

## The effect of sex ratio on size-assortative mating in two explosively breeding anurans

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**Abstract.** Size-assortative mating (SAM) is a widespread phenomenon related to individual fitness. In our study, we examined: (i) the appearance of SAM, and (ii) the effect of sex ratio on intensity of SAM in wild populations of two explosively breeding anurans, common frogs, *Rana temporaria*, and common toads, *Bufo bufo*. Despite a higher male-biased operational sex ratio (OSR) in toads, the body lengths of the paired males and females were significantly correlated only in frogs. Increasing male-male competition, assessed via the OSR, resulted in a stronger correlation also in frogs only. Thus, great variability in the presence and intensity of SAM has been observed within both studied species.

**Keywords:** *Bufo bufo*, correlation, explosive breeders, male-male competition, nonrandom mating, OSR, *Rana temporaria*, sexual selection.

### Introduction

Nonrandom mating patterns have been found in many populations of amphibian species (e.g., Arak, 1983; Halliday and Tejedo, 1995; Wells, 2007) as a consequence of mate choice or competition for mates (Andersson, 1994). Nevertheless, different causes of nonrandom mating play a role in prolonged and explosively breeding anurans. In prolonged breeders, whose reproduction period can extend over several months, females often choose larger males with better territories (Howard, 1978). This leads to a size-dependent mating pattern and males larger than average usually achieve higher reproductive success (Wells, 1977; Howard, 1978; Arak, 1983).

On the other hand, pairing in explosive breeders has been considered to result from male-male competition rather than female choice, because females have limited opportunities to choose potential mates among competitive males (Wells, 1977; Davies and Halliday, 1979;

Arak, 1983; Sullivan, Ryan and Verrel, 1995). In these species, mating and spawning takes only a few hours or days, as males struggle for females and often attack paired males, attempting to dislodge rivals from females and seize their positions (e.g., Davies and Halliday, 1978; Gittins, Parker and Slater, 1980a; Lu et al., 2008; Greene and Funk, 2009). Only a size-corresponding male (in relation to the female) can resist takeovers by other males under intense male-male competition (Wells, 1979; Arak, 1983). Such a mating pattern, wherein the body sizes of males and females correlate in pairs, is well known as size-assortative mating (SAM), the most documented nonrandom mating pattern in animals (Andersson, 1994). SAM can increase individual fitness (Burley, 1983), because pairs consisting of partners well matched for size should also have a higher proportion of fertilized eggs (Davies and Halliday, 1977; Bastos and Haddad, 1996).

The intensity of competition for mates in explosive breeders varies in time and space (Andersson, 1994; Wells, 2007), particularly due to changes in operational sex ratio (OSR, the ratio of sexually competing males to fertilizable females) (Emlen and Oring, 1977; Kvarnemo and Ahnesjö, 1996; Reynolds, 1996). It has been shown that an increasing male-biased OSR probably leads to more-intensive male-male

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competition (Tejedo, 1988; Höglund, 1989; Boll and Linsenmair, 1998; Lee and Park, 2009). If only pairs consisting of males and females well matched for size can resist takeovers by other males, then the increasing OSR and male-male competition should enhance the intensity of SAM within a population, expressed as the tightness of correlation between body size of males and females in pairs (Wells, 1979; Arak, 1983). Only a few studies have investigated the effect of OSR on the intensity of SAM in explosively breeding anurans (Arak, 1982; Elmberg, 1991; Lee and Park, 2009; Yu and Lu, 2010), and none of these have analyzed the statistical differences among the correlation coefficients obtained from samples with different OSRs.

Therefore, we examined the effect of different OSRs on SAM in wild populations of the most common explosively breeding European anurans, common frogs (*Rana temporaria*) and common toads (*Bufo bufo*).

## Materials and methods

### Study area

The study was carried out at Nový Tucharaz pond (1.37 ha), near Český Brod, Central Bohemia, Czech Republic (50°03'N, 14°51'E, 210 m a.s.l.). The pond had a permanent water column during the field work, with a maximum water depth of 1.1 m. Emergent vegetation covering 25% of the water surface was concentrated on shallow (mostly 0.6 m deep) parts of the pond, which also attracted most of the individuals in the two anuran populations under study here.

### Sampling of anurans

Both amplexant and single anurans were captured daily throughout the reproduction season from March to April 2004. Netting was performed using modified circle landing-nets until all observed frogs were caught. Pairs and singles were placed separately into plastic tanks with shallow water for a short period before measuring and marking. In total, we sampled 1013 adult frogs (460 single males, 83 single females and 235 pairs) and 2330 adult toads (1560 single males, 16 single females and 377 pairs). Snout-vent length (SVL) was measured to the nearest mm using a plastic ruler. All first-time captured anurans were uniformly marked, in order to prevent measuring of recaptured individuals. Marking was done by toe-clipping of only the last third of

the outer phalange and then the place of marking was wiped with alcohol. Marked anurans were kept for several minutes in plastic tanks and then released at the site of capture. All marked anurans behaved normally after release, so we assume that toe-clipping had no harmful impact on their breeding activity.

The breeding season (i.e., the time between appearance of the first and last individuals in the reproduction pond) of common frogs lasted 15 d (from 20 March to 3 April). However, 93% of paired individuals and 86% of singles were captured during a short period of 4 successive days between 30 March and 2 April (the main reproduction season, MRS). The breeding season of common toads was much longer (33 d, between 20 March and 21 April) with a 6-day period (from 3 to 8 April) representing the MRS, when 82% of pairs and 75% of singles were sampled. The OSR was calculated in both species for each sampling day of the MRS.

### Data analysis

The differences in the ratios of adult males and females (OSR) among sampling days of the MRS were analyzed using a chi-squared test of homogeneity. To compare OSRs during MRS between frogs and toads, we used the non-parametric Wilcoxon-Mann-Whitney *U* test. Because of the normal SVL distribution in all samples of both species, we examined the existence of SAM by Pearson's correlation coefficient. For each sampling day of the MRS, we calculated the correlation coefficient between the SVL of males and females in pairs, *r*, and the corresponding 95% confidence intervals for the population correlation coefficient,  $\rho$ , using the Fisher *z*-transformation (Zar, 1984). The Fisher *z*-transformation was also used to compare sample correlation coefficients within the MRS of each particular species.

All statistical analyses were performed in R statistical software, version 2.15.0 (R Development Core Team, 2009).

## Results

The OSR was always skewed in favor of males and varied significantly during the MRS from 1.41 to 3.44 in frogs (table 1; Chi-squared test,  $\chi_3^2 = 19.56$ ,  $P < 0.001$ ) and from 3.31 to 12.22 in toads (table 1;  $\chi_5^2 = 32.32$ ,  $P < 0.001$ ). The OSRs in common toads were significantly greater than in common frogs during the MRS (Wilcoxon-Mann-Whitney *U* test,  $W = 1$ ,  $P < 0.05$ ). In the case of SAM detection, the sample correlation coefficients, calculated for each sampling day of the MRS, were positive and significant in 3 of the 4 d with the higher OSR in common frogs, but these were not significant in any of the 6 d in common toads (ta-

**Table 1.** Examination of size-assortative mating in common frogs and common toads during the main part of the reproduction season (MRS).  $n_m$  = total number of males,  $n_f$  = total number of females,  $SVL_{m_p}$  = mean of snout-vent length of paired males,  $SVL_{m_{up}}$  = mean of SVL of unpaired males,  $SVL_{f_p}$  = mean of SVL of paired females,  $SVL_{f_{up}}$  = mean of SVL of unpaired females, OSR = operational sex ratio measured as total number of males to total number of females on each sampling day during MRS,  $n_p$  = number of pairs,  $r$  = correlation coefficients, 95% CI for  $\rho$  = 95% confidence limits for population correlation coefficient  $\rho$ ,  $R^2$  = coefficients of determination,  $t$  = test statistic,  $P$  = significance of correlation.

Date	$n_m$	$n_f$	$SVL_{m_p}$	$SVL_{m_{up}}$	$SVL_{f_p}$	$SVL_{f_{up}}$	OSR	$n_p$	$r$	95% CI for $\rho$	$R^2$	$t$	$P$
Common frogs													
30 March	109	58	7.56	7.29	7.14	7.83	1.88	41	0.34	0.04-0.59	0.12	2.27	0.029
31 March	111	79	7.30	7.21	6.89	7.76	1.41	62	0.11	-0.14-0.35	0.01	0.86	0.39
1 April	212	96	7.24	7.16	7.04	7.56	2.21	75	0.36	0.15-0.54	0.13	3.31	0.001
2 April	189	55	7.22	7.08	6.88	7.49	3.44	39	0.50	0.22-0.70	0.25	3.54	0.001
Common toads													
3 April	220	18	6.75	7.22	8.63	8.25	12.22	16	0.22	-0.31-0.65	0.05	0.85	0.41
4 April	298	90	6.71	6.70	8.48	8.53	3.31	87	0.12	-0.09-0.32	0.01	-1.08	0.28
5 April	192	50	6.74	6.76	8.55	8.35	3.84	48	0.17	-0.12-0.43	0.03	1.18	0.24
6 April	285	60	6.79	6.68	8.56	9.55	4.75	58	0.21	-0.05-0.44	0.04	1.63	0.11
7 April	259	69	6.79	6.71	8.56	8.45	3.75	67	0.14	-0.10-0.37	0.02	1.12	0.27
8 April	225	34	6.68	6.60	8.37	7.90	6.62	33	0.23	-0.12-0.53	0.05	1.29	0.21

ble 1). Furthermore, we found no significant differences among sample correlation coefficients either in frogs or in toads (frogs:  $P = 0.19$ ; toads:  $P = 0.99$ ).

## Discussion

### *The variability in presence of SAM*

We found evidence of SAM in common frogs (statistically significant correlation on most of the sampling days within the MRS) but not in common toads (no significant correlation). Furthermore, the presence of SAM varied also within the population of common frogs and occurred in three of the four day-samples with the higher OSR.

In explosive breeders, great variability in mating patterns has been observed among species (Duellman and Trueb, 1994; Sullivan, Ryan and Verrel, 1995; Wells, 2007), among populations of the same species (Davies and Halliday, 1977; Gittins, Parker and Slater, 1980a), and even over time within the same population (Olson, Blaustein and O'Hara, 1986; Wagner and Sullivan, 1995; Briggs, 2008). Apart from the effect of species-specific ecology, variability within a species or population in the intensity of SAM is probably af-

ected by differences in population densities and especially in OSRs (e.g., Emlen and Oring, 1977; Tejedo, 1988; Boll and Linsenmair, 1998; Lee and Park, 2009), and different results on the presence and intensity of SAM have been found within our both model species (e.g. Arak, 1983; Reading and Clarke, 1983; Elmberg, 1987; Höglund and Robertson, 1987; Ryser, 1989; Marco and Lizana, 2002).

Despite the significant positive correlation in most of the days in frogs, the coefficients of determination,  $R^2$ , were generally low here. Thus, relatively little of the (co)variance of male-female body size was explained (from 1% to 25%, table 1). Shine et al. (2001) have already pointed out that there is a great deal of "noise" in such relationships. Because the significance of the correlation is not a measure of the tightness of that correlation and depends not only on the value of the correlation coefficient but also on the sample size, we should not be satisfied merely with a significant correlation. For instance, a very weak correlation can provide a significant result if the sample size is large enough and, conversely, a relatively high correlation might not be revealed due to small sample size (Fisher, 1921; Zar, 1984). This implies that we must interpret the results with care and consider the amount of explained variation. It fol-

lows from the previous discussion that not only variability in the demographic parameters but also a misleading interpretation of correlation results may be the reason for ambiguous findings on the existence of SAM.

#### *The effect of OSR on size-assortative mating*

Although the OSRs were higher and varied greatly in toads, we detected positive correlation between the body length of males and females in pairs in frogs only. Furthermore, the sample correlation coefficients reflected increasing OSR only in frogs, but not in toads. Nevertheless, multiple comparisons among the correlation coefficients within the MRS showed non-significant differences in both frog and toad samples.

Although both common frogs and common toads are typical representatives of explosively breeding anurans (Arak, 1983), the differences in the existence and intensity of SAM related to OSR probably reflect their species-specific ecologies. For instance, the length of the MRS in frogs was shorter than in toads (4 vs. 6 d, respectively) in our study. The difference was even greater for the total length of breeding season (15 vs. 33 d). This is consistent with the findings of other authors (Arak, 1983; Elmberg, 1990). The length of the breeding season affects the population density and OSR at a breeding site. During the shorter reproduction period of common frog, the occurrence of reproductively active females is concentrated at a breeding site for only a few days. This could lead to relatively high daily proportions of females and a decline in OSR. During the longer reproduction period of common toads, on the other hand, there were probably fewer females at the breeding site on any one day. This is reflected in the heavily male-biased OSR (Emlen and Oring, 1977; Arak, 1983), and the OSR is usually higher in common toads, at between 2 and 8 (Davies and Halliday, 1979; Reading, 2001; Brede and Beebe, 2006), than in common frogs (in range 1-7, Elmberg, 1990, 1991). The intensity of competition could also be affected by

species-specific locomotion abilities and overall activity (Eshel, 1979; Crowley et al., 1991; Jirotkul, 1999).

It has been found that the higher the OSR within a population the more intensive is the sexual selection occurring through male-male competition (Arak, 1983; Tejedo, 1988; Höglund, 1989; Lee and Park, 2009). However, few studies have analyzed the effect of different OSRs on the intensity of correlation or on the presence of SAM in amphibians (Arak, 1982; Elmberg, 1991; Yu and Lu, 2010). Within those studies cited, moreover, the authors compared only the significances of correlations among different OSRs. As we point out above, the significance of a correlation is also affected by the sample size and only the correlation coefficient is a measure of the tightness of that correlation. Therefore, the comparison solely of significances is improper and yields size-biased conclusions. We conclude that a better way to analyze the effect of different OSRs on the presence of SAM is to compare correlation coefficients, as in our study.

#### **Conclusions**

Within both studied species, great variability in the presence and intensity of SAM has been observed. Despite prevailing significant correlations in frogs, the coefficients of determination,  $R^2$ , were generally low here, and explained relatively little of the (co)variance of male-female body size in pairs. We conclude that not only variability in the demographic parameters but also a misleading interpretation of correlation results may be the reason for ambiguous findings of SAM. To examine the existence of SAM, we should not be satisfied merely with a significant correlation, because its significance is also affected by the sample size. Only the correlation coefficient,  $r$ , is a measure of the tightness of that correlation. This is especially true for the comparison of existence of SAM among studies, and also for the analysis of the effect of different OSRs on the presence of SAM. The best

way is to compare correlation coefficients, as in our study. To avoid mistakes within studies on SAM, we should put emphasis on correct choice and interpretation of statistical methods.

**Acknowledgements.** We thank L. Čuhelová and V. Kos for their great help with the field work, as well as R. Healey and G.A. Kirking for useful comments on the manuscript. The study was supported by a grant from the Czech University of Life Sciences Prague (42900/1312/3166). The field work was performed on the basis of permission awarded by Prague City Hall.

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*Submitted: September 25, 2014. Final revision received: April 24, 2015. Accepted: April 26, 2015.*  
*Associate Editor: Sebastian Steinfartz.*