Male body size and parental relatedness but not nuptial colouration influence paternity success during scramble competition in *Rana arvalis*

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Abstract

Female mate choice promotes the development of male secondary sexual traits such as nuptial colouration, whereas scramble competition favours male traits which enhance their ability for access to females. In the explosively breeding moor frog (*Rana arvalis*), males express a conspicuous blue colouration during a short reproductive period characterised by scramble competition. In the present study we used controlled mating experiments and genetic markers to disentangle the effects of colouration, body size and pairwise genetic relatedness in determining paternity success. Males observed in amplexus with a female prior to spawning were larger than their competitors but did not differ from them in colouration. Polyandry occurred in 67% of the 18 analysed egg clutches, and amplecting males did not always achieve the highest siring success, most likely due to stray sperm. Successful mating pairs were characterised by higher genetic divergence between them than expected by chance. We confirm previous evidence that male nuptial colouration is not a trait selected by females, and provide evidence that male reproductive success is influenced by male size as well as genetic attributes.

Keywords

moor frog, blue body colouration, explosive breeder, male reproductive success, genetic relatedness.

1. Introduction

Female mate choice and male competition are the two major types of sexual selection (Andersson, 1994). When there is selection by female choice,

males often develop bright nuptial colours or ornaments during breeding, and females who choose the most attractive mating partner may gain a fitness advantage when male ornaments are associated with individual quality (Milinski & Bakker, 1990; Hill, 1991; Seehausen & Van Alphen, 1998; Polo-Cavia et al., 2013). In the case of male contests, males try to defend sexually receptive females against rivals, and large body size is assumed to be advantageous (Andersson, 1994). Scramble competition arises from cases when males are unable to defend a mating partner, for example because females are spatially dispersed or breed synchronously (Murphy, 1998). Under such circumstances, sexually selected male traits appear to be more subtle and difficult to study than those involved in individual contests (Schwagmeyer & Woontner, 1986), and can involve aspects like mate search ability and effort (Lane et al., 2009; Marmet et al., 2012).

Anuran amphibians show a wide range of mating systems (Wells, 2007). Female mate choice based on acoustic cues is assumed to be the most common mechanism of sexual selection (e.g., Gerhardt & Huber, 2002), although the number of species known to use multimodal acoustic and visual signals is increasing (Narins et al., 2003; Taylor et al., 2007; Gomez et al., 2009; Grafe et al., 2012; Preininger et al., 2013). In explosively breeding species, all individuals arrive almost synchronously at the breeding site and the reproductive period is short. The operational sex ratio (OSR) is usually male-biased, males actively search and scramble for mates, and the opportunity for pre-copulatory female choice is limited (Wells, 2007). In some species, competing males attempt to dislodge already mated rivals, and strong and/or large males can increase their mating success rate by achieving take-overs of mated females (Davies & Halliday, 1977; Hedengren, 1987; Höglund & Säterberg, 1989; Liao & Lu, 2011a, b). However, whether a mating advantage through size ultimately leads to higher proportion of offspring sired is largely unknown.

The European moor frog (*Rana arvalis*) is an explosively breeding anuran showing a remarkable dynamic sexual colour dimorphism: males develop a conspicuous blue body colouration during the short mating period while females remain dull brown (Ries et al., 2008). Male blueness was previously assumed to play a role in female choice and mating success (Hedengren, 1987; Hettyey et al., 2009b, c), and tadpoles sired by blue males have a higher survival rate in the presence of large predators than tadpoles from less intensively coloured fathers (Sheldon et al., 2003). Recent studies applying

spectrometry to measure male colour, however, found male blueness to be unrelated to the presence of females, mating success, or male size (Ries et al., 2008; Sztatecsny et al., 2012). Male blueness was shown to increase with water temperature (Hettyey et al., 2009c), and behavioural experiments suggested that it acts as a visual signal for instant sex recognition during scrambles (Sztatecsny et al., 2012).

Despite previous studies on the moor frog's mating behaviour, the effects of body size and body colouration on male siring success has not been investigated so far. Given that the moor frog is characterised by a male-biased OSR (Hedengren, 1987; Glandt, 2006), polyandry (Knopp & Merila, 2009), and limited control over mating by females, we suggest that large male body size should be advantageous for reproductive success. To test the influence of male body size and colouration on paternity, we performed mating experiments in which females were exposed to an equal number of large and small males that could permanently compete for access to females. By determining paternity of offspring using microsatellite genetic markers, we asked if body size or body colouration is more important in influencing siring success in male moor frogs. We also used the genetic data to infer whether there are any associations between mating success and pair-wise relatedness among mating partners.

2. Methods

2.1. Study species

The moor frog (*R. arvalis*) occurs from north-eastern Central Europe to Siberia and China, and breeds in standing or slowly flowing water bodies like swamps, moats and ponds (Glandt, 2006). Outside the breeding season, body colouration is generally reddish to greyish brown and indistinguishable between males and females (Glandt, 2006). During spring migration to the spawning site, the body colouration of males usually changes to a conspicuous blue maintained throughout the short breeding period. Males and females simultaneously arrive at the pond. Males establish large breeding aggregations which the females join for spawning (Glandt, 2006). Male calls are expected to attract females to spawning aggregations where male–male competition limits the opportunity for female mate choice. In our study site, spawning peaked during the warmest hours of the day and individual density at breeding aggregations reached 20 males per m^2 (Figure 1; M.S.,

Figure 1. A female moor frog (*Rana avails*, white arrow) within a group of bluely coloured males. This figure is published in colour in the online edition of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/journals/1568539x.

pers. obs.). Females lay one, sometimes two clutches per breeding season (Glandt, 2006). We counted approximately 70 egg clutches per m^2 at communal spawning sites.

2.2. *Experimental procedure*

Experiments were conducted between 25 March 2011 and 3 April 2011. We retrieved all study animals from a breeding pond in south-eastern Austria (47°10'N, 16°5'E), collecting 49 male and 21 female moor frogs from a drift fence with pitfall traps during immigration to the pond. All animals were in a similar physiological state and had not yet mated. We measured snout-urostyle length (SUL) to the nearest 0.1 mm with callipers and body mass (BM) to the nearest 0.01 g using a digital miniscale. Males were classified as large when they exceeded the average $\pm$ SE population SUL of 55.06 ± 4.50 mm, and as small otherwise. We marked males individually by waist tagging (for a detailed description, see Hettyey et al., 2009a), and placed three small and three large males together with three females in each of

seven round plastic tanks (55 cm diameter, 45 cm deep) covered with wire netting (1 × 1 cm mesh size) to prevent the animals from escaping. We filled the tanks with 15 cm of pond water and furnished them with a branch and reeds as egg laying substrate. Every two hours between 09:00 and 19:00 h we recorded which males were in amplexus with a female, and whether an egg clutch had been laid. We decided to observe the frogs' mating status only five times per day to keep disturbance at a minimum. By the end of the experiments all but three females had spawned, and we released all frogs into their breeding pond after removing their waist tags. We did not observe any adverse effects of the waist bands, and all individuals appeared healthy when they were released. All animals were collected under permits issued by the local government of the Austrian province of Styria (permit number FA13C-53S-7/2011-89).

2.3. Spectral data

To measure male body colouration, we took spectral reflectance scans of the tympanic membrane and the flank of each male (following previous protocols: Ries et al., 2008; Sztatecsny et al., 2012). The spectral measurements were obtained by an Ocean Optics Jaz spectrometer (Ocean Optics, Dunedin, FL, USA) with an internal pulsed xenon light source (Jaz-PX, Ocean Optics). All spectral data were collected in the range of 300 to 700 nm relative to a white reflectance standard (WS-1, Ocean Optics) and we averaged three measurements for each spot. Spectral reflectance data were collected immediately before starting the experiment and again after 24 h.

The spectral reflectance data were analysed using the program Avicol v.6 (Gomez, 2006). The raw spectra were smoothed using the triangular smoothing function and linearly interpolated with one value per nm in the range of 300–700 nm before calculating total brightness, hue, and UV-blue chroma (for a more detailed description, see Sztatecsny et al., 2012).

2.4. Genetic parentage identification

Prior to experiments, DNA from adults was sampled as buccal swabs and dried in sealed, sterile plastic tubes. To collect offspring DNA, we preserved five tadpoles of each clutch in 96% ethanol after rearing a subset of eggs from each clutch in 5 l plastic tubs filled with aged tap water. All remaining eggs and tadpoles were returned to the pond.

Table 1.

Characterisation of the four microsatellite loci for *Rana arvalis* used to determine paternity of tadpoles.

Locus	Repeat motif	Size range (bp)	Annealing temperature (°C)	A	H_E	H_O
WRA1-22	$(CT)_{12}$	142–156	54	7	0.79	0.76
WRA1-28	$(GT)_{55}$	106–166	52	11	0.84	0.87
WRA1-160	$(CA)_{10}$	262–292	60	8	0.80	0.86
WRA6-8	$(GTTT)_{18}$	164–184	50	6	0.68	0.61

Repeat motif refers to the sequenced clone. A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.

Total genomic DNA of 63 adult frogs and 90 tadpoles was extracted using a proteinase K digestion followed by a standard phenol-chloroform protocol. We genotyped all individuals at four polymorphic microsatellite loci (WRA1-22, WRA1-28, WRA1-160 and WRA6-8; Arens et al., 2007) (Table 1). A total of 10 μl PCR reaction volume contained approx. 10 ng of genomic DNA, 0.2 mM of each dNTP, 1 μM of each forward and reverse primer, 0.5 U of *Taq* DNA polymerase (Axon, Kaiserslautern, Germany; 4 × 250 U, 5 U/μl) and 1 μl of 10× NH_4 reaction buffer (Axon), at a final concentration of 1.5 mM $MgCl_2$. PCR programs consisted of an initial denaturation step at 94°C for 4 min followed by 39 cycles of 94°C for 45 s, the primer-specific annealing temperature (Table 1) for 45 s and 72°C for 45 s, with a final 72°C extension of 5 min. The amplified PCR products of each individual were multiplexed and diluted 1:20 with water. Then 1 μl of the diluted PCR product mix was added to 10 μl of a mix of Hi-DiFormamid and the internal size standard ROX500 (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3130xl sequencer. We used the program PEAKSCANNER 1.0 (Applied Biosystems) to score alleles. CERVUS 3.0 (Kalinowski et al., 2007) was used to determine expected (H_E) and observed (H_O) heterozygosities. Paternity assignments were performed with COLONY 2.0 (Wang & Santure, 2009), which implements a maximum likelihood-based method to assign the genealogy of sibships within a tank. Pairwise relatedness coefficients between successful parents in each tank were estimated using the Wang moment estimator as implemented in COANCESTRY (Wang, 2011), which has been shown to be particularly robust for small sample size and limited genotypic information (Wang, 2002). The estimate ranges between −1 and 1, with zero representing the population

mean; because competing males differ in their genetic makeup in each tank we treated all seven tanks separately for all analyses. The mean pairwise relatedness coefficient for successful mating pairs for each tank was calculated by weighing each pair depending on the number of offspring sired.

2.5. *Statistical analyses*

Using our observational data on mating success, we were able to determine males in amplexus with a female prior to egg laying and defined the following (non-exclusive) male categories: fathers (all males fertilising at least one egg of a clutch), and amplecting fathers (successful males last observed in amplexus with a female prior to spawning). All males that were not assigned to any offspring were treated as unsuccessful. As we were interested in differences between males fathering offspring or being in amplexus and their rivals within each tank, we applied General Linear Mixed Models (GLMMs) with binomial error distribution and included tank as random factor. Our sample size and block number (i.e., number of tanks) were small and we therefore wanted to keep our models simple, i.e., with a small number of factors (Bolker et al., 2009). To reduce the number of factors, we performed a principal components analysis in order to find a small set of compounds (principal components) capturing most of the variance of the original autocorrelated colour variables (Crawley, 2007). Only components that accounted for more variation than any individual variable (eigenvalue > 1) were considered and used as parameters for further analysis. To find the best model explaining paternity in male moor frogs we ranked candidate models based on their value for small-sample Akaike's Information Criterion AIC_c (Burnham & Anderson, 2002). AIC_c estimates the support that a model receives from the data. As the Pearson correlation coefficient between SUL and BM was 0.85 we did not include the two parameters in the same model. We also calculated Akaike weights (w_i) as a measure for posterior model probabilities (Burnham & Anderson, 2002), and used the lme4 package within the R statistical software version 2.15.2 (2014) to fit our models to the data.

3. Results

The first principal component (PC1), which accounted for 52% of the variation, was almost equally loaded by all variables except for hue at the beginning of the mating experiment (Table 2). Blue colouration in males was

Table 2.
Principal component (PC) loadings for colour variables measured from 42 male moor frogs at the start of the mating experiment and 24 h later.

Colour variable	PC1	PC2
Brightness	−0.34	−0.11
Hue	0.28	0.68
Chroma	−0.40	−0.47
Brightness 24 h	−0.48	0.26
Hue 24 h	0.43	−0.46
Chroma 24 h	−0.48	0.19
Explained variance (%)	51.97	23.17
Eigenvalues	3.12	1.39

expressed by low PC1 loadings because brightness and chroma were negatively but hue positively associated with the PC1 axis (Table 2). The second principal component (PC2), mainly loaded by hue and chroma at the begining and hue 24 h after the experiment had started, accounted for 23%. Eighteen out of 21 female moor frogs spawned 3–7 days after the onset of the experiment. Males amplexing a female 24 h after the beginning of the experiment had significantly lower scores for PC1 than unmated males (i.e., they were bluer, GLMM tank-wise comparison: $\beta = -0.828$, SE $= 0.374$; $Z = -2.213$, $p = 0.026$) but did not differ from other males in PC2, SUL or BM (GLMM: tank-wise comparison: $p > 0.1$ in all cases). Twenty-three out of 42 males (54.8%) were successful in fathering offspring with at least one female. Seven (30%) of these were not amplectant fathers. Of the 18 analysed egg clutches, three (17%) were fertilised by only one male, 12 (67%) were fathered by two males, and three males contributed to fertilising the eggs of three clutches. We found no differences in SUL, BM, PC1 or PC2 between fathers and unsuccessful males (GLMM tank-wise comparison: $p > 0.1$ in all cases; Table 3), and therefore restricted further analysis to amplectant fathers. Amplectant fathers were significantly larger and slightly heavier (Table 3) than their non-successful rivals. Paternity in amplectant fathers was best explained by the model containing only SUL (i.e., lowest AIC$_c$, highest Akaike weight, Table 4). The model including only BM ranked second but was three times less plausible than the best model. None of the models including the colour parameters PC1 and PC2 were well supported by the data.

Table 3.
Parameters tested for their influence on paternity in *Rana arvalis*.

	Parameter			
	SUL (mm)	BM (g)	PC1	PC2
Fathers (mean $\pm$ SE, $N = 23$)	55.66 ± 0.89	18.17 ± 1.08	-0.17 ± 0.39	0.19 ± 0.25
Unsuccessful males (mean $\pm$ SE, $N = 19$)	54.33 ± 1.11	16.74 ± 0.90	0.21 ± 0.38	-0.23 ± 0.26
Wald Z	0.957	0.990	-0.705	1.131
p	0.388	0.322	0.481	0.258
Amplecting fathers (mean $\pm$ SE, $N = 16$)	57.09 ± 0.97	19.19 ± 0.87	-0.05 ± 0.54	-0.16 ± 0.30
Unsuccessful males (mean $\pm$ SE, $N = 26$)	53.81 ± 0.86	16.49 ± 0.97	0.07 ± 0.30	0.10 ± 0.23
Wald Z	2.187	1.780	0.212	-0.674
p	*0.028*	*0.075*	0.832	0.500

Fathers included all males siring at least one tadpole from any clutch, amplecting fathers were the last males observed in amplexus with a female prior to spawning. Wald Z-statistic for the tank-wise comparison between successful and unsuccessful males as derived from a GLMM; p, significance level; N, sample size; italicised numbers indicate significant or near significant p-values.

Mean pairwise relatedness coefficients between successful mating partners were below the mean in six out of seven tanks (-0.06, -0.026, -0.12, -0.09, -0.16, -0.18, 0.12 for tanks 1–7, respectively). Twenty-six out of 33 successful mating pairs (78.8%) had a negative relatedness coefficient,

Table 4.
Ranking of models relating paternity of amplectant fathers in moor frogs to snout-urostyle-length (SUL), body mass (BM), and two principal components representing male colouration.

Model	$\log L$	K	AIC_c	ΔAIC_c	w_i
SUL	-25.00	3	56.63	0.00	0.693
BM	-26.20	3	59.03	2.40	0.209
SUL, PC1, PC2	-24.90	5	61.47	4.84	0.062
BM, PC1, PC2	-25.90	5	63.47	6.84	0.023
PC1, PC2	-27.70	4	64.48	7.85	0.014

Ranking is based on smallest AIC_c value; $\log L$, log likelihood; K, number of parameters in the model; w_i, Akaike's weight.
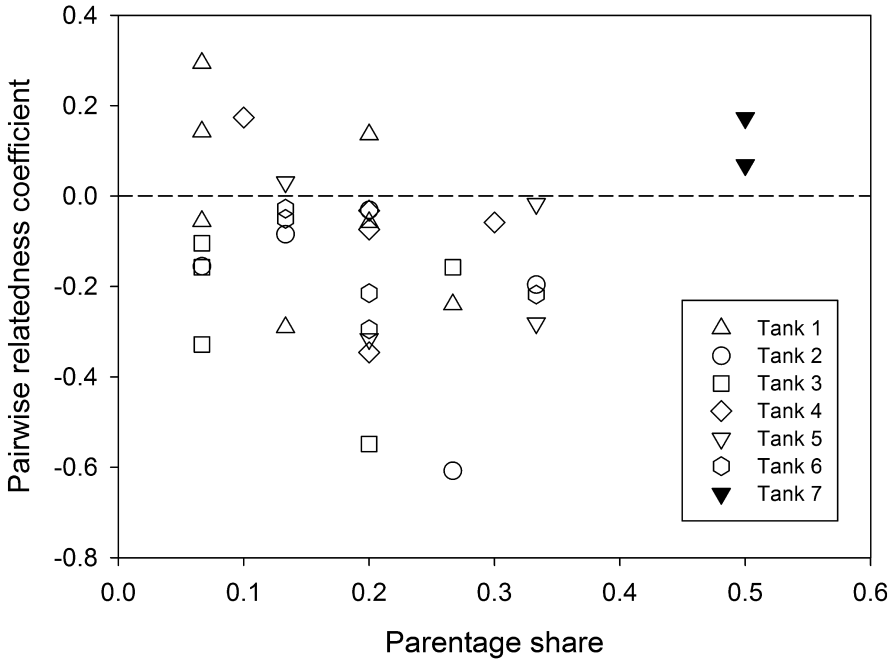
Figure 2. Relationship between inferred share of offspring sired and pairwise relatedness between mating partners for each of 7 experimental moor frog mating tanks. The pairwise relatedness coefficients are tank-specific, and we therefore refrained from overall correlational analyses.

which is significantly different from random expectations (Chi-square test, $p < 0.01$, Figure 2).

4. Discussion

Body colouration of male moor frogs influenced male mating at the beginning of the experiments but had no effect on paternity success. Overall, our experiments therefore support previous findings on mating success from free ranging animals (Sztatecsny et al., 2012). In large mating aggregations, a dynamic colour dimorphism can facilitate mate finding through instant sex recognition. Males not turning blue are visually more difficult to distinguish from females and will frequently be clasped by other males in search for a mate (Sztatecsny et al., 2012). Hence the blue nuptial colouration should improve an individual's mating opportunity without being a signal geared towards females. Blue colouration of mated males 24 h after the experiments

began, may have indicated early readiness to mate irrespective of later fertilisation success. Dynamic sexual dichromatism has been reported in numerous other anurans, but in most cases little is known about the species' mating systems (Sztatecsny et al., 2010, 2012; Bell & Zamudio, 2012).

We found amplectant fathers to be larger, and slightly heavier than their unsuccessful competitors. In other anurans, large males were shown to achieve take-overs of females from smaller competitors (Arak, 1983; Loman & Madsen, 1986; Andersson & Iwasa, 1996). Male body size and body mass could also affect search ability and search effort (e.g., large males may be fast swimmers and have high endurance). Mate-searching behaviour might be energetically demanding (Arak, 1983; Ryser, 1989), but whether body size affects searching success has not yet been investigated. Large male mating advantage has previously been reported for moor frogs (Hedengren, 1987) but was not confirmed in later field studies (Hettyey et al., 2009c; Sztatecsny et al., 2012). This coincides with other explosively breeding anurans for which no clear pattern of size dependence of mating has been revealed (Davies & Halliday, 1977; Berven, 1981; Höglund & Robertson, 1987; Howard, 1988; Höglund, 1989; Höglund & Säterberg, 1989; Greene & Funk, 2009). Male mating success as assessed in most studies may not guarantee fertilisation success, and males may adapt their mating tactics dynamically to population size, individual density, OSR, or time available for competition (Höglund & Robertson, 1988; Höglund, 1989; Byrne & Roberts, 2004; Zamudio & Chan, 2008). In our experimental tanks it took females at least three days to start spawning in a situation where they could not retreat from males. The probably more intense competition compared to natural settings may have increased the effect of male body size on paternity (Höglund & Robertson, 1987; Höglund, 1989).

Although our sample sizes were small, genetic paternity analysis revealed surprisingly high levels of polyandry. Studies on free ranging moor frogs and closely related common frogs (*R. temporaria*) found 14 and 29% (depending on the method applied) of eggs sired by additional males, respectively, and around 50% of egg clutches to be sired by more than one male (Laurila & Seppä, 1998; Knopp & Merila, 2009). In species where numerous males may simultaneously engage in amplexus, females may avoid drowning by spawning for convenience as soon as multiple males attempt mating (Sztatecsny et al., 2006). However, multiple amplexi in moor frogs are very rare, and convenience polyandry seems unlikely. Laurila & Seppä (1998) suggested

stray sperm or sperm leakage as a likely cause for polyandry in *R. temporaria*, which like moor frogs deposit their eggs in communal spawning assemblages where densities may reach 80–00 spawn clumps per m^2 (M.S., unpubl. data). Unmated *R. temporaria* males were observed to achieve reproductive success by releasing sperm on freshly laid clutches ('clutch piracy'; Vieites et al., 2004). As anuran spermatozoa can retain fertilising capacity over several hours (Hettyey & Roberts, 2006, 2007) or days (Edwards et al., 2004; Sherman et al., 2008), a female may expose her eggs to sperm of multiple males that compete over fertilisation without amplexus (Berger & Rybacki, 1992; Krupa, 1994; Roberts & Byrne, 2011). As our observations on male mating status were restricted to two hour intervals during daytime, males observed in amplexus could have been dislodged by rivals prior to egg deposition. However, given the relatively small volume of water in our experimental tanks (ca. 35 l), high stray sperm density is the most likely explanation for the observed high levels of polyandry and supported by the fact that the male actually observed in amplexus always remains as an individual contributing to the paternity share.

Fertilisation rates have previously been shown to differ between male moor frogs independently of their size (Sherman et al., 2010). In our experiment, successful fathers were in the majority of cases more distantly related to the female whose eggs they fertilised than expected by chance, irrespective of being in amplexus with the female or not. The exception was tank 7, in which no multiple paternities occurred, paralleling high relatedness among offspring-siring pairs. This evidence suggests that fertilisation success can be enhanced through genetic compatibility between mating partners which is inversely related with pairwise relatedness (as has been shown for internally fertilising amphibians, Jehle et al., 2007). The present study is however hampered by the rather small number of loci used, which was sufficient for unambiguous paternity assignment but provided only rather imprecise relatedness estimates. A recent review has suggested that, when direct pre-copulatory female choice is limited, cryptic post-copulatory female choice biasing paternity towards males that elevate offspring fitness might be more important than previously thought (Kvarnemo & Simmons, 2013). Our findings suggest that stray sperm or clutch piracy can be a cause of polyandry in moor frogs, and that genetic compatibility might influence the mating system in this species.

Acknowledgements

References

Andersson, M. (1994). Sexual selection. — Princeton University Press, Princeton, NJ.

Andersson, M. & Iwasa, Y. (1996). Sexual selection. — Trends Ecol. Evol. 11: 53-58.

Arak, A. (1983). Male–male competition and mate choice in anuran amphibians. — In: Mate choice (Bateson, P., ed.). Cambridge University Press, Cambridge, p. 181-210.

Arens, P., Sluis, T., Van't Westende, W.P.C., Vosman, B., Vos, C.C. & Smulders, M.J.M. (2007). Genetic population differentiation and connectivity among fragmented Moor frog (*Rana arvalis*) populations in The Netherlands. — Landscape Ecol. 22: 1489-1500.

Bell, R.C. & Zamudio, K.R. (2012). Sexual dichromatism in frogs: natural selection, sexual selection and unexpected diversity. — Proc. Roy. Soc. Lond. B: Biol. Sci. 279: 4687-4693.

Berger, L. & Rybacki, M. (1992). Sperm competition in European water frogs. — Alytes (Paris) 10: 113-116.

Berven, A.K. (1981). Mate choice in the wood frog, *Rana sylvatica*. — Evolution 35: 707-722.

Bolker, B.M., Brooks, M.E., Clark, C.J., Geange, S.W., Poulsen, J.R., Stevens, M.H.H. & White, J.-S.S. (2009). Generalized linear mixed models: a practical guide for ecology and evolution. — Trends Ecol. Evol. 24: 127-135.

Burnham, K.P. & Anderson, D.R. (2002). Model selection and inference, a practical information-theoretic approach. — Springer, New York, NY.

Byrne, P.G. & Roberts, J.D. (2004). Intrasexual selection and group spawning in quacking frogs (*Crinia georgiana*). — Behav. Ecol. 15: 872-882.

Crawley, M.J. (2007). The R book. — Wiley, Chichester.

Davies, N.B. & Halliday, T.R. (1977). Optimal mate selection in the toad *Bufo bufo*. — Nature 269: 56-58.

Edwards, D.L., Mahony, M.J. & Clulow, J. (2004). Effect of sperm concentration, medium osmolality and oocyte storage on artificial fertilisation success in a myobatrachid frog (*Limnodynastes tasmaniensis*). — Reprod. Fertil. Dev. 16: 347-354.

Gerhardt, H.C. & Huber, F. (2002). Acoustic communication in Insects and Anurans: common problems and diverse solutions. — University of Chicago Press, Chicago, IL.

Glandt, D. (2006). Der Moorfrosch — Einheit und Vielfalt einer Braunfroschart. — Laurenti Verlag, Bielefeld.

Gomez, D. (2006). AVICOL, a program to analyse spectrometric data. — Free program, available online at http://sites.google.com/site/avicolprogram/ (accessed 3 August 2012).

Gomez, D., Richardson, C., Lengagne, T., Plenet, S., Joly, P., Léna, J.-P. & Théry, M. (2009). The role of nocturnal vision in mate choice: females prefer conspicuous males in the European tree frog (*Hyla arborea*). — Proc. Roy. Soc. Lond. B: Biol. Sci. 276: 2351-2358.

Grafe, T.U., Preininger, D., Sztatecsny, M., Kasah, R., Dehling, J.M., Proksch, S. & Hödl, W. (2012). Multimodal communication in a noisy environment: a case study of the Bornean rock frog *Staurois parvus*. — Plos One 7: e37965.

Greene, A.E. & Funk, W.C. (2009). Sexual selection on morphology in an explosive breeding amphibian, the Columbia spotted frog (*Rana luteiventris*). — J. Herpetol. 43: 244-251.

Hedengren, I. (1987). Selection of body size, arm length and colour in male and female moor frogs (*Rana arvalis*). — University of Stockholm, Stockholm.

Hettyey, A. & Roberts, J.D. (2006). Sperm traits of the quacking frog, *Crinia georgiana*: intra- and interpopulation variation in a species with a high risk of sperm competition. — Behav. Ecol. Sociobiol. 59: 389-396.

Hettyey, A. & Roberts, J.D. (2007). Sperm traits in the quacking frog (*Crinia georgiana*), a species with plastic alternative mating tactics. — Behav. Ecol. Sociobiol. 61: 1303-1310.

Hettyey, A., Baksay, S., Vagi, B. & Hoi, H. (2009a). Counterstrategies by female frogs to sexual coercion by heterospecifics. — Anim. Behav. 78: 1365-1372.

Hettyey, A., Herczeg, G. & Hoi, H. (2009b). Testing the phenotype-linked fertility hypothesis in male moor frogs (*Rana arvalis*) exhibiting a conspicuous nuptial colouration. — Amphibia-Reptilia 30: 581-586.

Hettyey, A., Herczeg, G., Laurila, A., Crochet, P.-A. & Merila, J. (2009c). Body temperature, size, nuptial colouration and mating success in male moor frogs (*Rana arvalis*). — Amphibia-Reptilia 30: 37-43.

Hill, G.E. (1991). Plumage coloration is a sexually selected indicator of male quality. — Nature 350: 337-339.

Höglund, J. (1989). Pairing and spawning patterns in the common toad, *Bufo bufo* — the effects of sex-ratios and the time available for male–male competition. — Anim. Behav. 38: 423-429.

Höglund, J. & Robertson, J.G.M. (1987). Random mating by size in a population of common toads *Bufo bufo*. — Amphibia-Reptilia 8: 321-330.

Höglund, J. & Robertson, J.G.M. (1988). Chorusing behavior, a density-dependent alternative mating strategy in male common toads (*Bufo bufo*). — Ethology 79: 324-332.

Höglund, J. & Säterberg, L. (1989). Sexual selection in common toads: correlates with age and body size. — J. Evol. Biol. 2: 367-372.

Howard, R.D. (1988). Sexual selection on male body size and mating behaviour in American toads, *Bufo americanus*. — Anim. Behav. 36: 1796-1808.

Jehle, R., Sztatecsny, M., Wolf, J.B.W., Whitlock, A., Hödl, W. & Burke, T. (2007). Genetic dissimilarity predicts paternity in the smooth newt (*Lissotriton vulgaris*). — Biol. Lett. 3: 526-528.

Kalinowski, S.T., Taper, M.L. & Marshall, T.C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. — Mol. Ecol. Notes 16: 1099-1106.

Knopp, T. & Merila, J. (2009). Multiple paternity in the moor frog, *Rana arvalis*. — Amphibia-Reptilia 30: 515-521.

Krupa, J.J. (1994). Breeding biology of the great-plains toad in Oklahoma. — J. Herpetol. 28: 217-224.

Kvarnemo, C. & Simmons, L.W. (2013). Polyandry as a mediator of sexual selection before and after mating. — Philos. Trans. Roy. Soc. Lond. B: Biol. Sci. 368: 20120042.

Lane, J.E., Boutin, S., Gunn, M.R. & Coltman, D.W. (2009). Sexually selected behaviour: red squirrel males search for reproductive success. — J. Anim. Ecol. 78: 296-304.

Laurila, A. & Seppä, P. (1998). Multiple paternity in the common frog (*Rana temporaria*): genetic evidence from tadpole kin groups. — Biol. J. Linn. Soc. 63: 221-232.

Liao, W.B. & Lu, X. (2011a). Male mating success in the Omei treefrog (*Rhacophorus omeimontis*): the influence of body size and age. — Belg. J. Zool. 141: 3-12.

Liao, W.B. & Lu, X. (2011b). Proximate mechanisms leading to large male-mating advantage in the Andrew's toad *Bufo andrewsi*. — Behaviour 148: 1087-1102.

Loman, J. & Madsen, T. (1986). Reproductive tactics of large and small male toads *Bufo bufo*. — Oikos 46: 57-61.

Marmet, J., Pisanu, B., Chapuis, J.-L., Jacob, G. & Baudry, E. (2012). Factors affecting male and female reproductive success in a chipmunk (*Tamias sibiricus*) with a scramble competition mating system. — Behav. Ecol. Sociobiol. 66: 1449-1457.

Milinski, M. & Bakker, T.C.M. (1990). Female sticklebacks use male coloration in mate choice and hence avoid parasitized males. — Nature 344: 330-333.

Murphy, C.G. (1998). Interaction-independent sexual selection and the mechanisms of sexual selection. — Evolution 52: 8-18.

Narins, P.M., Hödl, W. & Grabul, D.S. (2003). Bimodal signal requisite for agonistic behavior in a dart-poison frog, *Epipedobates femoralis*. — Proc. Natl. Acad. Sci. USA 100: 577-580.

Polo-Cavia, N., López, P. & Martín, J. (2013). Head coloration reflects health state in the red-eared slider *Trachemys scripta elegans*. — Behav. Ecol. Sociobiol. 67: 153-162.

Preininger, D., Boeckle, M., Freudmann, A., Starnberger, I., Sztatecsny, M. & Hödl, W. (2013). Multimodal signaling in the small torrent frog (*Micrixalus saxicola*) in a complex acoustic environment. — Behav. Ecol. Sociobiol. 67: 1449-1456.

R Development Core Team (2014). R: a language and environment for statistical computing, 3.1.0. — R Foundation for Statistical Computing, Vienna, available online at: http://www.R-project.org (accessed 10 April 2014).

Ries, C., Spaethe, J., Sztatecsny, M., Strondl, C. & Hödl, W. (2008). Turning blue and ultraviolet: sex-specific colour change during mating season in the Balkan moor frog. — J. Zool. (Lond.) 276: 229-236.

Roberts, J.D. & Byrne, P.G. (2011). Polyandry, sperm competition, and the evolution of anuran amphibians. — Adv. Stud. Behav. 43: 1-53.

Ryser, J. (1989). Weight loss, reproductive output, and the cost of reproduction in the common frog, *Rana temporaria*. — Oecologia 78: 264-268.

Schwagmeyer, P.L. & Woontner, S.J. (1986). Scramble competition polygyny in thirteen-lined ground squirrels: the relative contributions of overt conflict and competitive mate searching. — Behav. Ecol. Sociobiol. 19: 359-364.

Seehausen, O. & van Alphen, J.J.M. (1998). The effect of male coloration on female mate choice in closely related Lake Victoria cichlids (*Haplochromis nyererei complex*). — Behav. Ecol. Sociobiol. 42: 1-8.

Sheldon, B.C., Arponen, H., Laurila, A., Crochet, P.A. & Merilä, J. (2003). Sire coloration influences offspring survival under predation risk in the moorfrog. — J. Evol. Biol. 16: 1288-1295.

Sherman, C.D.H., Uller, T., Wapstra, E. & Olsson, M. (2008). Within-population variation in ejaculate characteristics in a prolonged breeder, Peron's tree frog, *Litoria peronii*. — Naturwissenschaften 95: 1055-1061.

Sherman, C.D.H., Sagvik, J. & Olsson, M. (2010). Female choice for males with greater fertilisation success in the Swedish moor frog, *Rana arvalis*. — Plos One 5: e13634.

Sztatecsny, M., Jehle, R., Burke, T. & Hödl, W. (2006). Female polyandry under male harassment: the case of the common toad (*Bufo bufo*). — J. Zool. (Lond.) 270: 517-522.

Sztatecsny, M., Strondl, C., Baierl, A., Ries, C. & Hödl, W. (2010). Chin up: are the bright throats of male common frogs a condition-independent visual cue? — Anim. Behav. 79: 779-786.

Sztatecsny, M., Preininger, D., Freudmann, A., Loretto, M.-C., Maier, F. & Hödl, W. (2012). Don't get the blues: conspicuous nuptial colouration of male moor frogs (*Rana arvalis*) supports visual mate recognition during scramble competition in large breeding aggregations. — Behav. Ecol. Sociobiol. 66: 1587-1593.

Taylor, R.C., Buchanan, B.W. & Doherty, J.L. (2007). Sexual selection in the squirrel treefrog *Hyla squirella*: the role of multimodal cue assessment in female choice. — Anim. Behav. 74: 1753-1763.

Vieites, D.R., Nieto-Roma, S., Barluenga, M., Palanca, A., Vences, M. & Meyer, A. (2004). Post-mating clutch piracy in an amphibian. — Nature 431: 305-307.

Wang, J. (2002). An estimator for pairwise relatedness using molecular markers. — Genetics 160: 1203-1215.

Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. — Mol. Ecol. Resour. 11: 141-145.

Wang, J. & Santure, A.W. (2009). Parentage and sibship inference from multilocus genotype data under polygamy. — Genetics 181: 1579-1594.

Wells, K.D. (2007). The ecology and behavior of amphibians. — University of Chicago Press, Chicago, IL.

Zamudio, K.R. & Chan, L.M. (2008). Alternative reproductive tactics in amphibians. — In: Alternative reproductive tactics: an integrative approach (Oliveira, R.F., Taborsky, M. & Brockmann, H.J., eds). Cambridge University Press, Cambridge.