

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

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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 h 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

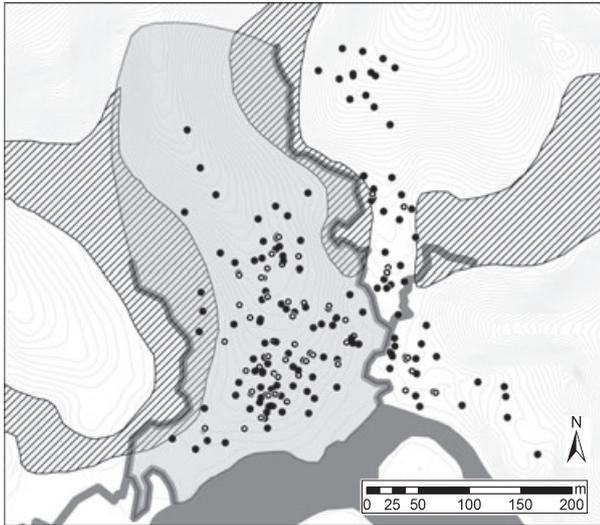


Fig. 1 Map of the study area: contour lines (1 m) in light grey; creeks and Arataye River in dark grey; study plot shaded in light grey; palm swamps hatched in black; males and females in 2008: filled and open circles, respectively.

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*: TCATTGAGGTCTTAGTTTCCA], 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.

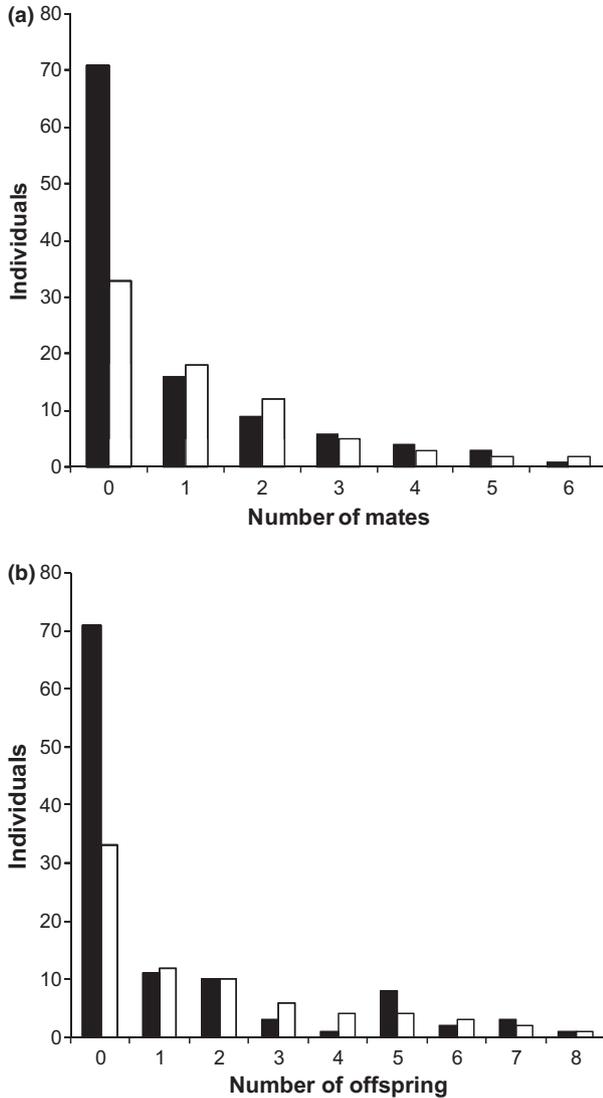


Fig. 3 (a) Distribution of mating partners per male (dark bars) and female (light bars); (b) Number of adult progeny per male (dark bars) and female (light bars) *Allobates femoralis*.

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.

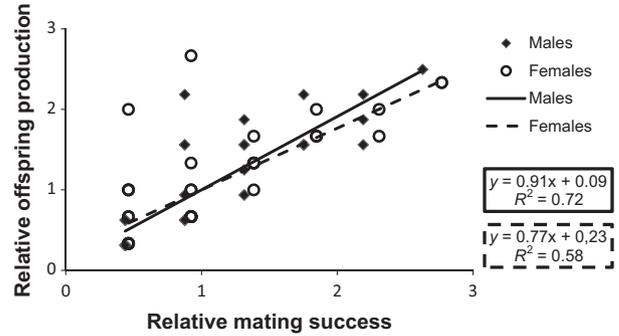


Fig. 4 Bateman gradients for males and females.

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 &

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	$I_{\text{♂}}^2/\text{mates} = 2.23$	$I_{\text{♀}}^2/\text{mates} = 1.56$	$I_{\text{♂}}^2/\text{mates} = 0.39$	$I_{\text{♀}}^2/\text{mates} = 0.43$
Number of offspring	$I_{\text{♂}}^2/\text{offspring} = 2.35$	$I_{\text{♀}}^2/\text{offspring} = 1.57$	$I_{\text{♂}}^2/\text{offspring} = 0.45$	$I_{\text{♀}}^2/\text{offspring} = 0.44$

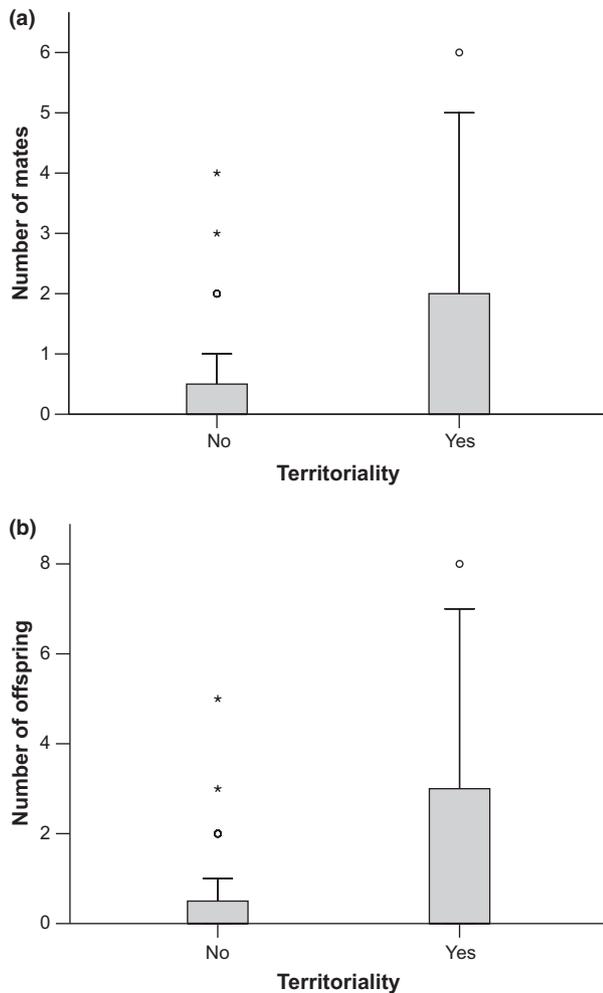


Fig. 5 Boxplots of (a) mating success and (b) offspring production of males with and without a territory.

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

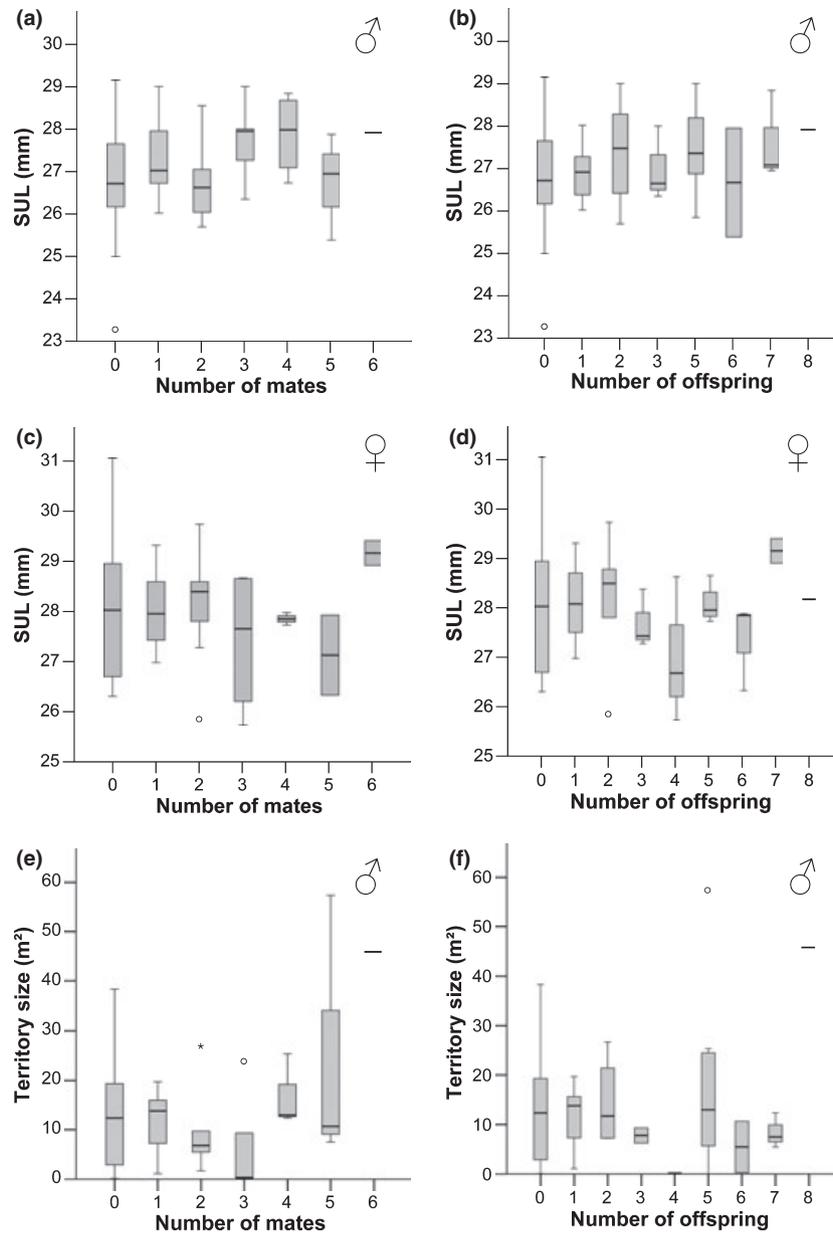


Fig. 6 Boxplots of the relationships between male and female reproductive success and specific traits: snout-urostyle lengths with respect to the number of mates per male (a) or female (c), snout-urostyle lengths with respect to the number of adult progeny per male (b) or female (d), and territory size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

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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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