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DISSERTATION

Titel der Dissertation

The genetic mating system and analyses of reproductive success in
Allobates femoralis

(Das genetische Paarungssystem und Analysen des
Fortpflanzungserfolges in *Allobates femoralis*)

Verfasserin

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angestrebter akademischer Grad

Doktorin der Naturwissenschaften (Dr.rer.nat.)

Wien, 2011

Studienkennzahl lt. Studienblatt: A 091 439

Dissertationsgebiet lt. Studienblatt: Zoologie

Betreuerin / Betreuer: ao. Univ.-Prof. Mag. Dr. Walter Hödl

*Was immer du tun kannst oder wovon du träumst - fang
damit an. Mut hat Genie, Kraft und Zauber in sich.*

Johann Wolfgang Goethe



photo: M. Ringle

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Preface

This doctoral thesis contains a total of five articles. Three of them have already been published/accepted in peer-reviewed journals; two are currently under review. They all deal with different aspects of the reproductive behaviour and life-history attributes in the Neotropical frog *Allobates femoralis*. The general introduction provides the framework for all contributing manuscripts and gives an overview of the background literature. Part 1 contains all my first-author manuscripts, where I conducted the main part of the data analysis, the interpretation of results, and the writing. Part 2 consists of two manuscripts I have co-authored, by assisting in the collection of data and writing of the manuscripts. They are tightly connected to my dissertation topic, since my thesis is embedded in a collaborative project with Max Ringler (Department of Evolutionary Biology, University of Vienna, Austria). We joined forces by integrating spatial and molecular data, and thus were able to address a broad range of population-wide research questions. While I contributed all molecular data, Max Ringler provided all spatial analyses. Field work was accomplished by both of us. My first-author articles are further co-authored by Robert Jehle and Walter Hödl. See the chapter 'Author Contributions' for details on the specific contributions of all manuscripts.

Work in the field and the laboratory was financially supported in 2008 by the 'Forschungsstipendium' and the 'Förderstipendium' from the University of Vienna, in 2008 and 2009 by the 'Nouragues Grant' from the French CNRS (PI Max Ringler), and also through the Austrian Science Fund grant FWF-18811 (PI Walter Hödl). In 2009 I received a two-year DOC-ffORTE scholarship from the Austrian Academy of Science. All necessary permissions for working, sampling and exporting tissue samples were obtained from the responsible French authorities (see Annex): the national centre for scientific research ('Centre National de la Recherche Scientifique', CNRS) which runs the field station 'Parare', and the regional environmental board ('Direction Regional de l'Environnement', DIREN). Fieldwork was conducted in compliance with current French and EU law according to the ASAB guidelines.

Summary

Anuran amphibians are often portrayed as generally congregating at confined aquatic sites to deposit rather large egg clutches during short reproductive bouts, but they actually show a remarkably wide array of different reproductive strategies. Neotropical poison frogs and their relatives (Dendrobatidae and Aromobatidae in the superfamily Dendrobatoidea), for example, hold an extraordinary position within amphibians due to their elaborate reproductive behaviour which is found across the whole taxon. Dendrobatoid frogs are characterised by prolonged breeding, territoriality of at least one sex, and uni- or biparental care. Hence, it can be expected that in this taxonomic group population genetics and reproductive dynamics also differ considerably from the 'temperate explosive breeding' situation. Dendrobatoid reproductive behaviour is well documented from behavioural observations, but very few molecular studies have been performed in this taxon so far.

The pan-Amazonian dendrobatoid frog *Allobates femoralis* is a highly suitable species for studies at the individual and also population level. Throughout the prolonged breeding period, which corresponds to the rainy season, males are highly territorial. Females are iteroparous and show strong site fidelity. Courtship and mating takes place inside the male territories. The aim of my dissertation was to assess the genetic mating system and to investigate the distribution and determinants of male and female reproductive success across an entire *A. femoralis* population. This thesis hence contributes to the knowledge about mating systems, parameters of mate choice, and effects of parental relatedness on reproductive success, particularly in Dendrobatoid frogs.

The *A. femoralis* population under study is located near the research station 'Saut Pararé' in the nature reserve 'Les Nouragues', French Guiana. Through molecular parentage analyses of two successive generations of adult individuals a highly polygynandrous mating system was identified. Despite significant differences in the reproductive behaviour of males and females, successful individuals of both sexes had similar numbers of mating partners and produced similar numbers of adult progeny. Furthermore, a high percentage of the males and females in the population produced progeny which survived until adulthood. Reproductive success was significantly higher in territorial males, but not related to territory size in males or body size in both sexes.

Parentage assignments of one offspring generation at two different life history stages were used to identify patterns of parental relatedness on reproductive success in *Allobates femoralis*. The stepwise spatial restriction of candidate males in our analysis allowed us to

discern between spatial and genetic effects of mate choice. There was no correlation between genetic and spatial distance. Females mainly (in 72%) chose their mating partners within a radius of 20 m. Our results show no evidence of behavioral inbreeding avoidance in *A. femoralis* but indicate a selective benefit of matings between partners of intermediate genetic divergence.

For the molecular analyses, we removed toe clips to gain sufficient DNA from all adult male and female *A. femoralis*. After a few months, the regrowth of the clipped toes was observed, a phenomenon, which was so far only occasionally reported from few anuran species. Furthermore, in almost all one- and two-year recaptured individuals the regrown toe discs showed a black instead of the original white colouration.

Territory occupancy, but not territory size, was identified as a significant determinant of reproductive success in *A. femoralis* males. Given the distinct difference between the core area that is occupied during calling and the area a male is willing to defend, results in two possible definitions of male territory size. Our finding that the extension of the defended area did not correlate with the area occupied during calling is of particular relevance to studies that investigate the effect of territory size on reproductive success.

Finally, we document a predation event on an *A. femoralis* male by a colubrid snake.

Zusammenfassung

Das Bild von Froschpopulationen, die als Brutgemeinschaften während kurzer reproduktiver Perioden an einzelnen Wasserstellen große Mengen an Laich ablegen, ist weit verbreitet. Innerhalb der Anuren finden wir jedoch ein breites Spektrum an verschiedenen reproduktiven Strategien. Neotropische Pfeilgiftfrösche (Dendrobatidae und Aromobatidae in der Überfamilie Dendrobatoidea) nehmen hinsichtlich ihres aufwändigen Reproduktionsverhaltens eine Sonderstellung innerhalb der Amphibien ein. Charakteristische Attribute aller Arten in dieser Gruppe sind lange Brutperioden, Territorialität zumindest bei einem der Geschlechter und obligate Brutpflege. Folglich kann man erwarten, dass sich Dendrobatidae im Bezug auf ihre Populationsgenetik und reproduktiven Dynamiken erheblich von „temperaten Explosivlaichern“ unterscheiden. Die Kenntnisse über reproduktives Verhalten von Pfeilgiftfröschen basieren Großteils auf detaillierten Verhaltensbeobachtungen, und wurden bisher nur durch wenige molekulare Studien belegt.

Die über ganz Amazonien verbreitete Pfeilgiftfroschart *Allobates femoralis* eignet sich sehr gut für Studien auf Individuen- bzw. Populationsniveau. Während der langen Fortpflanzungsperiode, die mit der Regenzeit einhergeht, sind die Männchen hoch territorial; die Weibchen sind iteropar und standorttreu. Balz und Paarung findet in den Territorien der Männchen statt. Das genetische Paarungssystem zu erfassen und die Verteilung des Fortpflanzungserfolges bei Männchen und Weibchen innerhalb einer *A. femoralis* Population zu untersuchen war das Ziel meiner Dissertation. Diese Arbeit trägt somit zum Wissen über Paarungssysteme, Parameter der Partnerwahl und über Effekte der elterlichen Verwandtschaft auf individuellen Fortpflanzungserfolg, insbesondere in Dendrobatoidea, bei.

Die untersuchte *A. femoralis* Population befindet sich in der Nähe der Forschungsstation 'Saut Pararé' im Naturschutzgebiet 'Les Nouragues', in Französisch Guyana. Anhand molekularer Elternschaftsanalysen zweier aufeinanderfolgender Generationen adulter Individuen konnte ein hoch polygynandrisches Paarungssystem identifiziert werden. Trotz erheblicher Unterschiede im reproduktiven Verhalten von Männchen und Weibchen, hatten erfolgreiche Individuen in beiden Geschlechtern sowohl eine ähnliche Anzahl von Fortpflanzungspartnern als auch adulte Nachkommen pro Individuum. Auch der Prozentsatz an Männchen und auch Weibchen in der Population, die erfolgreich Nachkommen produzierten, die bis zum Adultstadium überlebten, war sehr hoch. Der individuelle Reproduktionserfolg war signifikant höher für Männchen, welche ein Territorium besaßen, jedoch unabhängig von der Größe des Territoriums oder Körpergröße beider Geschlechter.

Elternschaftszuordnungen von Nachkommen zu zwei unterschiedlichen Zeitpunkten in der Entwicklung zu ihrer Parentalgeneration wurden verwendet, um die Effekte von genetischer Verwandtschaft auf individuellen Fortpflanzungserfolg in *A. femoralis* zu identifizieren. Die schrittweise räumliche Einengung der potentiellen männlichen Fortpflanzungspartner in unserer Analyse ermöglichte zwischen räumlichen und genetischen Effekten bei der Partnerwahl zu differenzieren. Räumliche und genetische Distanzen wiesen keinerlei Korrelation auf. Weibchen wählten ihre Fortpflanzungspartner meist (in 72%) innerhalb eines Radius von 20m. Unsere Analysen lieferten keinen Hinweis auf Inzuchtvermeidung bei *A. femoralis*, jedoch war die Varianz der genetischen Divergenz der erfolgreichen Fortpflanzungspaare signifikant kleiner, als bei Zufallsverpaarungen zu erwarten gewesen wäre.

DNA Proben für die molekularen Analysen wurden gesammelt, in dem wir von allen adulten männlichen und weiblichen *A. femoralis* Phalangenamputationen durchführten. Nach wenigen Monaten, konnte die Größenzunahme dieser Phalangenstümpfe beobachtet werden, ein Phänomen, das bisher nur fallweise bei einzelnen Froscharten beschrieben wurde. Außerdem wiesen die rasch nachgewachsenen Zehenscheiben in fast ausnahmslos allen Individuen, die nach einem bzw. zwei Jahren wiedergefangen wurden, eine schwarze statt der ursprünglichen weißen Färbung auf.

Der Besitz eines Territoriums, nicht jedoch dessen Größe, ist ein signifikanter Bestimmungsfaktor für den Fortpflanzungserfolg in *A. femoralis* Männchen. Jedoch resultiert der Unterschied zwischen der Kernzone, die ein Männchen während des Rufend okkupiert, und dem Raum, den ein Männchen aktiv verteidigt, in zwei möglichen Definitionen für „Territorium“. Das Ergebnis, dass in *A. femoralis* die Ausdehnung des verteidigten Areales nicht mit der Kernzone korreliert, ist von spezieller Relevanz für zukünftige Studien, die Effekte von Territorialität auf Fortpflanzungserfolg untersuchen.

Schließlich dokumentieren wir die Prädation eines *A. femoralis* Männchen durch eine Schlange der Art *Xenopholis scalaris* (Colubridae).

General Introduction

Dendrobatoid reproductive behaviour

Anuran amphibians show a remarkably wide range of reproductive behaviour with a variety of mating systems, ranging from scramble competition polygyny in explosive breeders to social and/or genetic monogamy in hyper-dispersed species (Arak 1983; Sullivan & Heatwole 1995; Haddad & Praho 2005; Wells 2007). Nevertheless, anuran amphibians are often portrayed as generally congregating at confined aquatic sites to deposit rather large egg clutches during short reproductive bouts. This might be due to the multitude of studies that have been conducted on explosive breeding species in temperate regions. Highly elaborate and complex reproductive behaviour, though, can be found in the Neotropical poison frogs (Dendrobatidae and Aromobatidae in the superfamily Dendrobatoidea, *sensu* Grant *et al.* 2006). Within this taxon various mating systems have evolved that are uncommon for the rest of anurans, such as social and genetic monogamy (Brown *et al.* 2010). Consequentially, also population genetics and reproductive dynamics in this taxonomic group can be expected to differ substantially from the 'temperate explosive breeding' situation. Dendrobatoid reproductive behaviour is well documented from behavioural observations (e.g. Donnelly 1989; Summers 1989, 1990, 1992; Roithmair 1992, 1994; Summers & Earn 1999; Pröhl 2002, 2005; Juncá & Rodriguez 2006), but there is little knowledge about the underlying genetic structures.

All dendrobatoid species that have been studied so far are diurnal and characterised by a prolonged breeding period and obligatory tadpole transport. Exceptions are the nocturnal *Aromobates nocturnus* (Myers *et al.* 1991) and a few species where metamorphosis takes place inside the clutch (Weygold 1987; Crump 1995; Lötters *et al.* 2007). After hatching, either single tadpoles, batches of tadpoles or entire clutches have to be carried to water for further larval development. In several species additional parental care such as clutch moistening, egg guarding or feeding of larvae has evolved (Lötters *et al.* 2007). Biparental care, which might be related to the evolution of social and/or genetic monogamy, is enforced by the nutrient-poor environment of small water bodies used for tadpole deposition (Caldwell 1997; Brown *et al.* 2010). The high variation in mating systems and reproductive behaviour renders Dendrobatoids a taxon that is particularly well suited for studies of behavioural and evolutionary ecology.

The study species

The Neotropical frog *A. femoralis* has a pan-Amazonian distribution and is forming disjunct local populations (Amézquita *et al.* 2009). A wide range of research on acoustic communication (Narins *et al.* 2003; Hödl 1987; Hödl *et al.* 2004; Amézquita *et al.* 2005, 2006; Göd *et al.* 2007), biogeography (Lougheed *et al.* 1998), phonotactic movement (Ursprung *et al.* 2009), and territoriality (Roithmair 1992) has already been conducted. The genetic mating system, reproductive contributions, as well as population genetic parameters in this species have not been investigated until now.

During the reproductive season, which coincides with the rainy season (Born & Gaucher 2001; Gottsberger & Gruber 2004), males are highly territorial and call from elevated structures, such as logs, roots, and fallen branches and palm leafs (Schlüter 1980; Amézquita *et al.* 2009). The advertisement call of male *A. femoralis* serves to attract females as well as to announce territory occupancy to potential male competitors (Hödl 1983, 1987). Territories are strongly defended by males against conspecific calling male intruders (Narins *et al.* 2003). A previous study found a relation between territory size and mating success in *A. femoralis* (Roithmair 1992). However, as this study was purely based on behavioural data, and mating and reproductive success do not necessarily correlate, parentage analyses based on molecular data are needed to verify the possible relation between territory size and male reproductive success. Females as well exhibit strong site fidelity, but show no defensive behaviour (Ringler *et al.* 2009). Pair-formation, courtship, and mating takes place in the territories of males (Roithmair 1992). Males never reject females and courtship can last up to several hours (Montanarin *et al.* 2011). Females often abandon males during courtship and therefore not all cases of courtship will result in successful mating (Roithmair 1994; Montanarin *et al.* 2011). Brood care and tadpole transport to bodies of water is mainly performed by males (Roithmair 1992, 1994; Weygoldt 1980, 1987; Caldwell & de Araújo 2005). The individually distinct ventral patterns of male and female *A. femoralis* (see Annex) allow for individual recognition within and between years. Hence, *A. femoralis* is a highly suitable species for behavioural, ecological, and genetic studies at the individual but also on the population level.

Mating System, sexual selection and determinants of reproductive success

Costs and benefits associated with potential reproductive rates, competition over resources and parental care have shaped reproductive strategies in males and females (Bateson 1983; Kokko & Jennions 2008), and led to the wide range of animal mating systems (Shuster & Wade 2003; Shuster 2009). The adult sex-ratio is an important determinant of the direction of mating

competition and the intensity of sexual selection (Emlen & Oring 1977). A biased ratio will make the more abundant sex compete for matings, and therefore the less numerous sex often has a greater opportunity to mate selectively (Bateson 1983; Kokko & Jennions 2008). In *A. femoralis*, a strong male-biased sex ratio has already been reported in former studies (Ringler *et al.* 2009), suggesting a high competition among males for access to females. Given that females are the choosing sex and actively approach calling males, they may select for specific male traits such as territory and male body size, or maybe base their choice on genetic factors (cf. Neff & Pitcher 2005). In my work I investigated the genetic mating system, the cross-generational reproductive contributions of males and females, and individual reproductive success in relation to several traits in a natural *A. femoralis* population.

Inbreeding has been shown to negatively affect the reproductive performance of individuals and populations in various vertebrate species (Keller & Waller 2002), as matings between genetically similar individuals will lead to an increase in individual and population-wide homozygosity (Freeland 2005). Inbreeding depression can significantly affect survival, reproduction, and individual resistance to pathogens and parasites (Keller 1998; Coltman *et al.* 1999; Slate *et al.* 2000). These negative effects may increase with environmental stress (Fox & Reid 2011) and might be more harmful in wild than captive populations, where certain selective pressures are missing (Crnokrak & Roff 1999; Halverson *et al.* 2006; but see Caughley 1994). Active avoidance of matings between close relatives has been found to occur in a wide range of species (Blouin & Blouin 1988; Pusey & Wolf 1996; see Garner & Schmidt 2003 for amphibians). However, behavioural inbreeding avoidance is assumed to evolve only, if inbreeding depression resulting from decreased survival of inbred offspring is high compared to the costs of mate desertion (Kokko & Ots 2006). On the other hand, mating between genetically very distinct individuals increases the risk of genetic incompatibility, due to outbreeding depression, which as well leads to a reduced reproductive output. Therefore, an intermediate level of genomic divergence between mating partners can be assumed to confer maximal fitness in terms of final reproductive success (Bateson 1983; Neff 2004; Richard *et al.* 2009). This selective benefit may even drive the evolution of a strong preference for compatible partners in the choosing sex (Tregenza & Wedell 2000; Mays & Hill 2004).

Despite the remarkable insights about inbreeding effects that laboratory studies have provided, information about in- and outbreeding in large populations in the wild is scarce and also biased towards bird and mammal populations (Keller & Waller 2002; Frankham 2005; Richardson *et al.* 2004; Townsend *et al.* 2009). Studies on wild populations in their natural habitats, including various taxa with differing ecology, are needed to obtain more general

estimates of natural levels of in- and outbreeding as well as of effects of in- and outbreeding depression (Keller & Waller 2002; Frankham 2005; Halverson *et al.* 2006). In the course of this doctorate thesis I have analysed patterns of pairwise parental relatedness across a natural *A. femoralis* population.

The prominent advertisement calling of *A. femoralis* males and the significant relation of territory possession and reproductive success (Ursprung *et al.* 2011a) renders the accurate assessment of territoriality and the precise measurement of territory sizes particularly important. As territorial behaviour often differs between a 'calling area' and a 'defended area', as it is also the case in *A. femoralis*, there are two different approaches of how to delimit territorial borders. However, these two different territory size estimates do not necessarily correlate. By using simulated point patterns we aimed to assess the minimum number of trials that need to be performed in the course of intrusion experiments in order to obtain reliable and meaningful territory size estimates. Furthermore, we tested the performance of different area estimators available.

Beside the studies on mating systems and reproductive success, this thesis covers two manuscripts that document two unexpected observations regarding the life history of *A. femoralis*. Both, the consistent regrowth of the toes, which were clipped in the course of this study in order to gain DNA samples, and the predation event by the colubrid snake *Xenopholis scalaris* have not been documented for this species so far.

Part 1

Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care

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Abstract

Our knowledge about genetic mating systems and the underlying causes for and consequences of variation in reproductive success has substantially improved in recent years. When linked to longitudinal population studies, cross-generational pedigrees across wild populations can help answer a wide suite of questions in ecology and evolutionary biology. We used microsatellite markers and exhaustive sampling of two successive adult generations to obtain population-wide estimates of individual reproductive output of males and females in a natural population of the Neotropical frog *Allobates femoralis* (Aromobatidae), a pan-Amazonian species that features prolonged iteroparous breeding, male territoriality and male parental care. Parentage analysis revealed a polygynandrous mating system in which high proportions of males (35.5%) and females (56.0%) produced progeny that survived until adulthood. Despite contrasting reproductive strategies, successfully reproducing males and females had similar numbers of mating partners that sired the adult progeny (both sexes: median 2; range 1–6); the numbers of their offspring that reached adulthood were also similar (both sexes: median 2; range 1–8). Measures of reproductive skew indicate selection on males only for their opportunity to breed. Reproductive success was significantly higher in territorial than in nonterritorial males, but unrelated to territory size in males or to body size in both sexes. We hypothesize that female polyandry in this species has evolved because of enhanced offspring survival when paternal care is allocated to multiple partners.

Keywords: *Allobates femoralis*, anurans, mating system, parentage analysis, reproductive success

Received 16 August 2010; revision received 24 January 2011; accepted 30 January 2011

Introduction

Reproductive opportunities, potential reproductive rates, competition over resources and patterns of parental care are important determinants of reproductive strategies in males and females (Emlen & Oring 1977; Bateson 1983; Kokko & Jennions 2008). The costs and benefits of these strategies depend strongly on the physical and cognitive abilities of individuals, the demographic structure of populations and the stability and predictability of environmental conditions (Alatalo *et al.* 1990; Fox & Rauter 2003; Kokko & Rankin 2006).

The use of molecular parentage analyses has substantially increased our knowledge about individual mating patterns and sexual selection and has enabled determining genetic mating systems across a wide range of taxa (Jones & Avise 1997; Fiumera *et al.* 2002; Griffin *et al.* 2003; Becher & Magurran 2004). When applied to whole populations over longitudinal scales, molecular data also enable the reconstruction of genealogical relationships across two or more generations. This has advanced the studies of wild populations at the interface between ecological and evolutionary research (Pemberton 2008; Clutton-Brock & Sheldon 2010). Cross-generational studies reveal the impact that specific mating strategies have on the structure of subsequent generations of breeders. Such an inference would

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not be possible solely through parentage assignments of premature offspring cohorts (cf. Rodríguez-Muñoz *et al.* 2010; Serbezov *et al.* 2010). Reconstructed pedigrees also find application in investigating the direct and indirect components of fitness, the degree and consequences of inbreeding and the heritability of traits under selection (Kruuk *et al.* 2000; Kruuk 2004; Szulkin & Sheldon 2008).

Anuran amphibians show a very wide array of reproductive strategies (Wells 2007). Neotropical poison frogs and their relatives (Dendrobatidae and Aromobatidae in the superfamily Dendrobatoidea) are characterized by highly complex reproductive behaviour that involves territoriality, prolonged breeding and parental care (Weygoldt 1987; Pröhl 2005; Lötters *et al.* 2007). Social and genetic monogamy, a so far unknown strategy in amphibians, has recently been documented in one dendrobatid species (Brown *et al.* 2010; Kokko & Jennions 2010). Most studies on the reproductive biology of dendrobatoids, however, have revealed polygamous mating in several species (Summers 1989, 1990; Pröhl 2002). These findings are based on behavioural observations, calling for corresponding molecular analyses of parentage. The range and complexity of reproductive behaviour in dendrobatoids make this taxonomic group highly suitable for comparative analyses of mating strategies.

Allobates femoralis is an aromobatid frog with a pan-Amazonian distribution, forming disjunct local populations (Amézquita *et al.* 2009). Both sexes are highly iteroparous within prolonged but rather discrete reproductive periods that coincide with local rainy seasons (Gascon 1993). Capture–recapture data have demonstrated that year-to-year survival in *A. femoralis* is below 20%, resulting in rather discrete generations in consecutive years (Ringler *et al.* 2009; Ursprung *et al.* 2011). During the reproductive period, males call from elevated structures on the forest floor to announce territory possession to male competitors and to attract females (Hödl *et al.* 2004). The intense calling activity and the immediate interception of intruders that call in a resident's territory indicate strong intrasexual competition for these multipurpose territories (Narins *et al.* 2003; Pröhl 2005). Females also exhibit site fidelity, but without aggressive behaviour towards conspecifics of either sex, and actively approach potential mating partners (Ringler *et al.* 2009). Pair formation, courtship and mating take place in the male's territory (Roithmair 1992). Here, externally fertilized terrestrial clutches of approximately 20 eggs are laid in the leaf litter. Females can deposit one clutch on average every 8 days (Weygoldt 1980; in captivity), whereas males were observed to attend to up to five clutches simultaneously (personal observation in the field). Tadpole transport to aquatic

sites, a synapomorphy of the Dendrobatoidea (Grant *et al.* 2006), takes place after 15–20 days and is mainly performed by males in *A. femoralis*, although occasional cases of transporting females have also been documented (Weygoldt 1987; Caldwell & Araújo 2005; personal observation). In *A. femoralis*, entire clutches are transported at once (Aichinger 1991). This might result in the patchy survival of full-sib groups. Despite paternal care, a higher potential reproductive rate for males than for females can be assumed. As male reproductive success is mainly limited by their access to females, strong intrasexual competition among *A. femoralis* males can be expected.

In this study, we use field observations and microsatellite data of two consecutive generations of adult individuals to identify the genetic mating system in *A. femoralis*. We compare the patterns of mating and reproductive success between males and females to reveal sex-specific differences in the strength of selection. We also investigate whether body size and male territory size are determinants of cross-generational reproductive success.

Material and methods

Study population

Our study population is situated in a lowland rainforest near the field camp 'Saut Pararé' (4°02'N, 52°41'W) in the nature reserve 'Les Nouragues', French Guiana. The study plot of about 8.25 ha was located on an ascending ridge, bordered by the river 'Arataye' to the south and two small creeks to the west and east (Fig. 1). To the north, we delimited the study plot at an altitude and inclination where no further individuals had been found for at least 100 m along the ridge. To account for offspring dispersal after metamorphosis and potential adult migration, we intermittently sampled an additional boundary zone of approximately 100 m beyond the eastern creek (cf. Krebs 2004). No sampling took place in the palm swamp to the west because this habitat is not used by *A. femoralis*.

Sampling

Sampling took place between 15 January and 30 April in 2008, and between 15 January and 15 March in 2009, within the reproductive period of *A. femoralis* in French Guiana, which usually lasts from December to May (Born & Gaucher 2001; Gottsberger & Gruber 2004). Surveying took place daily from 09:00 to 19:00 h, with an equal time effort spent per unit area. Individuals were identified with digital photographs of their ventral coloration patterns and sexed by the presence (male) or

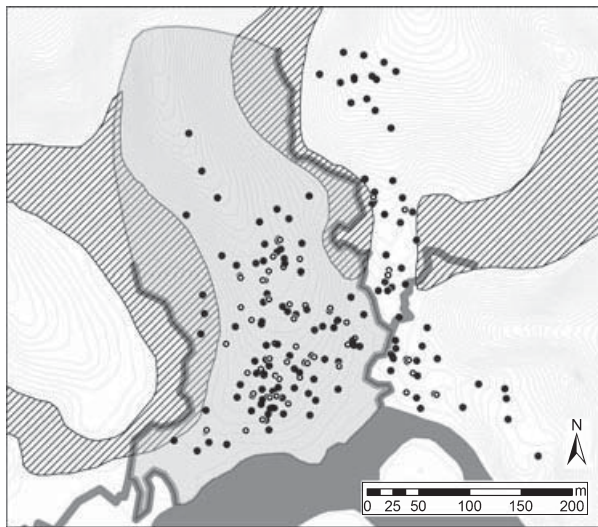


Fig. 1 Map of the study area: contour lines (1 m) in light grey; creeks and Arataye River in dark grey; study plot shaded in light grey; palm swamps hatched in black; males and females in 2008: filled and open circles, respectively.

absence (female) of vocal sacs. Body size (snout-urostyle length, SUL) was measured with standard callipers to the nearest 0.05 mm.

We attempted to sample all males and females in the study plot. Owing to different detection probabilities between the sexes, we determined the sampling coverage for each sex by calculating asymptotic population size estimates (MMMeans, Colwell *et al.* 2004), based on individual capture histories in 2008 (Figs S1 and S2 in Supporting Information), using EstimateS 8.2.0 (Colwell 2006). This estimator was chosen based on our sampling regime and the differential mobility and detectability between the sexes (Brose & Martinez 2004). The estimates were used as population sizes for males and females. This helps avoid bias in the measures of reproductive success that might arise from unequal sampling coverage (Marshall *et al.* 1998; Araki & Blouin 2005). Territorial status was assessed based on the spatial set-up of calling positions and on behavioural observations (Maher & Lott 1995). We followed the definitions of Ringler *et al.* (2009) and Gasser *et al.* (2009) and used a modified minimum area method (Harvey & Barbour 1965) to measure territory size. All spatial data were recorded in the field with the mobile GIS software ArcPad 7.0™ (ESRI) on pocket computers (Hewlett Packard iPaq™ HX4700) and further handled in ArcGIS™ 9.3 (ESRI).

DNA extraction and microsatellite genotyping

The third toe of both hind limbs of all newly encountered adults was removed and immediately preserved

in 96% ethanol (Ursprung *et al.* 2011). Genomic DNA was isolated using a Proteinase K digestion followed by a standard phenol–chloroform protocol. Microsatellite genotypes of seven loci (*Afem03*, *Afem05*, *Afem09*, *Afem12*, *Afem13*, *Afem15* and *Afem16*) were largely obtained using PCR primers and protocols described in the study by Jehle *et al.* (2008). For two loci, reverse primers differing from Jehle *et al.* (2008) provided more reliable amplification [*Afem15*: GTGCCGCTCATTAAGC TCAT; *Afem16*: TCATTGAGGTCTTAGTTTTCCTCA], yielding PCR products of 149–214 and 400–450 bp, respectively. The amplified products were diluted with water, mixed with internal size standard ROX500, run on an ABI 3130xl sequencer and analysed using PeakScanner 1.0 (Applied Biosystems). All loci were visually identified, and the final allele sizes were determined using the binning software Tandem 1.01 (Matschiner & Salzburger 2009). Ambiguous samples were re-genotyped up to five times. GENEPOP 4.0 (Rousset 2008) was used to determine expected (H_E) and observed (H_O) heterozygosities and to calculate probability tests for genotypic linkage disequilibrium. Departures from Hardy–Weinberg equilibrium at each locus were tested with the implemented Markov chain method (1 000 000 iterations) to obtain unbiased estimates of Fisher's exact test. We used CERVUS 3.0 (Kalinowski *et al.* 2007) to estimate the combined nonexclusion probability per parent pair and the null allele rate. The latter was also tested with the software MICRO-CHECKER (Van Oosterhout *et al.* 2004).

Pedigree reconstruction

Parentage assignment was carried out with COLONY 2.0 (Wang 2009), a likelihood-based method implementing a groupwise approach for sibship reconstruction to infer genealogies. The full likelihood model with medium precision and without setting a sibship prior was used, allowing for polygamous mating in both sexes. Individuals from 2008 were treated as potential 'fathers' and 'mothers', whereas all new encounters in the 2009 sample were treated as potential 'offspring'. Offspring that were inferred to be half sibs in the first analysis were re-analysed in half-sib groups. Only 'Best (ML) Configuration' assignments with the maximum likelihood obtained at the end of the computation (cf. COLONY user guide), and only parent–offspring triads for which at least one parent was found inside the study plot, were used for the subsequent analyses of reproductive contributions. Simulated parental genotypes as provided by COLONY were only included when they were identified as mating partners of parents from the study plot; for the general validity and reliability of our paternity data, see the corresponding

paragraph in the Discussion. A network graph displaying all inferred matings of parents from the study plot was constructed in CYTOSCAPE 2.6.3 (Shannon *et al.* 2003).

Measures for reproductive success and sexual selection

Based on our census size estimates (MMMeans), we used chi-square tests to test for differences in the number of mating partners and offspring between males and females. We also determined the accompanying Bateman gradients (the relative number of offspring in relation to the relative number of mating partners) for both sexes. The standardized variances in mating success (I_{mates}) and reproductive success ($I_{\text{offspring}}$) for males and females (Wade & Arnold 1980) were calculated to identify sex-specific differences in selection (AI , cf. Shuster 2009). To identify differences in the opportunity for selection among different subsets of individuals, variances in mating and reproductive success were calculated for the estimated population sizes of males and females and compared with variances obtained from successful reproducers only. Binomial skew indices B and the respective confidence intervals (Nonacs 2000) served as a measure of reproductive skew. Kruskal–Wallis and Mann–Whitney U -tests were used to test differences in male mating and reproductive success with respect to a male's territorial status, as well as differences in body size (for males and females) and territory size (males only) related to the number of mating partners and adult offspring per individual.

Results

We sampled a total of 204 and 232 adult *A. femoralis* in 2008 and 2009, respectively. Ninety-one males and 48 females were registered inside the study plot in 2008, with individuals being recaptured up to 16 times. Corresponding MMMeans estimators predicted a total of 110 males and 75 females in the study plot, corresponding to a sampling coverage of 82.7% for males and 64% for females and a sex ratio of 1.47 males per female. Sixty-two males of the parental cohort showed territorial behaviour. Median territory size was 11.01 m² (range: 0.03–57.33 m²). Three males without site fidelity were encountered several times across the study plot, and all other males were recorded only once or twice. In 2009, 22 males and 8 females from the 2008 cohort were recaptured in the study plot and the boundary zone, while 138 males and 64 females (87% of the total 2009 population) were new individuals and thus treated as potential descendants of the 2008 cohort. Mean body size (SUL)

in 2008 was 26.96 mm (SD = 1.07 mm) in males and 28.00 mm (SD = 1.10 mm) in females.

The microsatellite loci proved to be particularly polymorphic, bearing 11–22 alleles per locus (mean = 17.43) and having observed heterozygosities between 0.56 and 0.92 (mean = 0.85, Table 1). The loci *Afem05* and *Afem12* slightly deviated from Hardy–Weinberg equilibrium ($P = 0.03$ in both cases). The total proportion of loci typed over all individuals was 0.94; for more details on the microsatellite allelic data, see DRYAD entry doi: 10.5061/dryad.8714. No significant pairwise linkage disequilibria between loci were observed (P -values > 0.05). All loci had calculated null allele frequencies below <0.03 (Table 1). Accordingly, MICRO-CHECKER did not find significant evidence for large allelic dropouts or null alleles at any locus. The combined nonexclusion probability per parent pair was 5×10^{-8} .

Of the 202 putative descendants of the 2008 cohort, COLONY assigned 128 individuals (63.4%) to at least one known parent from the study plot. For 94 of these 128 individuals, both assigned parents came from the study plot. Within the 91 males and 48 females sampled in 2008, 34 males (37.4%) and 31 females (64.6%) produced at least one offspring that reached adulthood in 2009, with a resulting sex ratio in reproducers of 1.1 males per female. When including simulated parents from the COLONY analyses, and accounting for incomplete sampling by adjusting the total numbers of males and females to the MMMeans estimates (110 males and 75 females), 39 males (35.5%) and 42 females (56.0%) contributed to the following generation. The sex ratio among reproducers was 0.92.

The mating network revealed one large cluster of connected individuals and three small units of two or three individuals (Fig. 2). The number of mating partners and the number of offspring were equal among reproducing males and females (mates: median = 2, range

Table 1 Summary statistics for the seven microsatellite loci used for parentage analysis in *Allobates femoralis*

Locus	A	H_O	H_E
<i>Afem03</i>	11	0.883	0.857
<i>Afem05*</i>	17	0.555	0.613
<i>Afem09</i>	22	0.894	0.912
<i>Afem12*</i>	16	0.905	0.872
<i>Afem13</i>	20	0.897	0.905
<i>Afem15</i>	21	0.917	0.908
<i>Afem16</i>	15	0.893	0.906
Mean	17.43	0.849	0.853

A, number of alleles; H_E , expected heterozygosity; H_O , observed heterozygosity. 2008 individuals only ($n = 139$).

*Significant deviation from HWE.

1–6; offspring: median = 2, range 1–8; offspring per pair: median = 1, range 1–6).

The number of mating partners (Fig. 3a) and offspring (Fig. 3b) was marginally significantly different between males and females (chi-square test; mates: $\chi^2 = 8.895$, d.f. = 6, $P = 0.063$; offspring: $\chi^2 = 12.279$, d.f. = 8, $P = 0.077$). This can be attributed to a higher relative number of unsuccessful individuals in males ($\chi^2 = 7.648$, d.f. = 1, $P = 0.007$), whereas the number of mating partners and offspring within successful reproducers was indiscernible between the sexes (mating partners: $\chi^2 = 1.204$, d.f. = 5, $P = 0.715$; offspring: $\chi^2 = 4.472$, d.f. = 7, $P = 0.654$).

The standardized variance in mate acquisition (Table 2) was higher in males than in females ($\Delta I_{\text{mates}} = 0.67$), but the difference disappeared when considering only reproductively successful individuals ($\Delta I_{\text{mates}} = -0.04$). A similar pattern was found for the standardized variance in offspring production, which encompasses selection associated with mate acquisition and survival of offspring until adulthood ($\Delta I_{\text{offspring}} = 0.75$ for all individuals, $\Delta I_{\text{offspring}} = 0.01$ for successful reproducers, Table 2). Bateman gradients (Fig. 4) did not differ significantly between sexes ($F_{1,78} = 0.066$, $P = 0.798$), suggesting no difference in the intensity of sexual selection. B indices for skewness indicated that male and female reproductive success was significantly more skewed than would be expected at random mating ($B_{\text{♂}} = 0.0202$, 95% CI: 0.0139–0.9833; $B_{\text{♀}} = 0.0128$, 95% CI: 0.0074–0.0205) but did not differ from a ran-

dom distribution when considering only the successful individuals ($B_{\text{♂}} = 0.0035$, 95% CI: -0.0078 to 0.9666; $B_{\text{♀}} = 0.0025$, 95% CI: -0.0077 to 0.9684). No significant differences in reproductive skew were found between the sexes in either case because of an overlap of the confidence intervals.

The number of offspring and the number of mating partners were significantly higher for males that occupied territories compared with those that did not ($n_{\text{territorial/nonterritorial}} = 62/48$; offspring: $U = 1147.5$, $P = 0.016$; mates: $U = 1156.5$, $P = 0.019$, Fig. 5). SUL differed neither between males grouped by the number of mating partners or the number of offspring ($n = 89$; mates: $H = 8.994$, $P = 0.174$; offspring: $H = 6.146$, $P = 0.523$; Fig. 6a,b) nor between females grouped in the same way ($n = 46$; mates: $H = 5.686$, $P = 0.459$; offspring: $H = 7.279$, $P = 0.507$; Fig. 6c,d). Territory size also did not differ significantly between males grouped by number of mating partners or number of offspring ($n = 62$; mates: $H = 7.410$, $P = 0.285$; offspring: $H = 7.326$, $P = 0.502$; Fig. 6e,f).

Discussion

This study revealed individual genealogical relationships across two consecutive, exhaustively sampled generations of adult individuals in a wild anuran amphibian population. For *A. femoralis*, a Neotropical frog characterized by a male-biased adult sex ratio, terrestrial egg deposition and paternal care, we identified

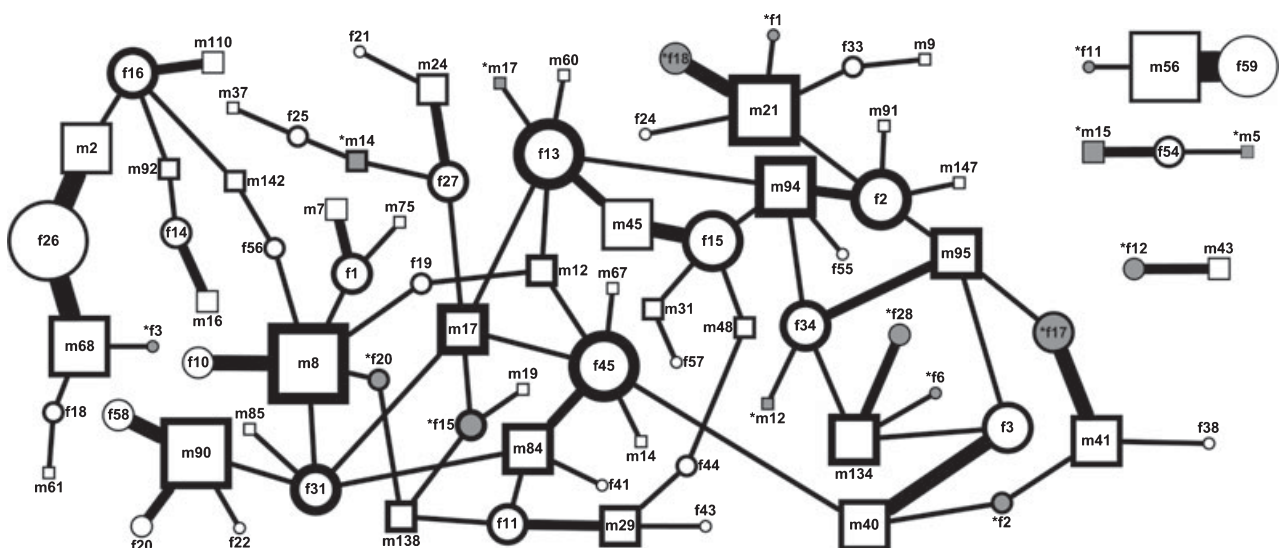


Fig. 2 Mating network in *Allobates femoralis*. Males (m) and females (f) are displayed as squares and circles, respectively; simulated genotypes (*m/*f) are shaded in grey. The symbol size represents the number of adult progeny per individual (1–8), the symbol outline width represents the number of mating partners per individual (1–6), and the width of the edges represents the number of adult progeny per parent pair (1–6). Distances and locations of nodes do not correspond to the actual spatial arrangement of individuals.

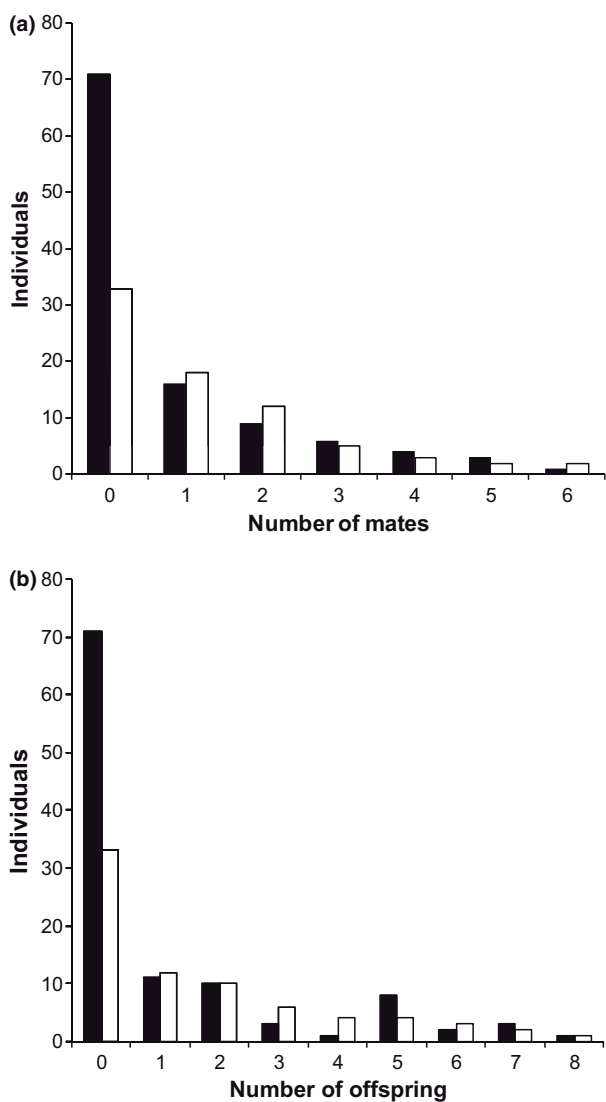


Fig. 3 (a) Distribution of mating partners per male (dark bars) and female (light bars); (b) Number of adult progeny per male (dark bars) and female (light bars) *Allobates femoralis*.

a highly polygynandrous genetic mating system with surprisingly equal numbers of reproductively successful individuals of both sexes contributing in equal measure to the following generation.

Table 2 Standardized variances in mating and reproductive success for male and female *Allobates femoralis*, calculated for all individuals and successful reproducers

	All individuals		Successful reproducers	
	♂	♀	♂	♀
Number of mating partners	$I_{\delta}^2/\text{mates} = 2.23$	$I_{\text{f}}^2/\text{mates} = 1.56$	$I_{\delta}^2/\text{mates} = 0.39$	$I_{\text{f}}^2/\text{mates} = 0.43$
Number of offspring	$I_{\delta}^2/\text{offspring} = 2.35$	$I_{\text{f}}^2/\text{offspring} = 1.57$	$I_{\delta}^2/\text{offspring} = 0.45$	$I_{\text{f}}^2/\text{offspring} = 0.44$

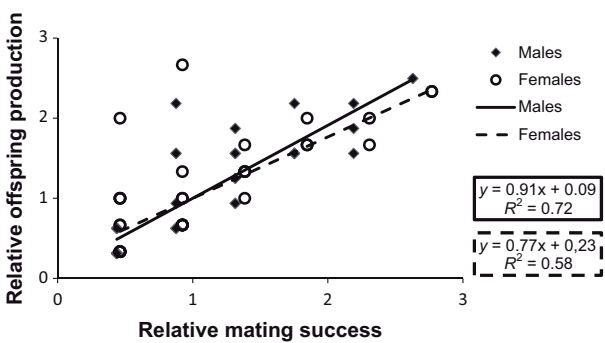


Fig. 4 Bateman gradients for males and females.

Methodological considerations

The natural boundaries of the study plot allowed us to treat the encountered individuals as a confined reproductive community. This was corroborated by the deviation of allele frequencies from Hardy–Weinberg equilibrium after including individuals from the boundary zone (heterozygote deficiency likely due to a Wahlund effect, data not shown) and by data on migration (Ringler *et al.*, in preparation). The year-to-year survival rates of 16.3% for males and 16.6% for females are similar to a neighbouring *A. femoralis* population (Ringler *et al.* 2009) and lead to rather discrete generations in consecutive years. We found no evidence for spatial differences in survival or reproductive rates throughout the study plot. By applying the survival rates to the estimated sampling coverages (males: 82.7%, females: 64%), the resulting probabilities of erroneously assigning individuals to the wrong generation are 2.8% and 6% for males and females, respectively. As none of the additional parental genotypes simulated by COLONY matched any genotypes of the 2009 cohort, we can assume that none of the unsampled parents from 2008 were still alive in 2009 and falsely assigned to the offspring cohort.

The high level of variation made the microsatellite loci sufficiently powerful for parentage assignments, despite a rather low overall number of loci used. We tested the reliability of COLONY, which is considered to generate fewer false assignments than other methods that use pairwise relationships (Wang 2004; Wang &

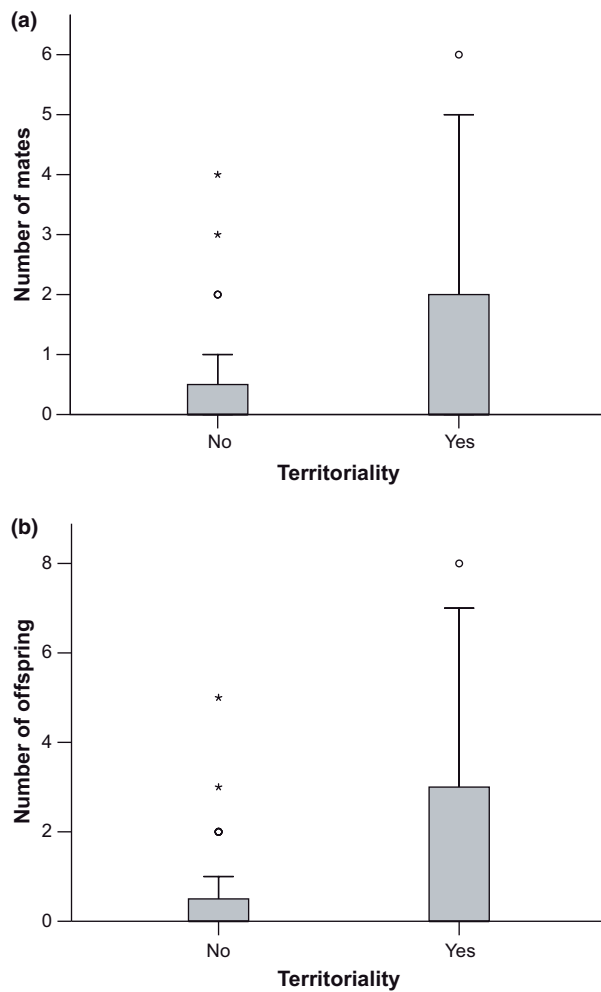


Fig. 5 Boxplots of (a) mating success and (b) offspring production of males with and without a territory.

Santure 2009; Walling *et al.* 2010). For this purpose, we analysed 133 tadpoles from 64 clutches that were haphazardly sampled in the study plot in 2008 and genotyped for a study on inbreeding avoidance (Ursprung *et al.*, in preparation). In all cases, identical parent pairs were found independently for tadpoles from the same clutch, providing no evidence for genotyping errors and supporting the reliability of the obtained parentage assignments.

The common measures for reproductive activity of individuals in populations are zygotes or early offspring stages. This helps avoid biases from later mortality and treats selection as a within-generation process. For our study, however, the sampling of eggs or larvae would have been inadequate, because our aim was to exhaustively quantify the cross-generational reproductive contributions of a large proportion of the entire population (see Rodríguez-Muñoz *et al.* 2010 and Serbezov *et al.* 2010 for a similar approach). The 64 clutches

encountered in 2008 (Ursprung *et al.* in preparation) represent only a fraction of the likely total number of clutches laid, taking into account the number of females in the population, their fecundity and the duration of the reproductive period. We are aware that, because of potentially differential survival of individuals from the zygote stage to adulthood, our measures of reproductive success do not necessarily equate with the actual number of offspring sired. However, our findings on the polygynandrous mating system, the equal reproductive contributions of both sexes and also the significance of territoriality on male reproductive success are not altered by these effects. We were able to evenly estimate population-wide cross-generational reproductive success solely by our approach of using an exhaustive sample of mature individuals of the following generation as the 'offspring cohort' (cf. Serbezov *et al.* 2010).

Mating system, cross-generational reproductive success and sexual selection

Both sexes in *A. femoralis* were highly polygamous, with very similar numbers of successful individuals contributing in almost equal measure to the following generation. Previous studies on population-wide measures of reproductive success in anurans were restricted to indirect measures derived from effective population size (N_e) estimates from temperate, pond-breeding species. They revealed values in the range of 1–10% of census population sizes (Schmeller & Merilä 2007; Broquet *et al.* 2009; Ficetola *et al.* 2010). For the first time in an anuran amphibian population, we directly identified the gene genealogies across two consecutive adult generations. In line with extended iteroparity during a long reproductive period and putatively enhanced offspring survival through parental care, we found high numbers of successful breeders in *A. femoralis* (35.5% of males, 56.0% of females). This suggests that 'typical' N_e values derived from temperate pond-breeding species might not be representative for anurans as a whole, in line with their wide array of reproductive strategies (Wells 2007).

Owing to our sampling of adult progeny, the observed levels of polygamy (up to six mating partners per male and female) constitute minimum values. The actual number of mating events is probably higher. Nevertheless, the average number of mating partners per female found in our study is one of the highest levels of polyandry reported in anurans so far (Pröhl 2002; Byrne & Keogh 2009). That 44% of females did not have mature progeny is likely due to offspring mortality rather than incompetency of these females in mate acquisition. This interpretation reflects the male-biased sex ratio and iteroparity during the long breeding

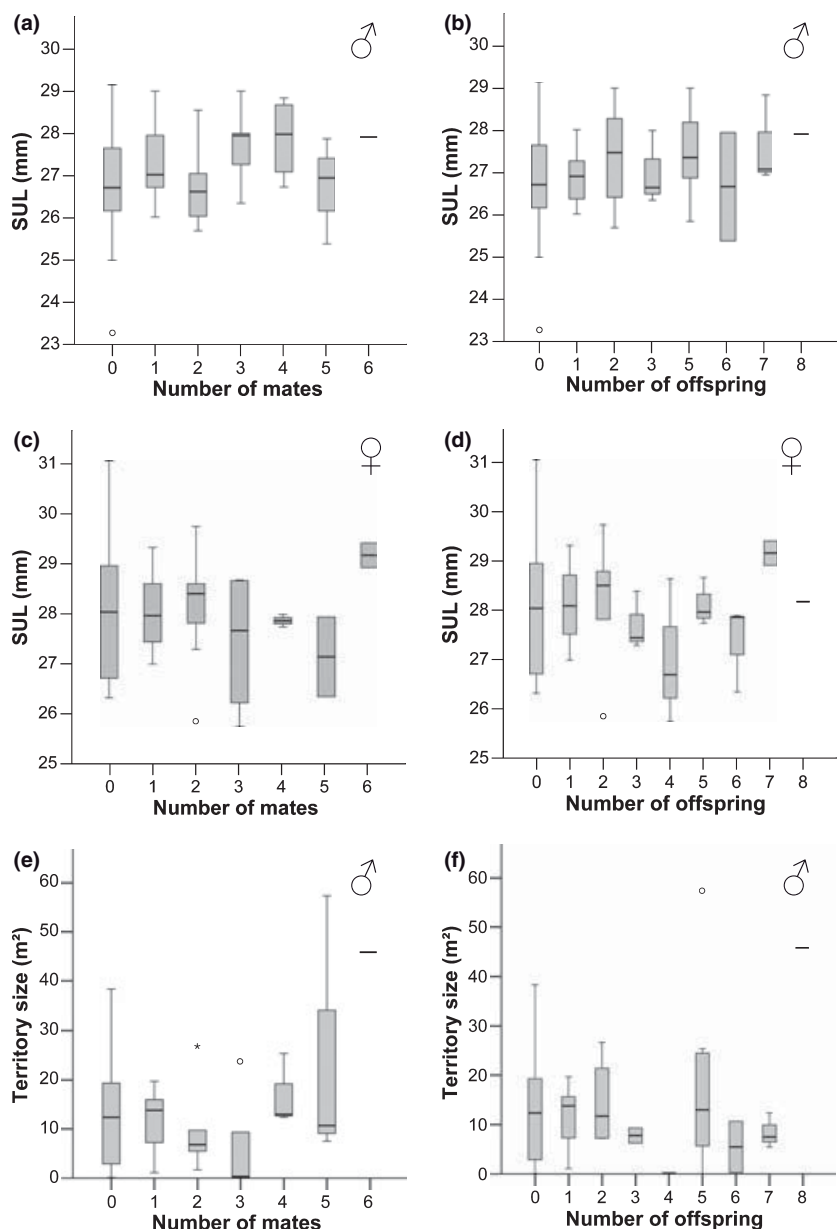


Fig. 6 Boxplots of the relationships between male and female reproductive success and specific traits: snout-urostyle lengths with respect to the number of mates per male (a) or female (c), snout-urostyle lengths with respect to the number of adult progeny per male (b) or female (d), and territory size in respect with the number of mates (e) and adult progeny (f) per male.

period. A similar amount of mortality acting on the offspring of males can be assumed.

Male and female *A. femoralis* have evolved highly contrasting mating strategies: territoriality and paternal care in males and the active approach of mating partners without maternal care in females. Nevertheless, we did not find significant differences in numbers of mating partners and adult progeny per successful male and female. The shape and location of the Bateman gradients (slopes ~ 1 , intercepts ~ 0) show that most matings are represented by only one offspring that

survived until adulthood. This contrasted with our expectation of finding larger clusters of full-sibs in the progeny, which would have resulted from patchy survival of conjointly deposited larvae from single clutches. Our unexpected result could reflect high competition or even cannibalism among tadpoles within the same pool (Summers & Earn 1999) or a bet-hedging behaviour of transporting adults where tadpoles are deposited across multiple pools (Ursprung *et al.* in preparation). Solely in one exceptional case, six offspring were assigned to the same parents. However, we

cannot discern whether these full-sibs resulted from a single mating event with incidental high survival of the whole clutch or from multiple mating events between this single parent pair.

The male bias in the standardized variances of reproductive success, calculated over all individuals of the population, suggests a higher opportunity for sexual selection to act on males than on females (Shuster 2009). Correspondingly, we found unequal binominal skew indices and marginally significant differences in the distributions of mates and offspring between males and females when considering all individuals. This effect disappeared when only actual reproducers were taken into account. Hence, we found no evidence for further sexual selection acting on successful males and females. This was also corroborated by their similar Bateman gradients. The observed differences between the sexes are because of uneven numbers of nonreproducing individuals (cf. Raffetto *et al.* 1990; Klug *et al.* 2010). This indicates that the opportunity to breed in males may be determined by binary rather than by gradual sexual selection.

Only territory possession was identified as a significant determinant of male reproductive success. The significantly higher success in territorial males corroborates previous findings that territoriality plays a fundamental role in dendrobatoid reproductive behaviour (Pröhl 2005 for the whole taxon; Roithmair 1992, 1994 for *A. femoralis*; but see also Born *et al.* 2010). For successful nonterritorial males that were only recorded once or twice, we cannot entirely rule out that actual territoriality went unobserved. Of the males without site fidelity that were observed several times in the study plot, none were successful. Potential alternative strategies of males, such as active sneaking, were never observed. Territorial advertisement might serve as an honest signal of male quality towards females, given the substantial energetic costs and risks of predation on males (Ryan *et al.* 1982; Wells 2001). For *A. femoralis*, however, we cannot discern whether females actively prefer territorial males or whether this apparent preference reflects an enhanced localizability of calling males by females, as territoriality and calling activity by males are inseparably linked.

Apart from territory possession, we found no other gradual predictors of reproductive success in *A. femoralis*. Body size did not influence mating and reproductive success in either sex, although in other anurans it is often positively related to fecundity in females (Cummins 1986; Tejedo 1992) and mate acquisition in males (Gerhardt & Huber 2002). We also found no effect of territory size on male reproductive performance, although this relation was documented in a previous field study for *A. femoralis* (Roithmair 1992). Sexual selection

appears to be based on territory possession, and females are likely to mate with any spatially proximate male that displays territory ownership (see Alonzo 2004 and Alonzo & Heckman 2010 for similar mating patterns in fish). The seemingly rather indiscriminate acceptance of females by males and the polyandrous mating of females are reflected in the highly interconnected mating network (Fig. 2).

Reciprocal polygamy

Polygamous mating occurs in a wide range of species (Shuster & Wade 2003), and molecular studies have shown that the actual levels of female polyandry can be higher than those estimated from behavioural observations (e.g. birds: Griffith *et al.* 2002; fish: Avise *et al.* 2002). Nevertheless, the underlying evolutionary mechanisms for female polyandry are still poorly understood and controversially debated (Hosken & Blanckenhorn 1999; Zeh & Zeh 2003; Simmons 2005). In dendrobatoids, polygamy has been described for several species (Summers 1989, 1992; Pröhl 2005). Moreover, it has been demonstrated that male polygyny can impose high costs on female reproductive success if the quality or quantity of male parental care declines as the number of mates per male increases (Halliday 1983; Summers 1990; Summers & Earn 1999). For *A. femoralis*, we assume that polygynandry evolved because of the likely direct and indirect benefits associated with sequential polygamy on offspring survival in unpredictable environments.

We assume that female *A. femoralis* can gain various direct and indirect benefits from sequential polyandry. Increased genetic quality or compatibility of mating partners and an increased genetic diversity among offspring (Yasui 1998; Jennions & Petrie 2000; Tregenza & Wedell 2000; Neff & Pitcher 2005; Sherman *et al.* 2009) would help insure against offspring mortality in unpredictable environments (Byrne & Keogh 2009; McLeod & Marshall 2009). Female mating strategies may also be related to male performance (sperm depletion and lower fertilization rates, Hettyey *et al.* 2009), given the potentially frequent matings during the prolonged breeding season. Finally, in *A. femoralis*, female reproductive success depends on male transporting performance and their ability to select suitable water bodies for tadpole deposition. Nonetheless, as males and females are spatially associated only during courtship, and because tadpole transport takes place several weeks after mating, we do not expect that females are able to base their mating decisions on a male's performance in parental care (cf. Yasui 2001). Rather, females can be expected to gain benefits through the sequential distribution of paternal care and the accompanying reduced risk of total brood loss through insufficient care or inadequate choice of

sites for tadpole development. Polyandry in *A. femoralis* thus might have evolved because of the reduced risk of reproductive failure and the enhanced overall offspring survival associated with the temporal and spatial spreading of eggs and tadpoles. Whether females actively allocate matings across multiple males and thereby enhance reproductive success, or whether this is merely a side effect of iteroparity and random mating, remains to be addressed in future studies.

Mating systems and parental care

The costs and benefits of parental care have been discussed in various contexts (e.g. Clutton-Brock 1991; Kokko & Jennions 2003; see Summers 1990 and Summers & Earn 1999 for dendrobatoids). The simultaneous occurrence of male parental care and female multiple mating can only evolve when the importance of paternal care for offspring survival is large in relation to the cost of lost mating opportunities and when the direct and indirect benefits of female multiple mating outweigh the direct costs (Ihara 2002). The high certainty of paternity, resulting from external fertilization and territoriality in *A. femoralis*, is a prerequisite for the evolution of male parental care (Clutton-Brock 1991; Ah-King *et al.* 2005). Given the discrepancy between potential reproductive rates and the numbers of sexually mature progeny we found in our study, future research in dendrobatoids should investigate and quantify how males and females contribute to their reproductive success beyond fertilization.

Acknowledgements

Work in the field and laboratory was funded by the University of Vienna ('Forschungstipendium' and 'Förderstipendium'), the 'Nouragues Grant' from the French CNRS, and supported through the Austrian Science Fund grant FWF-18811 (PI Walter Hödl). E Ursprung was supported by a DOC-FORTE scholarship from the Austrian Academy of Science. We want to thank Julia Felling and Hannes Kronaus for field assistance, Jinliang Wang for providing advice and running the COLONY computations, Norbert Milasowszky for statistical advice, Kyle Summers, Hope Klug, and three anonymous reviewers for valuable comments on this and a previous version of the manuscript. Kind regards to Michael Stachowitsch for language editing. Permissions and logistic help in French Guiana were provided by the CNRS and DIREN, notably Philippe Gaucher and Marc Henri Duffaud. Fieldwork was conducted in compliance with current French and EU law according to the ASAB guidelines.

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The authors are interested in a wide range of ecological and evolutionary questions, with a particular focus on amphibian populations. This manuscript is part of a collaborative PhD research project of E.U. and M.R. focussing on the reproductive biology of *Allobates femoralis*. Both PhD theses are supervised by W.H. and R.J.

Supporting information

Additional supporting information may be found in the online version of this article.

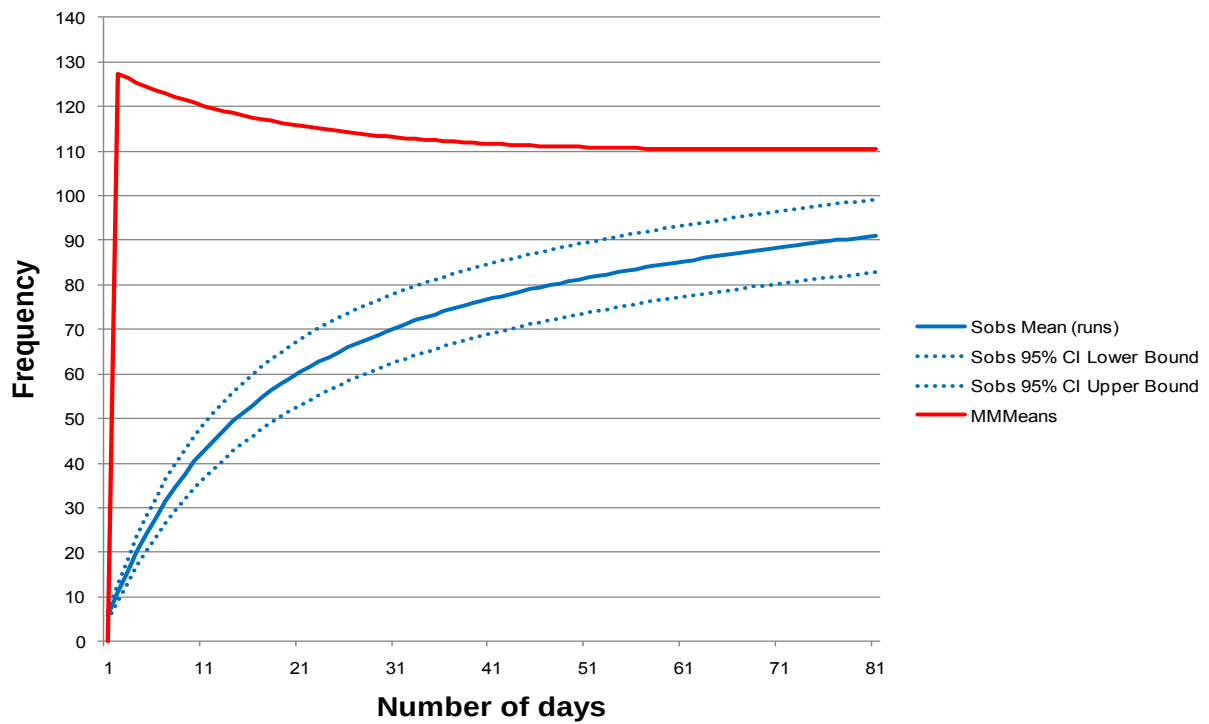
Fig. S1 Accumulation curves and estimates for male *Allobates femoralis* in the study plot in 2008.

Fig. S2 Accumulation curves and estimates for female *Allobates femoralis* in the study plot in 2008.

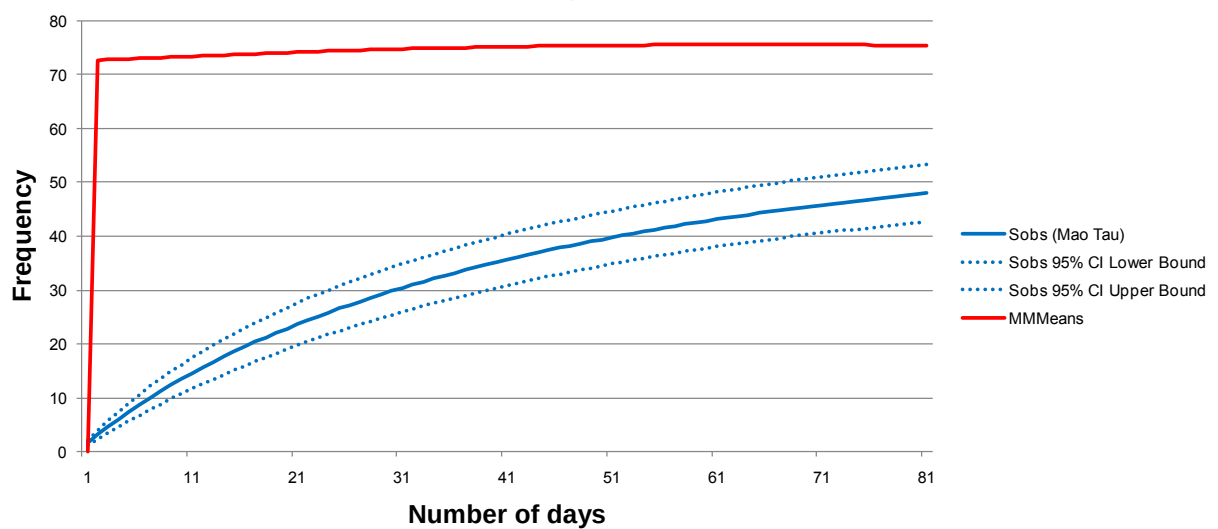
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Supplement 1

Accumulation curves and estimates for male *A. femoralis* in the study plot in 2008



Accumulation curves and estimates for female *A. femoralis* in the study plot in 2008



Selection against extremes? Parental relatedness across a natural population of a Neotropical frog

submitted to *Behavioral Ecology*: 28.07.2011

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Abstract

Strong in- and outbreeding can reduce the viability and fitness of offspring. Selection should therefore favor matings between individuals of intermediate genetic divergence. However, behavioral avoidance mechanisms are expected to evolve only if the negative effects of in- or outbreeding exceed the costs of selective mating. We used parentage assignments of embryos and adult offspring to identify patterns of parental relatedness in a natural population of the Neotropical frog *Allobates femoralis*. There was no correlation between genetic and spatial distance between adult individuals. Females mainly (in 72% of cases) chose their mating partners within a radius of 20 m. Mean pairwise relatedness of females to their inferred mating partners differed neither from the relatedness to the remainder of candidate males, nor from the values expected under random mating among all candidate males. Neither the number of clutches or embryos per clutch nor the number of adult offspring differed in respect to pairwise relatedness of mother–father dyads. Though, the variances of pairwise relatedness between females and their inferred mating partners were significantly smaller than the expected variances under random mating among candidate males. Moreover, heterozygosity was significantly lower in embryos than in the corresponding adult progeny from the subsequent year. Our results show no evidence for behavioral inbreeding avoidance in *A. femoralis*, but indicate a selective benefit of matings between partners of intermediate genetic divergence.

Keywords: *Allobates femoralis*, natural population, pairwise relatedness, reproductive success, inbreeding, outbreeding

INTRODUCTION

Mating between closely related individuals and the resulting increased homozygosity is commonly subsumed under the term ‘inbreeding’ (Jacquard 1975). Studies in the laboratory and in the wild have provided strong evidence that high levels of

inbreeding substantially reduce overall population fitness in naturally outbreeding species (Slate et al. 2000; Kruuk, Sheldon, and Merilä 2002; Frankham 2005; O’Grady et al. 2006; Johnson et al. 2010). Nonetheless, the highly varying levels of

inbreeding found in plant and animal populations do not necessarily correlate with deleterious effects (Keller and Waller 2002; Rioux-Paquette, Festa-Bianchet, and Coltman 2010; see also Crnokrak and Barrett 2002; Nebert et al. 2010). Mating between genetically very distinct individuals, on the other side of the spectrum, can also reduce reproductive success due to the risk of genetic incompatibility (Tregenza and Wedell 2000; Milinski 2003). For most natural populations, an intermediate level of genomic divergence between mating partners can thus be expected to confer maximal fitness (Bateson 1983; Neff 2004; Richard et al. 2009). In order to obtain more general estimates of natural levels of in- and outbreeding and to assess effects of in- and outbreeding depression in the wild, more research needs to be performed on populations in their natural habitats, as well as across various taxa of differing ecology (Keller and Waller 2002; Frankham 2005; Halverson, Skelly, and Caccone 2006).

A preference for mates of intermediate genomic divergence has already been documented in fish (Neff 2004), lizards (Richard et al. 2009), and birds (Arct et al. 2010). Active choice of suitable partners requires reliable detection of genetic compatibility, which might be assessed for example via olfactory cues related to MHC divergence (Penn 2002; Roberts 2009). However, most knowledge of assortative mating by relatedness has been gained from studies on inbreeding avoidance. The preference for mating partners of low genetic relatedness has frequently been documented in fish and mammals (Frommen and Bakker 2006; Archie et al. 2007; for a review see Pusey and Wolf 1996). Behavioral avoidance of close kin matings is expected to evolve only if the

negative effects of inbreeding to overall population fitness exceed the costs of searching for mates (Kokko and Ots 2006; Frère et al. 2010), and is particularly likely in species where kin recognition is also used in other aspects of social behavior (Penn and Frommen 2010). In general, the ability to avoid mating with close relatives is particularly important for monogamous species (Waser, Austad, and Keane 1986; Lehmann and Perrin 2003), small populations of long-lived species with overlapping generations (Keller and Arcese 1998), and for species with high philopatry (Wheelwright, Freeman-Gallant, and Mauck 2006). The urge to avoid mating with close relatives may therefore increase with the likelihood of close kin matings. Beside active avoidance of close-kin mates, sex-biased dispersal may also have evolved as an effective mechanism to minimize inbred matings (Lehmann and Perrin 2003; Szulkin and Sheldon 2008). Nevertheless, advantages of mating between close kin can override the effects of inbreeding depression and may even lead to a preference for inbred matings (Thünken et al. 2007).

Limited information is available on the prevalence of mate choice based on genetic relatedness in amphibians. In simultaneously polyandrous amphibians (i.e. explosively breeding or internally fertilizing species), multiple fertilizations insure against incompatibility of single mating partners (Jehle et al. 2007; Hettyey et al. 2010; Byrne and Whiting 2011), and thus may render selective mate choice, if possible at all, rather unnecessary. In externally fertilizing, prolonged breeding anurans, however, where mating events are typically spatially and/or temporally separated (Byrne and Roberts 2011), selective choice of genetically compatible partners

can provide substantial fitness benefits. So far, the recognition of kin is only known from larval and urodele amphibians (Villinger and Waldman 2008; Bos et al. 2009). But even if adult anurans are capable to discriminate between kin, a possible preference for a specific genotype or genomic divergence can still not be realized in the wild. Due to natal migration of full sib groups and/or small home range behavior of males and females, the preferred genotypes might not be among the candidate mates (Jennions and Petrie 1997).

Within anurans, dendrobatid frogs are a particularly interesting taxon for studies on mate choice and reproductive success, due to their complex reproductive behavior (Wells 2007). The Neotropical frog *Allobates femoralis* Boulenger 1883 (Dendrobatoidea, Aromobatidae) is widespread across Amazonia and forms disjunct local populations (Amézquita et al. 2009). During the prolonged reproductive period males are highly territorial (Roithmair 1992; Ringler, Ursprung, and Hödl 2009). They call from elevated structures to attract females as well as to announce territory occupancy to potential male competitors (Hödl, Amézquita, and Narins 2004), and show highly aggressive behavior against calling intruders (Narins, Hödl, and Grabul 2003). Female *A. femoralis* show site fidelity, but no aggressive behavior to individuals of either sex (Ringler, Ursprung, and Hödl 2009). Pair formation, courtship, and mating take place in the male's territory, where externally fertilized terrestrial clutches of approximately 20 eggs are laid in the leaf litter (Roithmair 1992; Montanarin, Kaefer, and Lima 2011). Tadpole transport is generally performed by males (Weygoldt 1980). A recent study showed that both sexes are highly iteroparous within a breeding season, and that

territory possession likely determines if males are actually considered as mating partners or not (Ursprung et al. 2011a).

In the present study, we used spatial data and parentage information obtained from embryo and adult offspring to infer patterns of parental relatedness in a natural *A. femoralis* population. The aim of this study was to (1) assess the relation between pairwise genetic relatedness and spatial distances across the entire *A. femoralis* population, (2) determine whether assortative mating by relatedness exists in *A. femoralis*, and (3) investigate possible effects of in- and outbreeding depression.

MATERIALS AND METHODS

Study population

Our study population is located in a lowland rainforest near the field camp 'Saut Pararé' (4°02' N, 52°41' W) in the nature reserve 'Les Nouragues', French Guiana. Sampling took place between 15 January and 30 April 2008, and between 15 January and 15 March 2009, during the reproductive period of *A. femoralis* (Born and Gaucher 2001; Gottsberger and Gruber 2004). The study plot was approximately 180 m x 450 m in size, naturally delimited by a river, two streams, and an ascending ridge (for more details see Ursprung et al. 2011a). In both years, surveys took place daily from 0900h to 1900h. We attempted total sampling of all males and females as well as of clutches in the study plot. Individuals were identified based on digital photographs of their ventral coloration patterns and sexed by the presence (male) or absence (female) of vocal sac folds. All spatial data on frogs and clutches were recorded in the field with the mobile GIS software

ArcPad 7.0™ (ESRI) on pocket computers (Hewlett Packard iPaq™ HX4700) and further handled in ArcGIS™ 9.3 (ESRI). Capture-recapture studies in this and another *A. femoralis* population have shown that year-to-year survival is below 20%, resulting in rather discrete generations in consecutive years (Ringler, Ursprung, and Hödl 2009; Ursprung et al. 2011a,b).

All necessary permissions were provided by the CNRS and DIREN. Fieldwork was conducted in compliance with current French and EU law with respect to the ASAB guidelines.

Tissue sampling, genotyping and parentage analysis

Detailed descriptions of the sampling procedures for all adult individuals are given in Ursprung et al. (2011a,b). To obtain the embryos required for the present study, we spent 10 min to search the leaf litter for clutches after every caught adult in 2008. Two embryos from every clutch that was found were preserved in 96% ethanol as soon as the yolk sac was no longer visible (10–15 days of development, Weygoldt 1980). Genomic DNA was isolated using a Proteinase K digestion followed by a standard phenol-chloroform protocol. PCR amplification of seven highly polymorphic microsatellite loci, genotyping and checking of genotyping errors followed the procedures described in Ursprung et al. (2011a).

We carried out all parentage assignments with the software COLONY v.2 (Wang 2009), a likelihood-based method implementing a group-wise approach for sibship reconstruction to infer genealogies. Parentage data from adults representing two successive generations (2008 and 2009), were already available from Ursprung et al. (2011a). For the present study, we used an

identical approach to infer parentage of embryos sampled from clutches to their parents in 2008. Each embryo was treated individually without prior information about assumed full sib relationship of tadpoles from identical clutches.

Relatedness estimates

Pairwise relatedness coefficients r (Queller and Goodnight 1989) for every possible male–female pair in the parental generation (2008) were determined with KINGROUP (Konovalov, Manning, and Henshaw 2004). This coefficient can be interpreted as a continuous measure of the overall genetic similarity between two individuals within a population. Values range from -1 to $+1$, with negative (positive) values indicating that two individuals have a lower (higher) probability of recent coalescence than random dyads within the population (Queller and Goodnight 1989; Blouin 2003; Konovalov and Heg 2008). We used the simulation function in KINGROUP, based on the allele frequencies of our genotype data, to estimate the expected relatedness among 100 full siblings, 100 half siblings, and 100 ‘unrelated’ individuals in order to obtain reference intervals for closely related individuals. Pairwise relatedness is expected to average 0.5 for full sibs, 0.25 for half sibs, and zero as the population mean (sensu Blouin et al. 1995 and Konovalov and Heg 2008). The overall performance of this coefficient is expected to increase with sampling coverage, and was found to be accurate even in cases where low numbers of loci with few alleles were used (Konovalov and Heg 2008). Given our attempt of total sampling of adult *A. femoralis* (see Ursprung et al. 2011a for details), we expected this estimator to be very suitable for our analyses. In order to evaluate the congruence of the parentage

and the relatedness estimates, we additionally calculated pairwise relatedness values between all adult individuals that were sampled in 2009. According to the sibship status inferred from the pedigree, we calculated mean parental relatedness of those individuals that were alleged full and half sibs, respectively, and compared the resulting measures to the values obtained by the KINGROUP simulations.

Spatial distribution of pairwise relatedness

To account for possible spatial effects on mating decisions (e.g. males that are spatially closer to a given female could have a higher probability of being chosen as a mate), we distinguished between three different sets of candidate males for each female. Initially, we treated all males inside the study plot as candidate males for each female ('all ♂'). Alternatively, we considered all males that were within 20 m of a female as candidate males ('♂ within 20 m'), a distance chosen based on observational data about female displacement from their resting sites during courtship (mean $\pm$ SD = 12.21 $\pm$ 5.14 m, range 5.24–23.48 m; Ringler, Ursprung, and Hödl 2009). Finally, for a conservative approach, we determined all immediate neighbors to each female ('♂ neighbors') by creating Voronoi tessellations (Voronoi 1907) based on all locations of males and females, respectively, with XTools Pro 7.1 (Data East 2007) in ArcGIS™ 9.3 (ESRI). All areas that belonged to a certain individual were topologically joined, in order to create a 'Voronoi area' for every individual. Only those males whose Voronoi area overlapped with that of a given female were assigned as direct neighbors to that female. Spatial distances between individuals were

calculated as the distance between the centroids of their Voronoi areas.

To identify a possible correlation between genetic relatedness and spatial distance, we compiled diagonal matrices of both values and tested for correlations between the two matrices using (partial) Mantel tests (Mantel 1967; Anderson and Legendre 1999). This approach allowed us to test all possible types of dyads (male–male, female–female, female–male) for significant correlations between spatial distance and genetic relatedness, while controlling for the effects of the dyads not of interest (cf. Wagner et al. 2007). All partial Mantel tests were calculated with the software zt (Bonnet and Van de Peer 2002), with 100,000 randomized permutations of the residual matrix.

Analyses of parental relatedness

To infer patterns of relatedness between successful reproducers, we used the embryo sample obtained for the present study as well as the cross-generational pedigree of adult individuals previously published in Ursprung et al. (2011). Hence, we were able to identify successful mating events of the parental generation from 2008 through immediate reproductive success and through sexually mature offspring recorded in 2009. Initially, we conjointly analyzed mating information that was gained through both datasets ('combined'), in order to use as much available mating information as possible. Since offspring assigned to the same parents could have resulted from single or from multiple mating events, only binary mating information, e.g. whether a male has been identified as a mating partner of a given female or not, was used. Alternatively, we also analyzed the parentage

information that was gained in each dataset ('embryo' and 'pedigree') separately to identify differences in parental relatedness across the two datasets. As single clutches could be treated as discrete mating events, information on repeated mating between identical partners in the parental generation was incorporated in tests involving only the embryo dataset.

To obtain means and unbiased variances of pairwise relatedness under random mating for each female, we generated 100,000 sets of mating partners randomly drawn from each of the three spatial sets of candidate males. The number of males per set equaled the actual number of actually observed mating partners of a given female. Randomizations without or with replacement were applied to generate values that correspond to the binary mating information or to allow for multiple mating between identical pairs. The permutations and calculations were performed in R (www.r-project.org, R Development Core Team 2011).

To investigate the possibility of assortative mating based on relatedness, we tested whether the observed mean relatedness of females to their actual mating partners differed from the relatedness of females to the remainder of the candidate males, or from what could be expected under random mating with all candidate males. Additionally, we tested whether the observed variances in pairwise relatedness of females to their actual mating partners differed from variances expected under random mating. All analyses were performed in a paired design using the Wilcoxon-*W* test, to account for the differences in pairwise relatedness between females and their respective candidate males.

We used Mann-Whitney *U* tests to investigate if the pairwise relatedness of successful pairs differed from the relatedness of females to the remainder of candidate males, or from randomly expected values. Furthermore, we tested for each female if the deviation of observed pairwise relatedness values from random values differed from the deviation of observed values from the mean relatedness of the entire population. This enabled to distinguish between effects of individual female preferences for specific genotypes and general differences in female relatedness to their actual mates, to the remainder of candidate males, and to randomly generated partners. For all these tests we used mating information obtained from the combined dataset to include as much individual mating information as possible, and mating information based on the embryo dataset to account for cases in which females chose specific males multiple times, respectively. All tests were performed across the three categories of candidate males. Finally, we investigated the relation between parental relatedness and the number of clutches observed, the number of embryos per clutch, or the number of adult progeny produced.

RESULTS

In 2008, we recorded 139 adult *A. femoralis* (91 males and 48 females) and found 63 clutches across the study plot. Clutches contained on average 14.46 embryos (min = 3, max = 22). The two embryos analyzed per clutch (126 in total) thus represent 17% of the embryos found.

Table 1. Summary statistics for the seven microsatellite loci used for parentage analysis in *Allobates femoralis*.

Locus	parents*			embryos			adult progeny		
	<i>N</i> = 139			<i>N</i> = 126			<i>N</i> = 128		
	<i>A</i>	<i>H_O</i>	<i>H_E</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>
Afem03	11	0.883	0.857	11	0.857	0.838	11	0.885	0.859
Afem05	17	0.555	0.613	16	0.437	0.48	14	0.63	0.625
Afem09	22	0.894	0.912	21	0.556	0.853	20	0.857	0.901
Afem12	16	0.905	0.872	16	0.881	0.854	17	0.935	0.882
Afem13	20	0.897	0.905	18	0.603	0.915	16	0.789	0.907
Afem15	21	0.917	0.908	18	0.643	0.891	18	0.839	0.891
Afem16	15	0.893	0.906	15	0.698	0.895	14	0.888	0.902

A, number of alleles; *H_E*, expected heterozygosity; *H_O*, observed heterozygosity

*data from Ursprung et al. (2011a)

The embryos showed significantly lower heterozygosities than the adult offspring (Wilcoxon-*W* test; *W* = -2.366; *P* = 0.018; Table 1), at non-significantly different numbers of alleles per locus (Wilcoxon-*W* test; *W* = -1.518; *P* = 0.129). The KINGROUP simulations calculated an average pairwise relatedness of $r \pm SD = 0.489 \pm 0.162$ for full siblings, $r \pm SD = 0.236 \pm 0.156$ for half siblings, and $r \pm SD = 0.003 \pm 0.127$ for ‘unrelated’ individuals (Figure 1). In comparison, the mean parental relatedness coefficients of identified full sibs and half sibs from 2009 were 0.41 (*N* = 44, *SD* = 0.18) and 0.21 (*N* = 89, *SD* = 0.14), respectively, well within the ranges obtained by the KINGROUP simulations.

Parentage assignments

COLONY always assigned both embryos from a given clutch to an identical parent pair. For 61

clutches (98.4%), paternity was assigned to the male that had been spatially closest at the presumed time it was sired (median father–clutch distance = 0.97 m); the two remaining clutches had father–clutch distances of 3.61 m and 4.39 m, respectively. Maternity was in 89% of cases (56 clutches) assigned to a spatially close female (median mother–clutch distance = 2.73 m), whereas in 11% of cases, mothers had remained unsampled and were simulated by COLONY. Both parents from 2008 had already been assigned to 128 adult individuals from the 2009 cohort in a previous study (Ursprung et al. 2011a). From both datasets, only mother–father dyads for which both parents were sampled were included in the subsequent analyses. We therefore considered 43 parental dyads, which were identified through the embryo dataset, and 66 parental pairs identified from the pedigree dataset. The parentage analyses

identified successful matings for 41 out of 48 (85.4%) females, and for 45 out of 91 (49.5%) males. On average, 64% of the mating partners per female were neighboring males, 72% were within 20 m distance (including neighboring males), and 28% were more than 20 m away. Four females

mated exclusively with partners that were neither direct neighbors nor situated within 20 m distance. Twenty-one females mated exclusively with males within 20 m distance, and 18 of these mated exclusively with direct neighbors.

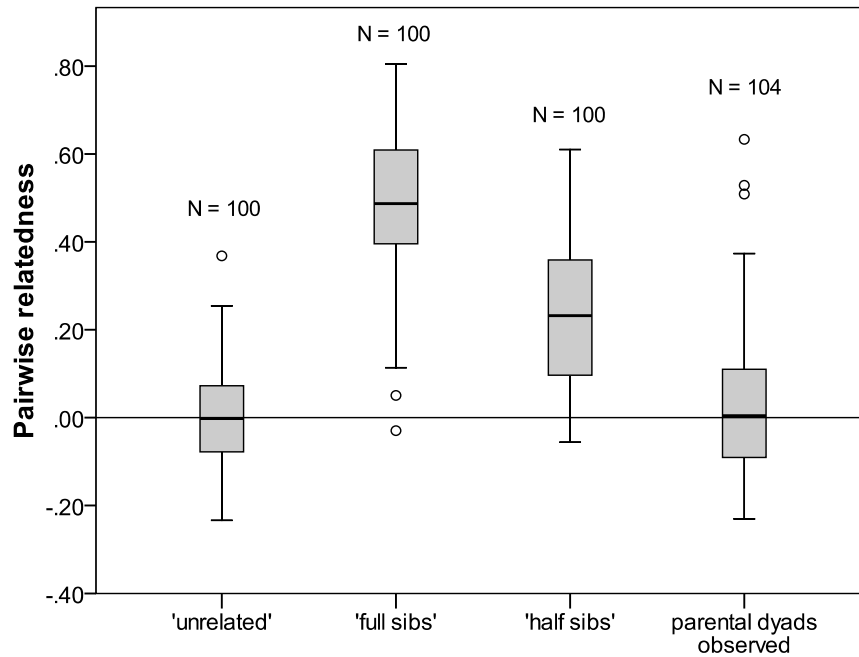


Figure 1. Pairwise relatedness values for the KINGROUP simulations ('unrelated', 'full sibs', 'half sibs') and for parental dyads observed in this study.

Spatial distribution of pairwise relatedness

There were no significant correlations between spatial distance and relatedness for the entirety of adult dyads in the parental generation (Mantel test; $r = -0.007$, $P = 0.344$), or when we separately analyzed the sexes (partial Mantel test; female–male: $r = -0.007$, $P = 0.347$, female–female: $r = -0.007$, $P = 0.344$, male–male: $r = -0.006$, $P = 0.357$). Mean pairwise relatedness for all 4368 possible male–female dyads within our population was -0.006 (s.d. = 0.153). In the whole population, 2% of candidate males per female were full sibs to

that female ('♂ within 20 m': 2.1%; '♂ neighbors': 2.4%), and 17% of candidate males were half sibs ('♂ within 20 m': 14%; '♂ neighbors': 15%). According to the pairwise relatedness values of parental dyads, 2.4% of matings took place between full sibs, 15.2% between half sibs, and 82.4% between 'unrelated' individuals.

The median pairwise relatedness values did not significantly differ between pairs that were inferred from the embryo sample and pairs that were inferred from the pedigree dataset (Mann-

Whitney U test, $N_{\text{embryo,pedigree}} = 43,66$, $U = 1238.5$, $P = 0.263$; Levene test, $F_{1,107} = 1.235$, $P = 0.269$). The median relatedness of actual parental dyads of neither sample differed from the relatedness of females to all males (Mann-Whitney U test; clutches–all, $N_{\text{embryos,all}} = 43,48$, $U = 988$, $P = 0.727$; pedigree–all, $N_{\text{pedigree,all}} = 66,48$, $U = 1439$, $P = 0.405$). Mean pairwise relatedness of females to their mating partners beyond 20 m distance differed neither from the relatedness to mating partners located within 20 m (Wilcoxon- W test; $N = 19$, $W = -0.563$, $P = 0.573$), nor from the relatedness to mating partners that were immediate neighbors ($N = 19$, $W = -0.121$, $P = 0.904$).

Analyses of parental relatedness

Mean pairwise relatedness of females to their chosen mating partners did not differ from the mean pairwise relatedness to the remaining candidate males and from mean values of randomly generated samples regardless of statistical approach and dataset (Table 2). The deviation of observed to random values per female did not significantly differ from the deviation of observed values to the mean relatedness of the entire population (Mann-Whitney U test; ‘all ♂’: $U = 1152$, $P = 1$; ‘♂ within 20 m’: $U = 684$, $P = 0.996$; ‘♂ neighbors’: $U = 641$, $P = 0.937$; Figure 2).

Table 2. Analysis of parental relatedness

dataset	Mean		Variance	
	combined	embryo	combined	embryo
all ♂	$N = 41$	$N = 30$	$N = 27$	$N = 16$
mate/non-mate	$W = -0.03$, $P = 0.97$ $U = 81$, $P = 0.75$	–	–	–
mate/random	$W = -0.06$, $P = 0.95$ $U = 94$, $P = 0.84$	$W = -0.59$, $P = 0.56$ $U = 37$, $P = 0.34$	$W = -3.39$, $P = 0.001$ $U = 132$, $P < 0.001$	$W = -3.52$, $P < 0.001$ $U = 1$, $P < 0.001$
♂ within 20m	$N = 37$	$N = 29$	$N = 19$	$N = 16$
mate/non-mate	$W = -0.49$, $P = 0.62$ $U = 84$, $P = 0.97$	–	–	–
mate/random	$W = -0.17$, $P = 0.86$ $U = 87$, $P = 0.99$	$W = -0.25$, $P = 0.804$ $U = 368$, $P = 0.54$	$W = 3.09$, $P = 0.002$ $U = 47$, $P < 0.001$	$W = -3.41$, $P < 0.001$ $U = 23$, $P < 0.001$
♂ neighbors	$N = 36$	$N = 27$	$N = 17$	$N = 15$
mate/non-mate	$W = -0.17$, $P = 0.86$ $U = 82$, $P = 0.84$	–	–	–
mate/random	$W = -0.36$, $P = 0.72$ $U = 78$, $P = 0.55$	$W = -0.36$, $P = 0.79$ $U = 346$, $P = 0.93$	$W = -2.23$, $P = 0.03$ $U = 38$, $P = 0.006$	$W = -3.18$, $P < 0.001$ $U = 26$, $P < 0.001$

Wilcoxon- W and Mann-Whitney U tests for differences in mean and variance in pairwise relatedness between females and their chosen mating partners and the remainder of candidate males (mate/non-mate), and randomly generated samples of equal size (mate/random), respectively, among all three candidate male categories.

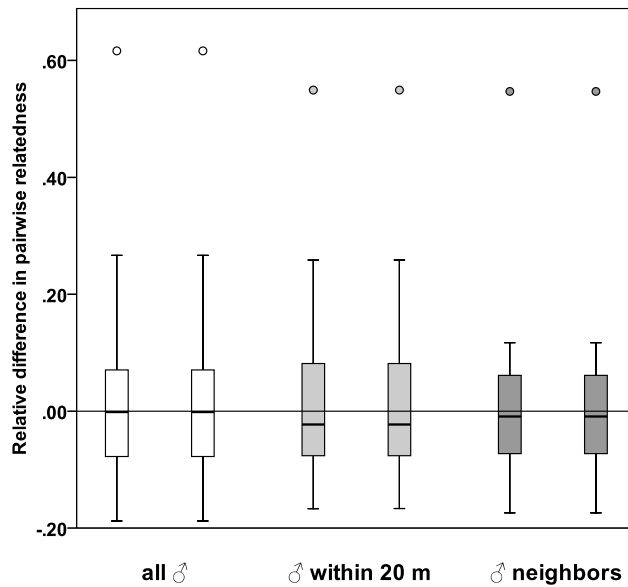
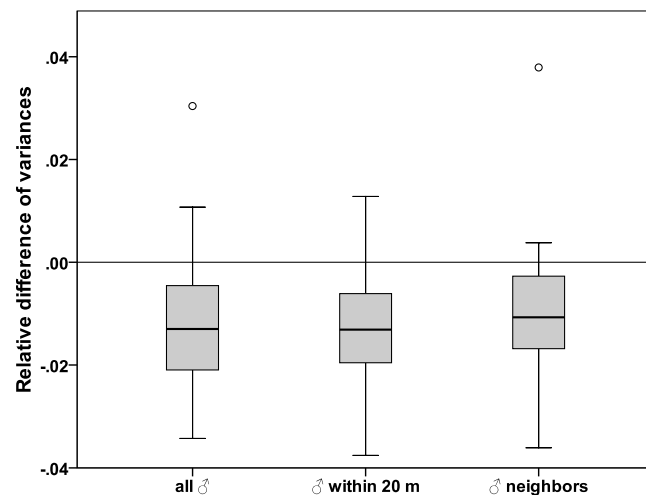


Figure 2. Differences in the deviation of mean pairwise relatedness.

Boxplots display the deviation of female relatedness to actual mating partners from random values per female (left column) and from the average mean value of pairwise relatedness within the whole population (right column), in all three spatial categories of candidate males (all ♂, ♂ within 20 m, ♂ neighbors). Zero indicating no difference of observed values to random values and the population mean, respectively.

Figure 3. Deviation of variances in pairwise relatedness of observed parental dyads from values expected under random mating.

Boxplots display relative differences between variances of pairwise relatedness of females and their mating partners and variances of randomly generated dyads for all three spatial categories of candidate males (all ♂, ♂ within 20 m, ♂ neighbors). Zero indicating no difference between observed and random variances.



The variance in pairwise relatedness was significantly lower between each female and her observed mating partners than what would be expected under random mating among candidate males (Table 2, Figure 3). Pairwise relatedness of mother–father dyads did not significantly differ in respect to the number of clutches (Kruskal-Wallis

test; $N = 43$, $H = 2.496$, $df = 2$, $P = 0.287$, Figure 4a) and the number of adult offspring produced ($N = 66$, $H = 3.743$, $df = 4$, $P = 0.443$, Figure 4b). The number of embryos per clutch did not correlate with pairwise relatedness of mates ($r = 0.044$, $P = 0.749$, Figure 4c).

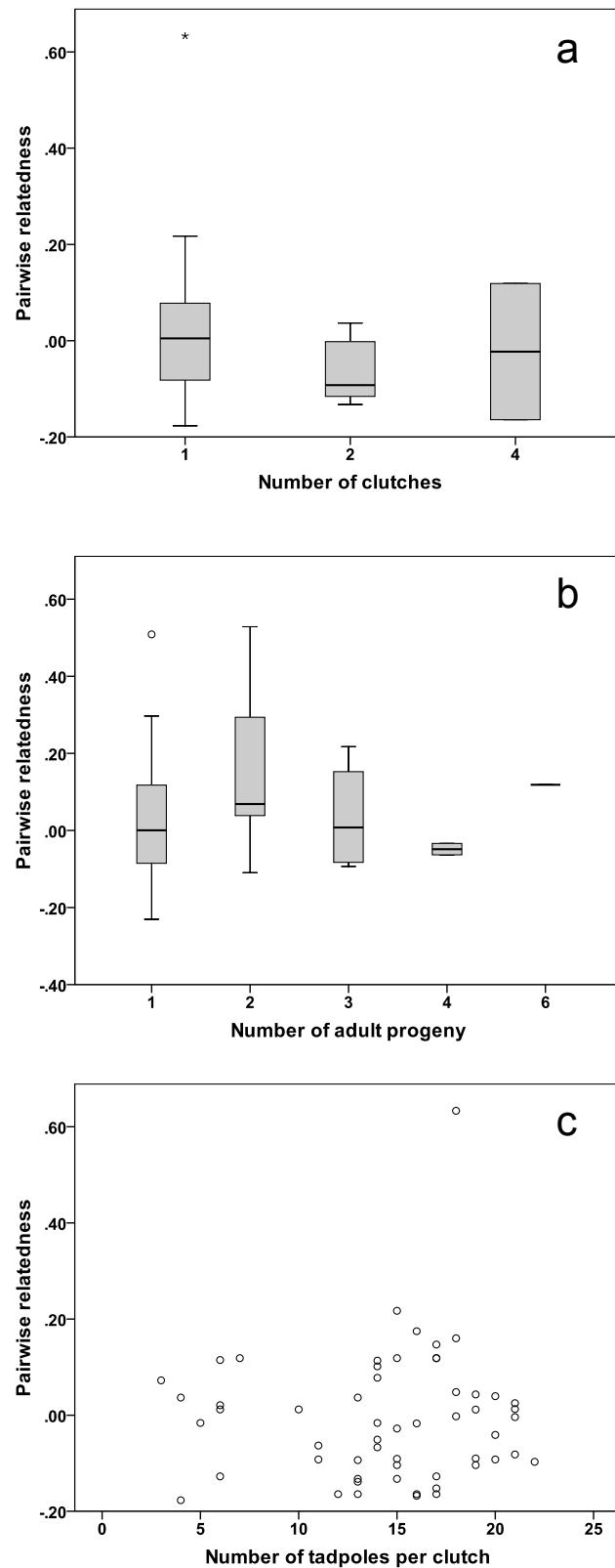


Figure 4 a) Boxplots of pairwise relatedness values of male-female dyads in regard to their number of clutches produced; b) Boxplots of pairwise relatedness values of male-female dyads in regard to their number of adult progeny produced, c) Scatterplot of pairwise relatedness of male-female dyads and their number of tadpoles produced.

DISCUSSION

The present study investigated patterns of parental relatedness across a natural population of the Neotropical frog *A. femoralis* across two generations. We inferred parentage at the embryo and adult stage to obtain two representative samples of reproductive success. The stepwise restriction of candidate males allowed us to discern between spatial and genetic effects on mating patterns.

COLONY always assigned identical parents to embryos from a given clutch, providing no evidence for multiple paternities within clutches. We assume that multiple fertilizations of clutches are largely precluded by the extended courtship behavior (cf. Montanarin, Kaefer, and Lima 2011), and by the fact that mating takes place in the territories of males from where competitors are vigorously repelled. We were able to identify mating success for 85.4% of females and 49.5% of males in the population. These values exceed the findings from our previous study based on adult offspring (Ursprung et al. 2011a: 56.0% and 35.5%, respectively), resulting from mortality between the embryo and adult stage. Females mainly mated with males within 20 m distance, which corroborates observational data on female movement during courtship (Ringler, Ursprung, and Hödl 2009). For the four females that mated exclusively with males further than 20 m, we cannot rule out that shifts of resting sites might have occurred prior to or after our observation period.

We found no correlation between genetic and spatial distance between individuals of the *A. femoralis* population. Pairwise genetic relatedness of females to males within 20 m distance and to

immediate neighbors did not significantly differ from the totality of males within the population. This indicates a random to hyper-dispersed distribution of genotypes within the population, likely caused by larval transport to water bodies and sexually unbiased dispersal of juveniles (Ringler, Ursprung, and Hödl 2009). However, males situated far from a given female are presumably less likely to be chosen as mating partners. To account for this effect, all analyses were performed across three spatial categories of candidate males. The spatial distribution of relatedness across all individuals suggests a rather low risk of inbreeding in the focal *A. femoralis* population. As large groups of full sibs are precluded by the high polygynandry and a low survival of embryos until adulthood (Ursprung et al. 2011a), the likelihood of full sib matings of about 2.4% is accordingly low. Previous studies also revealed that annual survival of adult *A. femoralis* is below 20% (Ursprung et al. 2011a,b; Ringler, Ursprung, and Hödl 2009). As a consequence, most parents will have died before their progeny have become reproductively active, and also parent–offspring matings will be likely rare.

In all analyses, mean pairwise relatedness of females to their mating partners did not differ from the relatedness to all other candidate males, or from what could be expected under random mating among candidate males. Also the mean values of pairwise relatedness of actual mates did not significantly differ from the overall pairwise relatedness across the population. This neither necessarily demonstrates that females are unable to recognize closely related individuals, nor does it exclude the possibility of a preference for unrelated or outbred males. Given that the

proportion of full and half sib matings corresponded to the number of full and half sibs among the candidate males, our results however show that females do not avoid full and half sibs as mating partners. Kin recognition in terrestrial amphibians is currently known only from larvae and early metamorphs, where it may have evolved due to the selective benefits of anti-predator behavior (Cornell, Berven, and Gamboa 1989; Blaustein and Waldman 1992; but see also Waldman et al. 1992). A previous study in *A. femoralis* has shown that the advertisement call might allow for individual discrimination (Gasser, Amézquita, and Hödl 2009). It remains to be investigated whether acoustic cues allow for kin recognition and whether females actually make mating decisions based on them.

Sequential mating with multiple partners can compensate for negative effects of single mating events (cf. Colegrave, Kotiaho, and Tomkins 2002; Roberts and Gosling 2003; Byrne and Roberts 2011). Thus, incestuous matings might still be preferable over not mating at all in iteroparous and polygamous species (Kokko and Ekman 2002; Kokko and Ots 2006; Thünken et al. 2007). In *A. femoralis* females, the cost of inbreeding avoidance in terms of lost breeding opportunities and the effort necessary to search for non-kin may exceed the costs of inbreeding (cf. Pärt 1996; Jamieson et al. 2009). We hypothesize that relatedness-based mate choice may not have evolved in *A. femoralis*, due to the low likelihood of mating with close relatives, and the comparatively high costs of active inbreeding avoidance (Kokko and Ots 2006; cf. Lampert et al. 2006 for *Physalemus pustulosus*).

Although no differences in mean values were found in all analyses (Table 2, Figure 2), the

variances of pairwise relatedness between parental pairs were significantly smaller than expected under random mating, regardless of whether analyses accounted for differences between females or not (Table 2, Figure 3). This suggests sexual and/or natural selection leading to a relative increase in reproductive success of mating partners with intermediate genomic divergence. Our study setup did not allow for distinguishing between active choice for mating partners based on their genetic suitability and natural selection acting against in- and highly outbred offspring. However, the significantly lower heterozygosity in the embryo compared to the adult progeny sample indicates that natural selection might act against offspring with in- and outbred genotypes. Interestingly, despite this selective process, the significantly lower variances of pairwise relatedness between parental dyads were already apparent in the embryo stage. Also the highly polyandrous mating behavior of *A. femoralis* females suggests random mating rather than sexual selection for mates of intermediate relatedness. The lack of a significant relation between parental relatedness and reproductive output in terms of clutches, embryos per clutch, or adult offspring produced might be due to the circumstance that only successful mating events could be considered in this study. We hypothesize that the polygynandry in *A. femoralis* may act as a bet-hedging mechanism that insures against negative effects of incompatible or inoptimal matings (cf. Tregenza and Wedell 2002; Byrne and Keogh 2009). Consequently, in- and outbreeding depression will probably have only a marginal effect on the reproductive performance of the whole *A. femoralis* population and thus exert a negligible selective pressure to trigger the

evolution of kin recognition in this species. As selection may differentially impact different larval stages (Ficetola et al. 2011), and because typically 80–95% of mortality in anuran amphibians occurs between hatching and metamorphosis (Vonesh and De La Cruz 2002), further studies under controlled conditions are needed to investigate the effects of parental relatedness on offspring survival at different life-history stages.

FUNDING

This work was supported by the University of Vienna ('Forschungsstipendium' and 'Förderstipendium'), the 'Nouragues Grant' from

the Centre national de la recherche scientifique, and the Austrian Science Fund ([FWF]: P18811 to W.H.). E.U. was supported by a DOC-fORTE scholarship from the Austrian Academy of Science.

ACKNOWLEDGEMENTS

We want to thank Julia Felling and Hannes Kronaus for field assistance and Philippe Gaucher for logistic help in French Guiana. Kind regards to Norbert Milasowszky and Hans Leo Nemeschkal for statistical advice, to Dustin Penn and Joachim G. Frommen for helpful comments on the manuscript, and to Michael Stachowitsch for language editing.

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Short notes

Toe regeneration in the neotropical frog *Allobates femoralis*

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Toe-clipping is a standard method for marking and tissue sampling in amphibians, and in most adult anurans such marks are permanent. Here we document the consistent regeneration of toes in the aromobatid frog *Allobates femoralis* during a three-year population study. The emergence of new toe discs was observed after about two months. After one year the regrown toes had recovered to 65.6%/63.8% (males/females) of the size of unclipped toes and after two years they had attained 74.0%/69.0%. Whereas toe discs before amputation were white dorsally, all but one regenerated toe discs were dark. We did not detect any malformations or infections of the digits. Recapture rates of toe-clipped individuals were indiscernible from those of a nearby population where no toe clips were taken. We discuss a possible link between toe regeneration ability and life-history attributes.

Key words: amphibians, Aromobatidae, dendrobatid,
toe clipping

In studies of natural amphibian populations, toe regeneration is commonly reported from newts (Smith, 1978) and salamanders (Ott & Scott, 1999; Davis & Ovaska, 2001), whereas adult anurans are generally assumed to lack this ability (Halliday, 1996). Nevertheless, field studies occasionally report the regeneration of toes in anurans in an anecdotal way (Ovaska & Hunte, 1992; Jungfer & Weygoldt, 1999; Richter & Seigel, 2002), and often only juveniles or just a small proportion of adults are involved (Hoffman et al., 2008; Campbell et al., 2009; Fong et al., 2010).

Toe-clipping is a common, cost-efficient procedure of marking amphibians in the field (e.g. Donnelly et al., 1994; Schmidt & Schwartzkopf, 2010), and removed toes can also be stored as tissue samples for DNA analysis (Gonser & Collura, 1996). In species that have the capacity for restitutive regeneration, toe-clipping does not offer a permanent mark for demographic studies (Ovaska & Hunte, 1992; Jungfer & Weygoldt, 1999). Furthermore, lower recapture rates in toe-clipped animals (McCarthy

& Parris, 2004; but see Lüddecke & Amézquita, 1999), as well as observations of infections and malformations of clipped toes (Golay & Durrer, 1994; Lemckert, 1996; Reaser & Dexter, 1996; Davis & Ovaska, 2001) have led to questioning of the ethics of toe-clipping in field studies (May, 2004). However, the extent of any negative impact may depend to a large extent on the species' morphology and behaviour (Lemckert, 1996; Funk et al., 2005; Liner & Smith, 2007), the number of toes clipped (McCarthy & Parris, 2004) and how clipping is conducted (Funk et al., 2005). While the use of buccal swabs with cotton buds, a tissue sampling method for DNA analysis, is easy only in larger species (Broquet, 2007), the lack of feathers, hair or sloughs in anurans offers no alternative to sampling methods that are to some extent invasive. In the present paper we document a case of consistent toe regeneration during a three-year population study on the aromobatid frog *Allobates femoralis*.

Allobates femoralis has a pan-Amazonian distribution with disjunct local populations. All activity in this species takes place on the forest floor (Hödl et al., 2004; Amézquita et al., 2009; Ringler et al., 2009). Our study population was located in a lowland rainforest near the Saut Pararé field camp (4°02'N, 52°41'W) in Les Nouragues nature reserve, French Guiana. The study plot comprised about 8.25 ha. Daily surveying took place from 15 January to 30 April 2008, from 15 January to 15 March 2009, and from 15 January to 20 March 2010, between 0900 and 1900. In all years we attempted to sample all males and females in the whole study plot. Although different years generally represent different generations due to low survival rates (Ursprung et al., 2011), the actual number of year-to-year survivors still enabled a comprehensive comparison of individuals of different age cohorts. Individuals were captured with plastic bags, recognized by distinct ventral coloration patterns, and sexed by the presence (males) or absence (females) of vocal sacs.

In the course of our study, the third toe of both hind limbs was removed from all newly encountered adult individuals using surgical scissors to cut the digit between the base and the first joint (Fig. 1b), and stored in 95% ethanol for molecular parentage analysis (Ursprung et al., 2011). Scissors were exposed to a flame to avoid DNA contamination between individuals, and no disinfectants or anaesthetics were used. We measured snout–urostyle length (SUL) as well as toe lengths of the individuals from 2010 (new encounters, one- and two-year recaptures) in the program ImageJ 1.43 (Abramoff et al., 2004), using digital photographs taken in front of a reference grid; repeated measurements revealed a precision of 0.15 mm on average.

From the first sampling period in 2008, 22 (15.3%) of the 144 registered males and eight (13.3%) of 60 registered females were recaptured in 2009, and six males (4.2%) and three females (5.0%) were again recorded in 2010. Of the 138 males and 64 females that were newly encountered in 2009, 12 males (8.7%) and 12 females (18.75%) were recaptured in 2010. A previous study on a population 11

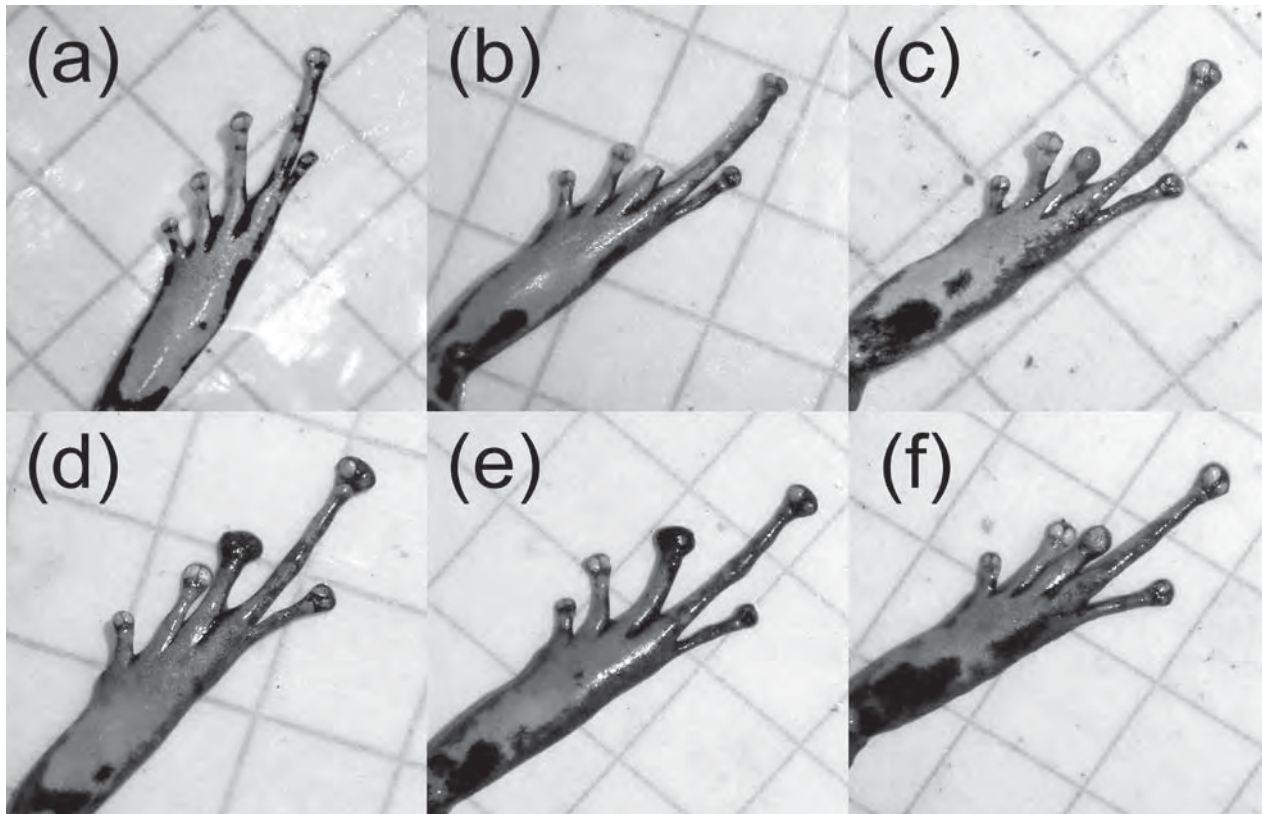


Fig. 1. Toes of *Allobates femoralis* (a) before clipping, (b) immediately after clipping, (c) after 6 weeks, (d) after 1 year, (e) after 2 years, (f) a toe-disc without colour change (remaining white instead of turning black).

km away from the site of the present study, where no toe clips were taken, revealed similar recapture rates (Ringler et al., 2009; one-year recaptures: 15.5/15.1% males/females; two-year recaptures: 2.4/2.1% males/females). We therefore have no evidence that toe clipping affected individual survival. Body size was slightly smaller in males than in females (mean SUL = 27.8/28.2 mm for newly encountered males/females), and increased with age in both sexes (males/females; one-year recaptures: 28.6/29.6 mm, two-year recaptures: 29.1/30.7 mm). The original toe length of the third toe of adult frogs was on average 4.07/3.9 mm for males/females (measurements from individuals in 2010). In all study years, we observed the regeneration of both clipped toes in all recaptured individuals after about two months (Fig. 1c). Regrown toes measured on average 2.67 mm (males) and 2.49 mm (females) in the individuals from 2009 recaptured after one year, while their length was on average 3.01 mm (males) and 2.69 mm (females) in the animals from 2008 recaptured after two years. Thus, regrown toes attained 65.6% and 74.0% (males), and 63.8% and 69.0% (females) of the average toe length of unclipped toes after one and two years, respectively. Whereas discs of unclipped toes are always white on the dorsal side (Fig. 1a), regrown toes possessed discs that were totally black (Fig. 1d,e), allowing for immediate identification of already marked

individuals. Only in one case was one regenerated toe disc white (Fig. 1f). We found no malformations or infections of the clipped toes at any time.

This is the first field study that has documented the consistent regrowth of toes and toe discs over two years in adult anurans. Surprisingly, our findings differ from a study that suggested the lack of regeneration ability in another population of *A. femoralis* (Roithmair, 1992). Although removed digits did regenerate, recaptured individuals could be immediately identified in the field due to the reduced size of the clipped toe and a pronounced change in toe disc coloration. The subsequent comparison of individuals' belly patterns offered a virtually error-free means of individual identification (see also Ringler et al., 2009).

The capacity for regeneration and tissue repair differs widely across the main vertebrate groups (Sánchez Alvarado & Tsonis, 2006; Galliot et al., 2008). The fact that the capacity for gene expressions that are involved in growth and pattern formation progressively decreases with age (e.g. Endo et al., 1997; Yokoyama et al., 2000 for studies on *Xenopus*) might explain the higher prevalence of regeneration in larvae and metamorphs (e.g. Richards et al., 1975). Considering the high mutilation risk of larval amphibians by fish or invertebrate predators (Ballenée & Sessions, 2009; Bowerman et al., 2010), a high degree of

regenerative ability appears to be especially advantageous at this stage. In adult amphibians, this ability is retained more widely only in urodeles and salamanders, while the actual mechanisms and reasons are not well understood (Sánchez Alvarado & Tsonis, 2006).

Despite its rarity, toe regeneration has been anecdotally reported in field studies from various anuran families, across a range of species with differing body size (e.g. Jungfer & Weygoldt, 1999; Richter & Seigel, 2002; Fong et al., 2010). The consistent toe regeneration in *A. femoralis* might be caused by the specific processes of cell proliferation and differentiation that allow for the lifelong growth of amphibians in general (Duellmann & Trueb, 1986), and the comparatively short life span of this species in particular. The difference in coloration between unclipped and regrown toe discs might in turn be due to the fact that differentiation and migration of pigment cells take place only in the early ontogenesis of amphibians (Erickson, 1993). However, quantitative and comparative as well as embryological studies of such possible effects remain to be conducted.

Finally, we would like to emphasize that at least for *A. femoralis* toe clipping has proved to be a very suitable method of tissue sampling for molecular analysis, due to the small amount of tissue needed and the high regenerative ability of this species.

Acknowledgements. Field work was funded by the University of Vienna (“Forschungsstipendium” and “Förderstipendium”) and the “Nouragues Grant” from the French CNRS, and supported through Austrian Science Fund grant FWF-18811 (PI Walter Hödl). E. Ursprung was supported by a DOC-ffORTE scholarship from the Austrian Academy of Science. We are grateful for field assistance to Julia Felling and Hannes Kronaus, and to Pedro Ivo Simões and an anonymous reviewer for their comments on the manuscript. Permissions and logistic help in French Guiana were provided by the CNRS and DIREN, notably Philippe Gaucher and Marc Henri Duffaud. Fieldwork was conducted in compliance with current French and EU law according to ASAB guidelines.

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Accepted: 20 October 2010

Part 2

Intrusion Experiments to Measure Territory Size: Development of the Method, Tests Through Simulations, and Application in the Frog *Allobates femoralis*

submitted to *PLoS ONE*: 02.06.2011

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Abstract

Territoriality is a widespread behaviour in animals and its analysis is crucial in several areas of behavioural, ecological and evolutionary research. Commonly, territory size is assessed through territory mapping and the application of simple area estimators such as minimum convex polygons. In the present study we demonstrate that territory size can be determined adequately with an active approach through intrusion experiments, a technique that is commonly used in behavioural research in other contexts. Tests with simulated data indicate that a minimum of twelve trials needs to be performed to establish reliable orders of relative territory size. To estimate absolute territory size, detailed hull techniques are most appropriate when analyzing point patterns of intrusion experiments, while the local convex hull estimator enables the construction of internal utilization distributions based on such point patterns. Additionally we suggest a 'stretch the centre' approach to emphasize the actual process of intrusion experiments in the construction of internal utilization distributions. To demonstrate the utility of the method, we apply all findings from the simulations to data from fieldwork with the model species *Allobates femoralis*, a territorial anurid frog from the lowland rainforest of French Guiana.

Introduction

The territory of an animal is usually defined as an area of intense and often exclusive use, which is announced and delimited by visual, acoustic, chemical and/or electric cues. In most cases a territory is also defended by physical aggression against conspecific, but sometimes also heterospecific intruders of either one or both sexes [1]. This opposes the concept of a territory

to that of a home range, which is defined as the entire area used by an individual in its regular activities [2]. The functional inequality of a territory and a home range has further implications regarding which data to use to adequately describe the one or the other. For example in birds, it was shown that singing locations alone do not provide an accurate estimate of space use [3] as the animals use and

defend much wider areas than those delimited by the sites that are preferred for singing.

Usually a territory contains one to several resources an animal needs to sustain its life, such as shelter or feeding resources, and/or to allow or support its reproduction, such as display sites, nesting sites or sites for egg or larval deposition [4]. It can be derived logically that, all other things being equal, territories of larger size are more likely to contain any of the resources mentioned, or to contain any one of these resources in higher quantities or qualities. In turn, higher resource abundance can allow for smaller territories, especially in the light of trade-offs between costs and benefits of large territories [5] (but see also [6]).

The ability of an individual to defend a territory of larger size has been shown to be a reliable indicator of an individual's quality and/or social status within a population, to be evaluated by conspecific competitors of equal sex and potential mating partners of the opposite sex [7,8]. Likewise, territory size has been shown to be linked to parameters of individual fitness like number of mates [9] or reproductive success [10] (but see [11] for contrasting findings in *Allobates femoralis*). While absolute territory size estimates might be of special interest for management and conservation purposes [12], individual-focussed correlational studies, for example on reproductive behaviour and sexual selection, at least need reliable estimators for relative territory size among a group of individuals [13,14]. Thus suitable estimators for these purposes have to produce concise and reliable rank orders of territory sizes, while 'true' territory size is often only of secondary interest.

The most widely used approach to assess territory extension is through 'territory mapping' [15], the observation of focal individuals and their marking and delimiting behaviour, as well as their interactions with other individuals. This yields points to define the centres of activity as well as points of interaction and delimiting behaviour at the periphery of the area an individual is defending [16,17]. Subsequently, these point patterns can be evaluated with a variety of area estimators, the most common ones (cf. [18], with a focus on home range studies) being minimum convex polygons (MCP) [19] and parametric (*sensu* [20]) kernel methods [21]. However, this purely observational approach to study territory size is susceptible to the observer's chance and ability to detect a sufficient number of peripheral locations for all individuals under study [22,23] to get individually unbiased estimates of territory extension. Additionally, the observations have to be situated in space and time in a way to allow for concise estimates of territory size without exceeding biases in either dimension [24–26].

Especially in situations where territorial individuals display territory ownership from central sites but defend wider areas against intruders [27], an active, systematic assessment of territory size can be deemed more adequate to obtain territory sizes for a larger number of individuals. For this purpose the respective territorial response in a species needs to be elicited actively by the researcher to observe territoriality 'in action'. This can be achieved through the performance of intrusion experiments, where adequate cues of fixed intensity are displayed towards focal individuals, or an according operational entity such as a breeding colony, at decreasing distances (Fig. 1A),

to find out about the reaction horizon, the distance where a territorial response can be elicited. This method can also be reversed, so that an adequate cue of a fixed intensity is presented at a distance where a response is reliable (e.g. in 95% of all trials) and subsequently the cue is removed until no response is observable anymore (Fig. 1B). Alternatively, the intensity of the cue can be increased at a fixed distance until a response is noticed (Fig. 1C), or reversely, decreased at a fixed distance, until no response can be elicited anymore (Fig. 1D). The data gained in a fixed cue-location setup then needs to be calibrated to allow the calculation of a reaction horizon (for various applications of the method cf. [28–35]). The actual approach taken depends on the type of cue that can be used to elicit territorial behaviour, the type of territorial response, and possible and sometimes long term, reactions like stress, hiding behaviour, or territory desertion of individuals of a given species to such experiments. Further also the time available in terms of territory stability as well as experimenter time, the number of experimenters available, and the characteristics of the environment where the territories are situated will influence the decision for or against one or the other approach.

Surprisingly, despite the regular appearance of intrusion experiments in the behavioural literature, to the best of our knowledge no consensus method for the evaluation of the point patterns typically produced in these experiments has been described so far. In general, a good territory estimator should be able to yield estimates with low bias and high precision,

independently of the actual behaviour of a certain study species [36]. Additionally, point pattern shape and sample size should not severely affect the estimator [37,38]. With detailed evaluations of existent estimators lacking and no new estimators that were developed explicitly for the use with point patterns from intrusion experiments, studies generally will fall back on MCPs as the most simple of all area estimators. However, this approach has two major drawbacks. MCPs, like all approaches that simply connect points, do not produce an internal utilization distribution as it is obtained by kernel methods, and they are rather sensible to the number of points in an analyzed pattern. Typically MCPs reach an asymptote well beyond the maximal number of intrusion experiments that reasonably can be performed on a single individual [39,38]. This can be alleviated by the use of detailed-hull techniques, where a certain set of restriction rules defines which points are connected to delimit a point pattern [40]. Thus, the assessment of 'true' territory size is also exacerbated by the general problem of information theory of how to fit shapes to a set of points in a plane [41,42].

In this study we describe the method of intrusion experiments to assess territory size and find the most suitable area estimators for the point patterns produced in these experiments. This is achieved by evaluating such point patterns from simulations and a dataset from fieldwork with the territorial dendrobatoid frog *Allobates femoralis*.

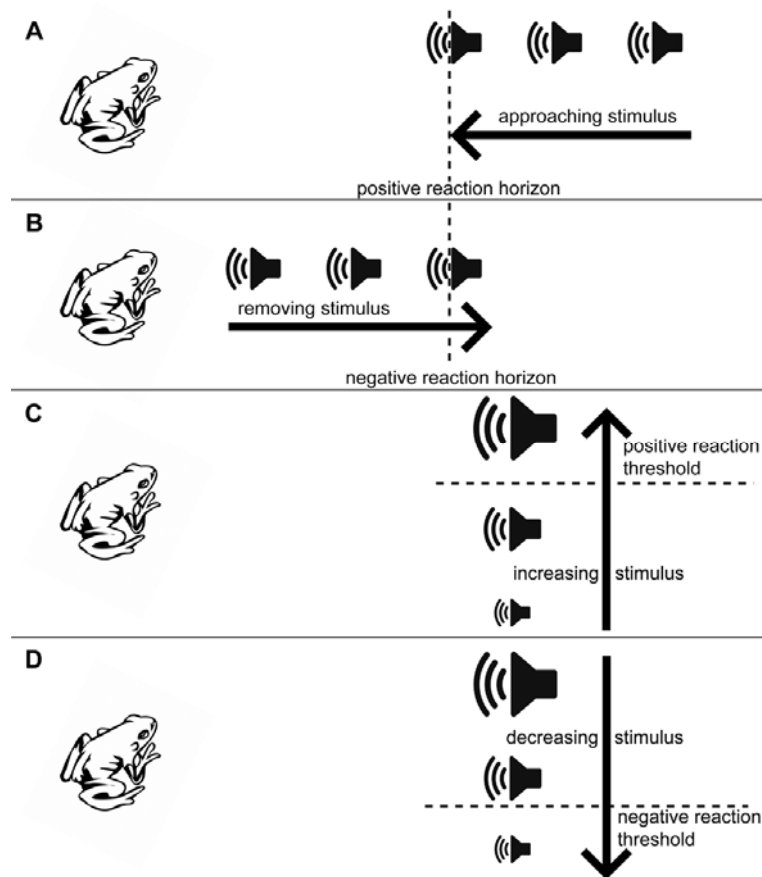


Figure 1. Setup of intrusion experiments.

There are four general setups for intrusion experiments with A) approaching or B) removing stimuli at fixed intensities, or C) increasing or D) decreasing stimuli at fixed distances.

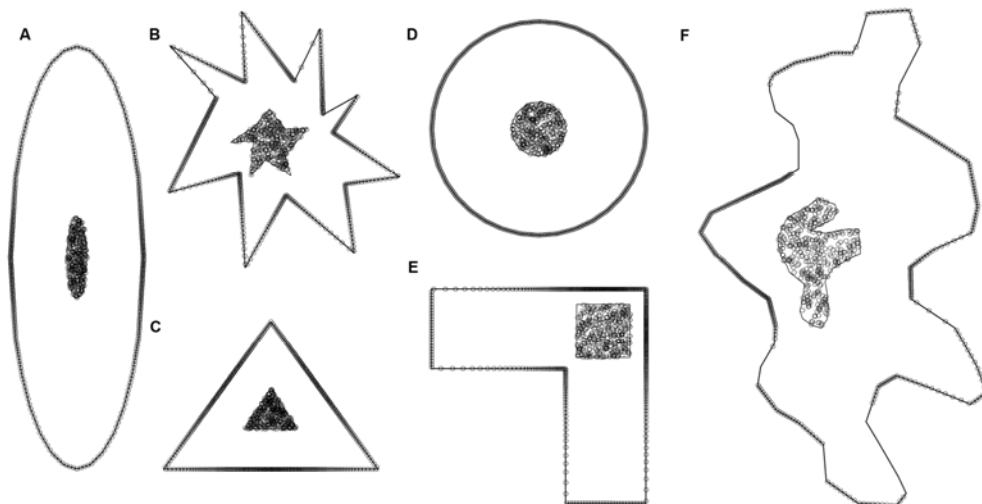


Figure 2. Shapes of the virtual territories.

A) ellipse, B) star, C) triangle, D) angle, E) circle, F) irregular. The territories comprise points from simulated intrusion experiments consisting of 360 randomly placed starting points in the central areas and an equal number of trial endpoints that were placed on the border of each territory, equiangularly in relation to the centroid points of the central areas.

A focus is given on free plug-ins in the ArcView®/ArcGIS® (ESRI) software environment, which are widespread among field biologists, to ensure the practical applicability of the method.

Material and Methods

Simulated data

To investigate the performance of different territory estimators in the analysis of point patterns as produced from intrusion experiments, and to decide on the actual number of trials to be performed in field experiments, we constructed six virtual territories (Fig. 2). The territories were drawn in ArcMap (ESRI) to represent a range of shapes from strictly convex to highly concave, and to span a range of sizes (ellipse: 243.58 units², star: 130.09 units², triangle: 88 units², circle: 200.04 units², angle: 155.63 units², irregular: 395.11 units²). Each territory consisted of a central area, representing assumed display and resting sites, and an outer region, representing a wider defended area. This mimicked the typical central-place territorial behaviour of many species, where potential intruders are detected and intercepted well before reaching an individual's area of concentrated use. The central areas of each territory were populated with 360 random points, using 'Hawth's Tools' for ArcMap [43], while along the edge of the outer regions 360 points were placed at 1°-intervals in relation to the centroid of the central area. Random pairs of one central and one edge point were grouped to represent simulated intrusion trials consisting of an initial position in the central area and a final position at the edge of the outer area (Fig. 3). For each of the six territories we described all 810 possible equiangular trial-subsets within the 360 directions

with numbers of trials that are integer divisors of 360 (i.e. 2, 3, 4, 5, 6, 8, 9, 10, 12, 15, 18, 20, 24, 30, 36, 40, 45, 60, 72, 90, 120, 180, 360). The rationale behind using equiangular trial sets, compared to trials in random directions, was to 'span up' the entire extension of a territory with a minimum number of trials to minimize time effort for experiments and disruptions and stress caused on the focal animals by excessive testing.

Frog data

Our research on *Allobates femoralis* in the CNRS field station "Saut Pararé" in the Nature Reserve "Les Nouragues", French Guiana was approved by the scientific board of the station (<http://www.nouragues.cnrs.fr/F-conseil.html>). No formal permits or approval ID"s were issued by this board, as our experiments did not involve killing or harming animals. All experiments were conducted according to French and EU law and followed the ASAB guidelines for the treatment of animals in behavioural research and teaching [44].

Study species

Allobates femoralis is a small, diurnal frog in the family Aromobatidae that inhabits the leaf litter throughout Amazonia and the Guiana shield [45]. The species forms disjunct local populations in lowland rainforests that are not exposed to regular inundations. Males announce their multi-purpose territories [46] by prolonged and intense calling during the reproductive season [47] and defend these territories against calling intruders by vigorous physical attacks [48]. Obviously the territory plays a vital role in the elaborate courtship that precludes mating and can last over several hours [49,50]. Females show a high degree of site fidelity but do not defend any territories

[51]. In a recent study [11], territory occupancy was shown to be the main determinant of male reproductive success, while territory size, measured like in previous studies by mapping encounter locations [46,51], did not influence the quantitative performance of actually reproducing males.

Study site & mapping

Field data were gathered during the rainy season from February 28th until March 16th, 2009 in a population of *A. femoralis* near the field station 'Saut Pararé' (4°02' N, 52°41' W; WGS84) in French Guiana, France in the course of a long term research project on reproductive behaviour and space use in this species. All spatial data were recorded in ArcPad 6.0 (ESRI) on PocketPCs (iPaq HX4700, Hewlett-Packard) using a detailed background map of all living trees (dbh > 10 cm), fallen trees and larger branches, and other structures on the forest floor that were used by frogs or constituted landmarks, to map the locations of the frogs.

Playback Trials

To assess the area of defended territories we conducted playback experiments on 15 neighbouring males in our study population (Fig. 4). Our approach took advantage of the stereotypic phonotactic behaviour of *A. femoralis* males [48] and used synthetic advertisement calls from a previous study on phonotactic approach patterns in this species [52]. The calls were

presented to the focal animals from WAV-files via a portable audio player (Maxfield G-Flash 512) and battery powered portable loudspeakers (Sony SRS-M30). All individuals were tested in twelve runs in a semi-random order. The number of twelve trials for each frog was chosen based on the results of the analysis of the simulated dataset (cf. results and discussion of the simulated data). The twelve playback trials per individual were conducted in a semi-random order towards every 30° (0° $\equiv$ north). We picked a random direction for the first trial for each individual, the second trial was performed in the complementary direction (+180°) and the third and fourth trial per individual were performed in random order to the right (+90°) and to the left (+270°). For the fifth trial a random direction was picked among the remaining directions and trials six to eight were performed in a similar manner than trials one to four. Finally, trials nine to twelve were performed accordingly within the remaining four directions. This protocol enabled us to uniformly 'span up' the territories of all individuals, thus avoiding unwanted temporal or spatial concentrations of data points for any individual over the trial period.

Before each trial, the initial position where the frog was spotted, usually when calling, was entered into the digital map. Then we played bouts of ten synthetic calls ('standard call' *sensu* [52]) towards the focal male from the selected trial direction and from a distance of three meters (± 30 cm, depending on vegetation structure).

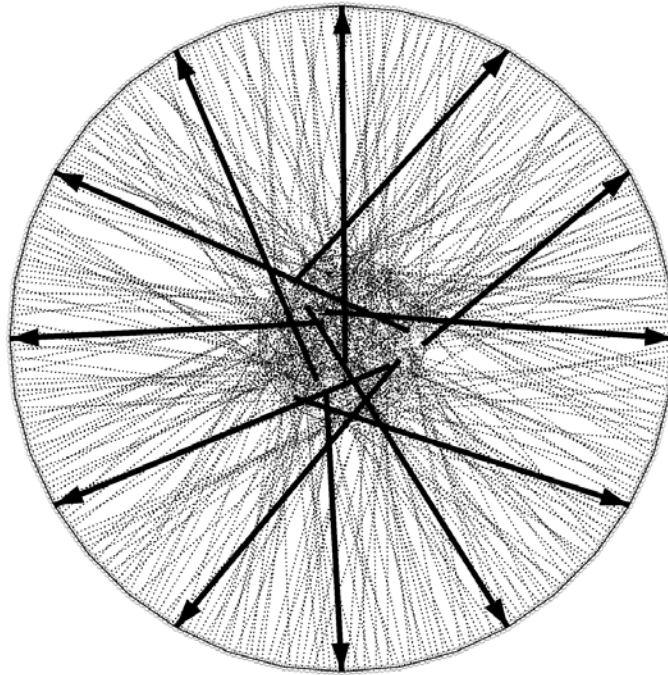


Figure 3. Circular virtual territory.

This virtual territory comprises 360 simulated intrusion trials (dotted lines) with the indication of an equiangular subset of twelve trials (bold arrows).

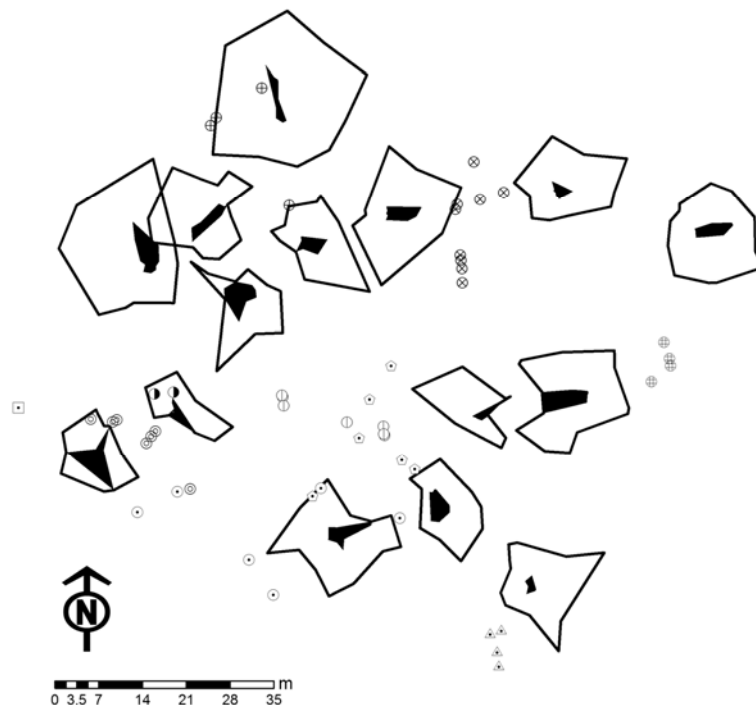


Figure 4. Spatial setup of 15 focal *Allobates femoralis* males during intrusion experiments.

Black areas shows the detailed hull of the calling positions, outer bold line shows the detailed hull of the endpoints of playback trials. Symbols indicate the encounter locations of ten other males that were found in the area during the intrusion experiments.

We kept the speaker 20 cm above ground level and adjusted to produce a SPL of more than 56 dB at the position of the focal individual to elicit phonotactic behaviour [53]. As soon as the frog started its phonotactic approach, the speaker was carefully withdrawn in the respective direction to maintain an equal distance between the focal individual and the speaker throughout the trial. Trials ended when a frog did not further approach the speaker during three consecutive bouts of the artificial call (~30 sec). The final location of the frog at the end of each trial was entered in the digital map, then the frog was caught, a picture of the ventral pattern was taken for identification, and finally the frog was released at its initial position. No frog was tested more than two times per day, and always with more than 4 hours between consecutive trials. When a frog did not respond in a playback trial, it was immediately tested in another direction. When the second trial resulted in a phonotactic approach, only the latter trial was recorded and a trial in the previous direction performed later. When the frog did not respond again, other trials in these directions were performed on later days. When the frog approached the speaker in these later trials, only the successful approaches were scored, otherwise only the initial locations were retained with no corresponding final locations.

Estimators of territory size and utilization distribution

Simulated data

Thirteen different territory estimators were calculated for all 810 equiangular simulated trial sets per virtual territory in the GIS programs ArcMap[®] 9.3.1 and ArcView 3.3[®] (ESRI) with

commonly used plug-in extensions. Minimum convex polygons (MCP) [19] and detailed hulls (DH) were calculated with the plug-in 'XTools Pro 4.2.0' [54] in ArcGIS. The detailed hull method as implemented in XTools Pro selects the points for hull construction similar to the point selection algorithm of [55]. Normal bivariate fixed kernel estimators [21,56] with least-squares-cross-validation (LSCV) and with an ad-hoc method (ADHOC) to find the smoothing parameter h were calculated with the 'Animal Movement Extension' (AM) [57] in ArcView. LSCV and a reference value (HREF) for the smoothing parameter were used for similar kernel calculation with the plug-in 'ABODE' [58] in ArcGIS, and LSCV, biased cross-validation (BCV) and HREF were used for fixed (f) and adaptive (a) kernel calculations with the plug-in 'Home Range Tools' (HRT) [59] in ArcGIS. Local convex hull (LoCoH) nonparametric kernels [60] were calculated as k -LoCoHs with the LoCoH-extension [61] in ArcView. The optimal value for the tuning parameter k for each territory was selected by applying the 'minimum spurious hole covering' (MSHC) rule [20] where k is selected manually to avoid any biologically, geographically or topologically unjustified holes or cutaways in the 100%-LoCoH of an individual.

To assess the performance of all estimators, we compared the final area estimates, based on 360 simulated trials per territory, with the known 'true' sizes of these territories and evaluated the asymptotic and rank-order behaviour of the estimators with increasing sample size. All polygon estimators, including LoCoH, were evaluated at their full extension, while all parametric kernel estimators were evaluated at the 95% isoclines, the most commonly used extension in home range studies [18]. The minimal number of equiangular

intrusion trials that generally has to be performed to reach concise conclusions about relative territory size was evaluated by looking at the asymptotic behaviour and rank-order of averaged area accumulation curves of the DH estimator for each shape. This estimator was chosen based on the previously described strong dependence of polygon estimators on the number of points used, where detailed hull methods better fit concave shapes compared to MCPs [62,63,40,42,64].

To compare the performance of the different estimators on random, bounded point patterns, we also calculated all estimators, besides the LoCoH estimator, for only the points from the central area with the points at the edge of the outer region omitted.

Frog data

We took a stepwise approach in the analysis of the frog data. First, we assessed the extent of the central areas of the territories ('calling territory') as well as of the defended outer areas ('playback territory'), using the DH method as it was the best performing estimator with the simulated data. In a second step we derived internal utilization distributions (UD) for the frog's territories, based on the entirety of central and peripheral (playback) positions. We used the LoCoH estimator, which was the best performing UD estimator with the simulated data, to emphasize UD calculation based on the entire set of points for a given individual. Additionally we developed a manifest 'stretch-the-centre' (STC) method to shape a parametric kernel of the calling positions to the outline of the playback territory, conceptually similar to 'elastic disc' models for central-place home ranges [65].

For STC only the initial positions of the intrusion experiments are used for kernel calculation, as they generally are arranged in a way where parametric kernels were shown to perform well (i.e. bounded random distributions) [56,66]. Kernels derived from these points are then expanded and reshaped to fit the outer locations from the intrusion trials, which emphasizes the process (i.e. moving animals) that led to the underlying point patterns. As the necessary spatial adjustment operations are not accessibly to automation in ArcGIS, we did not integrate this method in our tests with simulated data, due to the excessive manual manipulation required for this approach. Based on the evaluation of the different kernel estimators with the simulated central points (cf. results for simulated data), we calculated HRT-HREF(f) with all observed calling positions for each individual. We then used the 'rubbersheet' [67] function for spatial adjustment in ArcMap to reshape each central kernel to the edge of the corresponding defended area by stretching and jolting. For this purpose we linked the 99%-isocline of the central kernel along the axis of the twelve trial vectors (calling position → attracted position) with the corresponding end points of each trial as correction links and set all calling positions as identity links (cf. ArcGIS manual [68]). When an individual showed no response in a given direction, calling position and attracted position were taken to be identical, thus the transformation vector was set along the trial axis, pointing from the 99% isoclines towards the calling position. With all correction links set, we performed the spatial adjustment operation on all isoclines of the central kernel (Fig. 5 d, e). Similar to other studies that used utilisation distributions

from parametric kernels we subsequently analyzed the STC kernels at their 95% isoclines.

Statistical Analysis

All descriptive statistics were performed in SYSTAT 12[®] and SigmaPlot[®] 11.0. Normality of data was checked with the Shapiro-Wilk test as implemented in SYSTAT. Spearman rank-order correlations were calculated in SigmaPlot. The significance level was set at $p < 0.05$ in all cases.

Results

Simulated data

The both polygon estimators as well as the LoCoH estimator, but none of the parametric kernel estimators, showed stable and asymptotic behaviour with an increasing number of trials in the equiangular trial-subsets over all shapes (Figs. 6, 7, 8). When evaluated at the maximum number of trials (ie. 360 trials), the DH estimator reached the 'true' territory size most closely for all but the irregular shape, where ABODE-HREF performed slightly better. For strictly convex shapes (i.e. ellipse, triangle, circle) MCP, DH and LoCoH estimators produced identical percentages while with concave shapes (i.e. star, angle, and irregular) the DH estimator performed much better (Tab. 1). The DH estimator produced a stable rank order for all subsets with ten and more trials, with variation decreasing with an increasing number of trials (Fig. 7). Absolute territory size of the DH estimator did not increase more than 2% stepwise with an increasing number of equiangular trials from 20

trials upwards for all but the 'irregular' territory (Tab. 2). The rank order of the 100%-LoCoHs with MSHC-optimized k remained stable for all convex and the 'angle' and 'irregular' territories with twelve and more trials, while the estimator for the 'star' territory increased continually and changed its rank twice beyond twelve trials (Fig. 8).

For the central point distributions the only parametric kernel estimators with a reliable, non-erratic behaviour (Fig. 9) and producing an invariant rank order of territory sizes at a sample size of twelve and more locations were the fixed and adaptive HRT-HREF estimators with the former performing slightly better in terms of stability (Fig. 10). Accordingly we used this kernel estimator subsequently in our 'stretch-the-centre' approach on the frog data.

Frog data

In 179 playback trials, 15 *Allobates femoralis* males approached the loudspeaker over a mean $\pm$ SD distance of 7.07 ± 3.5 m (Fig. 11). In all trials where the males approached the loudspeaker, they unambiguously stopped at a certain point for an extended period of time, thus suggesting that they had reached the border of their defended area. Due to our experimentation protocol, for four of the 15 frogs we missed up to three final playback locations, corresponding to directions into which a frog apparently did not claim any territory possession. For one frog we could only perform eleven intrusion experiments due to time constraints, but still included the data in the final analyses.

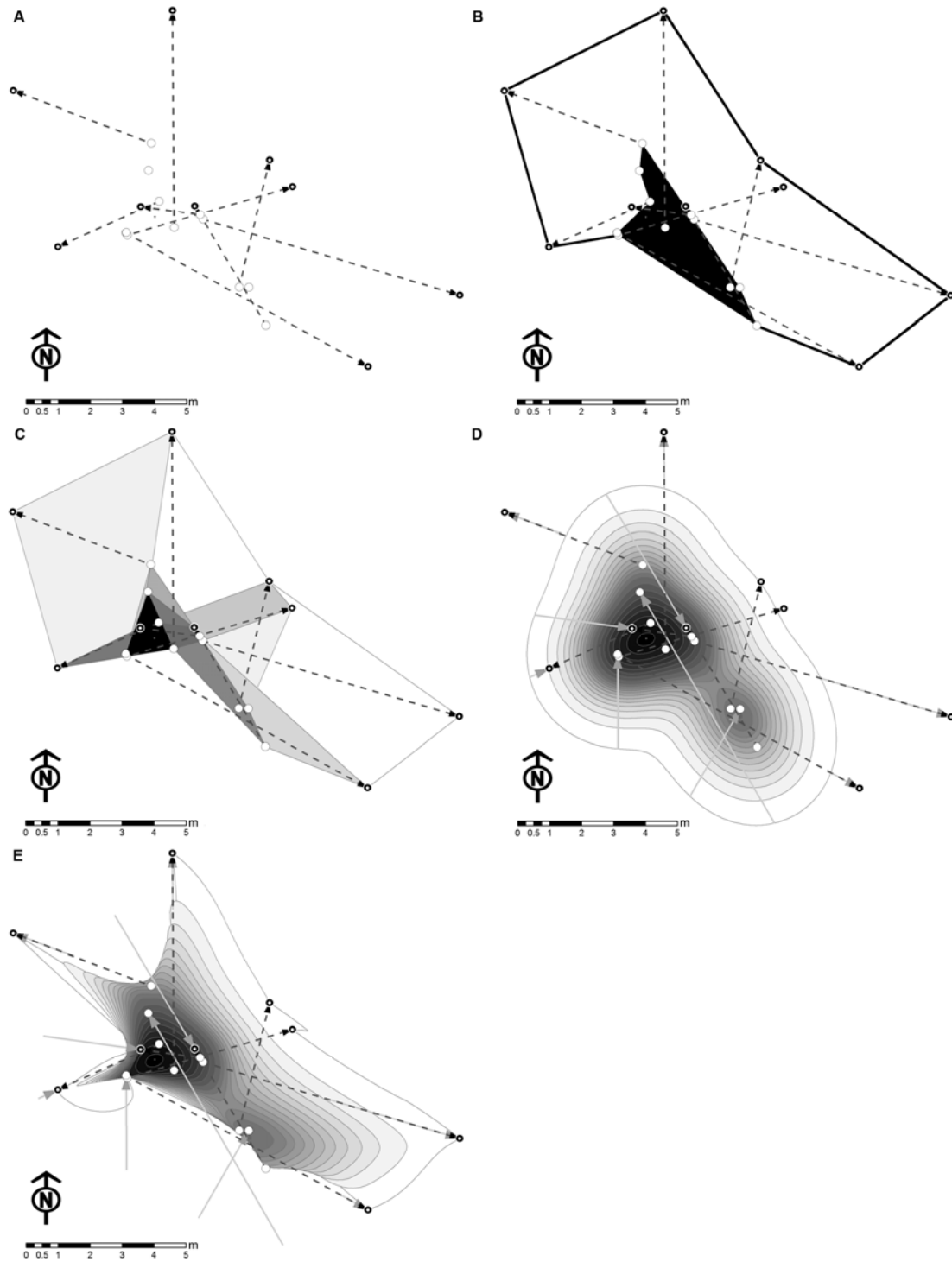


Figure 5: Intrusion trials and territory estimators for focal individual #08.

Hollow circles show calling positions, black circles represent final locations of playback trials, dashed lines show trial vectors and light grey lines indicate correction vectors for STC, areas between kernel isoclines and of $n\%$ -LoCoHs (99%, 95-5% (5%-steps), 1%) in incremental shades of grey; A) initial calling positions and final trial positions with according trial vectors, B) DH estimations for calling (black area) and playback territories (bold outline), C) LoCoH estimator with MSHC-adjusted k , D) kernel from HRT-HREF based on all calling positions, E) STC transformed kernel.

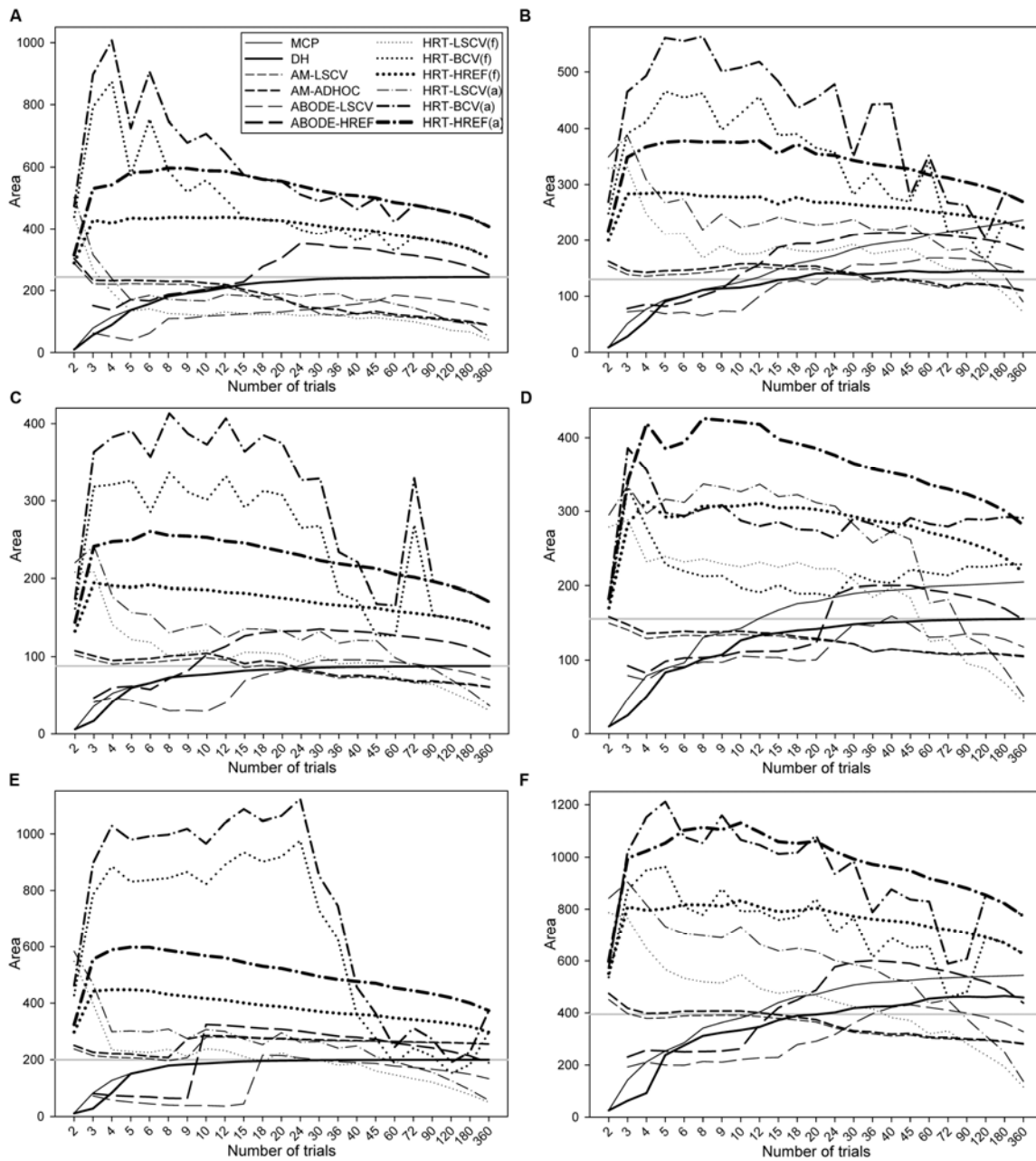


Figure 6: Average values of the different area estimators for equiangular subsets.

A) ellipse, B) star, C) triangle, D) angle, E) circle, F) irregular; horizontal grey line indicates true territory size; area in units².

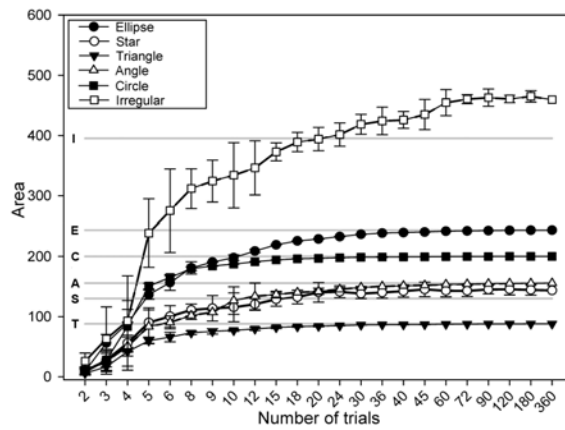


Figure 7. Performance of the detailed hull estimator.

Average values for equiangular subsets; horizontal grey lines indicate true territory sizes; error bars indicate standard deviations; area in units².

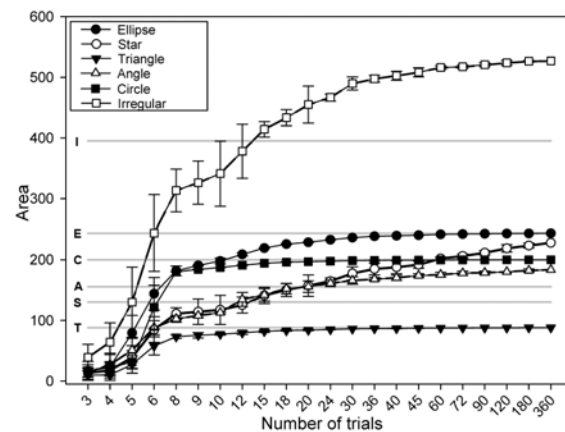


Figure 8. Performance of the *k*-LoCoH estimator with MSHC-adjusted *k*.

Average values for equiangular subsets; horizontal grey lines indicate true territory sizes; error bars indicate standard deviations; area in units².

Table 1. Performance of estimators based on 360 equiangular intrusion trials for all virtual territories.

Shape	TRUE (units ²)	MCP	DH	LoCoH	AM -LSCV	AM-ADHOC	ABODE-LSCV	ABODE- HREF
e	243.58	100%	100%	100%	35%	37%	56%	103%
s	130.09	182%	110%	175%	84%	85%	110%	140%
t	88.00	100%	100%	100%	68%	69%	80%	114%
a	155.64	132%	100%	118%	67%	68%	76%	99%
c	200.04	100%	100%	100%	127%	128%	66%	95%
i	395.11	138%	116%	133%	71%	71%	83%	111%

Shape	TRUE (units ²)	HRT- LSCV(f)	HRT-BCV(f)	HRT-HREF(f)	HRT-LSCV(a)	HRT-BCV(a)	HRT- HREF(a)
e	243.58	17%	125%	125%	21%	167%	167%
s	130.09	55%	171%	171%	69%	207%	207%
t	88.00	34%	155%	155%	41%	193%	193%
a	155.64	28%	147%	140%	33%	187%	181%
c	200.04	25%	148%	148%	28%	187%	187%
i	395.11	29%	159%	159%	35%	196%	196%

TRUE gives the known size of a virtual territory in units², all other columns give percentages of this area as estimated by the different methods; best performing estimators for a given shape in **bold**, best performing estimators with internal utilization distribution in *italic*; ellipse, star, triangle, angle, circle, irregular.

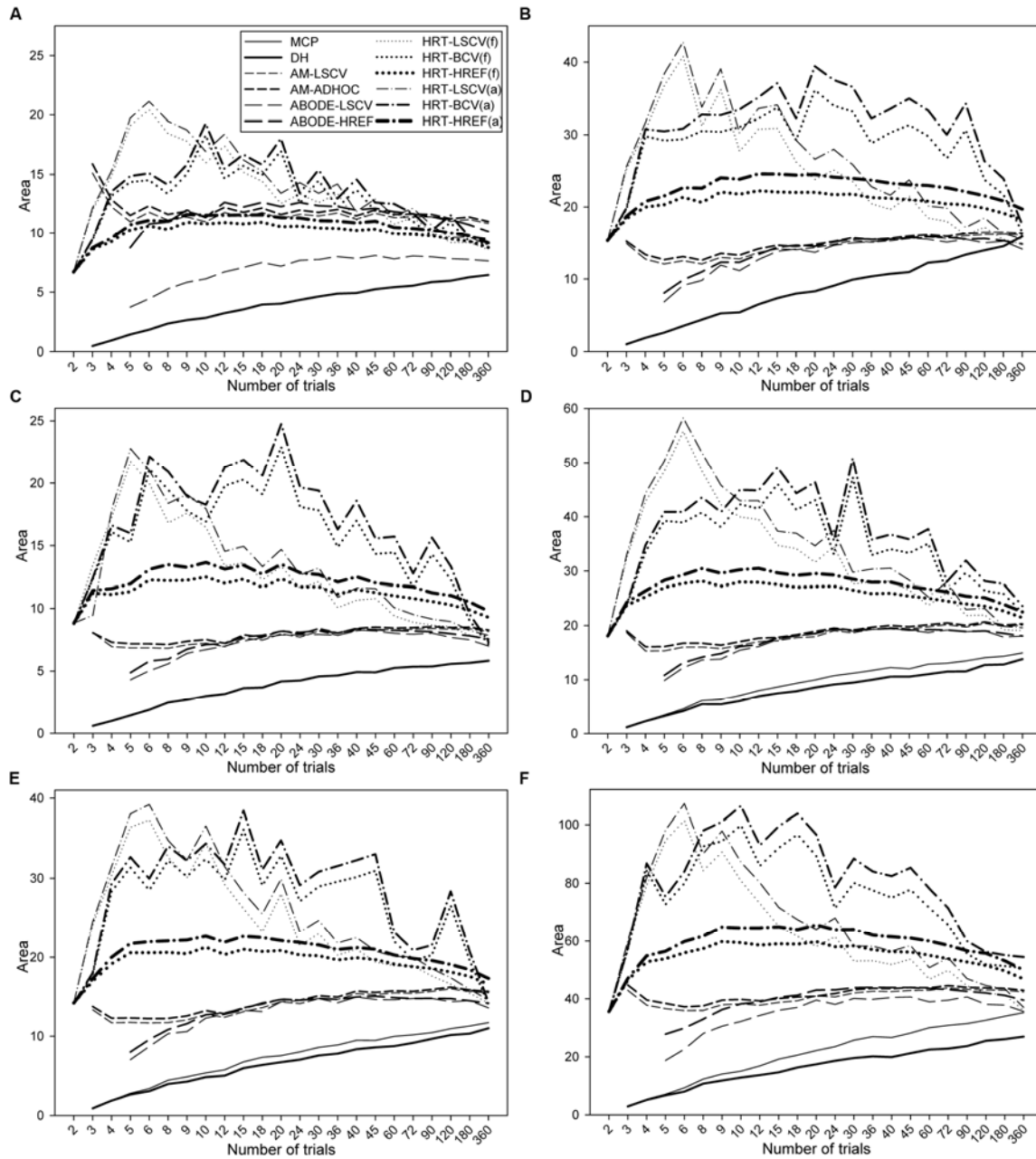


Figure 9. Average values of the different area estimators for point of the central area.

A) ellipse, B) star, C) triangle, D) angle, E) circle, F) irregular; area is given in units².

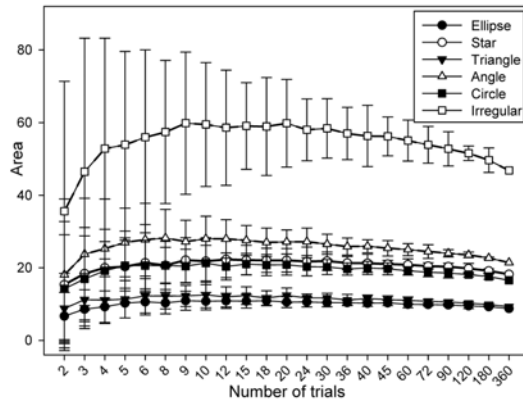


Figure 10. Performance of the HRT-HREF(f) estimator for the central points of the virtual territories.

Average values for equiangular subsets; error bars indicate standard deviations; area in units².

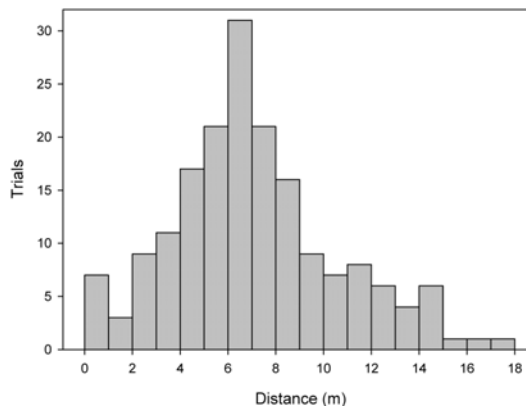


Figure 11. Histogram of the distances covered in playback trials.

Table 2. Relative change in territory size with increasing sample size, calculated with the DH estimator for all virtual territories.

Number of equiangular trials										
Shape	3	4	5	6	8	9	10	12	15	20
e	498%	58%	54%	15%	16%	5%	4%	6%	5%	3%
s	224%	102%	61%	11%	10%	2%	1%	4%	7%	6%
t	190%	149%	43%	10%	11%	3%	2%	3%	3%	2%
a	155%	102%	64%	8%	13%	5%	18%	5%	2%	2%
c	149%	201%	80%	10%	9%	3%	2%	2%	2%	0%
i	143%	47%	157%	16%	13%	4%	3%	4%	8%	4%
Shape	24	30	36	40	45	60	72	90	120	180
e	2%	2%	1%	0%	0%	1%	0%	0%	0%	0%
s	1%	-2%	1%	2%	2%	-1%	0%	2%	0%	-1%
t	1%	1%	1%	0%	0%	0%	0%	0%	0%	0%
a	2%	2%	1%	1%	1%	1%	0%	0%	0%	0%
c	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%
i	2%	4%	1%	0%	2%	5%	1%	1%	0%	1%

The last step-to-step change >2% in ***bold-Italic***; ellipse, star, triangle, angle, circle, irregular.

Ten other male *A. femoralis* that were encountered in the area during the trials (Fig. 4) were either found outside their territories during tadpole transport, did not show territorial behaviour, or left the trial area after one or two trials, probably because they had not yet established a territory at the time of the first trial and refused to settle after their encounter with the artificial caller. Two males in the centre of the study area were involved in prolonged territory disputes and did not show reliable phonotactic reactions at the onset of the experiments, so we excluded them from the study. None of the frogs ceased territory occupancy during the trials or showed any other evidence for enhanced levels of stress. All frogs started to call again within 10 minutes after being released on their original calling sites.

The DH estimations of the area of the calling territories (range: 3.02 – 22.89 m²; median = 10.82 m²;) and playback territories (range: 64.62 – 417.63 m²; median = 151.13 m²) varied considerably. All playback territories were larger than their accompanying calling territories by a median factor of 14.54 (range: 4.24 – 47.90). Calling and playback territory extension was not significantly correlated (Spearman $\rho = 0.024$, $p = 0.457$; Fig. 12). The 100% LoCoH (range: 65.06 – 424.7 m²; median = 164.36) and the 95% STC kernels (range 30.06 – 250.03 m²; median = 94.02 m²) showed similar variation among the individuals. The rank order of territory sizes remained the same for five individuals over all estimators, while for seven individuals the rank varied by one position, for two individuals the rank varied by up to two positions, and for one individual the rank varied for up to three positions among the different estimators (Fig. 13). The %-

isoclines of the LoCoH estimator showed expansion in stages, owing to the way isoclines are generated with this method [60], while the expansion of %-isoclines of the STC kernels was gradual (Fig. 14). There was a strong significant correlation between the extension of LoCoH and STC %-isoclines (Spearman $\rho = 0.923$, $p < 0.001$; Fig. 15). As it is intrinsically impossible to calculate a 100%-isocline for parametric kernels, the 99% isoclines of the STC estimator were used as an approximation in this correlation analysis. The median %-isocline where the extension of the calling territory (DH) was reached was 50% (range: 30% – 70%) for the LoCoH estimator and 40% (range: 15% – 65%) for the STC estimator. However, due to differential shapes, complete overlap on average was only reached at the 80% (median, range: 50% – 100%) isocline for the LoCoH estimator and the 90% (median, range: 75% – 99%) isoclines for the STC estimator.

Discussion

Simulated data

The analysis of point patterns from the simulated intrusion trials in virtual territories showed that central-place territories can be adequately evaluated by at least twelve equiangular intrusion trials. Based on this minimum number of trials, the detailed hull estimator produces reliable rank orders of territory size for further correlational analyses. To estimate absolute territory size however, the number of trials that is necessary to cause the area accumulation curve to reach an asymptote and thus to reflect the ‘true’ territory size, may exceed the number of trials that reasonably can be performed on individual animals.

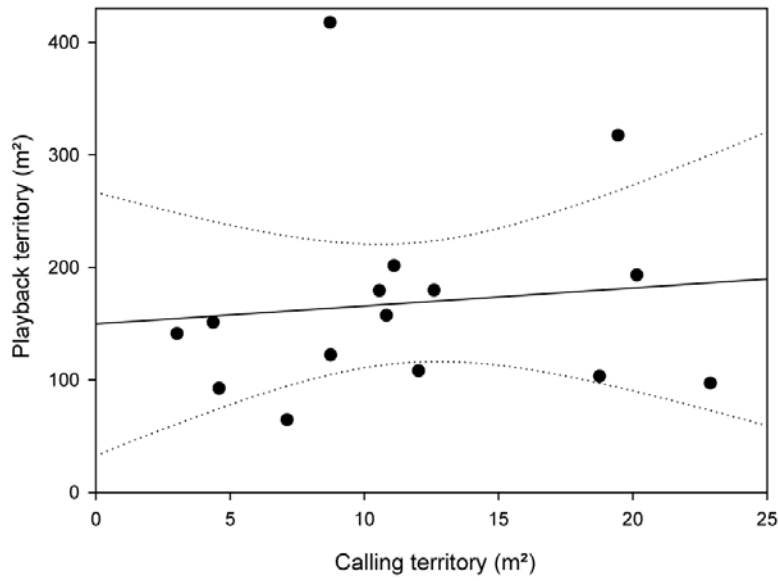


Figure 12. Scatter plot of the extensions of calling territories vs. playback territories.

Linear regression and 95% confidence intervals as continuous and dotted line, respectively.

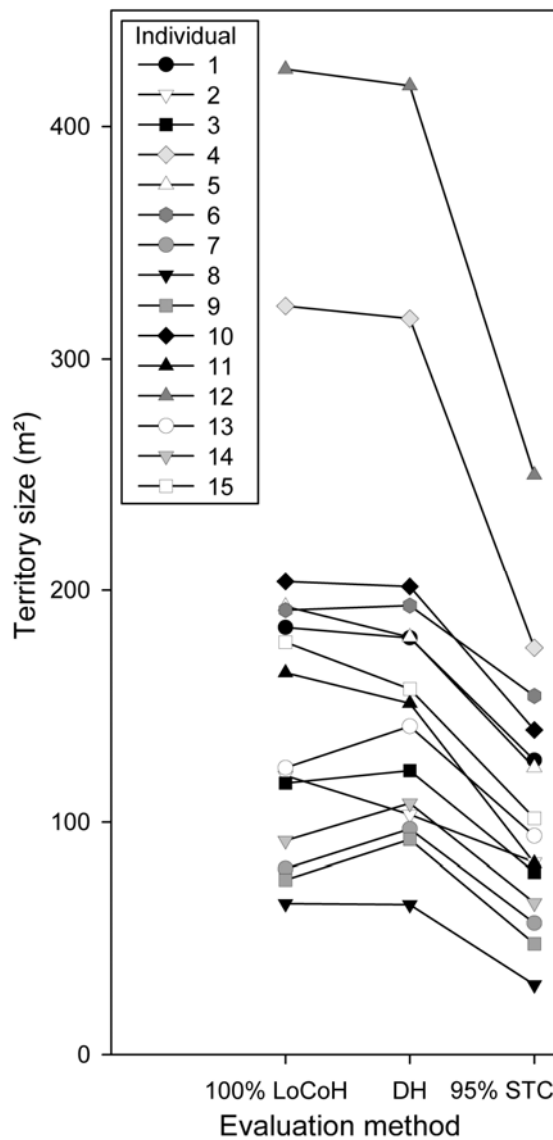


Figure 13. Rank order of 15 territories as evaluated by the LoCoH, DH, and STC estimator.

An animal's limited tolerance to the stress caused by repeated disturbance and conflict situations with simulated intruders during excessive testing could result in severely altered behaviour up to site abandonment and might prohibit the execution of a desirable number of trials. On the other hand, especially when activities that should take place in the time frame of the intrusion trials (e.g. reproduction) subsequently shall be related to territoriality and territory size, the individuals under study have to be given ample time besides defending their territories against simulated intruders. These factors force a trade-off decision between the accuracy of the territory estimates and the number of trials that can be sustained by the focal individuals. In this context the intended number of trials per individual has to be carefully chosen, and based on our simulated dataset we suggest twelve trials to be a good starting point for further considerations.

Our findings and recommendations concerning the use and performance of area and utilization distribution estimators with point patterns from intrusion experiments depend on the intended use of the estimates. The best performing estimator in terms of absolute territory size and rank order was the detailed hull estimator (Fig 5B, Fig. 16), which on the other hand faces two caveats. Unlike other detailed descriptors of point patterns, and due to the manufacturer's copyright policy, DH as implemented in 'XToolsPro' [54] is not 'open source', although some information regarding the underlying algorithm [55] was disclosed ([64] and personal communication with Data East). Nevertheless we decided for the use of this estimator in our current study, as it is the only

implementation of a detailed polygon descriptor that is readily available to field biologists. As other studies point out, it is likely that other, similar descriptors [63,40] will perform equally well or better [64]. We urge developers to make their methods and algorithms openly available and implement them for use in widespread software environments. The second drawback of DH, as with any simple point-connecting method, is the lack of an estimation of the internal distribution of space use. In the current context of intrusion trials this can be alleviated by the separate calculation of initial ('calling') and attracted ('playback') territories, which results in the separate delimitation of core and peripheral areas. However the explicit construction of internal utilization distributions remains desirable.

Among all estimators with internal utilization distributions, the non-parametric LoCoH estimator with MSHC adjustment of the tuning parameter k (Fig. 5C., Fig. 17) turned out to deliver the most concise results in terms of rank order and absolute territory size. However, regarding absolute territory size, the LoCoH estimator performed more similar to the MCP than to the DH estimator (Tab. 1). Both methods overestimated the extension of concave shapes with according fluctuations in rank order stability (Fig. 8). All parametric kernel estimators turned out to be unsuitable for the purpose of area estimation based on point patterns from intrusion experiments as all of them showed erratic fluctuations with increasing sample size (Fig. 6). We attribute this observation to a failure of the various algorithms to determine a sensible smoothing parameter h for the kernels.

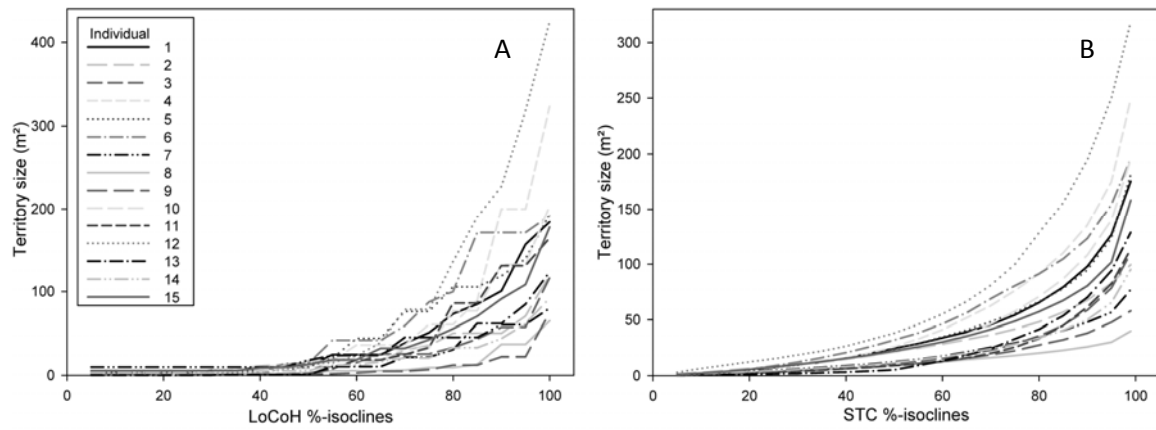


Figure 14. Extension of %-isoclines of the A) LoCoH and B) STC estimator for 15 individuals.

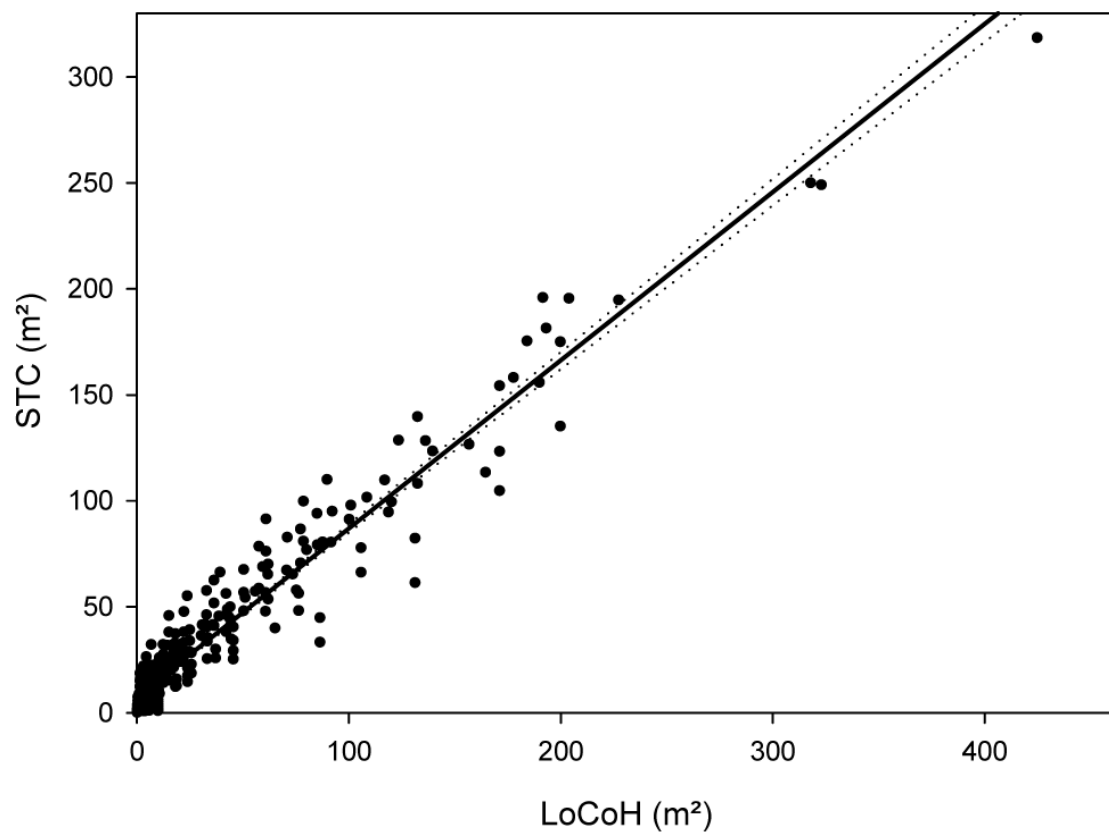


Figure 15. Scatter plot of the extension of LoCoH isoclines vs. STC isoclines.

Linear regression and 95% confidence intervals as continuous and dotted line, respectively.

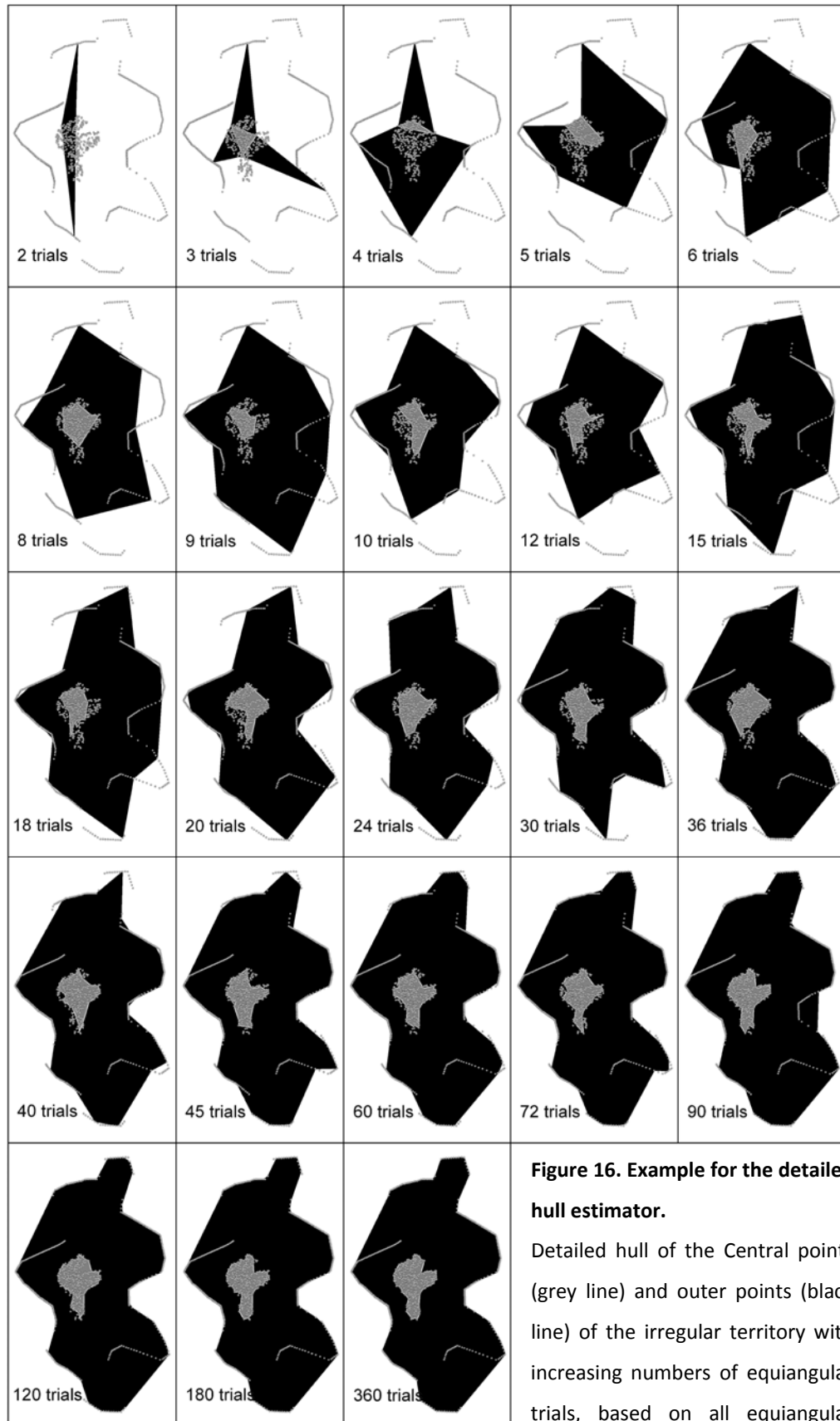
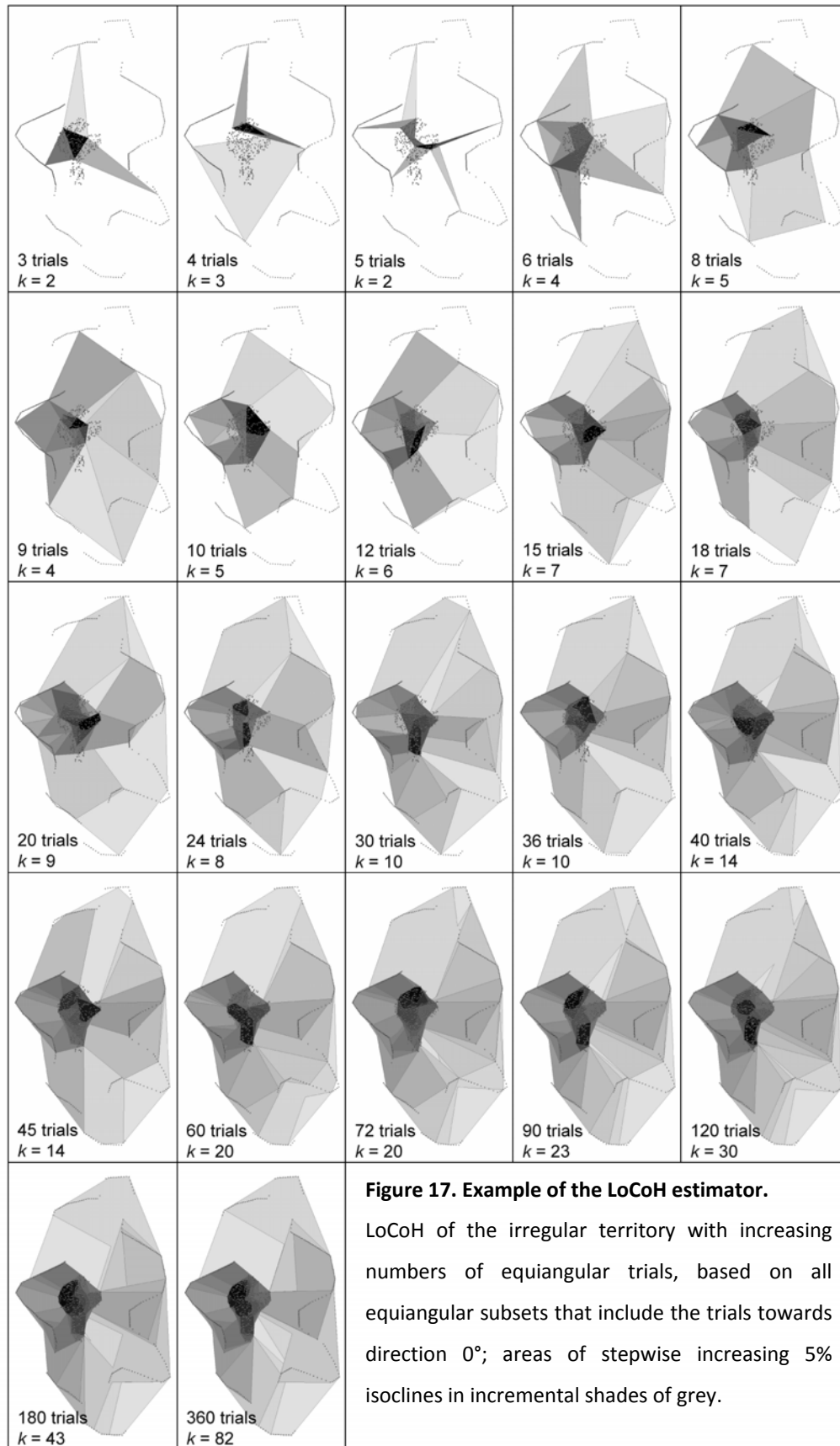
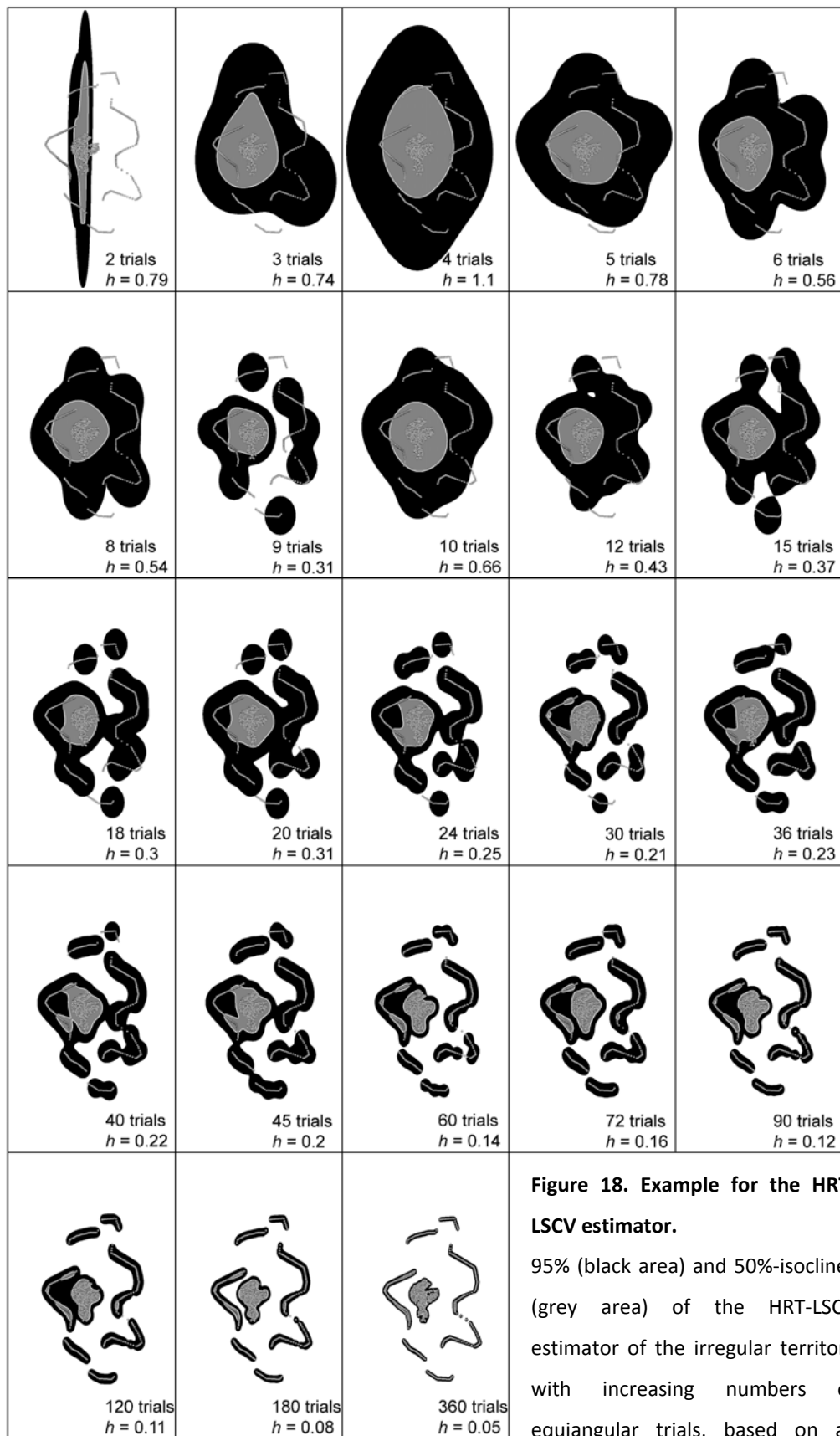


Figure 16. Example for the detailed hull estimator.

Detailed hull of the Central points (grey line) and outer points (black line) of the irregular territory with increasing numbers of equiangular trials, based on all equiangular subsets that include the trials towards direction 0° .





While all of these methods perform reasonably well with point patterns as they typically occur in home range studies (bounded, random patterns with some clumping), the bimodal clumping of points in the central area and along the edge of a territory, as it results from intrusion experiments, is likely to produce estimates of the parameter that are nonsensical, at least for the intended purpose (Fig.18). We also noted considerable discordances between different parametric kernel estimator plug-ins that pretended to employ the same algorithms for the calculation of the parameter h and for kernel calculation (AM-LSCV vs. ABODE-LSCV vs. HRT-LSCV(f); ABODE-HREF vs. HRT-HREF(f); Fig. 6). This observation was made already for home range studies [69–71] and also showed in our analyses of all points as well as of the restricted subset of central points only.

Frog data

Allobates femoralis males defend territories that are much larger than the area that comprises the calling locations. The analysis of playback trials of 15 individuals with the DH estimator revealed that the extension of the defended area, the ‘playback territory’, does not correlate with the area occupied during calling, the ‘calling territory’. This is of special interest in the context of previous studies where a correlation between the size of the calling territory and reproductive success in this species was initially found [46] but later abnegated [11]. In both studies, territory size was determined through mapping of calling positions over several months and the estimation by the modified-minimum-area method [72] to eliminate outliers. In the present study we intentionally did not evaluate the frog’s positions by the MMA method as our observation periods were

considerably shorter than those of previous studies, which would have rendered direct comparisons meaningless. Our present findings call for further studies of the effects of territory size on reproductive success in *A. femoralis* and other Dendrobatoids with a distinction between calling and defended territories, and an investigation into their differential roles in the reproductive behaviour of the species.

The comparison of the total extension of DH, LoCoH and STC estimators showed that all three methods produce essentially analogue rank orders (Fig 13.). Most differences originated from the variation in territory shape that resulted from the sensibility of the STC method to trials where tested males showed no reaction in a certain direction. The apparent lack of playback territory overlap (Fig. 4) indicates that this non-responsiveness in certain directions can be interpreted as an exclusion mechanism towards neighbouring territorial males. For only two individuals we could find a spatial overlap of playback territories, however the underlying playback trials were temporally separated by twelve days, which corroborates the notion of exclusive occupancy of territories with dynamic fluctuations over time.

In terms of extension of the isoclines our newly developed STC method yielded highly concordant results with the LoCoH estimator over the whole range of %-isoclines (Fig. 15). However, due to the effects described above, there was considerable variation in the shape of %-isoclines, especially at the centre of the territories. For the LoCoH and for the STC estimator, area equality with the DH of the calling territories was reached on average at the 50% and 40%-isoclines, respectively, however complete overlap was reached only as late as at the 80% and 90%-isoclines, respectively. Albeit

these promising results we are aware of some limitations to the STC approach at the moment. Clearly it would have been desirable to apply the ‘rubbersheet’ spatial adjustment directly to the underlying probability distributions instead of the resulting isoclines. However, when applied to the raster data of the distributions, the corresponding spline transformation for georeferencing (cf. ArcGIS manual [68] did produce severe artefacts far outside the DH playback territories, rendering the subsequent construction of probability isoclines meaningless. Thus the indirect approach to stretch and jolt isoclines of previous kernel calculations currently is the only practical way for the STC method. Given the highly promising results from comparisons with other estimators, we urge for the elaboration and further development of this approach and its

implementation in widespread software environments.

Our findings show that an active approach to assess territoriality and territory size can be more appropriate than traditional observational techniques, especially in the analysis of central-place territories. The fact that the extension of observed and defended territories does not correlate in *A. femoralis*, and presumably does not in other species as well, calls for further research on territorial behaviour, appropriate estimators of territory size, and the influence of territorial behaviour on fitness and reproductive success.

Acknowledgements

We are grateful for the help we received from Julia Felling and Hannes Kronaus during mapping of the study area and fieldwork.

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Predation on *Allobates femoralis*
(Boulenger 1884; Anura: Aromobatidae) by the colubrid snake
Xenopholis scalaris (Wucherer 1861)

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Subsumed under the vernacular name “poison frogs”, the superfamily Dendrobatoidea contains the “non-poisonous poison frogs” in the family Aromobatidae and the “true poison frogs” in the family Dendrobatidae (Grant et al., 2006). While the latter regularly feature bright, aposematic colouration and potent skin toxins, members of the Aromobatidae are usually cryptically coloured and rely on camouflage and rapid escape behaviour as anti-predator measures (Cooper, Caldwell

and Vitt, 2009). Despite intensive research on various aspects of dendrobatoid biology, little information is available on natural predators of this taxon (cf. Darst and Cummings, 2006; Saporito et al., 2007; Cooper, Caldwell and Vitt, 2009; Noonan and Comeault, 2009). *Allobates femoralis* is a semi-cryptic pan-Amazonian aromobatid frog (Amézquita et al., 2009) and possesses, at the most, only traces of skin toxins of the potent alkaloid classes known from dendrobatids (Daly, Myers and Whittaker, 1987). However, the species was found to be a Batesian mimic of poisonous species of the dendrobatid genus *Epipedobates* (Darst and Cummings, 2006), including *E. hahneli*, which is syntopic with *A. femoralis* at the observation site in French Guiana (Lescure and Marty, 2000; Born and Gaucher, 2001). Additionally, the skin

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Figure 1. Adult *Allobates femoralis* with attached HDF reflector.



Figure 2. Adult *Xenopholis scalaris* coiled under leaves.

excretions of *A. femoralis* are likely to make the frog unpalatable to some predators due to their bitter taste (pers. obs).

The focus individual, a male *A. femoralis* with a snout-urostyle length of 27.05 mm and a weight of 1.7 g, was used in field experiments to test the suitability of the harmonic-direction-finding (HDF) telemetry-technique (Pellet *et al.*, 2006) to study male tadpole transport and female mating commutation in this highly territorial (Ringler, Ursprung and Hödl, 2009) species. The trials took place in tropical lowland rainforest near the research station “Saut Pararé” in the nature reserve “Les Nouragues”, French Guiana (4.04° N, 52.68° W, WGS84). After some other individuals had been observed in captivity in order to optimise reflector attachment, on 28 February 2008 three individuals, including the focal male, were released inside their respective territories with attached reflectors to investigate their behaviour under natural conditions. The reflectors consisted of a Schottky diode that was soldered between two antennas made of 40 µm steel-strands forming a 6 cm by 12 cm T-shaped dipole with the braze points covered in shrinkable tubing. To attach the reflectors to the frogs we used waistbands (Fig. 1), made from latex-free condoms to prevent skin irritation (Gutleb *et al.*, 2001). Together with the waistband, the reflectors had a total mass of less than 0.05 g, which is



Figure 3. Partly digested *Allobates femoralis* recovered from partly skeletonised *Xenopholis scalaris*.

less than 5% of the body mass of adult *A. femoralis*.

The focal male was recovered with a RECCO HDF several times from 28 - 29 February and showed apparently normal behaviour during that time, including constant advertisement calls and regular movement and escape behaviour. On 1 March, with the first detection on that day at 1100 hours, we unambiguously retrieved the reflector's signal from an adult (aprox. 300 mm in total length) *Xenopholis scalaris*, which we discovered typically coiled in its protective position (Zug, Vitt and Caldwell, 2007) under some leaves (Fig. 2), still inside the territory of the focal male. To observe the further behaviour of the snake, we took it into captivity in a netted cage. During the night the snake died and was already partly skeletonised by ants when we discovered it the next morning. We opened the body cavity of the snake and recovered the partly digested frog, which apparently had been swallowed head first and whose ventral pattern was still recognizable for individual identification (Fig. 3). No apparent internal injuries from the reflector were visible and its antennae were folded smoothly in the intestinal tract of the snake. Hence we cannot be certain whether the death of the snake was caused by toxic skin excretions from the frog, direct effects of the swallowed reflector, or if it was attacked and killed by the ants that covered the corpse in the morning.

Xenopholis scalaris is a small (300-350 mm in total length) leaf-litter snake with an equally pan-Amazonian distribution as *A. femoralis* where it inhabits primary and old secondary rainforest in humid zones. It features a brick-red brown body with a dark longitudinal central line running along the body which is accompanied by perpendicular running, dark triangular shaped patterns (Fig. 2). The species is known to feed on frogs, and given its nocturnal activity (Starace, 1998), it is highly likely that the prey frog was taken during the night when *A. femoralis*, like almost all dendrobatoids, is not active. Snake predation on telemetered amphibians has been reported previously by Spieler and Linsenmair (1998) and Jehle and Arntzen (2000). However, as we did not witness the actual predation event, we can only speculate whether the attached reflector rendered the frog more conspicuous and made it more susceptible to fall prey to the snake, or if the reflector hindered the frog in escaping its predator.

Acknowledgements. Fieldwork was supported by the Austrian Science Fund grant FWF-18811 (PI Walter Hödl). We are especially grateful to Nicolas Perrin from the Department of Ecology and Evolution at the UNIL, Lausanne, Switzerland for the loan of the RECCO HDF. Permissions were provided by the CNRS and fieldwork was conducted in compliance with current French and EU law.

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Part 3

Concluding Discussion

The five original papers which constitute this doctorate thesis cover research on several aspects of the life history in the Neotropical frog *Allobates femoralis*. My aim was to investigate the genetic mating system and determinants of reproductive success in a population of the Neotropical frog *A. femoralis* at the study site 'Saut Pararé', French Guiana.

Methodological Considerations

Molecular techniques have become a powerful tool in the study of behavioural ecology and population genetics. Especially the possibility to infer genealogical relationships among individuals in a population has opened up various possibilities of research in behaviour, evolution and conservation (Jones & Ardren 2003; Pemberton 2008; Jones *et al.* 2009). Pedigrees directly disclose patterns of reproductive success across consecutive generations at the individual level. Therefore, pedigrees are extremely valuable for studies where representative observations of courtship and mating behaviour in the field are difficult to obtain, as it is the case in *A. femoralis* (cf. Montanarin *et al.* 2011). However, highly variable or large numbers of markers are required to perform parentage assignments and to reliably assess the genetic setup of a population (Blouin 2003). Furthermore, the urge to reconstruct family trees across all individuals in a given area is offset against considerable logistic constraints in terms of sampling effort. So far, the majority of currently available data sets have been compiled for birds and mammals (Pemberton 2008) with relatively few offspring, and whose family relationships can often be revealed through their extended parental care. Studies on other organisms have often been hampered by the absence of longer associations between mates and the complex life-histories of dispersed, long-lived, and/or cryptic individuals in large populations. Also most studies on amphibian populations have restricted their measurements to indirect, population-wide parameters such as effective population size, which cannot consider individual reproductive contributions to a following cohort (Araki & Blouin 2005; but see Broquet *et al.* 2009). The application of these indirect estimates is further constrained by the fact that several of their assumptions, such as a closed population, random mating, and discrete non-overlapping generations, are commonly violated in natural populations in the wild. To the best of my knowledge, the approach of pedigree reconstruction of two successive generations, which I used in order to calculate the number of (effective) breeders within the *A. femoralis* population, has never been taken on an entire anuran amphibian population so far.

Although ten microsatellite loci had been previously published for *A. femoralis* (Jehle *et al.* 2008) only seven of them actually could be used for the population in French Guiana. Moreover, for two microsatellite loci (Afem15 and Afem16) I had to design new primers in order to enhance PCR amplification rates. This was likely due to the circumstance that the markers were originally derived from individuals of a Brazilian population. The geographical distance between the two populations (more than 800 km; Amézquita *et al.* 2009) likely was associated with sequence differences at primer binding sites (effects of isolation-by-distance) that impeded the successful amplification at those loci. Despite the rather low overall number of microsatellite loci used ($n = 7$), the markers were sufficiently powerful for the parentage assignments and relatedness calculations, due to the high allelic diversity across loci. The broad range of statistical methods available for molecular-based parentage and kinship analyses makes it difficult to choose the best software for a particular dataset. I decided to perform all parentage analyses with COLONY, as this program is considered to generate fewer false assignments than other methods that use pairwise relationships (Wang 2004; Wang & Santure 2009; Walling *et al.* 2010). I evaluated the reliability of the COLONY assignments by analysing individuals with known parent-offspring relationships (see Ursprung *et al.* 2011a and Ursprung *et al.* submitted for details). For the analyses of relatedness the estimator implemented in the software KINSHIP (Konovalov *et al.* 2004) was chosen, since it was found to be accurate even in cases where low numbers of loci with few alleles were used (Konovalov & Heg 2008), and since the overall performance of this coefficient increases with sampling coverage. Given our attempt of total sampling of all adult *A. femoralis* in the population and the high variation across the microsatellite loci, I expected this estimator to be very suitable for our analyses.

Although statistical methods are permanently improving and genotyping is becoming faster and cheaper, enabling the precise assessment of pedigrees and relatedness estimates of huge datasets, genotype data alone does not provide sufficient information when investigating determinants of reproductive success. Therefore, we collected also behavioural and detailed spatial information for all individuals in the focal *A. femoralis* population. The integration of genetic and observational field data showed to be a powerful approach to investigate patterns of reproductive success in the *Allobates femoralis* population under study.

General Conclusions

Despite the special position of Dendrobatoidea within anuran amphibians owing to their elaborate reproductive behaviour across the whole group, few molecular studies on reproductive performance have been performed in this taxon. Thus, the knowledge about

reproductive behaviour and mating systems in the few dendrobatoid species that have been studied so far is almost exclusively based on observational data (Summers 1989, 1990, 1992; Roithmair 1992, 1994; Jungfer & Weygold 1999; Summers & Earn 1999; Pröhl 2002, 2005; but see Brown *et al.* 2010).

In my dissertation I investigated the genetic mating system and correlates of reproductive success in a natural population of the Neotropical frog *Allobates femoralis* (Aromobatidae). Microsatellite loci were highly diverse, with an average of 16.86 alleles per locus and a mean observed heterozygosity of 0.79. The analysis of parentage across two successive adult generations revealed a polygynandrous mating system in which high proportions of males (35.5%) and females (56.0%) produced progeny that survived until adulthood (Ursprung *et al.* 2011a). Hence, the observed genetic diversity was higher and the reproductive contributions of males and in females were much more even than what has been reported from most other studies in amphibian species (Scribner *et al.* 1997; Jehle *et al.* 2005; Schmeller & Merilä 2007; Beebee 2009; Ficetola *et al.* 2010; see also Neff & Gross 2001; Broquet *et al.* 2009). However, most of these studies were conducted on European pond breeding species, where severe bottlenecks caused by glaciations may have lead to the observed low levels of heterozygosity (Hewitt 2000; Rowe *et al.* 2006). The fact that genetic diversity and number of matings per reproductive season are often lower in amphibian species in temperate regions than in species in tropical environments may further be related to climatic factors and the according temporal constraints on reproduction. Reproductive behaviour in amphibians is strongly related to abiotic factors, especially precipitation and temperature. Additionally, biotic factors such as predation, inter-specific competition and depletion of food for tadpoles lead to temporal reproductive niche separation between syntopic species (Arak 1983; Morin *et al.* 1990; Lucas *et al.* 1996; McCauley *et al.* 2000). Given the need of sufficient time for the offspring to mature and to survive the first winter, this considerably restricts the time for reproduction that is actually available for species in temperate regions. As a consequence, the reproductive period of most temperate anurans seldom lasts longer than a couple of weeks per year, and females will only be able produce one clutch per reproductive season. Contrastingly, the absence of pronounced seasons in tropical environments allows for prolonged breeding and therefore enables sequential polyandrous mating of females.

As in *A. femoralis* females are the choosing sex, a special focus has been given on female mating behaviour in all further analyses of individual reproductive success. Parentage assignments revealed that females distributed consecutive matings across multiple partners. Despite contrasting reproductive strategies and the male biased sex-ratio, successfully

reproducing males and females had similar numbers of mating partners and similar numbers of offspring that reached adulthood. Consequentially, we wanted to investigate whether it is sexual selection that determines male reproductive success and to identify possible female preferences. By investigating the standardised variances of reproductive success over all individuals of the population, the results revealed a higher opportunity for sexual selection to act on males than on females (Ursprung *et al.* 2011a). As the difference between males and females disappeared when only actual reproducers were taken into account, this indicates that the opportunity to breed in males may be determined by binary rather than by gradual sexual selection. Given the strong male-biased sex ratio, the observed differences between the sexes were likely due to the differences in the numbers of non-reproducing individuals between the sexes (cf. Raffetto *et al.* 1990; Klug *et al.* 2010). Nonetheless, we additionally investigated actual male traits that females may potentially select for, such as male body size, territory size (Ursprung *et al.* 2011a), and also pairwise relatedness (Ursprung *et al.* submitted), to corroborate this hypothesis. Although a relation between territory size and mating success was found in a previous study in *A. femoralis* (Roithmair 1992), in our study we did not find an effect of territory size on reproductive success in terms of adult progeny produced. Reproductive success was significantly higher in territorial than non-territorial males, but unrelated to territory size in males or to body size in both sexes (Ursprung *et al.* 2011a). Given that mating frequency might not precisely predict actual reproductive output, as well as our recent finding that the size of calling and defended territories does not correlate in *A. femoralis* males (Ringler *et al.* submitted), more research is needed to carefully entangle the possible correlation between mating and reproductive success as well as their relation to male territory size in this species. Like for territory and male body size, our results also provided no evidence of assortative mating by relatedness (Ursprung *et al.* submitted). This might be due to the fact that for *A. femoralis* females the costs that are associated with in- and outbreeding avoidance in terms of lost breeding opportunities are likely higher compared to the costs of tolerating sub-optimal matings (cf. Pärt 1996). Incestuous or outbred matings might therefore be still preferable over not breeding at all (Kokko & Ekmann 2002; Kokko & Ots 2006). Likewise, pairwise genetic relatedness or kinship status might constitute a trait that is not assessable by female *A. femoralis* prior mating. In summary, we did not find any evidence of assortative mating of females by male body size, territory size, or pairwise genetic relatedness, but reproductive success was significantly biased towards territorial males. I interpret the observed patterns of reproductive success in *A. femoralis* as the result of inter- sexual selection through high male-male competition for territories and a subsequent, presumably random, mating of females among

territorial males. This also corroborates our previous findings on the patterns of the opportunity for selection in males.

Multiple direct and indirect benefits that can be associated with polyandry may have lead to the observed mating system in *A. femoralis*. Sequential mating with multiple partners may compensate for possible negative effects of single mating decisions (cf. Colegrave *et al.* 2002; Roberts & Gosling 2003; Byrne & Roberts 2011), and can therefore act as a bet-hedging mechanism against total reproductive failure (Birkhead 2000; Byrne & Roberts 2011). These 'negative effects' might be caused by genetic attributes (i.e. bad or incompatible mating partners), by behavioural factors (e.g. poor parental care), but also by natural selection in terms of predation or environmental threats. In species that breed in changing environments, where unforeseeable changes can significantly determine offspring survival, sequential polyandry is expected to be particularly beneficial owing to the spreading of risk (Ihara 2002; Fox & Rauter 2003; Byrne & Keogh 2009). Given their external fertilisation, amphibians are especially vulnerable to such unstable environmental conditions. In *A. femoralis*, I assume the threats associated with the transportation and development of tadpoles to constitute such unpredictable factors. As females move back to their resting sites after mating, and egg attendance and tadpole transport to water bodies are generally performed by males only (Weygold 1980; Caldwell & de Araújo 2005), females have no further influence or control over the survival of their offspring. Moreover, after the transportation to bodies of water where larvae stay until metamorphosis, tadpoles will be exposed to a certain predation risk (Werner & McPeck 1994), as well as to the risk of desiccation due to the lack of sufficient rain (Summers 1990). Iteroparous mating with multiple partners will hence temporally and also spatially allocate matings and consequentially parental care to several males. This will not only boost the genetic diversity among their progeny, but will temporally and spatially spread the risk of offspring mortality. Hence, by sequentially mating with multiple partners females can ensure against total reproductive loss and thereby enhance individual reproductive success (cf. Evans & Magurran 2000; Yasui 2001). For *A. femoralis*, this hypothesis is corroborated by the high percentage of females (but also males) with cross-generational reproductive output which was found in our study (Ursprung *et al.* 2011a). Finally, the evolution of female polyandry in *A. femoralis* is likely driven by the enhanced offspring survival when mating events and accordingly parental care are spread over multiple partners.

The variance of pairwise relatedness of females to their mating partners was significantly smaller than what could be expected under random mating. Furthermore, observed homozygosity was significantly higher in the embryo than in the adult cohort. This suggests

stabilizing natural selection acting on genetic divergence between mating partners for successful reproduction. Further studies under controlled laboratory conditions are needed to precisely investigate the effect of parental relatedness on the viability of offspring. Moreover, the observed high numbers mating partners per female raise the question whether females actively try to spread matings across multiple males, or if their mating behaviour is purely random.

Since in a previous study territory size was found to correlate with mating success (Roithmair 1992), we had a specific interest in accurately assessing territory size for the males in our *A. femoralis* population. We demonstrate that beside the common approach of simply mapping individual locations, territory size can be determined by an active approach through intrusion experiments. Simulations of intrusion experiments in virtual territories were used to assess the minimum number of trials required in order to obtain reliable size estimates. Furthermore, we analysed these simulated point patterns but also an empirical data set of playback experiments performed on *A. femoralis* males, to find the most suitable area estimator that is readily available to field biologists. The finding that the extension of the defended area, the 'playback territory', did not correlate with the area occupied during calling, the 'calling territory', is of particular importance to studies that investigate the relation between territory size and other traits of an individual. Thus, in future studies I will incorporate both, calling and playback territory size measures in the analyses of reproductive success and sexual selection.

The regrowth of the clipped toes in *A. femoralis* (Ursprung *et al.* 2011b) was totally unexpected by me. In amphibians, toe regeneration has been reported from newts (Smith 1978) and salamanders (Ott & Scott 1999; Davis & Ovaska 2001), whereas adult anurans were generally assumed to lack this ability (Halliday 1996). I hypothesise that the consistent toe regeneration in *A. femoralis* is caused by the specific cellular processes that allow for the lifelong growth of amphibians in general (Duellmann & Trueb 1986). Given that the capacity for gene expressions that are involved in growth and pattern formation progressively decreases with age (e.g. Endo *et al.* 1997 and Yokoyama *et al.* 2000 for studies on *Xenopus*), the short life span of *A. femoralis* may explain why adult individuals in this species retain the ability to regenerate toes. The difference in colouration between unclipped and regrown toe discs in turn, which facilitated detection of recaptured individuals within and between years, might be caused by the fact that differentiation and migration of pigment cells take place only in the early ontogenesis (Erickson 1993). However, quantitative and comparative as well as embryological studies of such possible effects remain to be conducted.

Also the predation event of a colubrid snake *Xenopholis scalaris* on an *A. femoralis* male, which has been observed in the course of this dissertation study, revealed new life-history

information. Despite intensive research on various aspects of the general biology of dendrobatoid frogs, there is very limited knowledge about natural predators of this taxon (cf. Darst & Cummings 2006; Saporito *et al.* 2007; Cooper *et al.* 2009; Noonan & Comeault 2009).

This thesis has employed an integrative approach by combining genetic, behavioural, and also spatial data to reveal the genetic mating system and explain patterns of reproductive success in *A. femoralis*. The five original papers that contribute to this thesis do not only provide comprehensive information about patterns of reproductive success in males and females, but also provide new insights regarding the life-history of this species. Thus, I believe my findings will be a valuable contribution to the general knowledge of mating systems in dendrobatoid frogs, but also enhance our general understanding about adaptive significance of individual reproductive behaviour that drives the evolution of mating systems in animal species.

References of General Introduction and Concluding Discussion

For the references of the manuscripts contributing to this doctoral thesis see the reference list of each paper.

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Author Contributions

The following authors contributed to the five studies of this doctoral thesis:

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- **Max Ringler (MR)¹⁾**
- **Walter Hödl (WH)¹⁾**
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Ursprung E, Ringler M, Jehle R, Hödl W (2011) Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. *Molecular Ecology* 20: 1759–1771.

EU and MR conceived the study. Field work was performed by EU and MR, with assistance from WH. EU did all molecular work and statistical analyses. MR performed all spatial analyses. Figures and tables were prepared by EU and MR. EU, MR and RJ wrote the paper. All authors read and approved the final manuscript.

Ursprung E, Ringler M, Jehle R, Hödl W (submitted to *Behavioral Ecology* – currently under review) Selection against extremes? Parental relatedness across a natural population of a Neotropical frog.

EU conceived the study. Field work was performed by EU and MR, with assistance from WH. EU did all molecular work and statistical analyses. All spatial analyses were performed by MR. Figures and tables were prepared by EU. EU wrote the paper with assistance from MR, RJ, and WH. All authors read and approved the final manuscript.

Ursprung E, Ringler M, Jehle R, Hödl W (2011) Toe regeneration in the Neotropical frog *Allobates femoralis* (Aromobatidae). *Herpetological Journal* 21: 83–86.

EU conceived the study. Field work was performed by EU and MR, with assistance from WH. EU did all measurements and statistical analyses. Figures and tables were prepared by EU. EU wrote the paper with assistance from MR, RJ, and WH. All authors read and approved the final manuscript.

Ringler M, Ursprung E, Magaña Mendoza D, Hödl W (submitted to *PLoS ONE* – currently under review) Intrusion Experiments to Measure Territory Size: Development of the Method, Tests Through Simulations, and Application in the Frog *Allobates femoralis*.

MR, DMM and WH conceived the study. Field work was performed by EU and MR. MR did all measurements and statistical analyses, and prepared all figures and tables. MR wrote the paper with assistance from EU and WH. All authors read and approved the final manuscript.

Ringler M, Ursprung E, Hödl W (2010) Predation on *Allobates femoralis* (Boulenger 1884; Anura: Aromobatidae) by the colubrid snake *Xenopholis scalaris* (Wucherer 1861), *Herpetology Notes* 3: 301–304.

MR conceived the study. Field work was performed by EU and MR. MR did all measurements and prepared all figures and tables. MR wrote the paper with assistance from EU and WH. All authors read and approved the final manuscript.

Annex

Ventral patterns used for individual identification of all adult *Allobates femoralis* encountered and genetically sampled in our research plot during both years of the study.



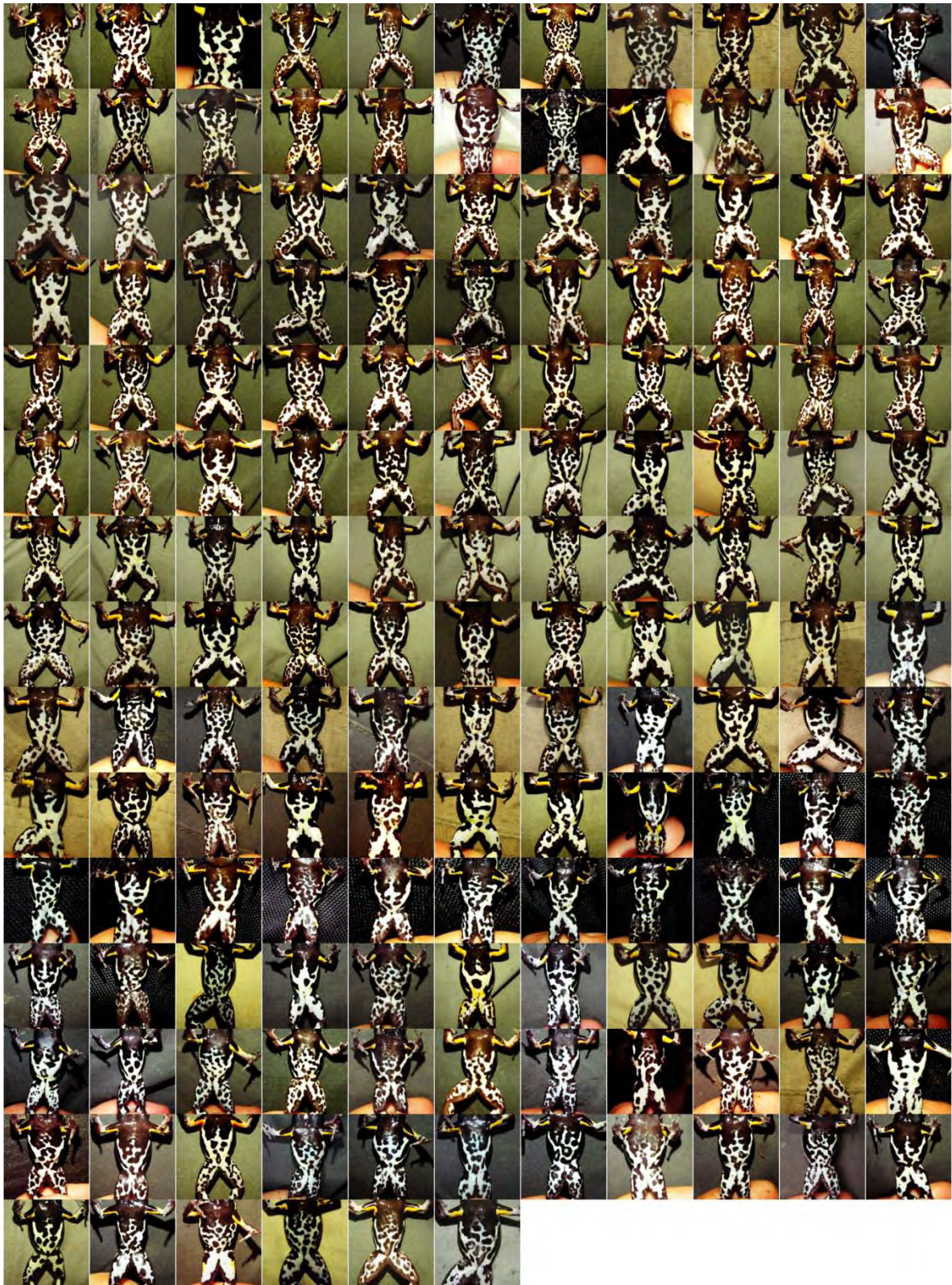
Female and juvenile *A. femoralis* sampled in 2008



Male *A. femoralis* sampled in 2008



Female and juvenile *A. femoralis* sampled in 2009



Male *A. femoralis* sampled in 2009

Genepop version 4.0.7: Hardy-Weinberg test

Number of populations detected: 1

Number of loci detected: 7

Estimation of exact P-Values by the Markov chain method.

Markov chain parameters for all tests:

Dememorization: 1000000

Batches: 100

Iterations per batch: 50000

Hardy Weinberg: Probability test

=====

Results by population

=====

Pop : femo08

Fis estimates

locus	P-val	S.E.	W&C	R&H	Steps
5	0.0351	0.0044	0.0957	0.1013	97777 switches
12	0.0327	0.0016	-0.0503	-0.0238	536516 switches
9	0.2335	0.0072	0.0198	0.0476	221085 switches
3	0.0599	0.0013	-0.0304	-0.0257	1132509 switches
15	0.0776	0.0037	-0.0092	0.0241	275466 switches
13	0.7783	0.0059	0.0086	0.0197	297663 switches
16	0.1073	0.0027	0.0148	0.0311	828495 switches

All (Fisher's method):

Chi2 : 32.9707

Df : 14.0000

Prob : 0.0029

Genepop version 4.0.7, Genotypic linkage disequilibrium

Markov chain parameters

Dememorisation : 1000000

Batches : 500

Iterations per batch : 50000

Pop	L#1	L#2	P-Value	S.E.	Switches
-----	-----	-----	-----	-----	
femo08	5	12	0.0589834	0.005356	104692
femo08	5	9	0.372995	0.013223	76787
femo08	12	9	0.663740	0.016194	28558
femo08	5	3	0.742696	0.009537	166606
femo08	12	3	0.568247	0.014011	69558
femo08	9	3	0.406665	0.014969	49396
femo08	5	15	0.380613	0.014409	76215
femo08	12	15	0.210542	0.013862	27923
femo08	9	15	0.537150	0.018437	19089
femo08	3	15	0.230091	0.012742	54820
femo08	5	13	0.825229	0.009436	78449
femo08	12	13	0.199510	0.013887	26189
femo08	9	13	1.000000	0.000000	18175
femo08	3	13	0.297290	0.014653	43295
femo08	15	13	0.323657	0.017608	18801
femo08	5	16	0.625743	0.011740	108906
femo08	12	16	0.322217	0.015239	36231
femo08	9	16	1.000000	0.000000	20385
femo08	3	16	0.085446	0.007434	55358
femo08	15	16	0.474633	0.018303	22940
femo08	13	16	0.116874	0.011626	21067

Normal ending.

CERVUS:

**** Summary statistics ****

Locus	k	N	HObs	HExp	PIC	NE-1P	NE-2P	NE-PP	NE-I	NE-SI	HW	F(Null)
5	17	137	0.555	0.613	0.595	0.762	0.570	0.348	0.168	0.486	NS	+0.0295
12	16	137	0.905	0.872	0.857	0.407	0.254	0.094	0.029	0.323	NS	-0.0227
9	22	123	0.894	0.912	0.901	0.312	0.184	0.055	0.015	0.300	ND	+0.0067
3	11	137	0.883	0.857	0.839	0.450	0.288	0.120	0.037	0.332	NS	-0.0165
15	21	132	0.917	0.908	0.897	0.321	0.191	0.058	0.017	0.302	ND	-0.0075
13	20	117	0.897	0.905	0.894	0.328	0.197	0.060	0.017	0.304	ND	+0.0011
16	15	121	0.893	0.906	0.894	0.328	0.196	0.061	0.017	0.303	ND	+0.0035

Number of individuals: 138

Number of loci: 7

Mean number of alleles per locus: 17.43

Mean proportion of individuals typed: 0.9358

Mean expected heterozygosity: 0.8535

Mean polymorphic information content (PIC): 0.8397

Combined non-exclusion probability (first parent): 0.00150024

Combined non-exclusion probability (second parent): 0.00005666

Combined non-exclusion probability (parent pair): 0.00000005

Combined non-exclusion probability (identity): 1.36E-0011

Combined non-exclusion probability (sib identity): 0.00043386

**** Locus 5 ****

Allele	Count	Heterozygotes	Homozygotes	Frequency	Frequency with null
97	3	3	0	0.0109	0.0110
99	5	5	0	0.0182	0.0184
101	1	1	0	0.0036	0.0037
103	166	66	50	0.6058	0.6075
107	2	2	0	0.0073	0.0073
109	5	5	0	0.0182	0.0184
111	1	1	0	0.0036	0.0037
117	9	9	0	0.0328	0.0334
119	3	3	0	0.0109	0.0110
123	10	10	0	0.0365	0.0372
125	13	7	3	0.0474	0.0372
127	23	17	3	0.0839	0.0758
129	26	20	3	0.0949	0.0877
131	4	0	2	0.0146	0.0073
133	1	1	0	0.0036	0.0037
135	1	1	0	0.0036	0.0037
193	1	1	0	0.0036	0.0037

Number of individuals typed: 137
 Heterozygotes: 76
 Homozygotes: 61
 Number of alleles: 17
 Observed heterozygosity: 0.5547
 Expected heterozygosity: 0.6132
 Polymorphic information content (PIC): 0.5946
 Average non-exclusion probability (first parent): 0.7618
 Average non-exclusion probability (second parent): 0.5703
 Average non-exclusion probability (parent pair): 0.3484
 Average non-exclusion probability (identity): 0.1678
 Average non-exclusion probability (sib identity): 0.4864
 Hardy-Weinberg equilibrium test
 Minimum expected frequency: 5.0
 Chi-square value (using Yates' correction): 0.0080
 Degrees of freedom: 1
 P-value: 0.9288
 Significance (with Bonferroni correction): NS
 Null allele frequency estimate: +0.0295

**** Locus 12 ****

Allele	Count	Heterozygotes	Homozygotes	Frequency	Frequency with null
114	47	35	6	0.1715	0.1628
118	4	4	0	0.0146	0.0147
126	5	5	0	0.0182	0.0184
130	5	5	0	0.0182	0.0184
134	17	17	0	0.0620	0.0641
138	71	61	5	0.2591	0.2799
142	5	5	0	0.0182	0.0184
146	16	12	2	0.0584	0.0524
150	11	11	0	0.0401	0.0410
154	28	28	0	0.1022	0.1080
158	18	18	0	0.0657	0.0680
162	22	22	0	0.0803	0.0838
166	13	13	0	0.0474	0.0486
170	7	7	0	0.0255	0.0259
174	4	4	0	0.0146	0.0147
178	1	1	0	0.0036	0.0037

Number of individuals typed: 137
 Heterozygotes: 124
 Homozygotes: 13
 Number of alleles: 16
 Observed heterozygosity: 0.9051
 Expected heterozygosity: 0.8722
 Polymorphic information content (PIC): 0.8574
 Average non-exclusion probability (first parent): 0.4065
 Average non-exclusion probability (second parent): 0.2544
 Average non-exclusion probability (parent pair): 0.0939
 Average non-exclusion probability (identity): 0.0287
 Average non-exclusion probability (sib identity): 0.3227
 Hardy-Weinberg equilibrium test
 Minimum expected frequency: 5.0
 Chi-square value (using Yates' correction): 2.7231
 Degrees of freedom: 1
 P-value: 0.0989
 Significance (with Bonferroni correction): NS
 Null allele frequency estimate: -0.0227

**** Locus 9 ****

Allele	Count	Heterozygotes	Homozygotes	Frequency	Frequency with null
149	1	1	0	0.0041	0.0041
173	1	1	0	0.0041	0.0041
177	6	6	0	0.0244	0.0247
181	32	28	2	0.1301	0.1305
185	27	23	2	0.1098	0.1074
189	6	2	2	0.0244	0.0164
193	16	16	0	0.0650	0.0673
197	6	6	0	0.0244	0.0247
201	3	3	0	0.0122	0.0123
205	4	2	1	0.0163	0.0123
209	20	16	2	0.0813	0.0761
213	8	8	0	0.0325	0.0331
225	9	9	0	0.0366	0.0373
229	28	24	2	0.1138	0.1120
233	34	32	1	0.1382	0.1446
237	29	27	1	0.1179	0.1212
241	6	6	0	0.0244	0.0247
249	3	3	0	0.0122	0.0123
261	1	1	0	0.0041	0.0041
265	1	1	0	0.0041	0.0041
269	2	2	0	0.0081	0.0082
277	3	3	0	0.0122	0.0123

Number of individuals typed: 123
 Heterozygotes: 110
 Homozygotes: 13
 Number of alleles: 22
 Observed heterozygosity: 0.8943
 Expected heterozygosity: 0.9123
 Polymorphic information content (PIC): 0.9015
 Average non-exclusion probability (first parent): 0.3115
 Average non-exclusion probability (second parent): 0.1844
 Average non-exclusion probability (parent pair): 0.0549
 Average non-exclusion probability (identity): 0.0155
 Average non-exclusion probability (sib identity): 0.2996
 Hardy-Weinberg equilibrium test: Not done
 Null allele frequency estimate: +0.0067

**** Locus 3 ****

Allele	Count	Heterozygotes	Homozygotes	Frequency	Frequency with null
192	10	10	0	0.0365	0.0372
196	32	30	1	0.1168	0.1203
200	24	22	1	0.0876	0.0878
204	27	27	0	0.0985	0.1039
208	20	18	1	0.0730	0.0719
212	48	42	3	0.1752	0.1805
216	71	53	9	0.2591	0.2600
220	24	22	1	0.0876	0.0878
224	2	2	0	0.0073	0.0073
228	14	14	0	0.0511	0.0525
232	2	2	0	0.0073	0.0073

Number of individuals typed: 137
 Heterozygotes: 121
 Homozygotes: 16
 Number of alleles: 11
 Observed heterozygosity: 0.8832
 Expected heterozygosity: 0.8572
 Polymorphic information content (PIC): 0.8387
 Average non-exclusion probability (first parent): 0.4497
 Average non-exclusion probability (second parent): 0.2876
 Average non-exclusion probability (parent pair): 0.1200
 Average non-exclusion probability (identity): 0.0367
 Average non-exclusion probability (sib identity): 0.3321
 Hardy-Weinberg equilibrium test
 Minimum expected frequency: 5.0
 Chi-square value (using Yates' correction): 0.0211
 Degrees of freedom: 1
 P-value: 0.8845
 Significance (with Bonferroni correction): NS
 Null allele frequency estimate: -0.0165

**** Locus 15 ****

Allele	Count	Heterozygotes	Homozygotes	Frequency	Frequency with null
109	1	1	0	0.0038	0.0038
149	7	7	0	0.0265	0.0269
153	2	2	0	0.0076	0.0076
157	36	36	0	0.1364	0.1472
161	35	31	2	0.1326	0.1340
165	7	7	0	0.0265	0.0269
169	3	1	1	0.0114	0.0076
173	1	1	0	0.0038	0.0038
177	5	5	0	0.0189	0.0191
181	4	4	0	0.0152	0.0153
185	16	12	2	0.0606	0.0545
189	25	21	2	0.0947	0.0913
193	30	28	1	0.1136	0.1166
197	41	35	3	0.1553	0.1561
201	13	13	0	0.0492	0.0505
205	8	8	0	0.0303	0.0308
209	10	10	0	0.0379	0.0386
213	11	11	0	0.0417	0.0426
221	3	3	0	0.0114	0.0114
225	1	1	0	0.0038	0.0038
233	5	5	0	0.0189	0.0191

Number of individuals typed: 132
 Heterozygotes: 121
 Homozygotes: 11
 Number of alleles: 21
 Observed heterozygosity: 0.9167
 Expected heterozygosity: 0.9084
 Polymorphic information content (PIC): 0.8974
 Average non-exclusion probability (first parent): 0.3208
 Average non-exclusion probability (second parent): 0.1911
 Average non-exclusion probability (parent pair): 0.0582
 Average non-exclusion probability (identity): 0.0166
 Average non-exclusion probability (sib identity): 0.3017
 Hardy-Weinberg equilibrium test: Not done
 Null allele frequency estimate: -0.0075

**** Locus 13 ****

Allele	Count	Heterozygotes	Homozygotes	Frequency	Frequency with null
192	1	1	0	0.0043	0.0043
196	2	2	0	0.0085	0.0086
200	6	6	0	0.0256	0.0260
204	5	5	0	0.0214	0.0216
208	8	4	2	0.0342	0.0260
212	11	11	0	0.0470	0.0482
216	16	14	1	0.0684	0.0663
220	9	7	1	0.0385	0.0348
224	37	33	2	0.1581	0.1628
228	31	25	3	0.1325	0.1278
232	15	15	0	0.0641	0.0663
236	10	10	0	0.0427	0.0437
240	42	36	3	0.1795	0.1835
244	15	15	0	0.0641	0.0663
248	9	9	0	0.0385	0.0392
252	6	6	0	0.0256	0.0260
256	7	7	0	0.0299	0.0304
260	2	2	0	0.0085	0.0086
264	1	1	0	0.0043	0.0043
268	1	1	0	0.0043	0.0043

Number of individuals typed: 117
 Heterozygotes: 105
 Homozygotes: 12
 Number of alleles: 20
 Observed heterozygosity: 0.8974
 Expected heterozygosity: 0.9052
 Polymorphic information content (PIC): 0.8936
 Average non-exclusion probability (first parent): 0.3282
 Average non-exclusion probability (second parent): 0.1965
 Average non-exclusion probability (parent pair): 0.0604
 Average non-exclusion probability (identity): 0.0174
 Average non-exclusion probability (sib identity): 0.3037
 Hardy-Weinberg equilibrium test: Not done
 Null allele frequency estimate: +0.0011

**** Locus 16 ****

Allele	Count	Heterozygotes	Homozygotes	Frequency	Frequency with null
405	12	10	1	0.0496	0.0465
409	9	9	0	0.0372	0.0379
413	8	6	1	0.0331	0.0294
417	21	17	2	0.0868	0.0819
421	46	42	2	0.1901	0.2023
425	18	16	1	0.0744	0.0729
429	26	26	0	0.1074	0.1139
433	11	9	1	0.0455	0.0422
437	17	17	0	0.0702	0.0729
441	27	21	3	0.1116	0.1046
445	24	20	2	0.0992	0.0955
449	9	9	0	0.0372	0.0379
457	7	7	0	0.0289	0.0294
461	6	6	0	0.0248	0.0251
465	1	1	0	0.0041	0.0041

Number of individuals typed: 121
 Heterozygotes: 108
 Homozygotes: 13
 Number of alleles: 15
 Observed heterozygosity: 0.8926
 Expected heterozygosity: 0.9059
 Polymorphic information content (PIC): 0.8944
 Average non-exclusion probability (first parent): 0.3284
 Average non-exclusion probability (second parent): 0.1961
 Average non-exclusion probability (parent pair): 0.0607
 Average non-exclusion probability (identity): 0.0173
 Average non-exclusion probability (sib identity): 0.3032
 Hardy-Weinberg equilibrium test: Not done
 Null allele frequency estimate: +0.0035

Acknowledgements

Here is the point where I'd like to say 'thank you' to so many people who guided, supported, encouraged, criticised, and supported me throughout all years of my doctorate studies.

First of all I want to express my deepest gratitude and acknowledgement to my partner and husband **Max Ringler**. You are the one who mainly introduced me to the field of science and the wonderful world of dendrobatoid frogs. You always encouraged and believed in me during all hard times I had to went through during the last years. Although it is sometimes challenging to separate professional and private life, especially when it comes to controversial discussions during field work or writing, I feel immensely privileged to be able to share all these experiences with you! Thank you for giving me so much love and inspiration. I love you.

Many thanks I'd like to express to my parents **Anna** and **Josef Ursprung**. Although you might not have always been able to relate to my passion for frogs and fundamental research, still you granted me with proud and support throughout all the years of my study.

Deep acknowledgements I like to express to **Walter Hödl**. You have not only inspired me to work with *Allobates femoralis*, but particularly gave me sufficient free scope for the creation of my own ideas and my scientific development. Thank you very much for your positive support, passionate encouragement, but also for your honest criticism and constructive feedback!

Many thanks to my co-supervisor **Robert Jehle**. You did not only introduce me into the molecular techniques at the University of Salford, but also provided substantial help and important assistance in the interpretation of results and writing of all manuscripts. I am very thankful for your rapidity (especially in replying to my e-mails) and your efficiency and comprehensiveness in your feedback.

High gratitude I'd like to express to the following people who assisted in the field work: **Hannes Kronaus, Julia Felling, Bibiana Rojas, Mathias Fernandez**, and all students of the field course in 2009 (**Stephan Böhm, Theresa Hebenstreit, Helena Naves Pinto, Michael Riedl, Johannes Schnepf, Fabian Sederl, Stefan Singer, Iris Starnberger, Mike Stiegler, Alexander Wagner, Claudia Zutz**).

Many thanks to **Philippe Gaucher**, who provided important logistic support in French Guiana throughout all the study years. Owing to the fact that we could always rely on your word and immediate assistance, we could purely focus on our work in the field.

Thanks to all the people who provided support in the lab: **Emiliano Dell'Ampio, Christian Baranyi, Daniela Bartel, Thomas Bauer, Silvia Burghesi, Harald Gruber**, and **Johannes Rath**.

Furthermore, I am very grateful to all people that provided comments on statistics, analyses, and writing, and thereby contributed in improving of the manuscripts included in this dissertation thesis: **Joachim Frommen, Hope Klug, Alex Kotrschal, Norbert Milasowski, Hans Leo Nemeschkal, Peter Nonacs, Dustin Penn, Bibiana Rojas, Michael Stachowitsch, and Kyle Summers.**

Finally, I like to acknowledge all authorities that financially supported my dissertation project:

Austrian Academy of Sciences (ÖAW) – granted me with a DOC-fORTE scholarship



FWF – supported the work in the field and in the laboratory



CNRS – supported field work in 2009



University of Vienna – awarded me with several grants for my dissertation project (see Curriculum Vitae)



L'ORÉAL Österreich in cooperation with **UNESCO Austria** and the **Austrian Academy of Sciences (ÖAW)** – awarded me with a „For Women in Science" scholarship



Curriculum Vitae

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Education:

1994 – 2002 AHS (high school) in Bruck/Leitha
20. 06. 2002 Matura with excellent success
2002 – 2007 University of Vienna – studies in biology and mathematics (curriculum for high school teaching)
Master thesis: ‘Phonotactic approach pattern in the dart-poison frog *Allobates femoralis* (Dendrobatidae) – spatial and temporal analysis of movement’
supervised by Ao. Univ.-Prof. Mag. Dr. Walter Hödl
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since 2007 University of Vienna, PhD study (Dr.-Studium der Naturwissenschaften Zoologie)
Dissertation topic: ‘The genetic mating system and analyses of reproductive success in *Allobates femoralis*’
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Research activities:

August-September 2005:	Excursion to Panama and Costa Rica including practical training at the tropical research station <i>La Gamba</i>
February-March 2006:	Field work at the tropical research station <i>Arataï</i> (French Guiana), diploma thesis project
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May 2008:	Laboratory work at the University of Salford (Manchester/UK) , dissertation project
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September 2010:	Practical training course at the Krakatau Islands (Indonesia): Rapid biodiversity assessment of birds and butterflies
February 2011:	Field work at the tropical research station <i>Saut Pararé</i> (French Guiana) – pre-work for future projects
June 2011:	Sampling at the tropical research station <i>Saut Pararé</i> (French Guiana) – pre-work and sampling for the planned FWF-project and the PhD Program (FWF/DK-Plus: W1234)

Functions in journals:

since 2010	member of the Advisory Editorial Board of <i>Amphibia-Reptilia</i>
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Teaching experience:

- 2004-2009: Collaboration at the Department for Pedagogics at the zoological garden 'Tiergarten Schönbrunn', Vienna
- SS 2007: Tutor in the practical course 'Biologische Einführungsübungen' at the University of Vienna
- WS 2007: Tutor in the practical course 'Diversität, Organisation und Biologie der Tiere' at the University of Vienna
- WS 2007-08: Tutor in the course 'UE Mathematikübungen für Lebensmittel- und Biotechnologie' at the University of Natural Resources and Applied Life Sciences (BOKU), Vienna
- since SS 2008: Lecturer in the course 'Freilanddidaktik – Marchegg' at the University of Vienna
- since WS 2008: Lecturer in the course 'Evolutionsbiologisches Labor' and 'Ethoökologie und Diversität neotropischer Amphibien - Projektpraktikum in Französisch Guiana' at the University of Vienna
- since SS 2009 Lecturer in the courses 'Zoologisches Labor' at the University of Vienna
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Active international congress and seminar presentations:

- 19.-23.09.2007 **14th congress of the European herpetological society** (Porto, Portugal) – Poster: 'Phonotactic Movement in *Allobates femoralis*'
- 17.-22.08.2008 **6th world congress of Herpetology** (Manaus, Brazil) – invited oral presentation in the symposium *Dendrobatoid Frog Biology*: 'Phonotactic Movement in *Allobates femoralis*'
- 15.-18.08.2009 Symposium **„The use of vertebrate model systems to study social evolution'**
(Adelboden, Switzerland) – oral presentation: '*Allobates femoralis* – a handy fellow for anuran socio-biology'
- 28.09.-02.10.2009 **15th European Congress of Herpetology** (Kusadasi, Turkey) – oral presentation: 'Population genetics and reproductive behaviour in the neotropical frog *Allobates femoralis*'

- 01.07.2010 Department seminar **UCLA** (Los Angeles, US) – oral presentation:
‘*Allobates femoralis* – a handy fellow for anuran sociobiology’
07. 07. 2010 Department seminar **Yale University** (New Haven, US) – oral presentation:
‘*Allobates femoralis* – a handy fellow for anuran sociobiology’
- 07.07.-12.07.2010 **2010 Joint Meeting of Ichthyologists and Herpetologists** (Providence/Rhode Island, US) – invited oral presentation: ‘Calling Activity, Territory Size and Reproductive Success in the Dedrobatoid Frog *Allobates femoralis* (Aromobatidae) – An Integrative Approach’ in the symposium ‘Acoustic communication in frogs and fishes: integrating physiology, environment, and behaviour’

Grants and Awards:

- 2005 University of Vienna (KWA) - support for fieldwork in Fr. Guiana (master thesis)
- 2005 University of Vienna (Förderstipendium) – support for fieldwork in Fr. Guiana (master thesis)
- 2005 University of Vienna (Leistungsstipendium) – academic achievement award
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- 2007 University of Vienna – grant for congress participation
- 2007 University of Vienna (Förderstipendium) – support for fieldwork in Fr. Guiana (dissertation thesis)
- 2007 University of Vienna (Forschungsstipendium) – grant for dissertation project
- 2007 University of Vienna (Leitungsstipendium) – academic achievement award
- 2008 CNRS (Nouragues Grant – PI: Max Ringler) – grant for dissertation project
- 2008 ÖFG - grant for congress participation
- 2009 CNRS (Nouragues Grant – PI: Max Ringler) – grant for dissertation project
- 2009 Austrian Academy of Sciences – DOCfFORTE scholarship
- 2011 Theodor Körner Preis – research grant
- 2011 CNRS (Nouragues Grant – PI: Eva Ursprung) – research grant
- 2011 L'ORÉAL Österreich – „For Women in Science" scholarship

Scientific Publications:

- Ursprung E**, Ringler M, Jehle R, Hödl W (submitted to *Behavioral Ecology*) Selection against extremes? Parental relatedness across a natural population of a Neotropical frog.
- Ringler M, **Ursprung E**, Magaña Mendoza D, Hödl W (submitted to *PLoS ONE*) Intrusion Experiments to Measure Territory Size: Development of the Method, Tests Through Simulations, and Application in the Frog *Allobates femoralis*.
- Ursprung E**, Ringler M, Jehle R, Hödl W (2011) Strong male/male competition allows for nonchoosy females: high levels of polygynandry in a territorial frog with paternal care. *Molecular Ecology* 20: 1759–1771.
- Ursprung E**, Ringler M, Jehle R, Hödl W (2011) Toe regeneration in the Neotropical frog *Allobates femoralis* (Aromobatidae). *Herpetological Journal* 21: 83–86.
- Ringler M, **Ursprung E**, Hödl W (2010) Predation on *Allobates femoralis* (Boulenger 1884; Anura: Aromobatidae) by the colubrid snake *Xenopholis scalaris* (Wucherer 1861). *Herpetology Notes* 3: 301–304.
- Ringler M, **Ursprung E**, Hödl W (2009) Site fidelity and patterns of short- and long-term movement in the brilliant-thighed poison frog *Allobates femoralis* (Aromobatidae). *Behavioral Ecology and Sociobiology* 63: 1281–1293.
- Ursprung E**, Ringler M, Hödl W (2009) Phonotactic approach pattern in the neotropical frog *Allobates femoralis* (Dendrobatidae)—A spatial and temporal analysis. *Behaviour* 146: 153–170.