



**University of
Zurich**^{UZH}

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 1999

Cryptic female choice: frogs reduce clutch size when amplexed by undesired males.

Reyer, H U ; Frei, G ; Som, C

Abstract: In species with internal fertilization, females can 'cryptically' choose (e.g. through sperm selection) which individuals sire their offspring, even when their overt preferences for copulatory partners are overrun by male-male competition and sexual coercion. The experiment presented here reveals that control of paternity after copulation has begun is also possible in species with external fertilization. Females of the hybridogenetic *Rana esonae*-*Rana esculenta* (LL-LR) waterfrog complex adjust their clutch size in response to mate type: they release fewer eggs when amplexed by hybrid LR males who jeopardize successful reproduction than when amplexed by parental LL males. This reduction in the number of eggs laid can increase a female's residual reproductive value through a second mating in the same breeding season or a larger clutch size in the next year. We argue that cryptic female choice through clutch size adjustment (i) may have evolved more often than previously assumed, and (ii) can arise even where females mate only once during a reproductive period.

DOI: <https://doi.org/10.1098/rspb.1999.0894>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-723>

Journal Article

Originally published at:

Reyer, H U; Frei, G; Som, C (1999). Cryptic female choice: frogs reduce clutch size when amplexed by undesired males. *Proceedings of the Royal Society B: Biological Sciences*, 266(1433):2101-2107.

DOI: <https://doi.org/10.1098/rspb.1999.0894>

**CRYPTIC FEMALE CHOICE: FROGS REDUCE CLUTCH SIZE
WHEN AMPLEXED BY UNDESIRED MALES**

Heinz-Ulrich Reyer¹, Gerhard Frei and Christian Som

Department of Zoology, University of Zürich, CH-8057 Zürich, Switzerland

¹*E-mail: ulireyer@zool.unizh.ch*

In species with internal fertilisation, females can “cryptically” choose (e.g., through sperm selection) which individuals sire their offspring, even when their overt preferences for copulatory partners are overrun by male-male competition and sexual coercion. The experiment presented here reveals that control of paternity after copulation has begun is also possible in species with external fertilisation. Females of the hybridogenetic *Rana lessonae*/*R. esculenta* (LL/LR) waterfrog complex adjust their clutch size in response to the mate type: they release fewer eggs when amplexed by hybrid LR-males - which jeopardise successful reproduction – than when amplexed by parental LL-males. This reduction in the number of eggs laid can increase a female’s residual reproductive value through a second mating in the same breeding season or a larger clutch size in the next year. We argue that cryptic female choice through clutch size adjustment (a) may have evolved more often than previously assumed and (b) can arise even where females mate only once during a reproductive period.

Keywords: Anurans, cryptic female choice, external fertilisation, male-male competition, sexual selection, clutch size adjustment

1. INTRODUCTION

Sexual selection theory predicts that individuals should choose mates that provide them with direct or indirect reproductive benefits (Kirkpatrick & Ryan 1991; Andersson 1994). Possibilities for female choice can, however, be severely restricted through male-male competition and sexual coercion (Qvarnström & Forsgen 1998). Here, the term sexual coercion refers to cases where males pursue, catch, and restrain females and forcefully copulate with them (Clutton-Brock & Parker 1995). Factors promoting such behaviour include both phenotypic traits, such as superior speed and strength or intromittent organs in males, and ecological conditions that lead to clumped aggregations where female movements are restricted and males cannot effectively control mating access to females (Smuts & Smuts 1993; Clutton-Brock & Parker 1995; Gowaty 1997).

Under these circumstances, selection will favour females that can reduce the probability of their offspring being sired by non-preferred males even after copulation has begun. One possibility is “cryptic” female choice, broadly defined as a female-controlled process that selectively favours paternity by males with a particular trait over paternity of males that lack the trait when the female has copulated with both types (Eberhard 1996). Such cryptic female choice has been reported for more than 100 species (Eberhard 1996), but in many cases alternative explanations were not excluded. Hence, there is an ongoing debate about the relative importance of female-controlled sperm selection and male-controlled sperm competition in skewing paternity (reviewed by Birkhead 1998).

Whatever the precise mechanism, most of the ca. 20 described mechanisms of cryptic choice are feasible only in species with internal fertilisation (Halliday & Verrell 1984; Jennions et al. 1992; Eberhard 1996; Halliday 1998). Yet, sexual coercion by males also occurs in species with external fertilisation. Typical examples

come from several frog and toad species in which females are often forcefully amplexed by other than the preferred males (Halliday 1983; Bourne 1993). Can coerced females of such species nevertheless exert mate choice?

European waterfrogs of the *Rana lessonae* / *R. esculenta* complex provide an excellent model system to study this question, because in this complex, mating with the “wrong” male will result in no reproductive success at all, rather than in a small reduction of fertility as has been reported for other anuran species (Davies & Halliday 1977; Ryan 1985; Robertson 1990). This is due to a rare reproductive mode known as „hybridogenesis“ (Schultz 1969). During gametogenesis, *Rana esculenta* (genotype LR) - which originated as an inter-specific hybrid between *R. lessonae* (LL) and *R. ridibunda* (RR) (Berger 1977) - eliminates its L genome and produces eggs and sperm containing only the R genome (Tunner 1974; Uzzell et al. 1980). As exclusion occurs prior to or during meiosis (Graf & Müller 1979; Uzzell et al. 1980; Tunner & Heppich 1981), recombination through crossing-over is normally prevented and the R-genome is transmitted closely (for exceptions see Uzzell et al. 1977, Hotz et al. 1992). In areas without *R. ridibunda*, LR x LR matings (i.e. hybrid x hybrid) lead to *R. ridibunda* tadpoles, but these usually die during the larval stage or shortly after metamorphosis, both under laboratory and nearly natural conditions (Berger 1976; Semlitsch & Reyer 1992). The cause of this low viability is assumed to be an accumulation of lethal factors on the clonally transmitted R genome (Berger 1976; Graf & Müller 1979; Uzzell et al. 1980). As a consequence, the hybrid can reproduce successfully only when it regains the lost L genome by mating with *R. lessonae*, its genetic host. *Rana lessonae*, by contrast, should avoid mating with the sexual parasite, since the resulting *R. esculenta* will again eliminate the L genome. Hence, in the LL/LR-system, a sexual parasite-host conflict is superimposed on the female-male conflict, which exists in all species with sexual coercion.

However, in this conflict the cost:benefit ratio from mating with LR-individuals is likely to be more detrimental to females than to males, because the repeatedly mating males only lose some time and relatively cheap sperm whereas females may lose the reproductive potential of a whole season (Abt & Reyer 1993). Hence, the ability of females to exert choice even under coerced mating would be beneficial. Indeed, preference for LL mates exists in both LL- and LR-females, but not in males (Abt & Reyer 1993; Engeler 1994). Female preference, however, is overrun by male-male competition: when exposed to three different LL/LR-ratios of interacting males, females formed amplexed pairs in proportion to the male ratio (Bergen et al. 1997). Yet, direct observations of amplexant pairs and analyses of clutches from ponds with known sex and genotype ratios among adults revealed that LL-males are significantly more often involved in successful amplexi than expected under the assumption of random mating (Engeler 1994; G. Abt, pers. comm. 1998; H.-U. Reyer unpubl. data). This suggests that females have some subtle means of mate choice, even when exposed to competing males and forcefully amplexed.

In this study, we investigate whether females adjust the number of eggs laid according to the genotype of their mates. This is one of the few cryptic choice mechanisms possible in species with external fertilisation; but it has never been clearly demonstrated (Eberhard 1996). Our experiment was designed: 1) to test for the possibility of *clutch size adjustment* (mating sessions A and B), 2) to investigate the consequences of such adjustment for *future reproduction* within (session B) and between years (session C) and 3) to analyse the *proximate mechanisms* underlying the trade-off between present clutch size adjustment and future reproduction. Although we did not allow females to choose mates (neither directly or indirectly), we can infer their preferences from the occurrence of clutch size adjustment and its fitness consequences.

2. Methods

(a) Natural history of waterfrogs

Waterfrogs reproduce in a wide variety of ponds, ranging from shallow ditches to small lakes. During the breeding period (May-July), males remain at ponds for several weeks and wait for the asynchronously arriving females. This results in a male-biased operational sex ratio (OSR), with severe competition among males and limited possibilities for female choice, as is typical for “prolonged breeders” (Wells 1977). Under these conditions competitively superior males can mate with more than one female. Ranid females, in contrast, are usually assumed to mate only once per season, and they may even skip a year of reproduction when their fat reserves are low and/or environmental conditions are not suitable (Burkardt 1912; Rastogi et al. 1983; Sjögren 1991, Bättig 1998; G. Abt, unpubl. data). Any eggs not released during one breeding season are resorbed in summer. From early September to the end of October, the newly acquired and resorbed resources are then invested in new follicles for the next breeding season (Burkardt 1912; Rastogi et al. 1983). This trade-off between present and future reproduction will be favoured only if females have a chance to breed again. This is true for waterfrogs: with annual survival rates of 55-80%, adult females have an expected lifespan of 2-5 breeding seasons, and some individuals survive to 8 years of age (Sjögren 1991; A.-K. Holenweg, unpubl. data).

(b) Experimental animals

During May and June of 1995, 1997, and 1998, male and female frogs were caught from a natural pond at night and were immediately weighed, measured and genotyped via albumin electrophoresis of lymph taken from a small incision between

two toes of a hind foot (Vogel 1973). In order to allow individual identification throughout the experiments (especially in communal holding pens), all frogs were marked with a transponder (TROVAN ID 100, Pamedia AG) introduced into their lateral lymphatic sac (Sinsch 1992). From body mass (M) and snout-vent length (SVL) we calculated female condition indices (CI) with the following equation: $CI = M \cdot 10^4 / (SVL)^3$ (Jørgensen 1992, p.442). Only females with a $CI \geq 1.15$ were used for the experiments, because they are likely to carry eggs and be ready to spawn (own observations).

(c) Mating experiments

All experimental animals went through two mating sessions (A, B) and some also through a third one (C). The setup for sessions A and B consisted of four identical ponds, each measuring 2.3 x 4.4 m, equipped with three water plants, and surrounded by drift fences. Video cameras, mounted above the ponds, recorded the position and behaviour of frogs day and night, every 10 minutes for 30 seconds.

Mating session A: One or two days after being caught, one pair of each of the four possible mate combinations (LL x LL, LL x LR, LR x LL, LR x LR; first letter = female, second letter = male) was introduced into each of the ponds. Pairs remained there for 1 or 2 days, depending on when the first spawning had occurred in any one of the four ponds.

Mating session B: Thereafter, all frogs were removed and females shifted to a new pond where each of them was matched with a male of the other type: LL- and LR-females which in session A had been together with an LL-male were now exposed to an LR-male and those females which had been together with an LR-male were exposed to an LL-male. For this session B, which also lasted for 1-2 days, we

used new males to eliminate potential effects of sperm depletion (Halliday 1978; Bourne 1993).

In both sessions A and B, frogs were assigned randomly to mates and ponds within the female/male SVL-ratio of 0.81-1.43 (mean \pm s.d.: 1.14 ± 0.16). Each of the 4 possible female-male combinations was replicated with freshly caught frogs 10 times (4 times in 1995, 5 times in 1997 and once in 1998), with all experiments occurring between May 29 and June 26. By putting only one pair into a pond we guaranteed that the female was amplexed by only one male which (a) mimics the usual situation in these frogs after a pair has formed and (b) eliminates direct male-male and sperm competition as confounding factors on measures of reproductive success. Whether or not amplexus had occurred was deduced from video surveillance and from the presence or absence of fertilised eggs in the clutch.

Overwintering: At the end of session B, frogs were separated by sex and genotype and transferred to communal holding pens where they remained until mid-October. In 1995 and 1998 they were then released into their pond of origin. In 1997, we kept 18 females, gradually cooled them from 15 to 5°C over a period of one week and kept them at this hibernation temperature until February 2, 1998. Thereafter, they were re-warmed to 20°C over 10 days and exposed to daylengths increasing from 10 to 16 hours within a month.

Session C: When the first overwintered females started releasing eggs without a male being present (March 12, 1998), the others were put together with one L-male each in 90 l aquaria, containing water plants, and allowed to spawn.

(d) Dependent variables

In all three sessions (A-C), ponds or aquaria were regularly searched for spawn, and all eggs were removed and transferred to 5-l dishpans containing aged

tap water. Eggs were counted and kept for several days to check whether fertilisation (and hence amplexus) had occurred. This posthoc test for successful mating works with all female x male combinations, even for the LR x LR pairs, because the resulting RR tadpoles die only later. Adult frogs were weighed before and immediately after mating, and also in fall, 8-12 weeks after spawning. Since egg numbers in frogs increase with body size and *R. esculenta* is, on average, bigger than *R. lessonae* (Berger 1977), all clutch sizes are expressed as the number of eggs per g female body mass.

In early September (1-3) and mid-October (14-16), of 1997 we drew small samples of blood (10-40 μ l) from an incision made between two toes of a hind foot. From these samples plasma titres of testosterone/dehydro-testosterone, which correlates positively with next year's egg production (Licht et al. 1983), were analysed through radioimmunoassay (RIA). Cross-reactions were measured with antibodies AK8/3 or AK8/5/10, which allow detection of hormone concentrations on the order of ≥ 1 ng/ml (based on 10 μ l plasma). Details of the analysis techniques have been described elsewhere (Fenske & Probst 1982).

(e) Statistics

Egg numbers and condition indices were related to genotypes of, and size ratios between, females and males by means of multi- and univariate analyses of variance. Various pairwise relationships between egg numbers, body condition and hormone titres were tested through regression analyses, after controlling for confounding genotype effects where necessary. Differences were considered significant at $p \leq 0.05$. Data are given as means \pm s.e. All analyses were performed with Systat 7.0 for Windows (SPSS Inc. 1997).

3. Results

(a) Spawning adjustment

Out of the 40 females used in both mating sessions, 11 did not release eggs in either experiment; the remaining 29 females spawned in either mating session A, B or both, 18 of them with and 11 without amplexus (Table 1). Considering only the cases of unsuccessful reproduction, their frequencies were not related to the pair combinations for either the 11 cases with no eggs or the 11 cases with eggs but no amplexus or all 22 cases together (all $p > 0.621$, Fisher exact probability test, 2-tailed).

A multivariate analysis of variance (MANOVA), based on the 29 cases where oviposition occurred, also revealed no effect of the specific pair combination on spawning, as indicated by the non significant values for size ratio ($p = 0.46$) and male x female interaction ($p = 0.51$; Table 2). The MANOVA did, however, show that clutch sizes depended on both female and male type (Table 2). In terms of female type, egg number per g body mass was 1.45 times higher for LR- than for LL-females (Fig. 1a, Table 1). In terms of male type, clutch size increased from females spawning without being amplexed through those mating with LR- to those mating with LL-males (Fig. 1b, Table 1).

(b) Multiple mating

Seventeen of the 29 spawning females released their first eggs during mating session A. Fourteen of these 17 females did not spawn again after mates were switched in session B, but three (17.6%) did. In 2 of these 3 cases, LR x LR matings in session A were followed by LR x LL matings in session B, with about equal proportions of eggs laid during both sessions (53 vs. 47% and 61 vs. 39%, respectively). In the third case, an LL x LL mating in session A was followed by an

LL x LR mating in session B, but this time the egg ratio between first and second mating was 90:10%. The other 12 of the 29 females did not spawn before session B and, hence, had no chance for multiple mating during the same season.

(c) Future reproduction

However, eggs withheld in one year increased reproductive output in the next (session C). Of 18 females that were held over winter in captivity nine spawned the next year while amplexed by LL-males. An ANCOVA ($R^2_{2,6} = 0.721$), controlling for the effect of female type, revealed a significant negative relationship ($p = 0.007$), between clutch size in 1997 and clutch size in 1998 (Fig. 2).

(d) Proximate mechanisms

This trade-off between present and future reproduction is probably mediated through body condition. Spring condition indices before session A did not differ among females (Table 2); but loss of condition after spawning, reflected the previous reproductive effort: it was least in females that had spawned alone, greater in those that had mated with LR-males and greatest in those mated with LL-males (Table 2, Fig. 1). The lower the number of eggs/g released during the breeding season, the better was the body condition (CI) in early September ($CI = -0.003 * \text{eggs} + 1.128$; $r_{1,27} = -0.456$, $p = 0.013$). September condition, in turn, was related to fall hormone titres (Fig. 3a), and to next year's reproductive output in mating session C (Fig. 3b).

4. Discussion

Our experiment shows that gravid LL- and LR-females release some of their eggs, even when not amplexed by males, but beyond this threshold they can (and do) adjust clutch size in relation to their mate preferences. When grasped by the

genetically „undesired“ RL-males, they laid fewer eggs than when exposed to the preferred L-males. Although the extent of clutch size reduction seemed to be slightly higher in LL- (40%) than in RL-females (30%) (Table 1), the overall response did not differ between the two females types, as indicated by the lack of a significant female x male interaction (Table 2). At present, we do not know whether clutch size adjustment also occurs when females are exposed to males with smaller quality differences between them (e.g. two LL-males). This, however can be tested with a similar experimental approach, which could be further improved by comparing the reproduction of females that were or were not allowed to choose a mate.

The only other anuran for which clutch size adjustment in relation to male partners has been reported is the red-eyed treefrog (*Agalychnis callidryas*), but in this species the adjustment is not related to mate preferences: females clasped by several males lay fewer eggs than those amplexed by only one male. This probably serves to reduce egg mortality which occurs when three or more males are clinging to a female (d'Orgeix 1996 cited from Halliday 1998).

In our waterfrogs, the reduction of present egg output can enhance future reproductive success in two ways. One way is to produce more than one clutch within the same season. Only 3 out of 17 females did this, a sample too small for making any general statement about the importance of this mechanism. Better supported is the second way: improved body condition in late summer and fall. Although our data demonstrate only correlations between clutch size in summer and body conditions and plasma levels of gonadal hormones in fall and egg numbers the following year (Figs. 2 and 3), evidence available from other studies strongly suggests causal relationships between these measures. From early September to the end of October, next year's reproduction is initiated; the number of vitellogenic and mature follicles increases markedly, and ovaries grow in weight (Rastogi et al. 1983;

Delgado et al. 1990). Resources for the fall investment are drawn from food uptake, fat bodies and resorbed follicles not spawned during spring and summer (Burkardt 1912; Jørgensen 1982; Rastogi et al. 1983). With decreasing food supply and/or fat stores, resorption - measured by the proportion of atretic follicles - becomes more and more important (Burkardt 1912; Rastogi et al. 1983). Thus, resorption of unreleased eggs probably provides the proximate explanation for the ultimately important link between reduced oviposition in one year and improved reproduction in the next year, which also has been found in the natterjack toad *Bufo calamita* (Tejedo 1992).

In the light of this interpretation the question arises: Why do females clasped by RL-males release eggs at all? One possible answer is that this is the only way to “satisfy” the male and terminate the amplexus as soon as possible. Another, not mutually exclusive, explanation is the following: eggs remaining in the oviduct are liable to swelling when water enters through the cloaca, and the resulting jelly mass can prevent subsequent oviposition or even threaten the female’s life (Günther 1990). Although ovulation can be triggered through environmental factors such as temperature and rainfall, it is often enhanced through amplexus (Duellmann & Trueb 1986, Jørgensen 1992). This may explain why the number of eggs released from the oviduct is higher in females clasped by RL-males than in those not amplexed at all.

Our study does not identify the particular cues used by females to distinguish between L- and E-males. However, given the definition of cryptic female choice mentioned in the Introduction, knowledge of these cues is not essential and, in fact, is also lacking in many studies of overt, i.e. precopulatory, female choice (summarised by Andersson 1994). Any cue from mating that originates in the male and is used by the female to selectively favour paternity by males which provide better stimulation than others could be subject to sexual selection (Eberhard 1996). Possible mechanisms include differences between males in behaviour during copulation, such

as calling (Licht 1969; Price & Meyer 1979), tapping, kicking, rubbing, squeezing and pressing the female (Rabb & Rabb 1960; Salthe & Mecham 1974), presenting her with nuptial gifts (Thornhill 1983; Sakaluk 1997) or stimulating her with secretions from specific glands (Duellmann & Trueb 1986) or through genital movements. Several of these mechanisms – some of which also occur in anurans – have been found to influence duration of copulation, transport of sperm to the fertilisation sites, ovulation, egg maturation, oviposition rate and other processes potentially linking female reproduction to male quality (for an extensive review see Eberhard 1996).

From a functional point of view, the female choice mechanism found in this study may be particularly important for waterfrog matings with their marked consequences on fertility; but the implications of our results go beyond this and the few similar systems reviewed by Dawley & Bogart (1989). The ability to adjust clutch size in response to male quality allows females to exert preferences, even when sexually coerced. Such adjustment would be beneficial in all cases where interbreeding between species, ecotypes, populations, families or other groups with differences in genetic compatibility leads to lower fitness than within group matings. Moreover, our study questions the common notion that cryptic choice can only be exercised if a female copulates with more than one male during any given fertile period (Eberhard 1996). Our finding that clutch reduction in relation to the copulatory partner of one year improves the residual reproductive value through larger clutches in the next season suggests that sexual selection on cryptic choice can operate even in seasonally monandrous species.

Thus, female choice through clutch size adjustment may be an underestimated mechanism in species with external – and perhaps also internal – fertilisation.

Potential candidates for investigating this mechanism include all species with large variations in clutch size, especially those with male coercion.

Acknowledgements. The study was supported by a grant of the Swiss National Science Foundation to H.-U. Reyer (No. 31-40688.94), and experiments were performed with permission and under the ethical guidelines of the “Kantonales Veterinäramt” (No. 133/96, 131/97 and 130/97). We are grateful to M. Roesli for overwintering the frogs, to I. Bättig for taking the blood samples and to I. Zerenner-Fritsche (University of Bayreuth, Germany) for performing the hormone analyses. The paper benefited from the comments made by T. Garner, T. Halliday, B. Hellriegel, A.-K. Holenweg, P. Pearman and especially J. vanBuskirk.

REFERENCES

- Abt, G. & Reyer, H.-U. 1993. Mate choice and fitness in a hybrid frog, *Rana esculenta* females prefer *Rana lessonae* males over their own. *Behav. Ecol. Sociobiol.* **32**, 221-228.
- Andersson, M. 1994. *Sexual selection*. Princeton University Press.
- Bättig, I. 1998. Fortpflanzungswahrscheinlichkeit bei weiblichen *Rana lessonae*, *Rana esculenta* und *Rana ridibunda*. Unpubl. MSC-Thesis, University of Zürich.
- Bergen, K., Semlitsch, R. D. & Reyer, H.-U. 1997. Hybrid female matings are directly related to the availability of *Rana lessonae* and *Rana esculenta* males in experimental populations. *Copeia* **1997**, 275-283.
- Berger, L. 1976. Hybrids of B-2 generations of European waterfrogs (*Rana esculenta* complex). *Ann. Zool. (Warsaw)* **33**, 201-214.
- Berger, L. 1977. Systematics and hybridization in the *Rana esculenta* complex. In *The Reproductive Biology of Amphibians* (ed. D. H. Taylor and S. I. Guttman), pp. 367-388. New York: Plenum Press.
- Birkhead, T. R. 1998. Cryptic female choice, criteria for establishing female sperm choice. *Evolution* **52**, 1212-1218.
- Bourne, G. R. 1993. Proximate costs and benefits of mate acquisition at the leks of the frog *Oloolygon rubra*. *Anim. Behav.* **45**, 1051-1059.
- Burkardt, L. 1912. Über die Rückbildung der Eier gefütterter aber unbegatteter Weibchen von *Rana esculenta*. *Archiv Mikroskop. Anatomie* **79**, 1-40.
- Clutton-Brock, T. H. & Parker, G. A.. 1995. Sexual coercion in animal societies. *Anim. Behav.* **49**, 1345-1365.
- Davies, N. B. & Halliday, T. R. 1977. Optimal mate selection in the toad *Bufo bufo*. *Nature* **269**, 56-58.

- Dawley, R. M. & Bogart, J. P. 1989. *Evolution and ecology of unisexual vertebrates*. Albany: The New York State Museum, Bulletin 466.
- Delgado, M. J., Gutiérrez, P. & Alonso-Bedate, M. 1990. Annual ovarian cycle and plasma levels of 17 β -estradiol in the frog *Rana perezi*. *Physiol. Zool.* **63**, 373-387.
- Duellman, W. E. & Trueb, L. 1986. *Biology of Amphibians*. New York: McGraw Hill.
- Eberhard, W. G. 1996. *Female control: Sexual selection by cryptic female choice*. Princeton University Press.
- Engeler, B. 1994. Partnerwahl im *Rana lessonae/Rana esculenta*-Hybridkomplex. Unpubl. MSc-Thesis, University of Zürich.
- Fenske, M. & Probst, B. 1982. Simultaneous release of androgens and progesterone by gerbil (*Meriones unguilatus*), Dispersed testicular interstitial cells under basal conditions and after stimulation with luteinizing hormone. *J. Endocrinology* **79**, 190-196.
- Gowaty, P. A. 1997. Sexual dialects, sexual selection, and variation in mating behavior. In *Feminism and evolutionary biology: Boundaries, intersections, and frontiers* (ed. P. A. Gowaty), pp. 351-384. New York: Chapman and Hall.
- Graf, J.-D. & Müller, W. P. 1979. Experimental gynogenesis provides evidence of hybridogenetic reproduction in the *Rana esculenta* complex. *Experientia* **35**, 1574-1576.
- Günther, R. 1990. Die Wasserfrösche Europas. Wittenberg Lutherstadt: Ziemsen Verlag.
- Halliday, T. R. 1978. The newt as an honest salesman. *Anim. Behav.* **26**, 1273-1274.
- Halliday, T. R. 1983. Do frogs and toads choose their mates? *Nature* **306**, 226-227.

- Halliday, T. R. 1998. Sperm competition in amphibians. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller), pp. 465-502. London: Academic Press.
- Halliday, T. R. & Verrell, P. A. 1984. Sperm competition in amphibians. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 487-508. New York: Academic Press, New York.
- Hotz, H., Beerli, P. & Spolsky, C. 1992. Mitochondrial DNA reveals formation of nonhybrid frogs by natural matings between hemiclinal hybrids. *Mol. Biol. Evol.* **9**, 610-620.
- Jennions, M. D., Backwell, P. R. Y. & Passmore, N. I. 1992. Breeding behaviour of the African frog, *Chiormantis xerampelina*, multiple spawning and polyandry. *Anim. Behav.* **44**, 1091-1100.
- Jørgensen, C. B. 1982. Factors controlling the ovarian cycle in a temperate zone anuran, the toad *Bufo bufo*, food uptake, nutritional state, and gonadotropin. *J. exp. Zool.* **224**, 437-443.
- Jørgensen, C. B. 1992. Growth and reproduction. In *Environmental physiology of the amphibians* (ed. M. E. Feder and W. W. Burggren), pp. 439-466. The University of Chicago Press.
- Kirkpatrick, M. & Ryan, M. J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* **350**, 33-38.
- Licht, L. E. 1969. Unusual aspects of anuran sexual behavior as seen in the red-legged frog, *Rana aurora aurora*. *Can. J. Zool.* **47**, 505-509.
- Licht, P., McCreery, B. R. & Pang, R. 1983. Seasonal and stress related changes in plasma gonadotropins, sex steroids, and corticosterone in the bullfrog, *Rana catesbeiana*. *Gen. Comp. Endocrin.* **50**, 124-145.

- Price, R. M. & Meyer, E. R. 1979. An amplexus call made by the male American toad, *Bufo americanus americanus* (Amphibia, Anura, Bufonidae). *J. Herpetol.* **13**, 506-509.
- Qvarström, A. & Forsgen, E. 1998. Should females prefer dominant males? *TREE* **13**, 498-501.
- Rabb, G. B. & Rabb, M. S. 1960. On the mating and egg-laying behavior of the Surinam toad, *Pipa pipa*. *Copeia* **4**, 271-276.
- Rastogi, R. K., Izzo-Vitello, I., Di Meglio, M., Di Matteo, L., Franzese, R., Di Costanzo, M. G., Minucci, S., Lela, L. & Chieffi, G. 1983. Ovarian activity and reproduction in the frog, *Rana esculenta*. *J. Zool., Lond.* **200**, 233-247.
- Robertson, J. G. M. 1990. Female choice increases fertilization success in the Australian frog, *Uperoleia laevigata*. *Anim. Behav.* **39**, 639-645.
- Ryan, M. J. 1985. *The Túngara Frog*. The University of Chicago Press.
- Salthe, S. N. & Mecham, J. S. 1974. Reproductive and courtship patterns. In *Physiology of the Amphibia* (ed. B. Lofts), pp. 309-521. New York: Academic Press.
- Sakaluk, S. K. 1997. Cryptic female choice predicated on wing dimorphism in decorated crickets. *Behav. Ecol.* **8**, 326-331.
- Schultz, R. J. 1969. Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. *Amer. Natur.* **103**, 605-619.
- Semlitsch, R. D. & Reyer, H.-U. 1992. Performance of tadpoles from the hybridogenetic *Rana esculenta* complex, Interactions with pond drying and interspecific competition. *Evolution* **46**, 665-676.
- Sinsch, U. 1992. Zwei Markierungsmethoden zur individuellen Identifikation von amphibien in langfristigen Freilanduntersuchungen, erste Erfahrungen bei Kreuzkröten. *Salamandra* **28**, 116-128.

- Sjögren, P. 1991. Extinction and isolation gradients in metapopulations. The case of the pool frog (*Rana lessonae*). *Biol. J. Linn.Soc.* **42**, 135-147.
- Smuts, B. B. & Smuts, R. W. 1993. Male aggression and sexual coercion of females in nonhuman primates and other mammals, evidence and theoretical implications. *Adv. Study Behav.* **22**, 1-63.
- Tejedo, M. 1992. Effects of body size and timing of reproduction on reproductive success in female natterjack toads *Bufo calamita*. *J. Zool.* **228**, 545-555.
- Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am. Nat.* **122**, 765-788.
- Tunner, H. G. 1974. Die klonale Struktur einer Wasserfroschpopulation. *Z. Zool. Syst. Evolutionsforsch.* **12**, 309-233.
- Tunner, H. G. & Heppich, S. 1981. Premeiotic genome exclusion during oogenesis in the common edible frog, *Rana esculenta*. *Naturwissenschaften* **68**, 207-208.
- Uzzell, T., Günther, R. & Berger, L. 1977. *Rana ridibunda* and *Rana esculenta*, A leaky hybridogenetic system (Amphibia, Salientia). *Proc. Acad. Nat. Sci. Philad.* **128**, 147-171.
- Uzzell, T., Hotz, H. & L. Berger. 1980. Genome exclusion in gametogenesis by an interspecific *Rana* hybrid, evidence from electrophoresis of individual oocytes. *J. Exp. Zool.* **214**, 251-259.
- Vogel, P. 1973. Elektrophoretische Untersuchungen der Serumproteine von Grünfröschen aus dem *Rana esculenta*-Komplex. Unpubl. MSc-Thesis, University of Zürich.
- Wells, K. D. 1977. The social behaviour of anuran amphibians. *Anim. Behav.* **25**, 666-693.

Table 1. Number of experiments (n) in „total“ and broken down into cases where females produced „no clutch“ or spawned „without amplexus“ and „with amplexus“, respectively. The numbers are further classified by mate combinations (female x male): LL = *Rana lessonae*, LR = *R. esculenta*. „eggs“ refers to the mean number of eggs / g body mass laid by females in the respective situation (adjusted least square means from the MANOVA).

Mate	total	no clutch	without	amplexus	with	amplexus
Combination	n	n	n	eggs	n	eggs
female x male						
LL x LL	10	1	4	7.4	5	53.5
LL x LR	10	1	4	6.0	5	31.9
LR x LL	10	6	1	10.5	3	71.6
LR x LR	10	3	2	13.1	5	49.9
sum	40	11	11		18	

Table 2. Summary statistics of an analysis of variance based on first ovipositions (in either session A or B) of 29 females. Egg numbers/g body mass, female condition in spring (prior to spawning) and condition loss due to spawning were related to two female types (LL, LR), three amplexus situations („male type“): no amplexus, amplexed by LR- or amplexed by LL-male as well as to the interaction of female and male type and the size ratio (snout-vent-length) of the (potential) mates. Shown are results from the multivariate model (a) and the univariate analyses (b), with significant *p* values shown on bold.

	a) multivariate			b) univariate						
	<i>df</i>	Wilks' λ		Egg number			Condition in spring		Condition loss	
		<i>F</i>	<i>p</i>	<i>df</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
<i>n</i> = 29										
Female type	3, 20	3.08	0.05	1, 22	4.51	0.04	1.84	0.19	0.87	0.36
Male type	6, 40	8.20	0.00	2, 22	39.45	0.00	0.30	0.75	7.10	0.00
Female x Male	6, 40	1.52	0.20	2, 22	0.70	0.51	2.76	0.09	0.49	0.62
Size ratio	3, 20	2.39	0.10	1, 22	0.57	0.46	1.75	0.20	2.54	0.13

Figure legends

Figure 1. Clutch size (eggs laid/g female body mass) and change in the female body condition due to spawning in relation to (a) two female genotypes and (b) three amplexus situations: females spawning alone, with *R. esculenta* males or with *R. lessonae* males. Change in body condition was calculated as the difference between the condition index in mid-September (8-12 weeks after reproduction) and that immediately before reproduction. Data are given as means \pm s.e.

Figure 2. Relationship between clutch sizes in 1997 and 1998 for females of *R. lessonae* (triangles) and *R. esculenta* (dots). Egg numbers in 1998 are estimates obtained from an ANCOVA which simultaneously controlled for female effects (LL, LR) on clutch size (cf. Table 1). Each data point represents one female that produced a clutch in 1998 while amplexed by a *R. lessonae* male. The regression line is given by the equation $y = -0.397x + 58.1$ ($r_{1,7} = 0.816$, $p = 0.007$).

Figure 3. (a) Testosterone titres (ng/ml plasma) in fall 1997 and (b) number of eggs spawned per gram body weight in 1998 in relation to female condition in fall 1997. In (b) egg numbers are estimates obtained from an ANCOVA which simultaneously controlled for male and female effects on clutch size. The regression line in (a) is given by the equation $y = 19.1x - 16.3$ ($r_{1,16} = 0.658$, $p = 0.003$), the line in (b) by the equation $y = 99.3x - 72.6$ ($r_{1,10} = 0.837$, $p = 0.001$).





