

Sex-biased predation by polecats influences the mating system of frogs

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In agile frogs, *Rana dalmatina*, an increase in male-biased operational sex ratio and in male abundance results in the emergence of alternative male mating behaviour in the form of searching. As a consequence, females are coerced into mating with multiple males, which in turn increases the level of conflict between the sexes. Selective predation on males by the European polecat, *Mustela putorius*, decreases the occurrence of polyandry. In ponds visited by polecats, the sex ratio is less male biased than in ponds where polecats are absent. As a result most males call to attract females and fewer males actively search for females. Females are able to choose between calling males and mate with a single male. Thus, predation by polecats is found to influence sex ratio, male abundance and sexual conflict in a frog mating system, restricting the opportunity for multiple mating.

Keywords: polecat; predation; polyandry; sexual conflict; sex ratio

1. INTRODUCTION

The asymmetrical interest of multiple mating is one of the main concerns in evolutionary biology. While males can increase their fitness by mating with more than one female, females were not supposed to maximize their reproductive success by mating with many males (Clutton-Brock & Vincent 1991; Arnold & Duvall 1994). The direct benefits gained by a female, such as gift or male parental care, give reasonable explanations for the occurrence of polyandry (Reynolds 1996; Fedorka & Mousseau 2002). Nevertheless, the reason why females consent to multiple mating is unclear when males provide only sperm. Polyandry may allow females to avoid genetic incompatibility or augment fertilizing efficiency (Keller & Reeve 1995; Newcomer *et al.* 1999). Alternatively, females may gain a fitness for their progeny through 'good genes' or increased genetic diversity (Yasui 1998; Jennions & Petrie 2000; Hosken & Stockley 2003).

However, superfluous mating may also carry significant costs, increasing the risk of predation, transmitted diseases or injuries due to competitive males (see Stockley 1997), and the reasons why polyandry occurs remained strongly debated. In frogs, genetic polyandry was evidenced in only five studies (D'Orgeix & Turner 1995; Laurila & Seppä 1998; Roberts *et al.* 1999; Prado & Haddad 2003; Lodé & Lesbarrères 2004). Numerous studies assumed that

polyandry is female driven. However, it is possible that changes in the operational sex ratio (OSR) could influence the occurrence of polyandry through its effect on male mating strategies. A male-biased sex ratio would increase the occurrence of alternative mating strategies resulting from competitive interactions. Male agile frogs *Rana dalmatina* adopt two mating strategies to attract females: stationary calling and searching. Searching males actively grasp females whereas males that call from stationary positions are approached by individual females. In male-biased choruses, searching occurs at a high frequency. When OSR is less skewed towards males, the number of males adopting searching behaviours decreases. These differences in the way in which males acquire mates might determine whether or not females mate multiply. Natural variations in OSR can occur through sex-biased predation. Agile frogs are preyed upon by polecats and the extent to which the sexes differ in their vulnerability to predation may influence the OSR.

We investigated whether predation and male-biased sex ratios can explain the occurrence of polyandry. By focusing on different patterns of mating behaviours we (i) examined the impact of carnivore predation on agile frog sex ratios, and (ii) determined whether the sex ratio influences genetic polyandry.

2. METHODS

We studied agile frog breeding congregations in 11 ponds. In each pond, we determined the nightly OSR and the mating tactic adopted by each male. We sampled a total of 95 clutches to infer genetic polyandry from eight allozymic loci. The impact of predation upon frogs was assessed by the radiotracking survey of 22 polecats (for detailed methods, see electronic Appendix A).

3. RESULTS

The total number of clutches was equal to the total number of females. The adult sex ratio (ASR) was male biased in every pond in all three breeding seasons averaging ASR = 1.84 (s.d. = 0.48, $n = 11$ ponds) with no significant differences among breeding seasons (Kruskall-Wallis $H = 4.501$, $p = 0.42$; see electronic Appendix B).

Male frogs totalled 83.5% of frogs preyed upon by polecats ($n = 249$). Given that 64.4% of frogs in ponds were males, the proportion of prey that were males was significantly different from that expected by chance ($\chi^2 = 26.7$, $p < 0.0001$). ASR and mean OSR were significantly correlated ($r_{\text{Spearman}} = 0.718$, $p < 0.012$, $n = 11$) and were significantly less male biased ($t_{\text{Welch}} = 3.64$, $p < 0.022$, d.f. = 9 and $t_{\text{Welch}} = 3.29$, $p < 0.011$, d.f. = 8, respectively) in ponds exploited by polecats (ASR = 1.50, s.d. = 0.08; OSR = 4.37, s.d. = 1.84) than in ponds without polecats (ASR = 2.25, s.d. = 0.45; OSR = 9.65, s.d. = 3.48). The proportion of non-calling males, i.e. searchers, averaged 35.4% while callers averaged 64.6% when data from all ponds in all years were pooled. The mean proportion of searching males present per night was significantly higher ($U_{\text{Mann-Whitney}} = 20.0$, $z = 2.45$, $p < 0.014$) in ponds without polecats averaging 38.0% ($n = 6$ ponds) than in ponds exploited by polecats (25.3%, $n = 5$ ponds). Similarly, when the polecats left the ponds, the frog sex ratio reached a significantly higher male-biased value the next year (comparison of mean proportions for two ponds: $z = -2.44$, $p = 0.024$) increasing to 40.6% (figure 1). By contrast, in two control ponds, the sex ratio did not differ significantly between years ($z = -0.30$, $p = 0.38$) suggesting that differences were not attributable to a temporal

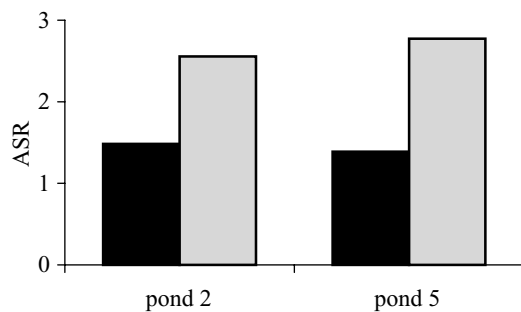


Figure 1. Variation in frog sex ratio (ASR) in ponds exploited by polecats (black bars) and in ponds left by polecats (grey bars).

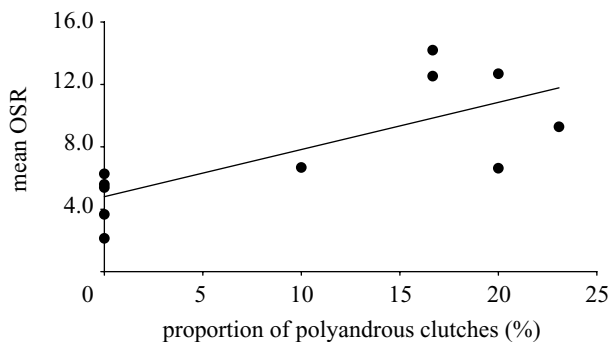


Figure 2. Correlations between mean OSR and occurrence of polyandry in 11 breeding ponds.

variation in ASR. Finally, male abundance in populations was significantly lower in polecat ponds compared to ponds without polecats ($U_{\text{Mann-Whitney}} = 3.0$, $z = -2.19$, $p = 0.028$).

On the 95 analysed clutches, monoandry was found in 82.1% of clutches while only 17 clutches (17.9%) were fertilized by at least two males evidencing multiple mating. All 17 multiple mating clutches were laid in ponds without polecats. The number of polyandrous clutches was correlated with ASR ($r_s = 0.680$, $p < 0.03$, $n = 11$) based on the 11 ponds from which ASR was obtained. Polyandrous clutches clearly coincided with the highest male-biased OSR and with the highest male abundance in ponds. Moreover, the proportion of polyandrous clutches was correlated with the average of nightly OSR with $r_s = 0.776$, $p < 0.005$ (corrected for ties, 11 ponds; figure 2).

4. DISCUSSION

The pattern of polecat predation on frogs was found to be sex selective, and as a consequence, determined the OSR and ultimately the mating pattern of the population(s). Male agile frogs space out in breeding aggregations by defending territories and advertise to females using soft repeated calls (Lesbarrères & Lodé 2002). In numerous other anurans (Halliday & Tejedo 1995), breeding males adopt either a stationary calling tactic to attract females or, alternatively, adopt a satellite strategy and sit near calling males and attempt to intercept approaching females. In agile frogs, 'satellite' males adopt an active searching behaviour. Polecat predation resulted in a decrease in the percentage of searchers, either owing to direct predation on searchers or predation on calling

males, thereby leaving vacant territories and allowing some searchers to switch roles to calling behaviour. In reality, demographic sex differences are common in wild species (Clutton-Brock & Vincent 1991), and many species endure male-biased predation (see the review in Magnhagen 1991).

Contrary to the assumption that high predation risk should increase the frequency of satellites (Lucas & Howard 1995), polecat predation increased the proportion of callers. The experimental manipulation of predator presence decreases the likelihood that these results are owing to confounding factors. Calling activity should be mainly influenced by competing interactions because males cannot assess how many females are present in the breeding pond.

Polyandry is often considered to be driven by females and acceptance of multiple partners can enhance female fitness (see Yasui 1998). However, these kinds of advantage are not always obvious, since multiple mating can also carry costs such as reduced fertilization success (Byrne & Roberts 1999). In agile frogs, if searching males are preyed upon more often than callers, females that mate with searchers face an increased risk of predation. Satellite males often are bad competitors, and in common toads were removed from female's backs by larger males (Davies & Halliday 1979). The success of males could be linked to male abilities to manipulate females and to force females to mate. This raises the question of whether polyandry arises as a consequence of coercion. In the agile frog, the male-biased OSR results in more searchers and as a consequence more attempts at forced mating by males. Indeed, we observed these searchers trying to intercept and to force females to mate. Numerous sexual phenomena could derive from a conflict of interest between sexes (Rice 2000; Gavrillets *et al.* 2001). A high proportion of searcher males were involved with multiple matings, which supports the suggestion that polyandry in this species results from male coercion. Multiple paternity may also be attributed to sperm diffusion between clutches such as in *Rana temporaria* (Laurila & Seppä 1998), but *R. temporaria* lay clutches in close proximity forming a large egg mass. Agile frogs lay clutches in male territories. Clutches are therefore well spaced and diffuse fertilization not possible.

This would suggest that multiple paternity in agile frogs occurs in one of three ways. (i) The first male to mate with the female is displaced by a second male while the female is depositing her clutch. The second male then ejaculates over the remaining eggs as they are extruded. (ii) Searching males behave like *Chiromantis* secondary males. Jennions & Passmore (1993) demonstrated the capability of sperm release by a second male in *Chiromantis*. The amplexed male tolerates the presence of other males in very close proximity to the point that secondary males are able to swing their cloaca inwards and release sperm over the eggs that are extruded by an already amplexed female. In agile frogs this may involve two or more searching males simultaneously grabbing a female or a searching male attaching himself to an amplexed pair. (iii) When a female mates with a chosen male, she can release her whole clutch, whereas she may release only a part of her clutch when a searching male forces. It was evidenced that leaf-folding frogs *Afraxalus delicatus* were

able to split clutches sequentially among males (Backwell & Passmore 1990).

Although the non-experimental nature of this work could include some confounding factors, the results are consistent with the hypothesis that sex-selective predation on males promotes monogamy in agile frogs, whereas reduced predation increased the skew in the OSR towards males and promotes polyandry and forced mating. Even if a searching male is not likely to have 'good' genes, the female may gain some genetic advantages by mating with genetically variable individuals in a kind of bet hedging (but see Yasui 1998). Nevertheless, the influence of polecat predation on polyandry through effects on OSR reveals the complexity of ecological interactions in evolution.

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