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Asynchronous arrival pattern, operational sex ratio and occurrence of multiple paternities in a territorial breeding anuran, *Rana dalmatina*

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Understanding why females mate multiply is a major issue in evolutionary ecology. We investigated the consequences of an asynchronous arrival pattern on male competition and multiple paternity in the apparently monoandrous agile frog (*Rana dalmatina*). The largest frogs arrived first and both males and females lost weight significantly during the spawning period. Asynchronous arrival at breeding sites resulted in a male-biased operational sex ratio (OSR). The OSR was more strongly male-biased at the beginning and at the end of the breeding period when the number of satellite males increased. All females mated only once, but multiple paternity within clutches occurred at the beginning and the end of the breeding period. The influence of asynchronous arrival and biased sex ratio suggests that reduced variance or bet-hedging promoting female fitness had only a reduced role in the evolution of polyandry, and polyandry is likely to be associated with male benefits. Polyandry in frogs can be explained either by forced mating as a result of sexual conflict or by clutch piracy. By modifying intrasexual competition, asynchronous arrival and changes in OSR may have a decisive influence upon the evolution of mating systems and favour both polyandry and stable coexistence of alternative mating behaviour. © 2005 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2005, **85**, 000–000.

1 ADDITIONAL KEYWORDS: alternative mating – multiple mating – polyandry.

INTRODUCTION

Following the differential allocation hypothesis (Burley, 1988; Andersson, 1994), mating systems basically result from the asymmetry in sexual investment by the two sexes. In most models of sexual selection, the limiting sex (typically females) exhibits the mating preference and the limited sex (typically males) displays the sexual ornaments (Lande, 1980; Kirkpatrick, 1982). An individual member of the limited sex is expected to maximize its fitness by multiplying the number of partners or by attempting to control access to mates. Whereas a male can produce sperm almost without restriction, a female may maximize her reproductive success by mating with only a single carefully selected male. By selecting high-quality males, females can enhance their fitness and, in turn, influence the evolution of male traits (Andersson, 1994). Nevertheless, because males compete for mates, the ability to obtain mates may be affected by the asynchronous arrival of breeders and by the respective proportion of receptive females and mature males at a given time, i.e. the operational sex ratio (OSR; Emlen & Oring, 1977). Thus, males could respond to competition by changing their reproductive patterns into alternative behaviours. Large males gain advantage in maintaining territories whereas small males, i.e. satellites, could only adopt non-territorial behaviours (Taborsky, 1994). For example, Hoglund (1989) showed experimentally that the degree of large male mating benefit increased with a male-biased OSR. Frequency-dependent mating success is one of the main mechanisms maintaining the stable coexistence of breeding behaviours (Maynard Smith, 1982; Gross, 1991). A change in male behaviour may be predicted when competitive interactions increased with male-biased sex ratio because the cost

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*Corresponding author. E-mail: thierry.lode@univ-angers.fr of defending a territory depends on the number of competing males. Furthermore, under 'good-gene' signalling it could be advantageous for a low-quality male to switch to alternative behaviours (Taborsky, 1994).

Divergence in genetic interest between males and females, i.e. the sexual conflict, may result in different reproductive strategies (Rice, 2000). By consenting to multiple mating, females may obtain advantages in the search for good genes or increase genetic diversity, but multiple mating often has a severe cost for females. Factors affecting female mating frequencies vary from energy expenditure required (Thornhill & Alcock, 1983), vulnerability to predation (Arnqvist, 2 1989), transmitted diseases (Hurst *et al*., 1995) and risk of injury by male aggressive behaviour (Stockley, 1998). Although multiple amplexus has been reported in numerous species, multiple paternity and sperm competition have still received little evidence in anurans (but see D'Orgeix & Turner, 1995; Laurila & Seppä, 1998; Roberts *et al*., 1999; Lodé & Lesbarrères, 3 2004) and there are some anuran species for which multiple amplexus has only rarely or never been observed so that they could be regarded as socially monoandrous species. Identifying the causes of polyandry is of major significance to several aspects of evolutionary biology ranging from sexual selection to genetic variability and gametic isolation (Burke, 1989; Zeh & Zeh, 1996; Birkhead, 1998; Jennions & Petrie, 2000; Wilmer *et al*., 2000; Zeh & Zeh, 2000).

In anurans, the pattern of male alternative strategies depends on the duration of the breeding period, and Arak (1983) differentiated a pattern of fighting and searching in explosive breeders with brief breeding congregations from a pattern of calling and satellite in prolonged breeders. Wells (1977) classified the agile frog, *Rana dalmatina*, as an explosive breeder on the basis of breeding period duration. However, Lesbarrères & Lodé (2002) showed that male calling showed a pattern of territoriality leading to opportunities for female mate choice and multiple amplexus was not reported in agile frogs. It could therefore be assumed that the territorial calling by the males results in monoandrous amplexus when the sex ratio is slightly male-biased. Because of the asynchronous arrival of breeders, the OSR may vary between the beginning and the end of the breeding period within a population. The precocious arrival to breeding sites by males is the most frequent sex-biased arrival pattern in vertebrates (Semlitsch *et al*., 1993; Hasselquist, 1998; Morbey, 2000). The timing of arrival can vary both intrasexually, when variation occurs due to individual quality (Møller, 1994; Kokko, 1999), and intersexually (advantage for the precocious or the late sex; Morbey & Ydenberg, 2001). The hypothesis of a side-effect of asynchronous arrival is supported for the blue tit, *Parus caeruleus*, under the term 'musical chair hypothesis': when the bird returns too late, its 'chair' could be already occupied, provoking divorce with its previous partner (Dhondt & Adriaensen, 1994). In frogs, asynchronous arrival mainly results in a biased sex-ratio and it may be expected that such arrival patterns may influence the occurrence of polyandry.

Investigating agile frog mating behaviour, this study aimed at: (1) determining the arrival pattern at the breeding ponds and related changes in the OSR and (2) investigating the occurrence of multiple paternity. We focused on asynchronous arrival and caller and satellite strategies to assess the evolutionary mechanisms influencing the coexistence of different patterns of mating behaviours.

MATERIAL AND METHODS

STUDY SPECIES AND SITES

Rana dalmatina is widely distributed in Europe, but breeding aggregations are often small (Schneider, Sofianidou & Kyriakopoulou-Sklavounou, 1988). During the breeding season from February to March, males call with low intensity in ponds and ditches. Amplectant pairs are always distant from each other. Females lay a single clutch per breeding season, clutches are spatially separated from each other and clutch division has never been reported. Metamorphosis occurs 2 months after hatching, after which the froglets scatter in the terrestrial environment (Geisselmann, Flindt & Hemmer, 1971). The study was conducted in western France, near Angers, during the breeding season 2002 in three breeding ponds numbered P1 to P3 (47°30′N, 0°45′W). All the ponds showed similar climatic conditions and habitat features.

FIELD STUDY: ARRIVAL PATTERNS

Capture design

Asynchronous arrival of breeders and temporal variation of the sex ratio was studied in two ponds (P1 and P2). The ponds were entirely surrounded from 21 January to 21 March. Frog migration occurred between 29 January and 10 March. We used a fence (i.e. green plastic canvas, 1.5 m high, placed at 50 cm from the edge of the pond) associated with pitfall traps (buckets). The fence was buried to a depth of 10 cm and maintained by stakes every 3 m. The pitfall traps, holding 2–3 cm of pond water and covered with a transparent and semi-rigid plastic film with a star-shaped opening at the centre, were buried up to ground level. They were placed every 10 m on each side of the barrier and facing each other (12 pitfall traps at P1, ten at P2). The fence and the pitfall traps were surveyed twice daily (each afternoon and each night), allowing recording of frog movements. The frogs were captured, sexed, measured (snout–vent length to an accuracy of 0.01 cm and body mass to an accuracy of 0.5 g) and marked individually by toe-clipping when they arrived at the breeding pond. The toes were transported to the laboratory and kept at –25 °C until laboratory analyses. The frogs were recaptured when they left the breeding pond.

Survey of studied populations

The total population size (N) corresponds to the total numbers of males (N_m) and females (N_f) marked during the whole breeding season. The number of new clutches per day (N_{cd}) was recorded every afternoon. Every clutch was marked with a natural colouring. Because females did not necessarily mate on their arrival day, we considered that the number of females per day (N_{fd}) should be different from the number of new clutches per day (N_{cd}). However, because each *R. dalmatina* female lays a single clutch per breeding season, the total number of clutches (N_c) should be equal to the total number of females (N_f) if all females mate at the pond. The number of males per day (N_{md}) and the number of calling males per day (N_{cmd}), estimated every night (21.00–04.00 h) by both auditory and visual localization, allowed the estimation of the number of satellite males (N_{sm}).

Two measures of sex ratio and two indexes were calculated:

- the adult sex ratio: $ASR = N_m/N_f$,
- the operational sex ratio: $OSR = N_{md}/N_{fd}$ (Emlen & Oring, 1977).
- the index of competition per day: 0 (0 male calls) $\leq IC = N_{cmd}/N_{md}$ (all males call), and
- the index of body condition for every individual: IBC = [body mass (g)/body size (cm)], which allowed us to estimate, for each sex, the temporal variation of the IBC index. The IBC index was also constructed by regressing log-transformed mass against log-transformed size following the method of Jakob *et al*. (1996).

LABORATORY ANALYSIS: MULTIPLE PATERNITY

Sample collection and standard rearing conditions

Pond P3 was not fenced and served as a control, i.e. to control that the capture design did not affect breeding and multiple mating. Ponds were surveyed every day. We collected a total of 37 clutches, i.e. 12, 12 and 13 at P1, P2 and P3, respectively. In each clutch, we randomly collected roughly 10% (105 eggs ± 5.47, range = 56–224, $N = 37$) of the total egg number, after which the rest of the clutch was released immediately in the pond.

Samples were placed in separate plastic containers (40 × 30 × 30 cm) filled with 7–8 L of pond water. The eggs were reared in the laboratory under natural photoperiod and constant environmental conditions at 17 °C. Container water was changed every 3 days. After reaching developmental stage 25 (Gosner, 1960), the tadpoles were stored at –25 °C until laboratory analysis.

General procedure: protein electrophoresis

Crude proteins used for horizontal starch-gel electrophoresis were extracted from tails of 740 tadpoles originating from the 37 collected clutches (20 offspring per clutch). Samples were homogenized in equal volume of distilled water and centrifuged at 12 000 ***g*** and 4 °C for 5 min. Migration was performed in 11% continuous Tris EDTA borate (pH 8) starch gels at 300 V and 4 °C for 3–5 h. Slices were stained to reveal specific enzymes using standard formulations (Pasteur *et al*., 1987). Six enzyme systems (AAT 2.6.1.1, α-GDH 1.4.1.3, LDH 1.1.1.27, MDH 1.1.1.37, MPI 5.3.1.8, 6-PGDH 1.1.1.44) encoded by eight polymorphic loci (*Aat-1*, *Aat-2*, *α-Gdh*, *Ldh-1*, *Ldh-2*, *Mdh-1*, *Mpi*, *6-Pgdh*) were successfully investigated.

Multiple paternity

Fstat 2.9 software (Goudet, 2002) was used to determine the allelic frequencies of every locus. We designated the most common allele by *100* and rare alleles by a numeral depending on its relative mobility compared with the most common allele. Multiple paternity was estimated using PAPA 1.0 (Duchesne, Godbout & Bernatchez, 2002). The parentage allocation method used in PAPA is based on the likelihood of a parental pair producing multilocus genotypes found in the tested offspring. PAPA embodies a simulator program that may be run prior to the collection of real parental genotypes, which allowed us to model the estimated proportion of parents in each clutch. The offspring genotypes of every clutch were analysed in PAPA each in turn to generate male and female parents using a sexed simulation and a preparental procedure. We set the number of pseudo-collected female parents equal to one because a clutch originates from a single female, and the number of pseudo-collected male parents as equal to one or more.

RESULTS

ASYNCHRONOUS ARRIVAL AND SEX RATIO

Among the 67 frogs captured at P1, 48 were males and 19 were females. In P2, 108 frogs were captured: 68 males and 40 females (Table 1). The total number of clutches (N_c) equalled the total number of females (N_f) and the ASR was male-biased (mean = 2.12 ± 0.41,

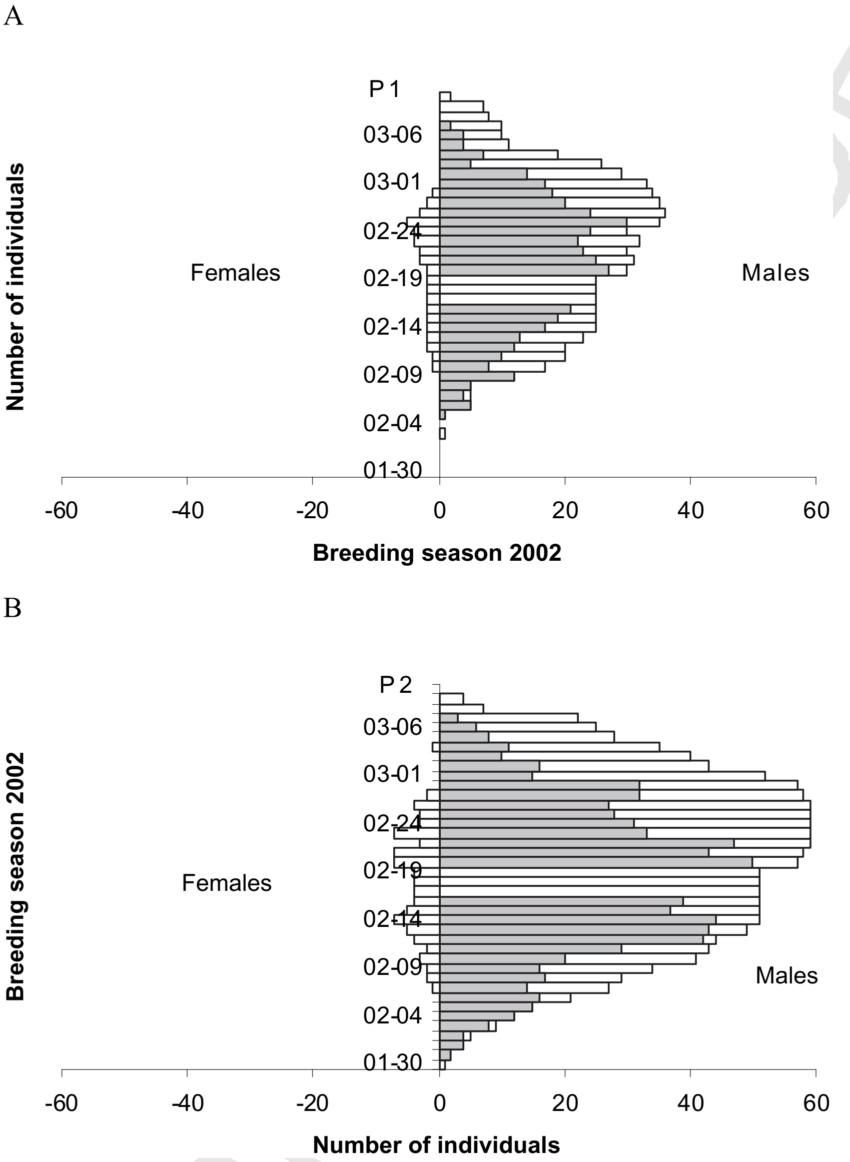


Figure 1. Number of females and males (calling, shaded; non-calling, blank) per day at breeding pond P1 (A) and P2 (B).

Table 1. Total population size (N), total numbers of males (N_m) and females (N_f), total number of clutches (N_c) and adult sex ratio (ASR) at ponds P1 and P2

Pond	N	N_m	N_f	N_c	ASR
P1	67	48	19	19	2.53
P2	108	68	40	40	1.7

$N = 2$; Table 1). Males reached a average size of 4.52 ± 0.05 cm and a weight of 15.2 ± 0.34 g ($N = 116$) whereas in females body size and weight were 5.67 ± 0.07 cm and 31.9 ± 0.9 g ($N = 59$) (mass prior to egg laying), respectively. Sexual dimorphism was 0.79 for size and 0.48 for body mass.

Males and females were together at breeding sites for a mean of 20 ± 1 days (range = 19–21, Fig. 1a, b). Males arrived at the ponds in 2002 from 30 January to 7 March, the largest males arriving first (Fig. 2). Females arrived at breeding sites in 2002 from 7 February to 27 February (except one at P2 that arrived on 4 March), those arriving earliest being larger (see Fig. 2). Both males and females lost weight during the breeding period (P1: Males $t = 14.57$, $P < 0.0001$, d.f. = 47; Females $t = 8.314$, $P < 0.0001$, d.f. = 18. P2: Males $t = 11.613$, $P < 0.0001$, d.f. = 134; Females $t = 9.931$, $P < 0.0001$, d.f. = 39, paired t-test). Thus,

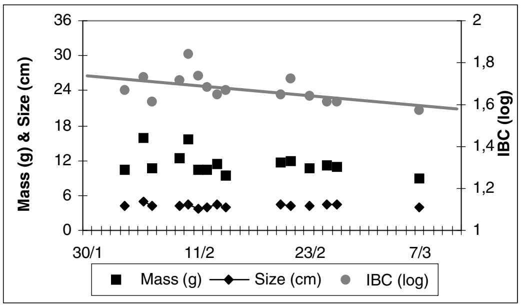
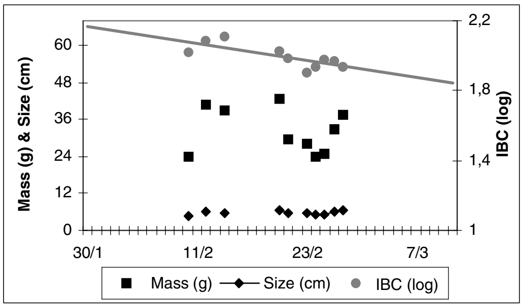
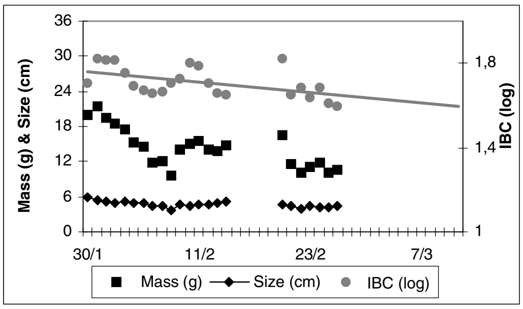
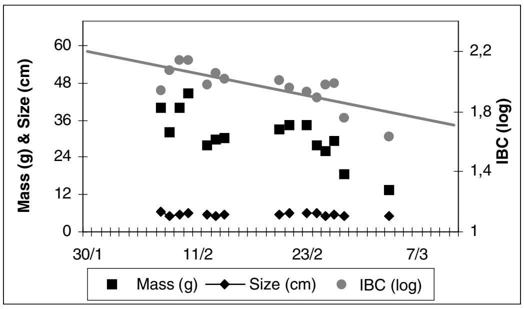
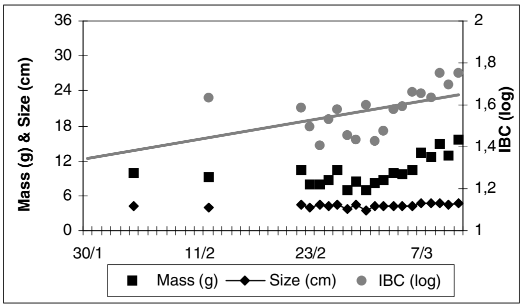
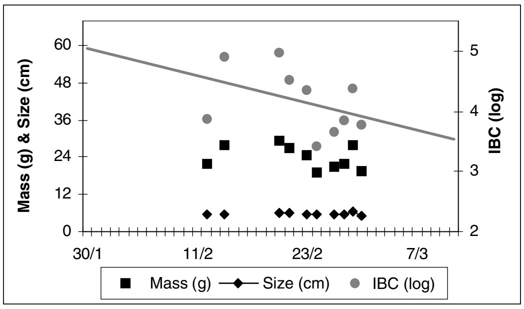
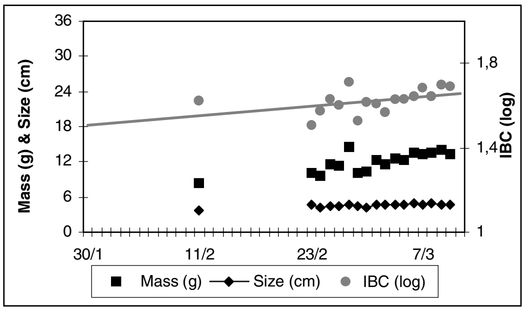
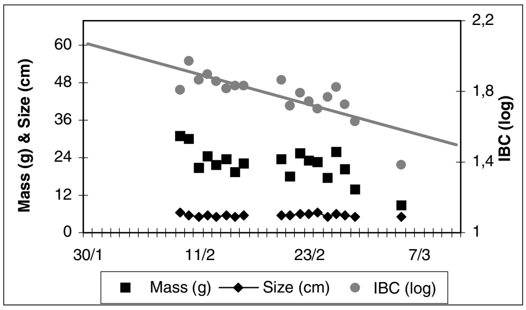
	MALES	FEMALES
ARRIVAL AT P1		
Correlations	Mass $R = 0.143$ *df*=13 $P = 0.617$ Size $R = 0.416$ *df*=13 $P = 0.123$ IBC $R = 0.534$ *df*=13 $P < 0.041$ *Significant*	Mass $R = 0.184$ *df*=8 $P = 0.446$ Size $R = 0.272$ *df*=8 $P = 0.610$ IBC $R = 0.763$ *df*=8 $P < 0.010$ *Significant*
ARRIVAL AT P2		
Correlations	Mass $R = 0.727$ *df*=21 $P < 0.0001$ *Significant* Size $R = 0.674$ *df*=21 $P = 0.0004$ *Significant* IBC $R = 0.523$ *df*=21 $P < 0.0010$ *Significant*	Mass $R = 0.345$ *df*=13 $P = 0.446$ Size $R = 0.740$ *df*=13 $P < 0.0016$ *Significant* IBC $R = 0.752$ *df*=13 $P < 0.0012$ *Significant*
DEPARTU RE FROM P1		
Correlations	Mass $R = 0.495$ *df*=13 $P < 0.027$ *Significant* Size $R = 0.479$ *df*=13 $P < 0.033$ *Significant* IBC $R = 0.485$ *df*=13 $P < 0.029$ *Significant*	Mass $R = 0.184$ *df*=8 $P = 0.446$ Size $R = 0.272$ *df*=8 $P = 0.610$ IBC $R = 0.419$ *df*=8 $P = 0.227$
DEPARTU RE FROM P2		
Correlations	Mass $R = 0.799$ *df*=15 $P < 0.0001$ *Significant* Size $R = 0.790$ *df*=15 $P < 0.0002$ *Significant* IBC $R = 0.435$ *df*=15 $P < 0.038$ *Significant*	Mass $R = 0.670$ *df*=16 $P < 0.0023$ *Significant* Size $R = 0.156$ *df*= 16 $P = 0.537$ IBC $R = 0.790$ *df*=16 $P < 0.0001$ *Significant*

Figure 2. Variations in mass, size and IBC according to date of arrival at breeding ponds and to the date of departure from breeding ponds (IBC calculated from log-transformed data).

males and females, respectively, lost 13.2 and 28.9% of their body mass.

At both ponds, the OSR (P1: range = 7–34, $N = 19$; P2: range = 7.29–35, $N = 22$) was strongly male-biased with two peaks at the beginning (P1: 10–11 February; P2: 7–9 February) and at the end (P1: 27–28 February; P2: 24–27 February) of the breeding periods (Fig. 3a).

The IC index showed similar temporal variations at both ponds (Fig. 3b). Before first female arrival, almost all males called (P1: $IC_{3/9\ Feb} = 0.80 \pm 0.16$, $N = 6$; P2: $IC_{30\ Jan/6\ Feb} = 0.81 \pm 0.12$, $N = 8$). After last female departure and until the last male departure, only one-quarter of males called (P1: $IC_{1/10\ Mar} = 0.25 \pm 0.06$, $N = 10$; P2: $IC_{28\ Feb/9\ Mar} = 0.25 \pm 0.05$, $N = 10$). Based on daily variations, the IC index correlated with the number of females (P1 $r = -0.419$, $N = 32$, d.f. = 30, $P < 0.002$; P2 $r = -0.339$, $N = 36$, d.f. = 34, $P < 0.043$), indicating that the number of calling males increased with the number of females. Unsurprisingly, the IC index was significantly negatively correlated with the variation in OSR (P1 $r = -0.554$, $N = 16$, d.f. = 14 $P < 0.026$; P2 $r = -0.648$, $N = 19$, d.f. = 17, $P < 0.003$). When the OSR was most biased, half of the males called (P1: $IC_{10/11\ Feb} = 0.48 \pm 0.01$, $N = 2$ and $IC_{27/28\ Feb} = 0.55 \pm 0.02$, $N = 2$; P2: $IC_{7/11\ Feb} = 0.55 \pm 0.04$, $N = 5$ and $IC_{22/27\ Feb} = 0.56 \pm 0.05$, $N = 6$). When the OSR was less biased, three-quarters of the males called (P1: $IC_{12/26\ Feb} = 0.74 \pm 0.03$, $N = 12$; P2: $IC_{12/21\ Feb} = 0.83 \pm 0.03$, $N = 7$).

MULTIPLE PATERNITY

The eight polymorphic loci revealed at least three alleles in clutches ($N = 37$) from the three ponds P1, P2 and P3. In the three studied ponds, 7/37 (18.9%) clutches were fertilized by at least two males (Table 2). In these clutches, 25.3% of eggs in a clutch were fertilized by one or several other males than that fertilizing most of the clutch. At P1, P2 and P3, multiple sired clutches were laid at the beginning (one, two and two, respectively) and at the end (one, one and one, respectively) of the breeding periods (Table 2). When testing for a temporal pattern by dividing the clutches into early/late season and middle season, the number of polyandrous clutches was significantly higher during the early/late than during the middle season (Table 3, Fisher's test $P < 0.008$, odds ratio 0.045). Multiple paternity mainly occurred at the

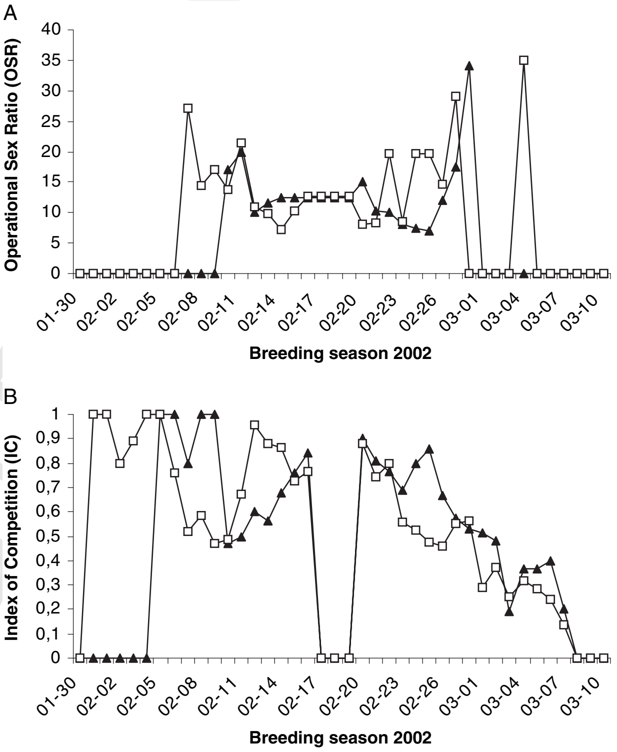


Figure 3. (A) Temporal variation of the operational sex ratio (OSR) and (B) variation of the index of competition (IC) during the breeding season 2002 at breeding pond P1 (black triangles) and P2 (open squares).

Table 2. Numbers of collected clutches, of multiple paternal clutches (%), of eggs in a clutch fertilized by one or several other males (%) and laying date of multiple paternal clutches at P1, P2 and P3 during the breeding season 2002

	Collected clutches			
Pond	No.	Multiple paternal clutches (%)	Percentage of eggs in a clutch fertilized by other male(s)	Laying date of multiple paternal clutches
P1	12	16.7	15.0 ± 0.00, $N = 2$	11 and 27 Feb.
P2	12	16.7	32.5 ± 2.50, $N = 2$	8 and 27 Feb.
P3	13	23.1	28.3 ± 1.67, $N = 3$	8, 10 and 25 Feb.
Mean (%)	–	18.9	25.3 ± 2.97, $N = 7$	–

beginning and at the end of call activity, corresponding to the two OSR peaks (Fig. 4). During the peaks of OSR in comparison with the rest of the breeding periods, the percentage of calling males decreased on average by 23.5% (Table 3).

DISCUSSION

Mate choice or intrasexual competition cannot alone explain polyandrous mating when females receive no obvious direct advantage (Fedorka & Mousseau, 2002). Conflicts of interest between the sexes in which females avoid associated mating costs and males increased their control over females have been 4 recently identified as a force driving sexual evolution 5 (Rice, 1996; Partridge & Hurst, 1998; Gavrilets, Arnqvist & Friberg, 2001; Stutt & Siva-Jothy, 2001; Arnqvist & Rowe, 2002). Asynchronous arrivals and changes in sex ratio within populations resulted in the exhibition of alternative behaviours, emphasizing the importance of considering sexual selection in a life-history perspective. It is especially remarkable that such alternative behaviours are time-dependent and favour the emergence of multiple paternities.

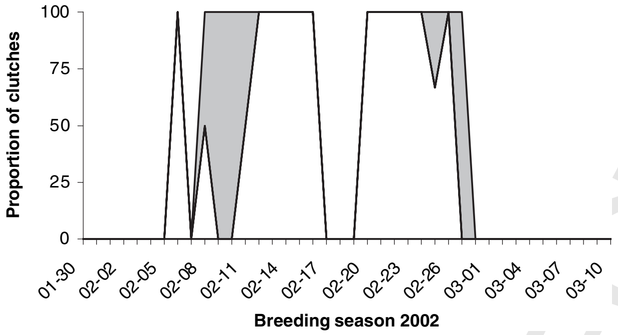


Figure 4. Percentage of single sired (blank) and multiple sired (shaded) clutches collected per day (data polled for the three ponds) during the breeding season 2002.

Seasonal variations in mating strategies are predicted because asynchronous arrival resulted in a male-biased OSR and increased competitive interactions at the beginning and at the end of the breeding season. The breeding period of *R. dalmatina* lasted on average 20 days. Based on this brief breeding period, Wells (1977) classified the agile frog as an explosive breeder. Nevertheless, male and female arrivals at breeding sites extended all along the breeding period with males arriving before females, and this asynchronous pattern is characteristic for prolonged breeders (Arak, 1983). Regardless, the timing of arrival was correlated with body size, the largest individuals arriving first. Such a pattern of arrival may be explained by the physical form of individuals, the largest being the earliest (i.e. constraint hypothesis; Peters, 1983) and the most able to resist the hostile environmental conditions that prevail at the beginning of the breeding season (i.e. susceptibility hypothesis; Morbey & Ydenberg, 2001). But these hypotheses are not supported from an intrasexual point of view. The mate opportunity hypothesis argues that males increase their mate opportunities via precocious arrival (Semlitsch *et al.*, 1993; Morbey, 2000). Both males and females showed a considerable decrease in body mass, emphasizing the importance of breeding investment. Most males called as soon as they arrived at the breeding sites. So when females arrived, they can select the males on the basis of call advertisements. In *R. dalmatina*, female reproductive strategy implies a precise mate choice, which can lead to the

Table 3. Number of polyandrous clutches vs. monoandrous clutches according to early/late breeding and middle season and differences between the mean values of the index of competition (IC) during the two peaks of OSR and during the rest of the breeding periods at P1 and P2

Breeding season	Early or late	Middle
Monoandrous clutches	12	18
Polyandrous clutches	7	0

	Mean IC index		
Pond	during the two peaks of OSR	during the rest of the breeding periods	Difference
P1	0.51 ± 0.02, $N = 4$	0.74 ± 0.03, $N = 12$	–0.23
P2	0.55 ± 0.03, $N = 11$	0.83 ± 0.03, $N = 7$	–0.27
Mean	0.54 ± 0.02, $N = 15$	0.78 ± 0.02, $N = 19$	–0.23

selection of a single mating partner. Moreover, this selection by females is reinforced by the exclusive territorial behaviour of calling males resulting in spacing of males. Thus, this reproductive strategy should mainly result in a monoandry and choosy females could obtain indirect benefit through a Fisherian or good gene process (Kirkpatrick, 1996). However, variations in the OSR may limit the ability of females to exert their selective mate choice.

Surprisingly, the precise female mate choice and the male territorial strategy did not prevent multiple paternity emergences. The proportion of clutches that present a multiple paternity is lower than the proportion of clutches that present a single paternity, which emphasizes the monoandrous character of *R. dalmatina*. Multiple paternity significantly occurred only at the beginning and at the end of the breeding period. With amplexus promiscuities, multiple paternity may be explained by simple sperm diffusion but, in *R. dalmatina*, the assumption of sperm diffusion may be rejected because 25.3% of eggs in a clutch with multiple paternity resulted from fertilization by another male. Furthermore, as the number of reproductive females per day is low, synchronization and the proximity of amplexus are quite unlikely. A second assumption to explain multiple paternities is synchronous multiple amplexus. In the Australian frog *Crinia georgiana*, multiple paternity results from a synchronous polyandry implying multiple amplexus (Roberts *et al*., 1999). Such polyandry is associated with a strong sexual dimorphism and a non-territorial breeding system. By contrast, *R. dalmatina* exhibit a weak sexual size dimorphism and defend widely spaced breeding territories. Another explanation could be found in the existence of parasitic fertilization by individual males such as in the common frog *Rana* 6 *temporaria* (Jennions & Passemore, 1993; Laurila & Seppä, 1998; Vieites *et al*., 2004).

Regardless, polyandry was more likely when partners arrived asynchronously on the breeding sites, entailing a biased sex-ratio and thus supporting the asynchronous arrival hypothesis as a cause of multiple mating. Male-biased OSR increased with arrival asynchrony, and multiple paternities may occur because potential partners are scarcer at the beginning and at the end of the breeding period. The close association between multiple paternity, a decrease in IC index and a higher male-biased OSR suggest that females could not display their direct choice. Although some studies provide evidence that females mate with multiple males in the search for good genes, the influence of breeding timing and sex ratio have been rarely evoked. Hoglund (1989) has shown that OSR modification, i.e. a stronger bias toward males, favoured the largest males. In males, the decrease in IC index is related to the change to a non-calling behaviour, i.e. satellite behaviour. Such switching may be explained by the increase of male–male competition, resulting in territory loss for the less competitive males (Lucas, Howard & Palmer, 1996). Satellite behaviour is initially an opportunistic behaviour to access females and could result in clutch piracy (Jennions & Passemore, 1993; Vieites *et al*., 7 2004). If male fitness chiefly depends on competition for mates rather than on female mate choice, benefits could be accrued by adopting satellite strategies, although such behaviours may result in multiple paternity.

Nevertheless, female preference may alter male advantage with a more balanced sex ratio leading to monoandry. Of considerable interest with regard to multiple paternity is the introduction of genetic diversity in the population. However, such a benefit is not always obvious. For example, polyandry can lead to reduced fertilization success (Byrne & Roberts, 2000) and Fedorka & Mousseau (2002) reported that polyandrous females suffered an approximate 29% decrease in longevity although they have highest fitness (good genes and increased genetic diversity were the two main hypotheses favouring multiple paternities and both hypotheses assumed the females' ability to discriminate among males. Polyandrous females may include a mechanism of bet-hedging by incorporating several genotypes into a single clutch but such bet-hedging remains improbable (Yasui, 1998). The influence of asynchronous arrival suggests, nevertheless, that bet-hedging promoting female fitness played only a reduced role in the evolution of polyandry in anuran while the major cause of polyandry is likely to be associated with male gains linked to increasing competitive interactions. Sexual conflict could be intensified by a male-biased sex ratio so that multiple paternities in *R. dalmatina* may be thought of as a result of either forced mating or clutch piracy.

Multiple paternity may be more frequent in anurans than otherwise expected. The role of females in the fitness of alternative behaviour needs to be highlighted because they may have a decisive role in the apparent monoandrous system in *R. dalmatina*. Asynchronous arrival may have a supplementary effect on the evolutionary dynamics driven by sexual conflict by increasing the occurrence of alternative behaviours.

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