1.4	2.4	1.6	1.5	2.2	12.2	5.4	31.7	14.0	9.1	3.2	1.2	2
ZFMK 62231	-	M	Andasibe	19.4	6.0	7.2	1.4	2.2	1.6	1.4	2.1	12.7	5.2	32.5	15.1	9.7	3.4	1.3	1
ZFMK 62232	-	M	Andasibe	19.9	6.2	7.5	1.2	2.2	1.6	1.5	2.3	12.9	5.7	33.6	15.5	10.4	3.5	1.7	1
ZFMK 62233	-	M	Andasibe	18.0	5.5	6.7	1.2	2.0	1.6	1.4	2.0	12.2	5.5	30.4	13.8	9.1	3.7	1.6	1

ted series of two to three similar short chirp notes (Table 2). In contrast, the undescribed species had three different note types: two longer pulsed notes and a short click note, combined in different ways (Table 3). Capture of specimens of the undescribed species was extremely difficult; only at one locality (Mandraka) did we succeed in capturing four male specimens which were all observed during call emission. At a nearby locality, Andasibe, we captured a further eight male specimens of *M. blommersae* (ZFMK 62226-62233) which were all observed emitting their typical calls.

DNA sequences were obtained from one specimen of each species in the *M. domerguei* group. The resulting phylograms (Fig. 3) did not group the two *M. blommersae*-like forms as sister species. Instead, *M. blommersae* was the sister species of *M. domerguei*, and the undescribed species was the sister species of *M. kely*. Bootstrap support for these groupings was high (89-98% in all cases). Alternative trees with *M. blommersae* and the undescribed species as sister groups needed 24 or 25 additional steps and were significantly worse under both Maximum Parsimony and Maximum Likelihood models (Kishino-Hasegawa tests; $P<0.05$). Analyses after exclusion of all hypervariable (loop) regions resulted in slightly lower support for the relevant groupings (bootstrap values 76-92%), but the alternative topologies could still be significantly excluded by Kishino-Hasegawa tests ($P<0.05$).

The undescribed species differed from its closest relative *M. kely* by 35 nucleotides (not considering indels) in the considered fragment (6.20%), while it differed from *M. blommersae* by 55 nt (9.73%) (Table 4); *M. blommersae* differed by 28 nt (4.96%) from its sister species *M. domerguei*. The high genetic differentiation and phylogenetic position of the undescribed species leave no doubt as to its distinctness; we therefore describe it formally in the following.

MANTIDACTYLUS SAROTRA SP. N. (FIGS. 1,2)

Diagnosis. A species assigned to the genus *Mantidactylus* based on the absence of nuptial pads and presence of femoral glands in males. Assigned to the *M. domerguei* group in the subgenus *Blommersia* based on small size, low relative hand length (ratio HAL/SVL <30%), a white horseshoe shaped marking on the throat, single subgular vocal sac, femoral gland morphology (single patch of similarly sized granules), and relatively elongated head (ratio HW/HL 76-80%). Within the *M. domerguei* group, *M. sarotra* is distinguished from *M. grandisonae, M. domerguei* and *M. blommersae* by a distinct white horseshoe shaped marking on the throat and by general structure of advertisement calls. It is further distinguished from *M. wittei*

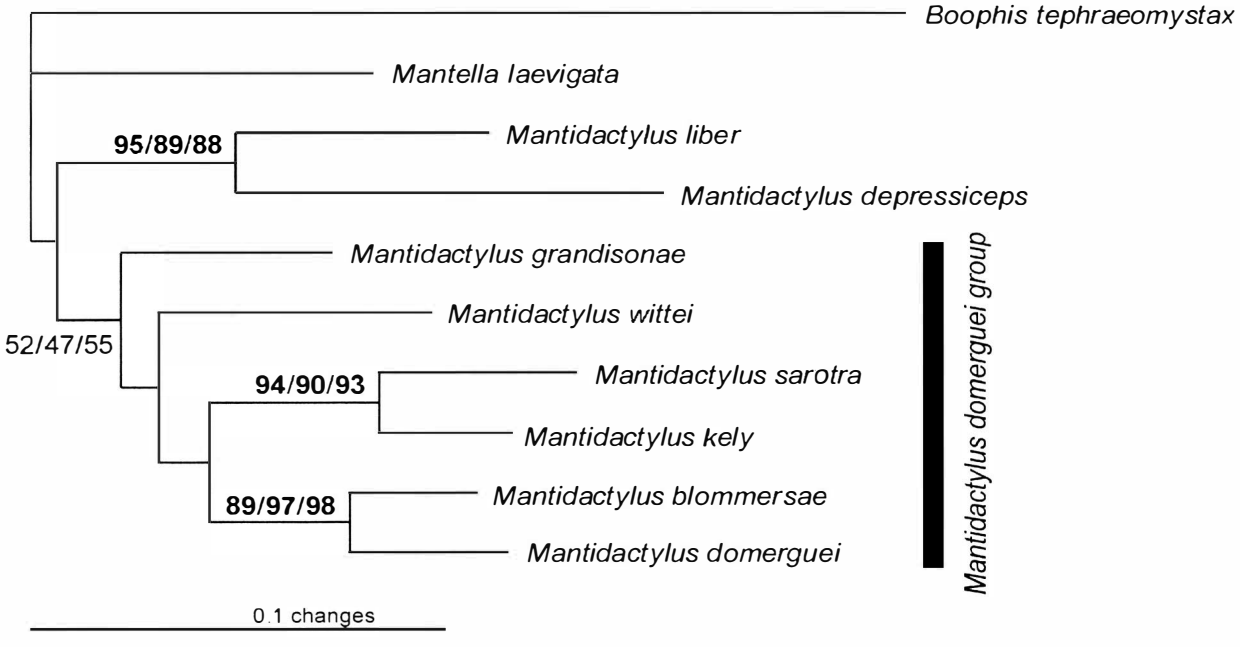

FIG 3. Maximum Likelihood phylogram based on 565 nucleotides of a fragment of the mitochondrial 16S rRNA gene in species of the *Mantidactylus domerguei* group, obtained using settings estimated by the program MODELTEST: Tamura-Nei substitution model with empirical base frequencies (A: 0.3154; C: 0.2237; G: 0.1833; T: 0.2776) and substitution rates (A-G: 2.7672; C-T: 8.7501; all other rates: 1), no invariable sites and a gamma distribution shape parameter of 0.1648. The topology agrees with that of the most parsimonious tree found by Maximum Parsimony analysis (396 constant and 97 parsimony-informative characters; 345 steps; consistency index 0.67, retention index 0.45) except for the arrangement of clades within the *M. domerguei* group (*M. wittei* placed as sister group of the *M. blommersae/M. domerguei* clade). Numbers at nodes are bootstrap support (2000 replications) in percent for Maximum Likelihood (left), Maximum Parsimony (middle) and Neighbour-joining (using LogDet distances; right) analyses, respectively. Bootstrap values below 50% in all three analyses are not shown. *Boophis tephraeomystax* was used as outgroup.

TABLE 2. Temporal and spectral call parameters in various populations of *Mantidactylus blommersae*.

	Ranomafana	Ankeniheny	An'Ala
Recording temperature	22°C	20.5°C	22°C
Recording date	29 February 1996	19 February 1994	11 February 1995
Note duration [ms]	29-65 (47±11,*n*=14)	45-129 (69±31,*n*=6)	113-187 (138±23,*n*=10)
Duration of interval between notes [ms]	26-52 (39±11,*n*=4)	42-49 (46±4,*n*=3)	13-33 (21±7,*n*=7)
Frequency [Hz]	3550-8850	4000-7100	4100-8300
Dominant freq. [Hz]	5900-6700	5800	5900

TABLE 3.Temporal and spectral call parameters in various populations of *Mantidactylus sarotra* and *M. kely*. Missing data refer to note types which may occur in the corresponding populations, but which were not recorded.

	M. sarotra	*M. sarotra*	*M. sarotra*	*M. kely*	*M. kely*
Recording information					
Locality	Mandraka	Andasibe	Ranomafana	Manjakatompo	Ambatolampy
Temperature	18.4°C	25.5°C	not recorded	18°C	23.2°C
Date	8 Feb. 2000	30 Jan. 1996	28 Feb. 1996	8 Jan. 1994	11 Feb. 2000
Notes of type 1					
Note duration [ms]	187-301 (244±80,*n*=2)	244-256 (250±6,*n*=3)	266-370 (321±34,*n*=10)	453-637 (564±60,*n*=10)	247-510 (399±91,*n*=10)
Number of pulses per note	35-55 (45±14,*n*=2)	44-46 (45±1,*n*=2)	37-50 (44±4,*n*=10)	23-36 (28±4,*n*=10)	19-35 (28±6,*n*=10)
Pulse repetition rate [1/s]	183-187 (185±3,*n*=2)	180 (*n* = 2)	130-145 (138±5,*n*=10)	39-61 (50±7,*n*=10)	55-84 (71±8,*n*=10)
Notes of type 2					
Note duration [ms]	96-138 (124±19,*n*=4)	118-172 (143±17,*n*=13)	87-202 (116±48,*n*=5)	- -	- -
Number of pulses per note	21-27 (24±3,*n*=4)	19-25 (23±2,*n*=6)	15-31 (19±7,*n*=5)	-	-
Pulse repetition rate [1/s]	181-218 (198±19,*n*=4)	136-182 (165±16,*n*=6)	153-185 (170±12,*n*=5)	-	-
Notes of type 3					
Note duration [ms]	11-14 (12±1,*n*=6)	5-13 (9±2,*n*=16)	4-7 (6±1,*n*=5)	- -	15-42 (24±8,*n*=10)
Interval duration					
between notes of types 1 and 2 [ms]	1126-1623 (1380±177,*n*=5)	751-1198 (912±158,*n*=13)	741-970 (890±70,*n*=11)	1565-2639 (1955±423,*n*=9)	1059-1485 (1266±230,*n*=4)
between notes of type 1 or 2 and notes of type 3 [ms]	29-43 (37±6,*n*=6)	22-44 (34±6,*n*=16)	41-50 (47±4,*n*=5)	- -	3-33 (18±11,*n*=10)
Frequency					
Frequency (of notes of type 1 & 2) [Hz]	2500-5750	2750-5350	3550-5850	3100-4650	3500-7000
Dominant frequency (of notes of type 1 &2) [Hz]	3750-5500	4000-5050	4350-4900	4100-4300	4650-4750

TABLE 4. Genetic differentiation between species in the *Mantidactylus domerguei* group. The values below the diagonal are numbers of pairwise substitutions in a fragment of the 16S rRNA gene (565 nucleotides); the values above the diagonal are total pairwise sequence divergences in percent. Indels were not considered.

	M. sarotra	*M. blommersae*	*M. domerguei*	*M. grandisonae*	*M. kely*	*M. wittei*
M. sarotra	-	9.73	9.22	7.61	6.20	10.09
M. blommersae	55	-	4.96	8.14	7.79	8.50
M. domerguei	52	28	-	9.20	8.67	8.14
M. grandisonae	43	46	52	-	8.85	7.43
M. kely	35	44	49	50	-	9.03
M. wittei	57	48	46	42	51	-

by absence of vomerine teeth (vs. presence) and smaller size (SVL 15-17 mm vs. 21-26 mm); from *M. grandisonae* by smaller size (SVL 15-17 mm vs. 18-23 mm) and by a uniformly brownish flank with only an interrupted line of indistinct white spots (vs. a sharp contrast between a continuous upper blackish and lower white line along the flanks); from *M. domerguei* by a uniform dorsum with only a poorly contrasted Y-shaped marking (vs. a contrasted pattern of three longitudinal dorsal bands); and from *M. blommersae* by smaller size (SVL 15-17 mm vs. 18-21 mm). By molecular analysis the closest known relative of *M. sarotra* is *M. kely*, which furthermore is the only species of the group sharing a white horseshoe shaped marking on the throat, and an advertisement call composed of a long and distinctly pulsed note which is followed by a short click note. However, the pulsed note of *M. kely* has a lower pulse repetition rate at similar temperatures (Table 3), and *M. sarotra* differs from *M. kely* by a different dorsal coloration (largely uniform light brown with only a poorly contrasted Y-shaped marking vs. dark brown with distinct blackish markings and a distinct yellowish vertebral stripe) and skin texture (smooth vs. granular).

Etymology. Derived from *sarotra* (Malagasy: difficult), making allusion to the difficulties involved both in capturing and correctly diagnosing the new species. The name is used as an invariable noun standing in apposition to the generic name.

Holotype. ZSM 351/2000, adult male, collected by F. Glaw and M. Vences on 8 February 2000 at Mandraka (18° 54' 44" S, 47° 54' 52" E, 1425 m altitude), central eastern Madagascar.

Paratypes. ZFMK 62887, adult male, collected by F. Glaw and M. Vences on 9 February 1994 at the type locality; ZSM 354/2000 and UADBA-FG/MV 2000.22, two adult males, collected by F. Glaw and M. Vences on 8 February 2000 at the type locality.

Description of the holotype. SVL 16.8 mm. For measurements, see Table 1. Body slender; head distinctly longer than wide, not wider than body; snout slightly pointed in dorsal and lateral views, nostrils directed laterally, not protuberant, nearer to tip of snout than to eye; canthus rostralis indistinct, straight; loreal region concave; tympanum distinct, rounded, 55% of eye diameter; supratympanic fold rather indistinct, slightly curved; tongue ovoid, slightly bifid posteriorly; vomerine teeth absent, maxillary teeth present; choanae rounded. Arms slender, subarticular tubercles single; metacarpal tubercles not visible; fingers without webbing; relative length of fingers 1<2<4<3, finger 2 distinctly shorter than finger 4; finger disks distinctly enlarged; nuptial pads absent. Hindlimbs slender; tibiotarsal articulation reaches snout tip; lateral metatarsalia connected; inner and outer metatarsal tubercles of similar size, small but distinct; only rudimentary webbing between toes; two phalanges of fifth toe (toe disk not counted)of web.

Skin on the upper surface smooth, without folds or ridges. No distinct enlarged tubercles in the cloacal region; ventral skin uniformly smooth. Femoral glands distinct, of type 2 sensu Glaw *et al.* (2000), consisting of 10 granules (diameter 0.4-0.7 mm) in internal view (after reflection of skin).

After about seven months in preservative, the back is light greyish brown with slightly darker, poorly contrasted though well-delimited pattern: a Y-shaped marking on the central dorsum and smaller spots and markings on the head and posterior dorsum. Also the flanks are slightly darker, although there is no distinct colour border between dark flanks and light back as in most *Mantidactylus* species of the subgenus *Chonomantis* or in several representatives of the genus *Mantella*. The hindlimbs are greyish brown with light crossbands: six on femur, five to six on tibia, up to eight on tarsus and foot including longest toe. Posterior to the eye, the head is laterally marked by a very conspicuous dark brown streak underneath the supratympanic fold, which includes the tympanum and ends sickle-like close to the forelimb insertion. Anterior to the eye, a narrower dark streak is positioned underneath the canthus rostralis. Ventrally uniformly cream-white except a very fine dark mottling along the lower lip and in the chest region, and few very fine dark spots on the hindlimbs. Colour in life was similar, except a distinct frenal stripe that ran below the dark lateral head marking, from the forelimb insertion along the upper lip to a point slightly anterior to the eye (not clearly recognizable in preservative). The general colour was light brown instead of greyish brown. The iris was dark in its lower two thirds, light yellowish brown in its upper third. Ventrally, the belly was rather translucent with a

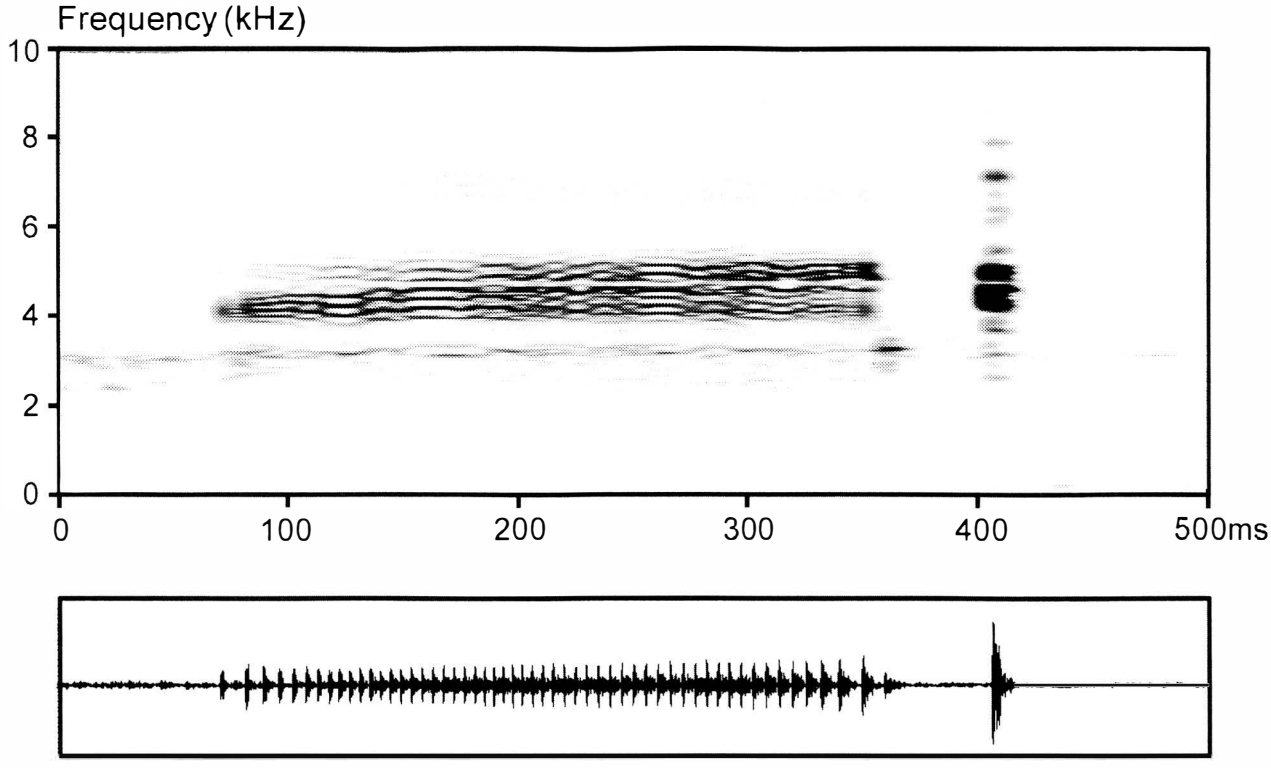

FIG. 4. Sonagram and oscillogram of a note of type 1 (left) and type 3 (right) of *Mantidactylus sarotra* (holotype) from Mandraka, recorded at 18.4°C. Notes of type 2 are similar to those of type 1 as shown in the sonagram, but have a lower number of pulses and therefore a shorter duration (see Table 3).

white central area. The throat was silvery white except for a pigmentless translucent central area.

Variation. The holotype and the three paratypes are very uniform in morphology and coloration (see Table 1 for measurements).

Natural history. Calls of *M. sarotra* were mainly heard during the day, at dusk, and early at night. Before dusk it was impossible to observe calling males, which obviously emitted their vocalizations hidden in the leaf litter or between the thin roots of the vegetation. At night, specimens were observed at Mandraka calling from positions a few centimetres above the ground close to a very small, slow-flowing ditch (10 cm wide, less than 3 cm deep). The inflated vocal sac was conspicuously white. Each note of type 1 or 2 was one expiration. Clutches possibly belonging to this species had been found attached to fallen leaves on the ground in a desiccated puddle (Glaw & Vences, 1994).

Vocalizations. Advertisement calls were recorded at Mandraka on 8 February 2000, at Andasibe on 30 January 1996, and at Ranomafana on 28 February 1996. Three note types could be distinguished. Notes of type 1 are unharmonious and distinctly pulsed (Fig. 4). They are often emitted at the beginning of a call. Notes of type 2 are similar to those of type 1, but of shorter duration. They are often emitted in series of 4-6 notes. Notes of type 3 are short clicks and often follow immediately after notes of type 1 or 2. A typical call is arranged as series of notes of the three types as follows: 1-3—2-3—2-3—2-3—2-3. Combinations as 1—1—1—1—1 or 1—1—1—1-3—1 can also occur. Temporal and spectral characteristics are given in Table 3.

Distribution. Reliably identified specimens were collected only at the type locality, Mandraka. Call records exist from Andasibe, the swamp Antorotorofotsy north of Andasibe, near Moramanga, and Ranomafana. Glaw & Vences (1994) mention a further locality, Tolagnaro in SE-Madagasçar for *Mantidactylus* sp. b. The corresponding vouchers (ZFMK 53698-53699 from Manantenina, close to Tolagnaro) are larger than the type specimens (17-20 mm SVL) and show a different coloration. As no reliable call recordings are available from this locality, we do not consider the vouchers as conspecific with *M. sarotra.* Their status pends further study.

NEW DATA ON *MANTIDACTYLUS BLOMMERSAE*

Occurrence of *Mantidactylus blommersae* could be confirmed at the localities Ranomafana, Andasibe, Ankeniheny and Mandraka (Blommers-Schlösser & Blanc, 1991; Glaw & Vences, 1994). The species was also found at An Ala, a further mid-altitude locality in central eastern Madagascar. Calls from these localities were similar to each other. They were always composed of only a single chirp-like note type without regularly

pulsed structure, notes being arranged in series of two or three notes. Specific assignation of the populations from the Chaînes Anosyennes (Blommers-Schlösser & Blanc, 1991) is currently uncertain. Locality of the holotype is 25 km south of Moramanga (see also Blommers-Schlösser & Blanc, 1991). The paratypes have all been stated to originate from Andasibe (Guibé, 1975; Blommers-Schlösser & Blanc, 1991), but according to the MNHN catalogue one paratype (MNHN 1975.06) appears to have been collected at Ranomafana.

NEW DATA ON *MANTIDACTYLUS KELY*

Mantidactylus kely has so far only been reported from the type locality in the high altitude forest of Manjakatompo (19°21'30"S, 47°18'50"E; altitude ca. 1700 m). In February and March 2000, we heard the typical calls of this species also in an unforested swamp area close to Ambatolampy, about 15 km from Manjakatompo (19°21'54"S, 47°26'01"E, altitude 1595 m). Males were calling during the day from the dense vegetation along stagnant-water ditches. Recording temperature at this site was higher than at Manjakatompo, but pulse repetition rate in notes was still distinctly lower than in *M. sarotra* (Table 3). An explanation of the higher pulse rate of *M. sarotra* by temperature effects could thus be excluded. Tadpoles collected in the same water bodies could be assigned to *M. kely* by DNA sequences; they were of the generalized type typical for the *M. domerguei* group (Blommers-Schlösser, 1979) and will be described in detail elsewhere. We also noted a possible difference in the diel activity rhythms of syntopic *M. kely* and *M. domerguei* at Manjakatompo. On the six days of observation in March 2000, *M. kely* calls were mainly heard during the day. In contrast, *M. domerguei* calls were almost exclusively nocturnal, although they were often heard during the day at other localities. Only at dusk did we hear simultaneous calling of both species. The new analysis of the recordings from Manjakatompo revealed that *M. kely* also produces click notes (type 3). These were always heard at the end of the long pulsed notes (type 1). In some cases, the click was merely a slightly different final pulse; in other cases, it was a double pulse or a distinct, long click-note; this variability accounts for the high standard deviations of the corresponding temporal measurements (Table 3).

Mantidactylus kely was described as having separated lateral metatarsalia. This state, which does not refer to the metatarsal bones but to the investing tissue, was the main character used to distinguish the artificial genera *Gephyromantis* and *Mantidactylus* sensu Guibé (1978). Blommers-Schlösser (1979) doubted the phylogenetic value of this character and joined all involved species in one genus, *Mantidactylus*. The subgenus *Blommersia* contains species with apparently separated (*M. grandisonae, M. wittei*) and connected (*M. blommersae, M. domerguei*) metatarsalia. In the new material of *M. kely* available to us, the state of the metatarsalia is difficult to assess, and the decision (connected or separated) depends on the method of examination. Pulling the fifth toe laterally and using strong light, the metatarsalia appear separated, but without this drastic method, they appear connected. The same is true in *M. sarotra*. This indicates that the states of this character are not unequivocal in the small species of the subgenus *Blommersia*, and corroborates their doubtful phylogenetic value in the genus *Mantidactylus*.

DISCUSSION

This paper presents the first data on the degree of genetic differentiation among sibling species of Malagasy anurans. All species of the *M. domerguei* group are remarkably similar in morphology, but well distinguished by advertisement calls. Their similar general appearance strongly suggests their monophyletic origins; nevertheless, monophyly was only weakly corroborated by the molecular data.

The phylogenetic relationships of the included species as presented here were recovered from mitochondrial sequences of single specimens, as is usual in similar analyses (e.g Richards *et al.*, 2000). The main assumption in such studies is that the mitochondrial gene tree represents the phylogenetic species tree, which means that such factors as haplotype polymorphism and recent introgression by hybridization play no relevant roles. Although a few examples of high intrapopulational mitochondrial haplotype polymorphism appear to exist in other organisms, in most cases such phenomena are uncommon (Avise, 2000). In the extensive amphibian data set available through Genbank and our own research (except for hybridogenetic species), no examples of intrapopulational polymorphism affecting more than only a few mutations in the rather conservative 16S gene are known to us. In *Blommersia,* we obtained additional sequences of three *M. wittei* populations and of additional specimens of both *M. kely* and *M. domerguei* (to be included in forthcoming publications), which corresponded to the sequences included in the present study. We therefore believe that the degree of haplotype polymorphism in this group is low, and that the mitochondrial gene tree actually corresponds to the species tree. Considering the high degree of differentiation among all taxa, any possible introgression must have occurred soon after the main cladogenetic events. If the recovered phylogeny represents correctly the relationships of species of *Blommersia,* some conclusions regarding speciation and its timing in this group are possible.

First, the genetic differentiation revealed by the DNA sequences was higher than expected in a group of such morphologically similar species. Pleistocene glaciations led to modifications in the distribution of vegetation types in Madagascar (Burney, 1996). For

example, the currently isolated montane floras were in broad contact along the central mountain chain. Two species of the *M. domerguei* group (*M. domerguei* and *M. kely*) occur mainly at higher altitudes (up to 1700-2000 m). Hence, it could be hypothesized that speciation in the group was partly triggered by the Pleistocene climatic and habitat shifts, as may have been the case in other representatives of the herpetofauna (Raxworthy & Nussbaum, 1996; Vences & Glaw, 1999). Molecular clock estimates in the 16S gene range from ca. 0.3-0.7% pairwise divergence per million years in amphibians and reptiles (e.g. Veith *et al.*, 1998; Caccone *et al.*, 1997; Carranza *et al.*, 2000). Even assuming an accelerated rate of 1% per million years, the youngest split in the *M. domerguei* group (between *M. blommersae* and *M. domerguei*; divergence 4.96%) would be estimated to have occurred five million years before present, and thus at the Miocene/Pliocene boundary. Hence, speciation events among the known species of this group may have taken place well before the Pleistocene, a trend similar to other tropical (Clough & Summers, 2000) and non-tropical vertebrate groups (Avise *et al.*, 1998; Avise, 2000).

Second, morphological differentiation does not appear to be crucial for speciation in these frogs; factors favouring syntopic occurrence of different species are probably ecological (different breeding habitat of *M. blommersae* and *M. sarotra*; possibly different diel activity cycles in *M. domerguei* and *M. kely*) and bioacoustic (different advertisement calls of all species). The low bootstrap support for most relationships within the group does not allow us to formulate a hypothesis for call evolution in the group. The two well-supported groupings, however, are contradictory: *M. kely* and *M. sarotra* (6.20% sequence differentiation) have structurally similar calls, while *M. blommersae* and *M. domerguei* (4.96%) strongly differ in general call structure. More genetic data on other Malagasy anuran groups are necessary to assess the main mechanisms that led to the high number of morphologically similar sibling species (Glaw & Vences, 2000) in this speciose monophyletic radiation (Richards & Moore, 1998; Richards *et al.*, 2000; Vences *et al.*, 2000*a*).

ACKNOWLEDGEMENTS

We are grateful to Annemarie Ohler and Alain Dubois (MNHN), and Wolfgang Böhme (ZFMK), who allowed examination of specimens held in their care. Nirhy Rabibisoa, Domoina Rakotomalala, Olivier Ramilison, Noro Raminosoa, and Fara Ranaivojaona assisted in the field. The work in Madagascar was made possible by a cooperation between the UADBA and the ZSM. The Malagasy authorities are acknowledged for collection and export permits. The work in Ambatolampy and Manjakatompo was technically supported by the Gesellschaft für Technische Zusammenarbeit; thanks are especially due to Guido Besmer and Jürgen Gräbener. The research was supported by the 'Deutsche Forschungsgemeinschaft' (to F. G.) and the 'Deutscher Akademischer Austauschdienst' (to M. V.). DNA sequencing was financially assisted by the 'Freunde der Zoologischen Staatssammlung e. V.'. The constructive comments of two anonymous referees helped to substantially improve an earlier version of this manuscript.

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Accepted: 14.8.01

HERPETOLOGICAL JOURNAL, Vol. 12, pp. 21-28 (2002)

WHEN CROWDED TADPOLES (*RANA ARVALIS* AND *R. TEMPORARIA*) FAIL TO METAMORPHOSE EARLY AND THUS FAIL TO ESCAPE DRYING PONDS

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Do moor frog (*Rana arvalis*) and common frog (*R. temporaria*) tadpoles increase developmental rate if there is a risk of their pond drying up before metamorphosis? To study this, I performed an experiment designed to mimic natural conditions in many drying ponds. The number of tadpoles per tank was constant during the experiment but the water level was lowered in experimental tanks so that crowding increased. Experimental tadpoles grew and developed more slowly than control tadpoles that were in constant water volume. Also, metamorphosis was delayed (i.e. a smaller proportion had metamorphosed when the experiment was concluded on 1 August) and the metamorphs were smaller. I conclude that, due to crowding, the tadpoles in this experiment were not able to speed up development rate adaptively. Performance of the tadpoles in the experiment was compared to that of *R. temporaria* tadpoles in the field. These lived in a pond where desiccation resulted in division of the water body into a small pool and a large pool. The small pool dried out completely before the rest of the pond. Tadpoles in this pool were smaller and had relatively smaller hind legs, suggesting slower development. This pattern confirms the result of the experiment, supporting my suggestion that the experimental set-up mimicked many natural situations. Of particular interest is the fact that other studies – carried out both in the same geographical area and elsewhere – have shown *R. temporaria* to have the ability to respond adaptively to pond drying. The fact that it did not do so in this particular experiment, as well as in the field pond studied here, shows that care must be exercised when extrapolating from one study to the properties of a species. Different conditions, both in the field and in experiments, may well give different responses.

Key words: amphibian development, competition, frog tadpoles, drying ponds

INTRODUCTION

Ponds used by breeding frogs range from permanent waters to temporary. In dry years, frogs breeding in temporary ponds face the risk of losing all offspring (Wilbur, 1984; Semlitsch & Wilbur, 1988; Tejedo & Reques, 1994, Griffiths, 1997; Barandun & Reyer, 1997). This habitat is used by many aquatic organisms that have evolved various strategies to survive (Wiggins, Mackay & Smith, 1980). Strategies to counter the risk of pond desiccation should be under strong selective pressure.

A facultative shortening of development time would be one such strategy suitable for frogs. This is likely to involve a trade-off between early metamorphosis at a small size and late metamorphosis at a large size (Merilä *et al*., 2000*b*). The optimum balance may depend on information concerning the quality of the breeding pond (including expected hydroperiod) as well as the expected quality of the future terrestrial habitat (Wilbur & Collins, 1973; Werner, 1986). Plasticity in timing of metamorphosis has indeed been demonstrated for amphibians in experiments by Semlitsch & Wilbur (1988) for *Ambystoma talpoideum*; Crump (1989) for *Hyla pseudopuma*; Newman (1989) for *Scaphiopus couchii*; Tejedo & Reques (1994) for *Bufo calamita*; and Laurila & Kujasalo (1999) for *Rana temporaria*. A pattern of size at metamorphosis and time to metamorphosis in the field that supports this plasticity has been demonstrated for a salamander *Ambystoma talpoideum* (Semlitsch *et al*., 1988). Extreme plasticity has been demonstrated in *Scaphiopus hammondii* tadpoles that were not only able to accelerate development as water levels decreased but were also able to slow development when water levels were raised again (Denver, Nooshan & Phillips, 1998). A similar level of plasticity in mosquitoes has also been shown (Juliano & Stoffregen 1994). In contrast to the studies above, tadpoles of the *Rana esculenta* complex did not exhibit a plastic response in time to metamorphosis when exposed to experimental pond drying (Semlitsch & Reyer, 1992).

Growth rate and developmental rate both tend to increase with decreased competition, as shown by Wilbur (1976; 1977*a*) and modelled by Hentschel (1999). This is likely to be an effect of increased food resources. Thus, such responses are not necessarily adaptive. Growth rate and development rate are not necessarily coupled but may respond to different cues (Smith-Gill & Berven, 1979). If tadpoles in drying ponds accelerate developmental rate, this may proximately be due to tadpoles somehow directly sensing the decrease in water level. Alternatively, tadpoles may sense increased competition and respond to this, either because it is an indication of a dangerously decreasing water volume or because in itself it constitutes an adverse condition, mo-

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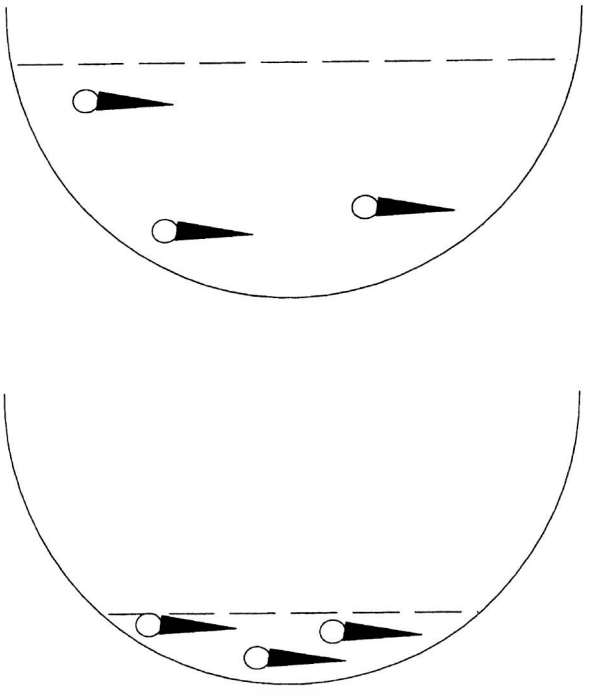

FIG. 1. Cross section of the experimental tanks. Decreasing water level not only reduces water volume but also substantially reduces the area available for algal growth.

tivating a quick escape onto land. Both these responses are adaptive (Gotthard & Nylin, 1995) and only differ in proximal cues.

In Sweden, breeding ponds used by *Rana arvalis* (moor frogs) and *R. temporaria* (common frogs) are variable in terms of the risk of drying. Many frogs breed in ponds that contain adequate water in most years but that do dry in some years, causing catastrophic mortality. Thus, drying of a pond is a mortality factor of some importance for tadpoles of these species (Cooke, 1985; Kutenkov & Panarin, 1995; Loman, 2001). The present study is an attempt to investigate wheter *R. arvalis* and *R. temporaria* exhibit plasticity with respect to developmental rate.

This study has two parts. The first is an experiment where manipulation of water level was done in large tanks. Water level was decreased without any change of tadpole numbers. Due to the shape of the tanks, this also reduced the area available for algal growth and thus led to reduced resources (Fig. 1). Any effects on development rate could thus have been due either to effects of crowding (combined with reduction of resources) or to direct responses (if so, presumably adaptive) to decreased water level. This treatment should mimic a natural pond with little mortality. The second part is a field study of a shallow natural pond. During spring and early summer, part of the pond was naturally cut off and dried out more quickly than the main basin. Performance of tadpoles in the two parts of the pond were compared and results compared to those of the experiment.

TABLE 1. Manipulation of water volume (litres) in tanks with different water reduction schemes. "Dry 1", "Dry 2" and "Dry 3" are considered experimental tanks.

	Start	Day of treatment			
	23 April	7 May	21 May	5 June	17 June
Control	80	80	80	80	40
Dry 1	80	40	20	10	5
Dry 2	80	20	10	5	2.5
Dry 3	80	10	5	2.5	1.25

MATERIALS AND METHODS

Collection and Introduction of Tadpoles

Newly hatched tadpoles (not more than two days old) were collected during April 1990 from four breeding ponds; two with *R. arvalis* and two with *R. temporaria*. They were situated within 20 km of each other in the province of Skåne in southernmost Sweden. The samples contained tadpoles from several clutches at each pond. After collection, all tadpoles from one site were mixed. After two days (when their condition had been monitored and any weak or dead individuals replaced), on 23 April, tadpoles were introduced into 20 tanks. At the start of the study there were 80 tadpoles in each tank. All tadpoles in one tank were from the same pond.

Tanks

The 20 tanks were located outdoors at Lund University Ecology Department's field station, 17 km east of Lund (55° 40' N, 13° 30' E). They were made from 200 litre plastic barrels, cut in half lengthwise. Two weeks before the introduction of the tadpoles, tanks were filled with 80 litres of tap water and "inoculated" with about 2 litres of pond water. Some vegetation (strings of *Elodea canadensis*, *Ranunculus aquatilis*, and *Chara* sp.) was added to ensure an adequate oxygen level in the tanks. No more food was added. Tadpoles fed exclusively on resources produced in the tank; mainly algae growing on vegetation and on the wall of the tank. Tanks were covered with lids of fine mesh netting to prevent colonization by predaceous insects. Above the tanks I suspended a thin layer of textile material to provide shading from the sun. This reduced temperature extremes.

Experimental Design and Analysis

The two species were sampled in different ponds and each housed in 10 tanks. Of these ten tanks, four were control tanks (two from each pond) and six experimental (three from each pond). The effects of source pond and species were not within the scope of the study. These effects had the nature of blocking factors and

were included as factors in the tests, but the effects of species and pond *per se* were not further considered. Testing tadpole performance (variables in Table 3), mean measurements for each tank were used as data for dependent variables. Testing metamorphosis success, percentage metamorphosing in each tank (Table 2, "% metam.") was used as data for the dependent variable. Some tadpoles were lost. Lost tadpoles (dead or possibly escaped as metamorphs) were excluded from the total when computing the percentage metamorphosing. In tanks with reduced water, depth was at most 5 cm during the period of metamorphosis. In these tanks all tadpoles were clearly visible and any lost tadpoles could be attributed to mortality. In the control tanks, water level was always at least 20 cm and, as visibility in some of the tanks was reduced due to algal blooms, some of the lost tadpoles may have reached metamorphosis unnoticed and escaped. For this reason, control tanks were not used for analysis of survival.

Experimental Treatments of Water Level

Two control tanks from each pond (i.e. eight tanks in total) had constant water levels almost throughout the experiment. Only towards the end of the experiment was the water reduced to 40 litres. This reduction was made to facilitate the capture of metamorphs. In 12 experimental tanks, the water level was successively lowered to mimic a drying pond. The water in these tanks was lowered according to three different schemes; "Dry 1", "Dry 2" and "Dry 3" (Tables 1, 2). Three degrees of drying regime were used to guard against threshold effects. The full results are shown in the figures, but for statistical analysis a conservative approach was chosen and all three levels pooled. An exception is the analysis of lost (dead) tadpoles. Because the control tanks were not used, the three degrees of water reduction were contrasted in this analysis.

Tadpole Densities

Tadpoles that died during the course of the experiment were not replaced. However, on 30 May – when all tadpoles were counted (and measured) – few were missing. The tank with the highest mortality actually contained 71 tadpoles out of the original 80 (Table 2). The experiment was terminated on 1 August. All remaining tadpoles were counted (Table 2).

TABLE 2. Summary of the material used in the study. *R. a., Rana arvalis*; *R. t., Rana temporaria*. 'Dry', water level reduction scheme (1, moderate; 2, medium; 3, drastic). 'P', Original pond. On 30 May the tadpoles were measured. The experiment was concluded on 1 August when all remaining tadpoles were counted and measured. 'Lost' represents tadpoles dead since the last count of numbers as well as possible escapes of metamorphs. '% metam.' is the percentage recorded metamorphosing of all tadpoles that were present at the count on 30 May and not later lost.*/ Metamorphs in these two tanks were difficult to find due to algal blooms. Most of the lost tadpoles may have escaped.

Species	Treatment	Dry	P	Initial number	30 May	1 August			
					Found	Metamorphs found	Remaining tadpoles	% metam.	Lost
R. arvalis									
	Control		A	80	80	28	49	36.4	3
			A	80	78	29	42	40.8	7
			B	80	78	61	3	95.3	14
			B	80	78	67	5	93.1	6
	Reduced water	1	A	80	77	18	54	25.0	5
		1	B	80	78	65	4	94.2	9
		2	A	80	77	3	47	6.0	27
		2	B	80	71	52	6	89.7	13
		3	A	80	77	2	24	7.7	51
		3	B	80	74	28	22	56.0	26
R. temporaria									
	Control		C	80	78	19	0	100.0	59 */
			C	80	80	52	0	100.0	28 */
			D	80	78	60	9	87.0	9
			D	80	79	55	17	76.4	7
	Reduced water	1	C	80	80	59	0	100.0	21
		1	D	80	78	54	14	79.4	10
		2	C	80	76	36	19	65.5	21
		2	D	80	80	35	21	62.5	24
		3	C	80	75	46	20	69.7	9
		3	D	80	74	29	23	55.8	22

TABLE 3. Testing effects of treatments on tadpole performance. This was done with a 3-way hierarchical ANOVA. The factors included were treatment, species and pond (nested under species). All interactions were non significant and removed before final test. Data are average values for tanks.

	Treatment			Species			Pond		
	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Tadpoles - body length	1,15	14.51	0.002	1,15	2.59	0.13	2,15	6.91	0.007
Tadpoles - leg development	1,15	3.74	0.072	1,15	0.03	0.86	2,15	22.8	<0.001
Metamorphs - body mass	1,15	18.38	0.001	1,15	0.05	0.83	2,15	2.13	0.1

EXPERIMENTAL PROCEDURES

The first experimental reduction of water level (on 7 May) took place when tadpoles were about two weeks old. This was early in development and the tadpoles had no visible limb buds,or early limb buds only (Gosner stage 25 to 27; McDiarmid & Altig, 1999). This was designated the start of the experiment, as prior to this date the tanks only served to house the growing tadpoles.

Therefore, to minimize any variation that was due not to the experimental treatments but to random tank effects, the following procedure was used on 7 May. The tadpoles were removed from the tanks and all 400 tadpoles (i.e. five tanks containing 80 tadpoles each) from one source pond were sorted into three or four size classes. One fifth of the tadpoles from each size class was assigned at random to each of the five tanks and reintroduced. The same procedure was repeated for each of the four pond-specific tank sets. Thus, tanks with tadpoles of the same species and source ponds started the experimental phase with samples of similarly sized tadpoles.

MEASUREMENTS

On 30 May all tadpoles were measured alive and returned to the tanks. Measurements taken were body length (snout-vent) and hind leg length. The latter was used to calculate "relative leg length" – leg length divided by body length. This was considered a measure of development rate; a tadpole with longer legs for its size being closer to metamorphosis.

The first metamorphs appeared on 17 June (*R. temporaria*) and 20 June (*R. arvalis*) respectively. After this, tanks were checked at least every other day. Tadpoles that had a tail shorter than body length were close to metamorphosis and were removed, weighed, and had their body length measured.

FIELD DATA

In 1995, tadpoles of *R. temporaria* were also collected in the field. This was from a pond, part of which had been naturally isolated due to a decreased water level following a dry spring. The two parts of the pond separated completely on 30 May, when the tadpoles were about five weeks old. The smaller and shallower part dried completely on 22 June, the larger (and deeper) part dried on 2 July. No tadpoles survived in either part of the pond that year. Tadpoles were collected from the smaller pool and from the main body of water on both 6 June and 20 June. Total area of the pond was about 500 m^2 at the time of spawning, and about 300 m^2 when the smaller pool (about 10 m^2) was separated. Collected tadpoles were humanely killed and preserved, and body length and hind leg length were measured. Relative hind leg length (hind leg/body length) was used as an index of development.

RESULTS

EXPERIMENTAL TREATMENT

Tadpoles in tanks with reduced water were significantly smaller than those in control tanks (Fig. 2, Table 3). These tadpoles also had a tendency to have less developed hind legs on 30 May (Fig. 3, Table 3). Out of the tadpoles that did metamorphose, those from control tanks were larger than those from experimental tanks (Fig. 4, Table 3).

In control tanks, more tadpoles had metamorphosed by the time the experiment was concluded on 1 August than in the tanks with reduced water (Fig. 5, Table 2). The effect of treatment was significant (three-way

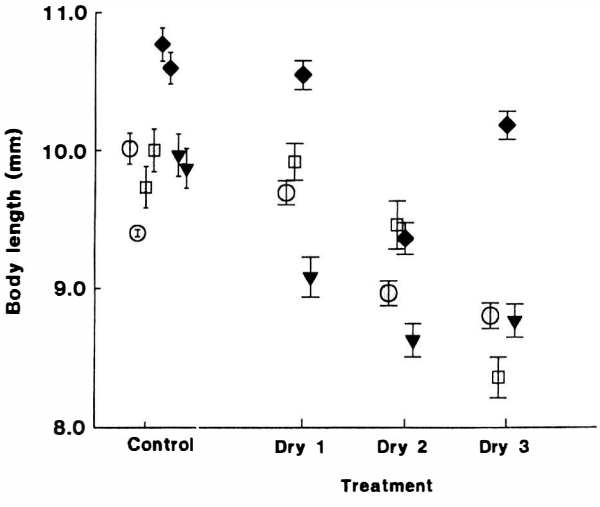

FIG. 2. Body lengths of tadpoles from different treatments. For each tank, mean length is given and standard error of mean indicated by the error bars. Number of measurements for each tank is given by Table 2. The four different symbols represent different source ponds. Open symbols are for *Rana arvalis* and filled symbols for *R. temporaria*.

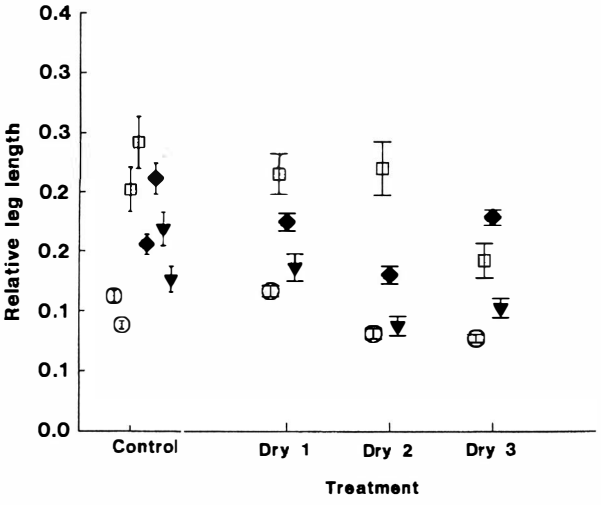

FIG. 3. Relative leg lengths (hind leg length/body length) for tadpoles from different treatments. Symbols as for Fig. 2.

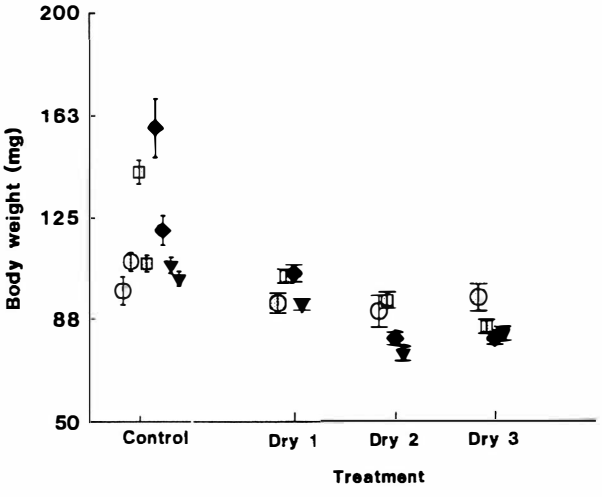

FIG. 4. Body weights of metamorphs from different treatments. Symbols as for Fig. 2.

ANOVA, controlling for species and pond (nested under species): F=11.73, df=1,15, P=0.004). For none of the above variables was there an interaction between species and treatment effects (all P>0.25). For *R. arvalis*, the proportion dead increased with the severity of drying (Fig. 6, two-way ANCOVA, F=14.2, df=1,3, P=0.036). Variation between ponds was not significant (F=2.67, df=1,3, P=0.20). There was no treatment effect for *R. temporaria*. The difference in slope between the two species was significant (three-way ANCOVA with species, pond [nested under species] and treatment, F=6.81, df=1,6, P=0.040).

FIELD DATA

R. temporaria tadpoles from the smaller and shallower part of the pond were smaller than those found in the rest (Table 4). Also, tadpoles from the small shallow part had less – developed hind legs. These results were true for both sampling periods.

DISCUSSION

Tadpoles subject to drying seemed to develop more slowly than those in control tanks. This is indicated by the tendency for such tadpoles to have more slowly developing legs. Also, few of the surviving tadpoles in the experimental tanks had metamorphosed before the end of the experiment compared to those in the control tanks. This conclusion is valid regardless of the undecided fate of the lost tadpoles in the control tanks. If they had died, the conclusions refer to surviving tadpoles in all tanks. If they had escaped, they should be added to those metamorphosing before 1 August, making the case even stronger. Extrapolating the tendency that fewer (*R. arvalis*) – or similar (*R. temporaria*) – numbers died in tanks with moderate water reduction compared to the control tanks, suggests that most of the large number lost in two such tanks were actually escapes. Also, the conclusion that metamorphs in experimental tanks were

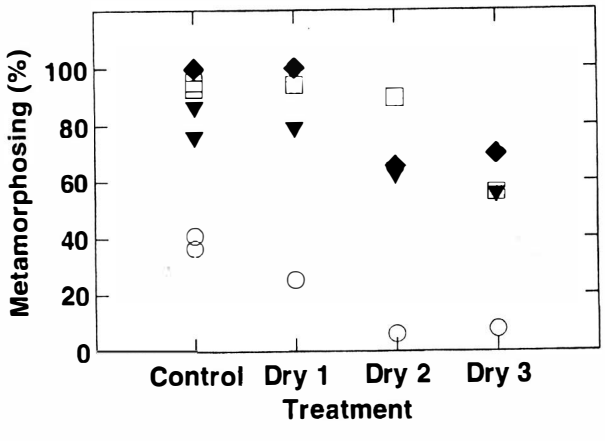

FIG. 5. Proportions of all tadpoles that metamorphosed before 1 August in the different treatments. Proportions based on all metamorphs found and all remaining tadpoles on 1 August. Symbols as for Fig. 2.

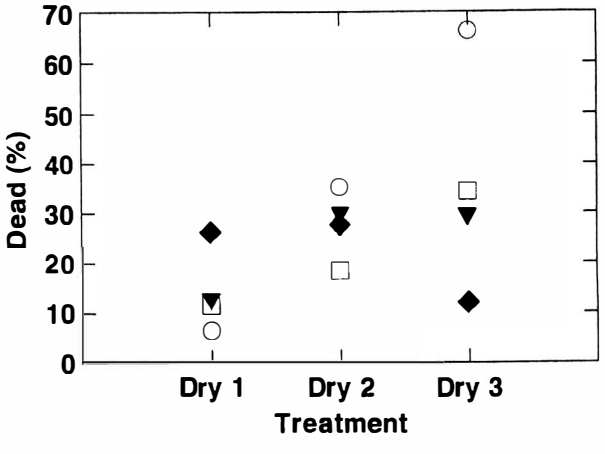

FIG. 6. Proportions of tadpoles that died in the different treatments. Control tanks were excluded because some of the lost tadpoles in these may have escaped rather than died. Symbols as for Fig. 2.

TABLE 4. Comparison of the performances of *Rana temporaria* tadpoles sampled from two parts of a natural drying pond.

	Deep part	Shallow part	t	P
Body length (mm)				
6 June	10.7 (n=39)	7.5 (n=8)	7.1	<0.001
20 June	12.3 (n=17)	8.6 (n=8)	7.3	<0.001
Relative hind leg length				
6 June	11 % (n=39)	6.0 % (n=8)	4.0	<0.001
20 June	24.4 % (n=17)	9.3 % (n=8)	4.4	<0.001

smaller is robust with respect to the problem of lost tadpoles. There is no reason to expect a size bias with respect to escaped tadpoles; all measures of metamorphs – in control and experimental tanks alike – are thus an unbiased measure of the size of tadpoles that had metamorphosed by 1 August. The measures of tadpoles were unaffected by the losses after 30 May. Before that, all losses were small and due to mortality. Effects found on tadpole size were thus not affected by mortality and escapes.

This effect of drying on development rate is contrary to what would be expected if tadpoles modified development rate adaptively and attempted to escape a drying pond. It is also contrary to what is expected if the method used led to a higher temperature in the shallow experimental tanks. The result is thus not caused by a temperature bias. Because these tadpoles not only developed but also grew more slowly, they may simply not have reached the minimum size required for metamorphosis early enough to leave the water by the time the control animals metamorphosed. This supports the notion of a minimum size, as proposed by the model of Wilbur & Collins (1973). However, because those slow-growing experimental animals that did indeed metamorphose did so at smaller size than the others, it is even better support for the model of Harris (1999, pp 283–284).

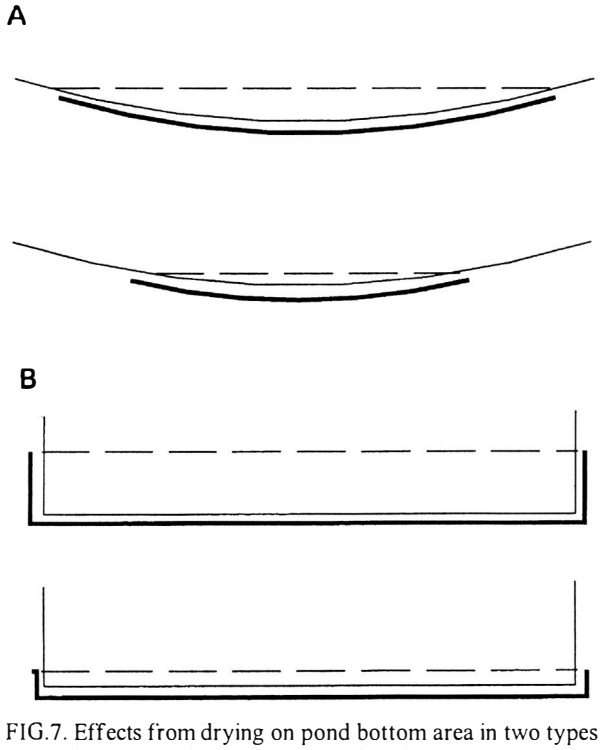

FIG.7. Effects from drying on pond bottom area in two types of pond. A, in ponds with sloping sides, drying results in less area available for tadpole feeding. B, in ponds with steep slopes, drying and reduction of water level may have small effects on the area available for feeding. Although tadpoles may be crowded by volume, the effects on resources per tadpole are small.

The reason for slower development was probably related to the crowding experienced by tadpoles in experimental tanks. Also, the slower growth and higher mortality suggests that the tadpoles experienced crowding. Decreased growth, as a trade-off, had been expected if the tadpoles developed faster (Merilä *et al.*, 2000*a,b*). However, as this was not the case here, crowding is the most likely explanation. Such effects of crowding on growth and development rate have been shown in several studies of tadpoles (Wilbur, 1977*b*; Dash & Hota, 1980; Semlitsch & Caldwell, 1982; Cummins, 1989; Beck, 1997; Merilä *et al.*, 2000*a*). Also, Wilbur (1987) found an effect of crowding on development in *Bufo americanus*: this led actually to the elimination of crowded tadpoles in tanks with a short hydroperiod.

In a similar experiment (with *Bufo calamita* tadpoles), Reques & Tejedo (1997) found a variation between sibships in their response. One reacted similarly to the pattern observed in this study, but most showed no response to hydroperiod, regardless of tadpole density. Also, Brady & Griffiths (2000) report a similar experiment. They found no effect of the water level treatment on time to metamorphosis in either of the three species' studied (*Rana temporaria, Bufo bufo*, and *B. calamita*). However, size at metamorphosis was smaller for tadpoles in drying tanks, and they also concluded that crowding had prevented any acceleration of development.

The experiment cannot disprove that, under some conditions, tadpoles are capable of an adaptive response to pond desiccation. Indeed, Laurila & Kujasalo (1999), Loman (1999) and Merilä *et al.* (2000*b*) have demonstrated experimentally that *R. temporaria* can increase development rate adaptively when threatened by drying. However, this study demonstrates there is a limit to this ability. It had definitely been in the crowded tadpoles' best interest to abandon the drying tanks. This is because such conditions in nature had been an indication that the pond would dry completely in the immediate future, and also because the crowding by itself reduced the available food resources. However, if crowding is sufficiently intense, the tadpoles are apparently unable to increase development rate.

The experiment by Loman (1999), that did demonstrate a plastic development rate in response to drying in

R. temporaria, was similar in set-up to the present experiment. However, there were two differences that both contributed to reduced competition in that instance. First, the experiment used 80 or 20 tadpoles per tank (rather than 80 as in the present study). Second, water was not reduced until very late, i.e. 23-26 June. This was when the earliest developing tadpoles' front legs emerged (Gosner stage 41-42; McDiarmid & Altig, 1999), and more than a month later than in the present study. There was indeed a plastic response at both tank densities, but the effect was only significant when both were combined. This comparison supports the conclusion that competition can prevent a plastic response. The comparison between the two experiments also shows that *R. temporaria* tadpoles are capable of reacting plastically even if the cue appears very late in development.

What is the situation tadpoles in drying ponds experience in nature? This depends on the shape of the pond. If the remaining water stays in one or a few small depressions (Fig. 7A), it is common for these to contain a high density of tadpoles, mimicking the experiment (Brady & Griffiths, 2000). If so, my results suggest that they may not be able to perform an "emergency" metamorphosis. The situation in the natural pond studied here conformed to this scenario and the result was similar to that in the experiment. Although no measures of densities could be made, the picture was the same in both. Under drying conditions, tadpoles were both smaller and less developed, apparently unable to escape desiccation by accelerating development rate.

On the other hand, if the original density is low or if the water depth is even, so that the area of water is independent of water depth (Fig. 7B), it is possible that tadpoles can experience drying without substantial resource reduction. Whether tadpoles of these species under such conditions do speed up development in the field remains unsettled.

So, if reduced water level leads to increased density of tadpoles, which is likely to happen in many natural situations, this results in crowding effects that oppose tendencies to increase development rate.

ACKNOWLEDGEMENTS

The present study was carried out with support from the Swedish Council for Forestry and Agricultural Research. Mats Wirén assisted me in measuring the animals. Bodil Enoksson, Ralph Tramontano, Johan Elmberg, Thomas Madsen, Richard Seigel and James Sutherland helped me improve the presentation.

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Accepted: 12.09.01

HERPETOLOGICAL JOURNAL, Vol. 12, pp. 29-35 (2002)

HOW DOES A NEWT FIND ITS WAY FROM A POND? MIGRATION PATTERNS AFTER BREEDING AND METAMORPHOSIS IN GREAT CRESTED NEWTS (*TRITURUS CRISTATUS*) AND SMOOTH NEWTS (*T. VULGARIS*)

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Migration patterns across a drift fence with pitfall traps were studied between 1997 and 1999 at a breeding pond with populations of great crested newts, *Triturus cristatus*, and smooth newts, *T. vulgaris*, at a study site in south-central Sweden. Metamorphs and older newts emigrated from the pond non-randomly and seemed to avoid exiting where open fields adjoined, but were oriented towards a patch of forest immediately to the east of the pond. Movement patterns changed slightly over the years, but metamorphs were more dispersed and less concentrated than older newts, and did not choose directions identical to those of older newts. Older great crested and smooth newts showed similar directional orientation. Great crested newt metamorphs dispersed towards both edges of the forest patch, and possible explanations for this are discussed. The results suggest that orientation in relation to cues from the surroundings of a breeding pond may be used by newts to make migratory decisions.

Key words: Amphibia, behaviour, conservation, dispersion, circular statistics

INTRODUCTION

Pond-breeding newts of the family Salamandridae are capable of remarkable homing and orientation (e.g. Cummings, 1912; Grant, Anderson & Twitty, 1968; Joly & Miaud, 1993), and make interesting models for studies of migration (see Glandt, 1986; Sinsch, 1991; Dingle, 1996). Adults often show high breeding site fidelity and individuals frequently return to their natal site (Griffiths, 1996), possibly due to their constraining demands for complex landscape structures with high connectivity (Fahrig & Merriam, 1994; Hanski, 1999; Marsh & Trenham, 2001). For example, it has been demonstrated that the occurrence and abundance of newts is related to the presence and width of uncultivated habitat sectors (Oldham, Keeble, Swan & Jeffcote, 2000; Joly, Miaud, Lehmann & Grolet, 2001), which connect or constitute primary landscape elements. When newts are leaving a breeding pond they usually travel in straight lines and seem to move towards favourable habitat patches in the vicinity (Verrell, 1987; Sinsch, 1991; Macregor, 1995; Jehle, 2000; Jehle & Arntzen, 2000). This indicates that individual newts try to optimize the use of available spatial units (Sinsch, 1990) and that suboptimal choices can be costly. An important mechanism to facilitate such migratory behaviour is presumably the ability to use chemical cues (see Joly & Miaud, 1993; Joly *et al.*, 2001). Hayward, Oldham, Watt & Head, (2000) suggested that metamorphs of great crested newts, *T. cristatus*, detect and follow cues left by piloting adults, which could be an important strategy enabling individuals without any experience of the surroundings to find suitable habitats. This requires, however, that adults can identify areas favourable for dispersal or for terrestrial activities. The present study focuses on questions related to this problem. In particular, are migration patterns directed towards habitat patches that are preferred by newts in different life stages? Furthermore, do newts have stronger directional responses to such habitats as they get older, and are there detectable differences in orientation and dispersion between newts in different stages of life, or even between closely related species? I studied migratory movements across a drift fence with pitfall traps from 1997 to 1999, at a pond in south-central Sweden, as part of a population study of great crested and smooth newts (*T. cristatus* and *T. vulgaris*, respectively). The questions addressed here may be interesting from a general biological perspective, but detailed knowledge about migratory behaviour can also prove critical for conservation efforts (Sutherland, 1998; Marsh & Trenham, 2001).

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METHODS AND MATERIALS

The site used for this study is a circular cattle pond located in Lanna (59°15'N 14°56'E, altitude 110 m), 25 km W of Örebro in south-central Sweden. It contains breeding populations of great crested and smooth newts, and has a surface area of 300 m^2, with a maximum water depth of approximately 1.8 m in spring and 0.8–1.0 m in warm and dry summers. The central part of the pond has a dense floating mat of water moss (*Drepanocladius*), with a small stand of reedmace (*Typha latifolia*) to the west. An open littoral zone dominated by broad-leaved pondweed (*Potamogeton natans*) and submerged grasses is present around the pond. Within 100 m of its perimeter (Fig. 1A) the pond has open pasture and meadow on its western half, and a spinney of birch (*Betula pubescens* and *B. pendula*), ash (*Fraxinus excelsior*) and aspen (*Populus tremula*) on its eastern half. This forest element, which consti-

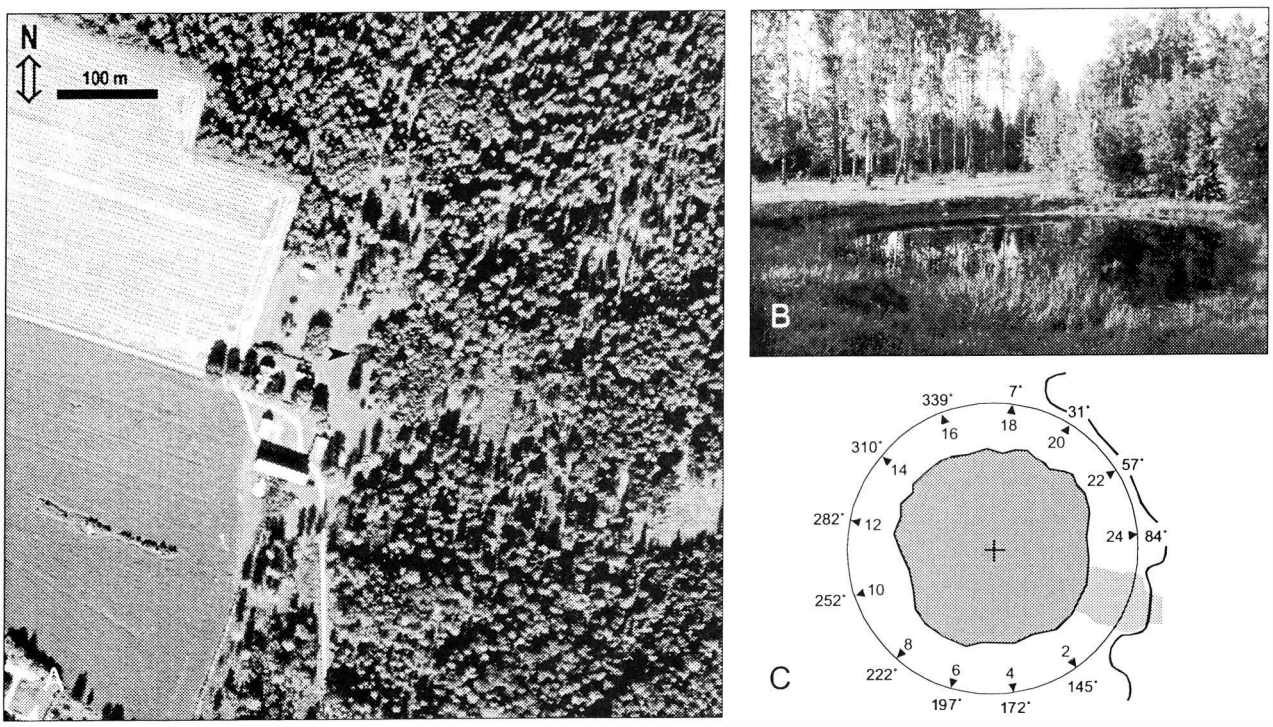

FIG. 1. A, aerial photo of the study site, with the pond at centre (arrow). Photo courtesy of the Swedish National Road Administration. B, photo of the pond taken from the south in the season before drift fences and pitfall traps were installed. C, plan of the pond, with triangles depicting trap locations with associated numbers and compass bearings relative to the pond centre. A trap was not placed in the seasonally flooded southeastern segment (shaded). To compensate for this, the midpoints of trap grouping intervals were used for traps 2 and 24 (136.5° and 92.5° respectively) in all analyses. The outline of the forest sector immediately adjacent to the eastern half of the pond is drawn into the figure.

tutes a circular patch with a diameter of 100–120 m, covers a sector from approximately 9° to 137° and lies 1–3 m from the perimeter fence. There are a few, single, large birches located in the pasture directly to the north. At a distance of approximately 100 m further to the north, south and east of the pond, a wide band of old forest dominated by spruce (*Picea abies*) and hardwood (see above) replaces the old pasture. At the same distance to the west, the open pasture and meadowland is replaced by extensive arable fields.

The breeding pond was completely encircled by a drift fence with pitfall traps and has been monitored continuously from 1997. The fence was constructed of UV-resistant plastic sheets stretched between poles with wire and presents a barrier 50–60 cm above ground and 10-20 cm below. Plastic 10-litre buckets, buried with the rim at ground level and placed firmly against the fence, were used as pitfall traps (n=25). Traps were placed alternately on the inside (facing the pond, n=12) and outside of the fence (n=13). The distance between traps on each side of the fence was 6 m, and the total length of the fence was approximately 75 m. A single interruption of the even distribution of traps was caused by a boggy and seasonally flooded area at the south-eastern segment of the fence (between traps 2 and 24, which were separated by 12 m; see Fig. 1C). The average distance between the fence and the pond shoreline varied between 3 m and 4 m over the season. The traps had a 10 cm depth of water at all times and were checked every 1–3 days, when the water was changed and trapped animals were registered. The data presented in this paper are based on newt captures from pitfall traps on the pond side of the fence during three years of outward migration episodes (1997–1999). Great crested and smooth newts were counted, size-classed and sexed before being released on the outside of the fence. Only two size- or age-classes (hereafter referred to as life-stage classes) are used in the analyses presented here – *metamorphs* (i.e. young of the year) and *all other ages* pooled (i.e. post-metamorphs, including juveniles and mature adults).

Since captures were made at a circular fence with a fairly even distribution of pitfall traps, single observations of migrating individuals can be treated as vectors for certain directions. I therefore used circular statistics (Mardia, 1972; Batschelet, 1981; Upton & Fingleton, 1989; Fisher, 1993) to analyse migration patterns in population samples. I assumed that individuals wandered in a fairly straight line from the pond to meet the fence and fell into one of two traps closest to their point of exodus, which is a common supposition of drift fence studies. All traps were further assumed to be equally effective at catching newts, so producing a statistically valid sample of emigrating newts for all directions. Compass bearings for all traps were established and confirmed in winter, relative to the pond

TABLE 1. Emigration dates for great crested and smooth newts (*Triturus cristatus* and *T. vulgaris*) from 1997 to 1999 at a breeding pond in south-central Sweden. Onset (first observed emigrant), end (last observed emigrant) and duration (number of days) of emigration for each year, species and life stage class (metamorphs and all other ages).

	Class	Onset	End	Duration
T. cristatus				
1997	Older	10 June	8 August	59
	Meta.	27 July	23 October	88
1998	Older	21 May	29 July	69
	Meta.	18 June	3 November	138
1999	Older	1 June	19 July	48
	Meta.	30 June	21 October	113
T. vulgaris				
1997	Older	16 June	16 September	92
	Meta.	3 August	16 November	105
1998	Older	1 June	10 September	101
	Meta	9 August	29 October	81
1999	Older	24 May	23 September	122
	Meta.	23 August	21 October	59

centre. The data were treated as grouped (with 12 angular orientations) in all analyses, and to compensate for the gap in the distribution at the south-eastern segment of the fence, traps 2 and 24 were assigned the values of the midpoints of their grouping intervals (Fisher, 1993), which corresponded to 136.5° and 92.5°, respectively. These bearings were used in all analyses reported here. This procedure contributes statistically to minimizing the effect of too large sample intervals, but may sacrifice intuitive interpretation. Sample distributions were assessed both graphically (by linear histograms and uniformity plots) and formally (test of randomness against any alternative) to establish modality (Fisher, 1993). Rayleigh's uniformity test was used to test the hypothesis of random dispersal against the alternative of preferred directions for samples with unimodal distributions. Samples that had bimodal or multimodal distributions were tested with Rao's spacing test for one-sidedness against the alternative of random dispersal, implemented as a special case of empirical coverage permutation tests (Mielke, 2001), with the statistical software 'Blossom' (Cade & Richards, 1999). This test is an alternative to Rayleigh's test for detecting departures from uniform distributions when multimodality is suspected. However, it may be sensitive to grouped data, so interpretations from the results must be made with caution. Tests of differences in directional orientation between samples were performed through a multi-response permutation procedure (MRPP). This method is based on distance functions (Mielke, 2001), has the advantage of not being sensitive to the underlying modality of the data, and compares grouped data in a way that is analogous to a one-way analysis of variance. The null hypothesis tested with MRPP is that circular distributions are identical for the samples compared. The test detects departures under the alternative hypothesis, due to differences in mean and median angles, angular dispersion, number of modes or any combination of these effects, where the samples are non-identical. MRPP analyses were run in 'Blossom' by class (metamorphs, all other ages), and for pooled samples of class and species within and across years. The hypothesis of differences in the strength of directionality between samples was tested through a separate test for the concentration parameter, following Fisher (1993). Moreover, chi-square tests were used to test whether the samples were distributed towards the forest element immediately adjacent to the eastern half of the pond, and Student's *t*-test was used to test for differences between classes in the mean duration of the emigration episodes.

RESULTS

A total of 8600 observations of newts leaving the pond were made at the drift fence during the course of the study (recaptures included). Great crested newt observations numbered 1926 (22%), with metamorphs comprising 63% and all other ages 37%. Smooth newt observations numbered 6674 (78%), divided into 83% metamorphs and 17% all other ages. Onset, duration and end of emigration episodes varied over the years (Table 1). The duration of outwards migration was significantly different between the classes of great crested newts: the mean (± SE) for metamorphs was 113±27 days, while that for older newts was 59±12 days (two-tailed *t*-test: t=3.47, df=4, P=0.0256). The overlap of the two classes' periods of emigration varied from 12 days to 41 days. Smooth newts did not show a similar difference between classes in the duration of emigration periods (t=1.46, df=4, P=0.2181): metamorphs emigrated over periods of 82±25 days and older newts over periods of 105±17 days. The overlap in emigration periods of newly-metamorphosed and older smooth newts varied between 31and 44 days. Observations of actual dispersal from the pond were mostly made at dusk, but metamorphs and older newts of both species were occasionally observed at the fence during rainy days. Whether classes and species left the pond at different hours was beyond the scope of this study, mostly due to time and resource constraints.

Dispersal of great crested and smooth newts from the breeding pond was non-random in both life stages and in all years (Fig. 2). The null hypothesis of uniform distributions could be rejected for all samples in favour of modal differences among life stages after graphical assessments and formal tests. All samples of older newts, as well as metamorphs of both species sampled in 1997, had approximately unimodal distributions with preferred directions towards the forest patch (Rayleigh's test, all P<0.0001), whereas samples of metamorphs from both species over 1998 and 1999 dispersed one-sidedly (Rao's spacing test, all P<0.0001) but tended to

have distributions that were either bimodal (*T. cristatus* 1998, 1999) or even multimodal (*T. vulgaris* 1998, 1999). The proportion of newts caught in traps on eastern (traps 18–4 pooled, n=6) vs. western (traps 6–16 pooled, n=6) halves of the fence perimeter (i.e. towards or away from the adjacent forest edge, respectively) differed significantly (chi-squared tests, all P<0.0001). Thus, there was a strong grouping of newts in traps on the eastern half, facing the adjacent forest (metamorphs 66–79%, adults 81–90%). A similar, but slightly weaker difference was detected in the proportion of newts caught at northern (traps 12–22 pooled, n=6) vs. southern (traps 24–10 pooled, n=6) halves of the fence (chi-squared tests, all P<0.05), with the northern half being preferred (metamorphs 53–67 %, adults 58–83 %). Overall, this shows that there was a tendency in all samples for newts to orientate towards the forested area immediately adjacent to the pond (which covers bearings 9°–137°, roughly corresponding to the sampling intervals included by traps 18, 20, 22, 24 and 2), as is evident from Fig. 2. Older newts had a strong directional response towards this specific habitat patch, with the exception of smooth newts sampled in 1999. When the life stage classes were compared for the concentration parameter (Table 2), metamorphs consequently showed a significantly weaker directional response than older newts, again consistent in all groups except for smooth newts in 1999 (Fig. 2). Thus, metamorphs were in general more dispersed than older newts, which suggests that age-related factors affect how strongly newts are drawn to certain directions. Furthermore, metamorphs tended to disperse towards the edges of the adjacent forest patch, rather than straight into it as older newts did. This pattern was particularly evident in great crested newts (Fig. 2). A comparison between great crested and smooth newts revealed no consistent interspecific difference in the strength of the directional response (using the concentration parameter), either in metamorphs or in older newts.

Significant differences in movement patterns were observed in both species when circular distributions of emigration directions were analysed within and among life stage classes (Table 3, Fig. 2). Differences were consistent both when the classes were compared for single years and across years (MRPP, all P<0.01). Analyses from MRPP and the circular histograms show that older newts of both species were distributed towards the forest patch to the east. The differences between years within this life stage, regardless of species, indicates that the angular distributions in at least one year differs from the others with small chance that this is due solely to sampling variability. In great crested newts, a larger proportion of post-metamorphs moved to the east in 1998 and 1999 than they did in 1997, when the larger proportion moved east-northeast. The difference in older smooth newts is explained by the observation that in 1999 a larger number of newts dispersed towards the edges of the forest patch than they did in 1997 and 1998. Similar reasoning can be applied to analyse test results from MRPP on metamorphs, where the proportion of individuals moving towards the forest edges comprise the largest contributing factor to explain the differences. When data for all years were pooled, there were differences in the distributions of great crested and smooth newt metamorphs emerging from the pond (MRPP, P<0.0001). The major difference among years appears to be due to differences in the proportions of newts that emerged from the pond in relation to the edges or the centre of the adjoining forest patch. The only comparison of distributions where great crested and smooth newts did not differ was when data on all years were pooled for post-metamorphs (MRPP, P=0.1572). This observation suggests that older great crested and smooth newts had similarly strong preferred orientations towards the north-eastern sector of the pond perimeter as a route for emergence.

DISCUSSION

Great crested and smooth newts of all categories emigrated from the pond after breeding or metamorphosis in non-random directions, tending to leave the pond where forest adjoined, rather than open fields. The data suggest that cues from the surroundings were important for orientation, and there were clear age-effects in the way newts selected a route to leave the pond. Metamorphs – which had no prior experience of the terrestrial environment – were more dispersed and less concentrated than older newts, but were still mostly distributed along the eastern half of the pond, towards the forest sector. Older newts, who had previous experience of the surroundings, left the pond from the north-eastern sector, where the fence perimeter adjoined the forest patch most closely. Their strong directionality and consistency over the years in doing so suggest that they were piloting towards familiar habitats and that the forest patch to the east served as either a preferred habitat or a corridor funnelling individuals towards the more extensive forest beyond. That both great crested and smooth newts shared the same general tendency to emigrate in this direction over the years gives some support to this conclusion. Furthermore, that the directionality was greater in newts that had spent at least one year on land than in naïve, newly-metamorphosed newts, may be the result of accumulated experience of the habitat and local conditions, and by effects of natural selection during the first few years of life. Experiments on alpine newts, *Triturus alpestris,* (Joly & Miaud, 1989; Joly & Miaud, 1993) and on radio-implanted marbled newts, *Triturus marmoratus*, and great crested newts, *T. cristatus,* (Jehle, 2000; Jehle & Arntzen, 2000) have demonstrated that adult *Triturus* newts may be faithful to breeding ponds and terrestrial sites, and have navigational abilities during migrations. The results obtained here suggest that the strong directional orientation towards an adjoining forest patch may indeed be a response towards a favourable habitat.

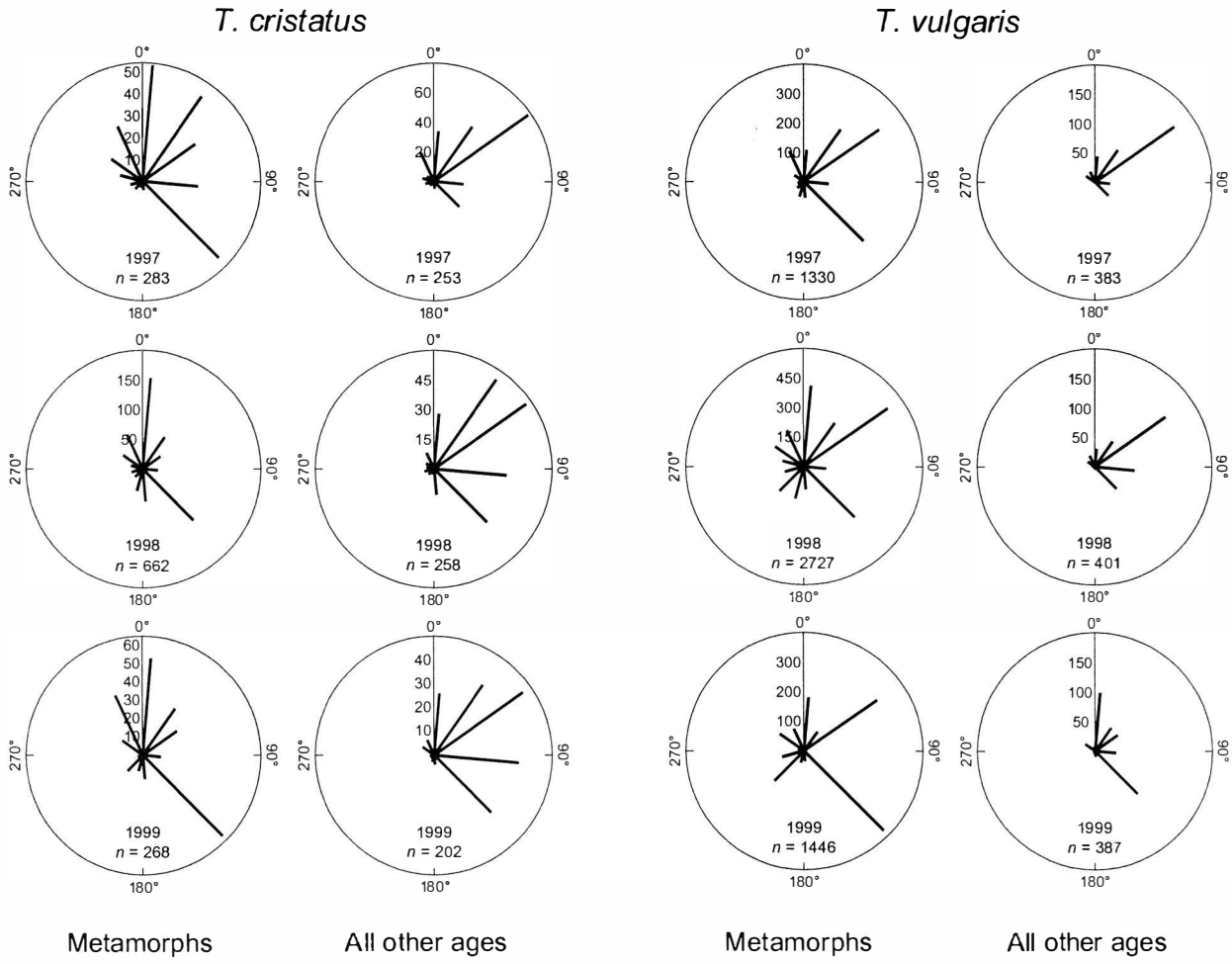

FIG. 2. Circular histograms of migration patterns after breeding and metamorphosis in great crested and smooth newts – *Triturus cristatus* and *T. vulgaris* respectively – at a pond in south-central Sweden for three consecutive years, showing patterns for life stage classes and species. The length of each line radiating out from the circle centre indicates the number of newts moving in that direction. Study year and sample size (*n*) for each sample are given.

Results from a study of nine radio-implanted adult great crested newts during an emigration episode at the study site during four weeks in July and August 2001 (*unpubl.*) also support this. The latter resulted in 30 point localizations of preferred microhabitats, of which 26 (87%) were situated within the adjoining circular forest patch (max. dia. 120 m) to the east of the pond. Overall, the data indicate that directional preferences by adult newts are potentially reliable indicators of where suitable terrestrial habitats are located.

Even though post-metamorphs of the two species had similar directionality when pooled across years, movement patterns (in terms of circular distributions) changed slightly from one year to the next for all samples. Furthermore, metamorphs did not migrate in directions identical to those taken by piloting post-metamorphs. Instead, metamorphs had a tendency to be distributed in traps adjoining either edge of the eastern forest patch, and this behaviour seemed to be more developed in great crested than in smooth newts.

TABLE 2. Summary results from tests between life stage classes and species, based on the concentration parameter – which is an indicator of the directionality strength. Horizontal and vertical lines connect concentration values for the sample pairs tested, and asterisks denote the associated probability for the null hypothesis that two samples have equal concentrations. NS = not significant ($P>0.05$), * $P<0.05$, ** $P< 0.01$, *** $P<0.001$.

	1997			1998			1999		
	Metamorphs		All other ages	Metamorphs		All other ages	Metamorphs		All other ages
T. cristatus	0.99	**	1.48	0.45	*	1.34	0.64	***	1.56
	NS		NS	**		**	NS		***
T. vulgaris	0.98	***	2.17	0.53	***	1.87	0.42	NS	1.06

Hayward *et al.* (2000) found that *T. cristatus* metamorphs had the ability to detect and trail chemical cues left by piloting adults in experiments, and postulated that tracking may be an important mechanism for finding suitable terrestrial habitats. The observations reported here do not clearly confirm this, but rather seem to indicate that great crested newt metamorphs possibly detect and avoid using the same migration routes as more experienced individuals, even though the latter are demonstrably reliable indicators of where favourable habitats are situated. Since there is a distance of 3-4 m between the pond perimeter and the fence, the metamorphs do not lack opportunies to track older newts. Alternatively, metamorphs in the field may not be using cues left by older newts at all but following odour cues from other metamorphs, or using visual or chemical cues from the environment as information sources. A second alternative is that metamorphs are prone to migrate through the scrub along the edge of a forest patch, whereas older newts have more to gain by returning to well-known territory as quickly as possible. The tendency to follow trails left by adults may also be density-dependent, and the patterns observed by great crested newts in this study could reflect a strategy by metamorphs to minimize competition with older individuals over resources (e.g. food or hiding places). Trailing behaviour is likely to account for some of the variation in natural systems, since adults and metamorphs have a period of overlap in the timing of emigration from a pond, but it may be more complex than previously believed. For example, only metamorphs that emerge early are likely to benefit from trailing older newts if the overlap between emerging metamorphs and older newts returning to terrestrial habitats is limited. If this is taken into account, the data from this study suggest that when the temporal overlap was short (1997) a larger proportion of great crested newt metamorphs emerged in the same directions as older newts, whereas during 1998-99 – when the classes had a greater overlap – metamorphs were more dispersed towards the forest edges. That is, when cues from older newts were likely to be more available, the metamorphs responded with avoidance (i.e. negative feedback). Emigration patterns for smooth newts do not seem equally complex, but rather suggest that large proportions of the metamorphs of this species either followed older individuals or emigrated without taking notice of where newts had travelled before, since many dispersed in the same general direction as older newts. Further experimentation in this field may provide valuable insights into the actual determinants of orientation and terrestrial habitat selection in newts.

TABLE 3. Comparisons of the directional orientation within and among life stage classes and species for great crested and smooth newts (*T. cristatus* and *T. vulgaris*, respectively) within and among years. Standardized test statistics results from multi-response permutation procedures (MRPP). The *P*-value is the associated probability that two samples have equal circular distributions.

Comparison	Standardized test statistic	*P*
T. cristatus		
Metamorphs vs. all other ages		
1997	-5.68	0.0012
1998	-27.37	< 0.0001
1999	-14.62	< 0.0001
1997-9	-52.72	< 0.0001
Life stage classes among years		
Metamorphs	-6.15	0.0004
All other ages	-5.44	0.0007
T. vulgaris		
Metamorphs vs. all other ages		
1997	-44.82	< 0.0001
1998	-75.35	< 0.0001
1999	-24.76	< 0.0001
1997-9	-121.59	< 0.0001
Life stage classes among years		
Metamorphs	-59.11	< 0.0001
All other ages	-30.72	< 0.0001
T. cristatus vs. *T. vulgaris*		
Metamorphs	-13.82	< 0.0001
All other ages	-0.84	0.1572

Patterns of aquatic microhabitat selection may obscure emigration distributions. However, the vegetation structure and topography of the pond does not suggest that newts were inhibited from emerging in certain directions, but examination of this aspect was beyond the scope of this study. Hayward *et al.* (2000) showed that metamorphs moved away from their larval sites before metamorphosis and concluded that they had begun to be influenced by an orientation mechanism at this stage. They also found that females moved around widely in the pond during breeding and egg-laying, and before leaving the pond (see also Madison, 1998). It appears plausible that this is also applicable here and that orientation towards surrounding habitat elements already takes place before the newts leave the pond.

Although this study is based on observations at a single, isolated breeding pond, which may not be typical, it identifies topics that are in need of further investigation. For example, it seems crucial to determine mechanisms and cues that are involved in producing the non-random orientation relative to the surroundings observed here in all life stages and both species, and I have introduced some possible explanations. I also propose that valuable information can be extracted from drift fence studies where migration patterns in different life stage classes are analysed in relation to habitat availability. Both great crested and smooth newts do seem to have similar requirements in terms of the qual-

ity of the surroundings for dispersal, and their directions may be used as indicators of the presence of suitable habitat patches. The data suggest that newts may become more directional as a forested sector adjoining a pond becomes narrower, or conversely, that dispersal approaches uniformity when a pond is entirely surrounded by equally favourable forest habitat. If this can be demonstrated it could prove useful for conservation purposes and show how important well-designed forest and terrestrial habitat management practices may be in the conservation of newt populations.

ACKNOWLEDGEMENTS

I thank Christer Tikkala and Per Bülow for assistance at the study site, and Johan Elmberg, Sven-Åke Berglind and Daniel Gustafson for constructive comments on early manuscript drafts. Two anonymous referees, Clive Cummins and Brian Cade also contributed with comments that sharpened the focus of the manuscript considerably. Permits for the study were acquired from the Swedish National Board for Laboratory Animals (Tierp, C 149/97, C 84/0) and the Swedish Environmental Protection Agency. The study was funded in part by grants from Helge Ax:son Johnsons Fund for Scientific Research, the Swedish Environmental Protection Agency and the Royal Swedish Academy of Sciences.

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Accepted: 28.9.01

SHORT NOTES

HERPETOLOGICAL JOURNAL, Vol. 12, pp. 37-38 (2002)

THE OCCURRENCE OF THE ARTHROPOD ENDOPARASITE, *RAILLIETIELLA NAMIBIENSIS* (PENTASTOMIDA: CEPHALOBAENIDA), IN THE LUNGS OF AGAMID LIZARDS OF WINDHOEK, NAMIBIA

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Key words: parasite, Pentostomida, agama, lizard

Pentastomid arthropods are obligate endoparasites found in the respiratory systems of reptiles, birds and mammals (Meglitsch, 1972; Riley *et al*., 1985). A single intermediate host, usually an insect, is present in their life-cycle (Meglitsch, 1972). Lavoipierre & Rajamanickam (1973), for example, showed that the life-cycle can be completed experimentally with the cockroach *Periplaneta americana* as intermediate host.

During a study of the reproductive biology of the lizard species *Agama aculeata aculeata* and *Agama planiceps planiceps* in Windhoek, Namibia (Heideman, 1992), a new pentastomid species was discovered in their lungs. The species, *Raillietiella namibiensis*, was recently described by Riley & Heideman (1998). Following its discovery, the lungs of all lizards collected monthly from 1987 to 1990 for the reproductive study were also routinely examined for the pentastomid.

Agama a. aculeata is widely distributed throughout sandveld areas in semi-desert and savannah biomes of southern Africa, while *A. p. planiceps* is found only in rocky outcrops in semi-desert and arid savannah areas in Namibia (McLachlan, 1981; Branch, 1988). Both are common around Windhoek (22°34'S; 17°06'E), which lies in the seasonal tropics at an altitude of ca. 1725 m above sea-level, in a cool steppe region.

The prevalence and abundance of the pentastomid in males and females of each species were calculated using the definitions of Margolis *et al.* (1982), while the parasite's dispersion was determined according to the definition of Anderson & Gordon (1982). Prevalence refers to the number of infected hosts as a percentage of the total number of hosts examined. Abundance, on the other hand, is the total number of pentastomids found in a sample divided by the number of hosts examined. The number of parasites per host, the parasitaemia, was also calculated for each sex. The dispersion of the parasite in each sex was calculated by expressing the mean parasitaemia as a ratio to its variance. Snout-vent length (SVL) of the lizard specimens examined was compared using Student's *t*-test. Pearson's correlation analysis was used to test for a significant relationship between parasitaemia and SVL. All analyses were carried out using the computer programme STATISTICA 5.1 (StatSoft Inc., USA) with the significance level for all tests set at P=0.05. All lizard specimens were eventually deposited in the Namibia National Museum, Windhoek.

Snout-vent length of *A. a. aculeata* males was significantly greater than that of females (94.5 mm vs. 88.5 mm, t=2.192, df=255, P<0.05), while *A. p. planiceps* males and females did not differ significantly (97.6 mm vs. 94.6 mm, t=1.34, df=257, P > 0.05). No significant difference in SVL was found when comparing the males of the two species (t=0.91, df=264, P>0.05), but SVL of *A. p. planiceps* females was significantly greater than that of *A. a. aculeata* females (t=3.57, df=248, P<0.001).

The prevalence of *R. namibiensis* in *A. a. aculeata* males and females was almost identical, perhaps suggesting a similar degree of contact with the vector(s) of the parasite and a similar likelihood of infection (Table 1). In *A. p. planiceps*, on the other hand, prevalence of the parasite in females was almost twice that in males (Table 1), suggesting either greater contact between females and the vector(s) of the parasite or greater resistance to infection among males. Interspecific differences between corresponding sexes may have similar explanations, but such hypotheses remain to be tested in follow-up studies. The prevalence of *R. namibiensis* in the two lizard species studied here was lower than that reported for pentastomids in other lizard species. For example, Pence & Selcer (1988) reported a 44% prevalence of *Raillietiella frenatus* in the Mediterranean gecko, *Hemidactylus tursicus*, in Texas, while Riley *et al.* (1988) reported figures ranging from 19.8% to 32% for *Raillietiella teagueselfi*. The dispersion factor of less than 1 in all cases reflects the uneven distribution of *R.*

TABLE 1. *Raillietiella namibiensis* infection of *Agama aculeata aculeata* and *Agama planiceps planiceps* males and females in Windhoek, Namibia. Parasitaemia is given as the mean ±1 SD; N=total sample examined; n=number of infected specimens.

	A. a. aculeata		*A. p. planiceps*	
	Males	Females	Males	Females
N	138	119	128	131
Prevalence	16.7%	16.8%	12.5%	23.7%
Abundance	2.4	1.4	0.6	1.3
Parasitaemia	14.4± 14.9 (n=23)	8.2± 10.56 (n=20)	5.1± 5.10 (n=16)	5.5± 5.52 (n=31)
Dispersion	0.06	0.07	0.2	0.2

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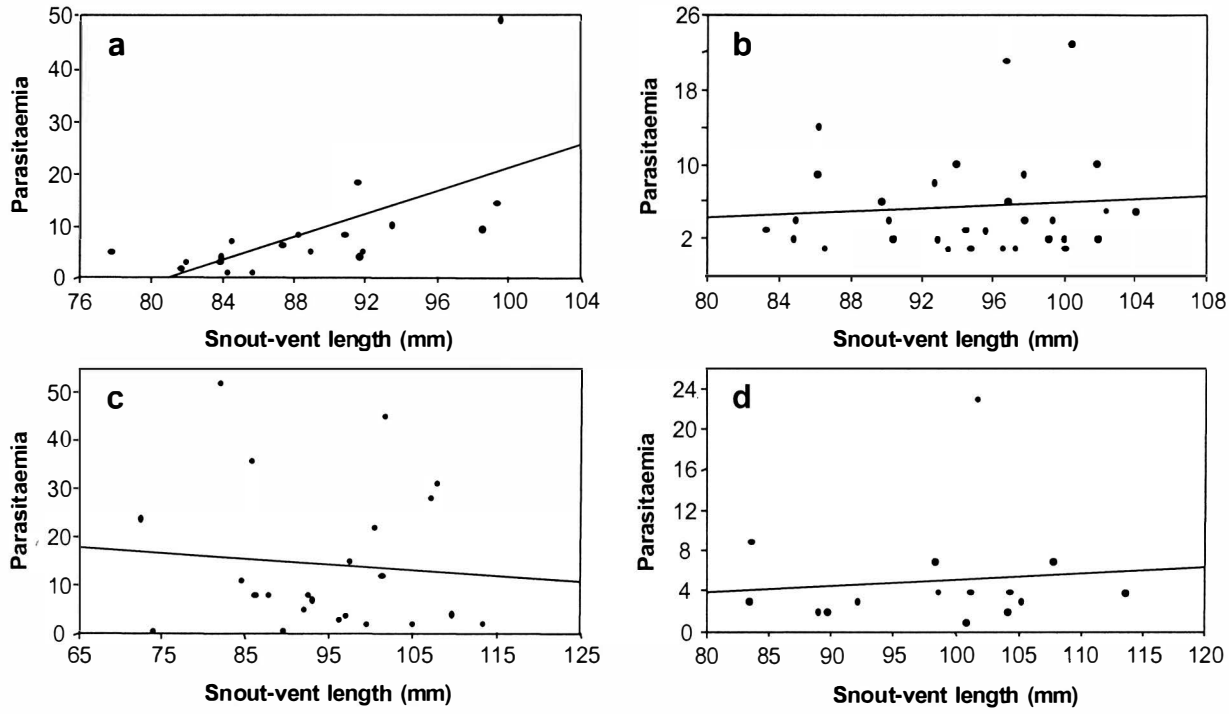

FIG 1. The number (parasitaemia) of *Raillietiella namibiensis* in the lungs of agamid lizards from Windhoek, Namibia and the snout-vent lengths of the lizards: (a) female *Agama aculeata aculeata*; (b) female *Agama planiceps planiceps*; (c) male *A. a. aculeata*; (d) male *A. p. planiceps*. Fitted linear regressions: (a) parasitaemia = 1.09xSVL-88.85 (P<0.001); (b) parasitaemia = 0.083xSVL-2.41 (N.S.); (c) parasitaemia = -0.12xSVL+26.01 (NS); (d) parasitaemia = 0.063xSVL -1.077 (NS).

namibiensis among its hosts. The abundance of the parasite in males and females of the two *Agama* species was low in all cases and showed no consistent pattern (Table 1).

Parasitaemia in relation to lizard SVL is shown in Fig. 1. A significant positive correlation was found between the number of parasites per host and SVL only in *A. a. aculeata* females (R = 0.959, $P < 0.001$). It could be hypothesized that larger (older) females have higher infection levels than smaller (younger) individuals because they have had longer exposure to potential infection. However, the absence of such a relationship in the rest of the lizards does not support this hypothesis in general. The reasons for the observed differences in prevalence, abundance and parasitaemia may lie in differences in the host species' and sexes' diets, habitats or behaviours. *Agama a. aculeata* lives in sandy areas, whereas *A. p. planiceps* lives on rocks. Further investigation of these differences is thus required.

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Accepted: 20.9.01

HERPETOLOGICAL JOURNAL, Vol.12, pp. 39-42 (2002)

DIET COMPOSITION OF *LIOLAEMUS BIBRONII* (IGUANIA: LIOLAEMIDAE) IN SOUTHERN RIO NEGRO PROVINCE, ARGENTINA

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Key words: *Liolaemus*, iguanid lizard, diet, Argentina

The new world lizard genus *Liolaemus* is very diverse in the southern part of South America, where approximately 170 species have been described (Cei, 1986, 1993; Etheridge, 1995; Avila *et al.* 2000). In spite of this diversity, the biology and ecology of only a few species have been studied. Vitt & Zani (1996) commented on the need to collect basic natural history data on neotropical lizards because they are very important in our understanding of the ecological relationships between species. This information is also important for the formulation of realistic and testable hypotheses, and for the design of appropriate experiments for studying species interactions in the complexity of ecological systems. Additionally, this basic information can be useful in the evaluation of the conservation status of some poorly known species (Reca *et al.*, 1994).

Liolaemus bibronii (Bell, 1843) is a small lizard, widely distributed within Andean habitats of mid-west and Patagonian habitats of southern Argentina as well as a small portion of Chilean Patagonia. The only study on the diet of this species was made by Videla (1983), in sub-andean habitat of Mendoza province, near the northernmost edge of its distribution. The purpose of this study is to describe the diet of *Liolaemus bibronii* in a typical, cold desert habitat of Patagonia, in the central part of its distributional range.

Lizards were collected in Ingeniero Jacobacci, (41°18´ S, 69°36´ W), 25 de Mayo Department, Río Negro Province, Argentina. Phytogeographically, the area is included in the Provincia Patagónica (Cabrera, 1994), and the study site was a shrub-dominated slope of a basaltic plateau. Representative elements of the flora are *Mullinum spinosum*, *Nassauvia axillaris*, *Prosopis patagonica*, *Verbena tridactyllites*, *Berberis empetrifolia*, *Colliguaya intergerrima*, *Stipa patagonica* and *Poa bonariensis*. The climate is dry; annual precipitation is less than 200 mm, and most falls as snow. The annual mean temperature is 9.3 °C and the monthly mean temperatures range from 16.8 °C (January) to 2.2 °C (July). This is one of the coldest and windiest areas of Patagonia.

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Thirty-three lizards were collected by hand between November 1991 and March 1992. Because of their small size and the likely stress involved in handling, we decided not to attempt the evacuation of stomach contents of live lizards. To minimize the effect of sampling on the study population, only a small sample of lizards was collected to provide a representative sample of dietary composition (Maury, 1981). These were killed humanely in the laboratory, fixed in 20% formaldehyde, transferred to 70% ethanol, and deposited in the herpetological collections at the Instituto de Herpetología, Fundación Miguel Lillo (San Miguel de Tucumán) and LJAMM collection (CRILAR-CONICET), Anillaco (Argentina).

Stomachs were later removed for diet analysis and stored separately in 70% ethanol in small vials. Whole stomachs were dissected and contents were examined using a stereoscopic microscope, identified, counted, and placed into food resource categories. Prey items were identified to the lowest practicable taxonomic level – usually family – based on entire items or identifiable fragments. We only considered items found in the stomachs themselves as these were the least digested. The developmental stage of prey was recorded (e.g. larvae, adults) and percentages of each prey type (by item number, volume and occurrence) were calculated. Volume was estimated by measuring length and width of each item to the nearest 0.1 mm with a dial calliper, and approximating the prey body to a prolate spheroid, following Dunham (1983). Trophic diversity was calculated with Shannon's Index, and breadth of the trophic niche was calculated with Levins' index (Krebs, 1989). Four lizards had empty stomachs, but a sample of 20 to 25 stomachs was considered sufficient to stabilize the diversity curve (H=2.05) and show the diet composition of *Liolaemus bibronii*.

Twenty-four categories of prey comprising 556 prey items were found in the stomachs, and these reveal that *Liolaemus bibronii* had fed mainly on small leafhoppers and ants. Table 1 shows the number, volume and frequency of each prey category in terms of total number and percentage. Numerically, Cicadellidae (45%), Formicidae (18%) and Coccoidae (11%) were most important. In terms of volume, Cicadellidae and Formicidae were again predominant (26% and 20%, respectively), followed by Scarabaeidae (13%), Lepidoptera (11%) and Curculionidae (9%). Cicadellidae provided 59 % of items taken, followed by Ixodidae (31%) and Formicidae/Salticidae (28%).

Of the 556 prey items, 213 were active insects and 343 were motionless or very slow moving arthropods, larvae or plant material. Of the latter, 150 Cicadellidae – a slow-moving type of insect – were found in a single stomach. The mean number (±SD) of prey items per stomach was 19.17±50.02; range = 1–163, with only two individuals containing a single prey category. Aver-

TABLE 1. Diet composition of *Liolaemus bibronii* (*N*=29), with prey categories presented as percentage by frequency (*n*=number of lizards whose stomach contained one or more prey; %= percentage of the lizards sampled), number (number of prey items and % of the total number of prey) and volume (in mm^3 and percentage of volume total).

	Frequency		Number		Volume	
	n	%	*n*	%	mm^3	%
HYMENOPTERA						
Formicidae	8	27.59	101	18.17	459.35	20.05
COLEOPTERA						
Scarabaeidae	4	13.79	12	2.16	296.14	12.92
Curculionidae	5	17.24	13	2.34	216.96	9.47
Carabidae	4	13.79	5	0.90	90.31	3.94
Chrysomelidae	1	3.45	3	0.54	48.39	2.11
Larvae	5	17.24	5	0.90	124.27	5.42
HEMIPTERA						
Lygaeidae	3	10.34	2	0.36	3.12	0.14
HOMOPTERA						
Cicadellidae	17	58.62	253	45.50	588.43	25.68
Coccoidae	3	10.34	62	11.15	34.43	1.50
Aphididae	6	20.69	5	0.90	76.42	3.33
DIPTERA						
Staphylinidae	1	3.45	2	0.36	0.05	0.00
Tabanidae	1	3.45	1	0.18	0.60	0.03
Stratiomyidae	1	3.45	1	0.18	0.47	0.02
Larvae						
LEPIDOPTERA	2	6.90	2	0.36	254.15	11.09
ACARI						
Ixodidae	9	31.03	43	7.73	23.98	1.05
Oribatidae	2	6.90	2	0.36	0.52	0.02
ARACHNIDA						
Salticidae	8	27.59	10	1.80	58.87	2.57
Tomicidae	2	6.90	2	0.36	0.86	0.04
OTHERS						
Pupae	1	3.45	1	0.18	5.81	0.25
Eggs	1	3.45	1	0.18	0.16	0.01
Unidentified larvae	1	3.45	1	0.18	0.25	0.01
PLANT MATERIAL						
Fruits	3	10.34	10	1.80	5.70	0.25
Seeds	4	13.79	12	2.16	0.03	0.00
Flowers	1	3.45	7	1.26	0.65	0.03
Vegetative parts	7	24.14			1.63	0.03

age prey length was 4.05±0.7; range = 0.7–19.4 and mean prey volume was 107.9±83.1 mm^3. Twenty-four lizards were found to have eaten active insects, 28 had eaten slow-moving arthropods and 14 had eaten non-mobile prey. One lizard contained only plant materials (volume = 153.45 mm^3).

Liolaemus bibronii is predominantly insectivorous and in our study area it fed largely on Cicadellidae, Ixodidae, Formicidae, Curculionidae and Scarabaeidae, with plant material as the other major dietary component. The high frequency of plant material can be attributed to accidental ingestion when lizards caught their prey, because plant material volume is very low. Some dietary items add a large amount in volume (Lepidoptera) but they are eaten in very low frequency. Other items are very important in number or frequency, but their contribution in volume is very small and less important (Ixodidae or Salticidae). The generalization in the diet reflects, in part, a sedentary foraging strategy, as the diversity of arthropods in the diet is characteristic of sit-and-wait predators (Schoener, 1969, 1971; Huey & Pianka, 1981). This seems to correspond with the secretive behaviour of *L. bibronii* (Acosta *et al.*, 1996*b*), as this lizard is frequently found under stones and close to small thorn bushes, where it forages and thermoregulates. Nevertheless, the high frequency of slow-moving prey could also indicate a strategy of actively searching for food. *L. bibronii* may possibly

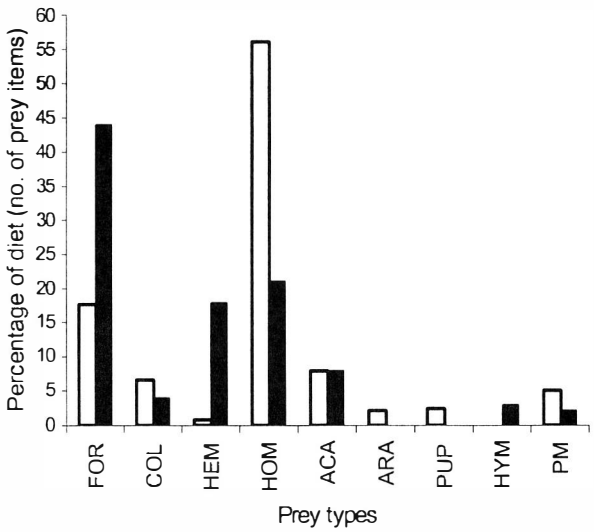

FIG 1. Comparison of the diet of *Liolaemus bibronii* in this study [open bars] and that reported by Videla (1983) [filled bars].The percentage of the diet made up by each prey category is shown on the basis of numbers of prey items. FOR = Formicidae, COL = Coleoptera, HEM = Hemiptera, HOM = Homoptera, ACA = Acari, ARA = Arachnida, PUP = pupae, HYM = Hymenoptera, PM = plant materials.

change its foraging strategy according to food availability, like other lizards in the genus. Data not presented here indicate that *L. bibronii* is an opportunistic predator (Levins' index = 4.5), taking the most abundant prey item found in its habitat.

Ingestion of plants by lizards is often regarded as an accidental consequence of the capture of arthropod prey within vegetation. However, the fact that plant parts (leaves, flowers, fruits and seeds) were found in 48% of stomachs suggests that ingestion was not entirely accidental. According to the literature, the use of plant material as a significant part of the diet is not common within small species of lizards. Pough (1973, 1983) suggested that, because of morphological and physiological constraints, small lizards are usually insectivorous whereas larger species are carnivorous, omnivorous or herbivorous. However, this suggestion may not be apply in cold habitats, such as those at high altitudes or in cold deserts like Patagonia. The consumption of plants by *Liolaemus bibronii* agrees with the suggestion of Rocha (1989) that ingestion of plant material by small lizards may be more widespread than previously believed. In these habitats, food items such as invertebrates can sometimes be very scarce. In *L. boulengeri*, a sympatric species, Acosta *et al.* (1996*a*) found a significant volume and frequency of plant materials, and – in the previous study of *L. bibronii* – Videla (1983) found a small portion of vegetable matter, while Formicidae was the most important food category. In some habitats, such as at Videla's (1983) study site, ants can be very common and comprise an important part of total biomass. Fig. 1 shows a comparison between the main items found by Videla (1983) and in this study.

In summary, our analysis indicates that *Liolaemus bibronii* has a generalist diet but that a few prey categories are very important; these features of its foraging behaviour can be viewed as adaptations to the variability of food resources in a highly unpredictable desert environment (Maury, 1995) – in this case, an arid and cold steppe desert, the Patagonian.

ACKNOWLEDGEMENTS.

M. Archangelsky helped to identify prey and provided useful comments and corrections to the English manuscript. J. C. Acosta and G. Blanco provided the lizards used in this study and ecological data. M. Morando and D. Perez helped with the field work, and recorded some biological and ecological traits; and J. A. Corronca helped to identify prey. We thank J. Sites for reading and correct the English version of this manuscript.

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Accepted: 9.10.01

BOOK REVIEWS

Amphibians and Reptiles of Madagascar and the Mascarene, Seychelles, and Comoro Islands. Friedrich-Wilhelm Henkel & Wolfgang Schmidt. (2000). 324 pp. Krieger Publishing Company, Florida.US$64.50 (cloth).

I like Madagascar. I like its people, its music and its wildlife. I especially like Malagasy herpetofauna. It should therefore come as no surprise that I am always excited about the prospects of a new book on the subject. So, despite its (many) faults, I was determined to find something positive in this new work by Henkel and Schmidt.

There are a number of publications that describe Madagascar's amazing collection of reptiles and amphibians. Unfortunately only a relatively small number are available in English. Perhaps the most respected book in English is that by Glaw & Vences (1994). However, there is still an opening for an accessible guide that provides more thorough descriptions on individual species than Glaw and Vences' rather short accounts, particularly one that includes additional species from other islands within the region. This new book by Henkel and Schmidt is a basic guide to the Malagasy herpetofauna with descriptions of 240 species of reptiles and amphibians (not nearly all of them but since there are over 200 described amphibians and over 300 described reptiles, this is understandable for this type of book), together with information on vivarium care and general information on each of the four geographical areas covered. The geographic descriptions are basic but cover most of the important issues. The text is translated from the original German version, which has led to occasional lapses in style and grammar

The bulk of the book is devoted to the individual species accounts. For each species, information is provided on distribution, habitat, description, biology and vivarium care. Each species is also accompanied by a colour plate. The photographs are generally of very high quality. Unfortunately they do not necessarily illustrate the correct species. Within the Preface there is an onerous warning that there may be inaccuracies in species identifications. Indeed there are! The plate accompanying *Calumma malthe*, for example, is an excellent photograph of a male *C. brevicornis*. Unfortunate, but mistakes like this can happen with publishers, who often lose, juggle, smear or mislabel an author's carefully arranged collection of transparencies (I speak from personal experience). Yet it appears that the publishers are not to blame for the mistakes in this book. The species account for *C. malthe* quite clearly describes the *C. brevicornis* photograph. If only the authors had checked with Glaw & Vences (1994) they would have immediately realised their error. This mistake led me to look at the text in more detail. Apparently, *C. malthe* (or is that *C. brevicornis*?) is only rarely found in dense bushes lower than 5 m. This does not correspond with my own observations of either species. Other mistakes and inconsistencies in the text abound. *C. gastrotaenia* is apparently a pure rain forest dweller that is only found in dense forest regions. But reading further on, this statement is apparently contradicted by another that says 'hunting juveniles go into nearby meadows and cultivated land'. Other interesting comments on chameleon natural history abound, but it is often difficult to distinguish fact from anecdote. According to Henkel and Schmidt, 'the light, vivid, sand-coloured round spot on the forehead of [*Brookesia minima*] enables these animals to locate one another in their natural habitat.' There are several reliable published accounts of chameleon behaviour in the literature (e.g. Raxworthy & Nussbaum, 1995), but as far as I am aware the only published description of *Brookesia* behaviour details their defensive response and does not mention anything about spots. Despite the presence of a bibliography, the sources of many other snippets of information are unclear. I also have a grievance about the sections on 'Vivarium care'. For many species the authors quite rightly point out that 'nothing is known'. For several species (e.g. *Amphiglossus polleni*) they then provide vivarium care information. If nothing is known about a species' biology, how can it be possible to provide guidance on its care in captivity? It is also interesting that vivarium care information is given for species that are currently included on a CITES trade moratorium. For the Malagasy chameleons, Abate (1999) claims there are very few captive animals remaining alive since the 1996 ban (other than the four permissible trade species; *F. pardalis*, *F. oustaleti*, *F. lateralis* and *F. verrucosus*). I could find no discussion of this important issue within the book.

To summarise, this book contains some excellent colour photographs that accompany not so excellent text. The species accounts do not really provide any more useful information than that already available in Glaw & Vences (1994), which is likely to remain the recommended text on this topic for the foreseeable future.

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Snakes. Peter Stafford (2000). 112 pp. The Natural History Museum, London £9.95 (paper).

Snakes are a group of reptiles that have a peculiar fascination for many people – and not just for biologists. The recent upsurge in documentary television programmes on snake natural history, in addition to a huge increase in the availability of the animals in the pet trade, has led to an ever-increasing demand for information about their biology and husbandry. The market has responded with a plethora of publications, the quality of which varies greatly, particularly when they are in the lower price bracket. A few of these nevertheless do manage to provide a valuable insight into the lives of snakes at a very modest price; this work by Peter Stafford is one of these books.

According to the author, the main objectives of the book are to answer some of the questions surrounding the unusual morphology and biology of snakes in addition to surveying the major snake families and their diversification. Descriptions of the various snake families begin with the primitive burrowing blind snakes (Leptotyphlopidae, Typhlopidae and Anomalepididae), pipe snakes (Aniliidae, Cylindrophiidae and Anomochilidae) and shieldtailed snakes (Uropeltidae). This is followed by the sunbeam snakes (Loxocemidae and Xenopeltidae), boas and pythons (Boidae), Round Island boa (Bolyeriidae), dwarf boas (Tropidophiidae) and file snakes (Acrochordidae). The more advanced forms, burrowing asps (Atractaspididae) rat snakes, racers, garter snakes etc (Colubridae) cobras and their allies (Elapidae), and vipers (Viperidae) complete the survey.

A chapter introducing us to the structure and lifestyle of snakes precedes these sections. The author draws attention here to the gaps in knowledge about the sensory mechanisms in snakes – for instance our knowledge of snake vision and hearing is still far from complete. The methods of measuring the toxicity of different types of venom and their usage in medicine are also briefly discussed. Throughout the book there are supplementary text boxes that provide additional snippets of information on, for instance, *the trade in snake skins*, *how snakes move*, *giant snakes*, *convergent evolution in snakes*, *mimicry* and *which snake is the most dangerous*. One text box describes the unusual relationship that exists between blind snakes and screech-owls, of which I was completely unaware. Apparently, the nesting parent owls capture Texas blind snakes (*Leptotyphlops dulcis*) and take them back to the nest. They do not necessarily kill the snakes, but release them into the nest – at least in some cases. The snakes that survive in this way live in the nest and feed on the larvae of parasitic insects that are a major cause of brood failure. Young owls with resident blind snakes often grow faster and suffer lower mortalities than those in nests without snakes. The author does point out, however, that in other areas blind snakes are eaten by owls and so it is not yet known whether the relationship is indeed a case of mutualism or simply fortuitous.

There are 112 photographs in the book, most of which are of very good colour quality. A photograph showing the hemipenes of a coral snake is the only one in black and white. I think that photographs that are able to show animals in their natural environment are more informative than those that do not, and in this respect I particularly liked the photograph on page 105 (apparently supplied by Chris Mattison) of a western diamondback rattlesnake partially basking in its desert environment. Others that I found interesting were those comparing the neck vertebrae of an extinct giant palaeophid snake and a 6.6 m reticulated python, and that of a caterpillar (*Hemeroplanes triptolemus*) that mimics a pit viper (*Bothriechis schlegelii*). There are several useful drawings mostly illustrating the structure and jaw mechanisms of snakes. Finally, there are brief sections giving sources of further reading, addresses of herpetological societies and, in keeping with modern times, internet resources.

This is a book that is both easy to read and informative and therefore manages to maintain the high standard set by a previous publication on snakes by the Natural History Museum (Parker & Grandison, 1977). The quality of production is good and surpasses many more costly publications – indeed I can only think of one herpetological publication that exceeds it in value for money and that is the work on South African reptiles by Branch (1988). I think in the main the author has achieved his objectives which were to answer some of the morphological and biological questions asked about snakes and at a very reasonable cost. If I have a criticism of the work at all, it is that the chapter on structure and lifestyle could have been expanded to provide a little more detail, even if this added a few pounds to the cost.

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THE HERPETOLOGICAL JOURNAL

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(revised July 2000)

1. The *Herpetological Journal* publishes a range of features concerned with reptile and amphibian biology. These include: *Full Papers* (no length limit); *Reviews* and *Mini-reviews* (generally solicited by a member of the editorial board); *Short Notes*; controversies, under *Forum* (details available from the Editor); and *Book Reviews*. Faunistic lists, letters and results of general surveys are not published unless they shed light on herpetological problems of wider significance. Authors should bear in mind that the *Herpetological Journal* is read by a wide range of herpetologists from different scientific disciplines. The work should therefore appeal to a general herpetological audience and have a solid grounding in natural history.

2. *Three* copies of all submissions, and illustrations, should be sent to the Scientific Editor. All papers will be subject to peer review by at least two referees. Authors are invited to suggest the names of up to three referees, although the editor may choose alternative referees to those suggested. Papers will be judged on the basis of the reports supplied by referees, scientific rigour, and the degree of general interest in the subject matter. The Editor's decision will be final.

3. Authors should consult a recent issue of the Journal regarding style. Papers should be concise with the minimum number of tables and illustrations. They should be written in English and spelling should be that of the *Oxford English Dictionary*. Papers should be typed or produced on a good-quality printer, and double-spaced with wide margins all round. The journal is typeset direct from the author's computer diskette, so all manuscripts should be prepared using a wordprocessor (preferably on a PC-compatible microcomputer). If figures are prepared using computer graphics, they should be supplied separately and NOT embedded in the text of the wordprocessor file. Preferred formats are MS Word for Windows (text) and MS Excel, Bitmap, TIFF, or JPEG files (graphics). It is not necessary to submit a computer diskette with the initial manuscript, but this will be required in the event of the manuscript being accepted for publication.

4. For all papers the title page should contain only the following: title of paper; name(s) of the author(s); address of the Institution where the work was done; a running title of five words or less, and the name and address of the corresponding author with (if available) an email address. The text of the paper should begin on page 2 and be produced in the following order: Abstract, Keywords, Text, Acknowledgements, References, Appendices. Full papers and reviews should have the main text divided into sections. The first subhead will be centred in capitals, the second shouldered in lower case, and the third run on in italics. Footnotes are not permitted. *Short Notes* (generally less than six manuscript pages and accompanied by a single data set) should be produced as continuous text. A *sans serif* font (e.g. Universe or Helvetica) is preferred.

5. The usual rules of zoological nomenclature apply.

6. Tables are numbered in arabic numerals, e.g. TABLE 1; they should be typed double spaced on separate sheets with a title/short explanatory paragraph above the table. Horizontal and vertical lines should be avoided.

7. Line drawings and photographs are numbered in sequence in arabic numerals, e.g. FIG. 1. Colour photographs can only be included at cost to the author. If an illustration has more than one part each should be identified as (a), (b), etc. The orientation and name of the first author should be indicated on the back. They should be supplied camera-ready for uniform reduction of one-half on A4 size paper. Line drawings should be drawn and fully labelled in Indian ink, dry-print lettering or laser printed. Illustrations produced using other types of computer printer are not usually of suitable quality. A metric scale must be inserted in micrographs etc. Legends for illustrations should be typed on a separate sheet.

8. References in the text should be given as in the following examples: "Smith (1964) stated —"; "—as observed by Smith & Jones (1963)." "—as previously observed (Smith, 1963; Jones, 1964; Smith & Jones, 1965)". For three or more authors, the complete reference should be given at the first mention, e.g. (Smith, Jones & Brown, 1972), and *et al.* used thereafter (Smith *et al.*, 1972). For the list of references the full title or standard abbreviations of the journal should be given. Articles 'submitted' or 'in prep' may not be cited in the text or reference list. The following examples will serve to illustrate the style and presentation used by the Journal.

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9. Final acceptance of a paper will depend upon the production by the author of a typescript, illustrations and computer diskette ready for the press. However, every assistance will be given to amateur herpetologists to prepare papers for publication.

10. Proofs should be returned to the Managing Editor by return of post. Alterations should be kept to the correction of errors; more extensive alterations will be charged to the author.

11. Twenty-five offprints and one complimentary copy of the Journal are provided free of charge. Further copies (minimum of twenty-five) may be purchased provided that they are ordered at the time the proofs are returned.

12. All submissions are liable to assessment by the editorial board for ethical considerations, and publication may be refused on the recommendation of this committee. Contributors may therefore need to justify killing or the use of other animal procedures, if these have been involved in the execution of the work. Likewise, work that has involved the collection of endangered species or disturbance to their habitat(s) will require full justification.

THE HERPETOLOGICAL JOURNAL

Volume 12, Number 1 2002

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Herpetological Journal vol. 11, no. 4 was published on 19 February 2002