ELECTRONIC APPENDIX

This is the Electronic Appendix to the article

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

by

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Proc. R. Soc. B **271** (Suppl. 6), 399–401 (doi:10.1098/rsbl. 2004.0195

Electronic Appendix A:

DETAILED METHODS

Mating and sex-ratio

We studied 11 breeding ponds in western France from 1999 to 2002 (four ponds studied for two successive years and seven ponds studied for one year, see electronic Appendix B). Adult agile frogs gathered in breeding congregations from February to March, and mature frogs were captured when they arrived at the breeding pond. The ponds were entirely enclosed by a plastic canvas associated with pitfall traps. Additionally, a wire fencing coupled to the canvas prevented most of frog predators from accessing the bottom of the canvas but frogs could easily go through. The frogs were sexed and marked by toe-clipping and all toes were transported at the laboratory and kept at -25°C (for two months maximum). The number of males per night and the number of calling males per day were estimated every night by both auditory and visual localization using night optics. In most frogs, the term satellites refers to those individuals that sit near calling males, but in agile frogs, while each territorial male defends a calling place against any intruders, other males actively search for females and were called *searchers*. The number of searching males was determined from the difference between the number of callers and total males present in the pond. Male abundance was calculated as the ratio males/total frogs in ponds. The adult sex-ratio (ASR, total n males/total n females) and the operational sex-ratio (OSR,. nightly ratio of males to fertilizable females) were calculated (Emlen & Oring 1977) and the number of new clutches per day was recorded. Correlations between ASR and OSR averaged over all nights were performed based on 11 ponds. Tests of Chi^2 were performed using Yates correction, differences were estimated with Welch alternate *t* test or Mann-Whitney U test, and we used Spearman rank correlations. It is unlikely that OSR was overestimated because Agile frogs do not forage during the breeding season, mate only one to three times (minimum sperm depletion) and only breeding adults gathered on breeding ponds (no immature individuals). After breeding, agile frogs returned to winter sites for a latency period before going to their summer sites in May.

Polecat predation

The European polecat *Mustela putorius* is the main nocturnal predator of breeding agile frogs (Lodé 2000), and twenty two polecats were radiotracked. At six of the enclosed ponds, polecats were allowed access via

inclined ladders. The rungs were spaced at a distance that prevented the use of the ladders by frogs. Prey remains provided evidence for polecat predation upon frogs around the ponds and polecat predation upon frogs was easily and always evidenced by prey remains (Lodé 2000). Because polecats left two surveyed ponds in 2001, the impact of polecat predation was studied both from horizontal comparisons (*i.e.* different ponds with and without polecat predation) and vertical comparisons (*i.e.* the same ponds, during polecat predation and after polecat departure).

Genetic evidence for multiple mating

We sampled 95 clutches, of which 73 were from 10 ponds of 11 studied ponds, and 22 clutches were from three other ponds. Regarding ethical considerations, we randomly collected 10% of eggs, as our goal was only to demonstrate multipaternity. A female produced a single clutch, and the distance between the selected clutches was such (from 2.5 to 5 m) that we avoided collecting from mixed clutch (territorial breeder). Proteins used for horizontal starch-gel electrophoresis were extracted from toes of adults and from tails of 2210 tadpoles (20-22 offspring per clutch). Migration was performed in 11% continuous Tris EDTA Borate starch gels. Seven enzyme systems encoded by eight polymorphic loci (*Aat-1, Aat-2, α-Gdh, Ldh-1, Ldh-2, Mdh-1, Mpi, 6-Pgdh, Pgm*) were successfully investigated. From the electrophoretic pattern, multipaternity was estimated using PAPA 1.0 (*Duchesne, P., Godbout, M.- H. & Bernatchez, L. 2002.* PAPA -Package for the Analysis of Parental Allocation-: A computer program for simulated and real parental allocation. Version 1.0. *Laval, Québec: Université de Laval.*). The parentage allocation method used in PAPA is based on the likelihood that a parental pair produces multilocus genotypes found in the tested offspring. PAPA is a simulator program that may be run prior to the collection of real parental genotypes, which allowed for the estimation of the minimal number of genitors for each clutch. Two types of simulations were performed, *i*) including potential males and *ii*) excluding parents. Unfortunately 5 polyandrous clutches were found in ponds without sex-ratio estimate.

Electronic Appendix B: Total number and adult sex-ratio (ASR) of frogs breeding in 11 ponds in western France

		Breeding period	Males	Females	ASR
Ponds exploited by polecats.	Pond 1	1999	28	19	1.474
	Pond 2	2000	46	31	1.484
	Pond 3	2000	9	6	1.500
	Pond 4	2000	17	11	1.545
	Pond 5	2000	25	18	1.389
	Pond 6	2001	31	19	1.632
n			***156***	***104***	
Ponds without polecat predation.	Pond 7	2002	48	19	2.526
	Pond 8	2002	68	40	1.700
	Pond 9	2001	82	29	2.828
	Pond 10	2001	33	17	1.941
	Pond 11	2002	29	13	2.231
n			***260***	***118***	
Ponds left by polecats.	Pond 2	2001	61	22	2.773
	Pond 5	2001	46	18	2.556
Control ponds	Pond 3	2001	8	6	1.333
	Pond 4	2001	19	13	1.462