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Mating and sexual selection in the threatened crayfish, *Austropotamobius italicus*

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Abstract
Mating by the threatened crayfish *Austropotamobius italicus* was studied in the laboratory during October-November 2002. For two hours we observed the interactions occurring in 17 groups composed of three differently-sized adult males and one receptive female and we checked, in the subsequent three days, for spermatophore deposition by males and for spawning by females. Results showed: (1) the "reluctant" and "choosy" behaviour of the females, (2) the large-male size advantage, and (3) the occurrence of a form of "sperm competition". In our study, we did not find an ultimate explanation for female preference of large males. Indeed, male size is a good predictor of the outcome of intrasexual competition for the access of mates. If males have genes that enable them to attain the larger size, females mating with big males might produce big sons and therefore gain better reproductive success through their sons' progeny.

KEY WORDS: sexual selection / mate choice / *Austropotamobius italicus* / "sperm competition" / large-male size advantage

Comportamento riproduttivo e selezione sessuale nel gambero minacciato *Austropotamobius italicus*
Alcuni aspetti del comportamento riproduttivo del gambero di fiume minacciato *Austropotamobius italicus* sono stati analizzati in laboratorio nel periodo compreso tra ottobre e novembre 2002. Sono state osservate per due ore le interazioni di 17 gruppi composti ciascuno da tre maschi adulti, di differenti dimensioni, e una femmina ricettiva. Nei tre giorni successivi sono stati eseguiti controlli per verificare l'eventuale presenza di uova nelle femmine e/o la deposizione di spermatofore da parte dei maschi. Dall'analisi dei risultati è emerso che: 1) le femmine sono generalmente "riluttanti" ed "esigenti", 2) i maschi di maggiori dimensioni hanno più successo nella competizione intrasessuale e 3) anche nei gamberi è presente una forma di competizione spermatica. Non è stata trovata una spiegazione definitiva della preferenza della femmina per i partner "grandi". Le dimensioni del maschio sono comunque un buon indice della riuscita nella competizione per l'accesso alla femmina. Se i maschi hanno geni che permettono loro di ottenere una taglia maggiore, le femmine che si accoppiano con questi potranno generare individui di grandi dimensioni e ottenere un miglior successo riproduttivo attraverso la loro progenie.

PAROLE CHIAVE: selezione sessuale / scelta del partner / *Austropotamobius italicus* / "competizione spermatica" / vantaggio del maschio di maggiori dimensioni

INTRODUCTION

DARWIN (1871) was the first author to differentiate between two processes, the first favouring 'the power to charm the females' ("intersexual" or "epigamic" selection; HUXLEY, 1938a,b), and the second favouring 'the power to conquer other males in battle' ("intrasexual" selection; HUXLEY, 1938a). Subsequent studies showed that intersexual and intrasexual selections often act simultaneously to mould the mating behaviour of a species. However, except for a few examples (e.g. CHRISTY, 1982; FUKUI, 1995; VILLANELLI & GHERARDI, 1998), this issue has been neglected in the crustacean decapod literature.

The white-clawed crayfish, *Austropotamobius italicus*, is endemic to Italy (FRATINI *et al*., 2004). As part of the *A. pallipes* complex, this species is under protection by the European Council Directive 92/43/ECC. However, the introduction of highly competitive non-indigenous species and other factors such as pollution, diseases, over-fishing, river engineering, floods, and drought are leading to the narrowing of its

pattern of distribution.

Mating in this species occurs between late October and the first half of November. During this restricted period, the behaviour of this species changes; locomotion activity and dispersal increase (GHERARDI *et al*., 1998), possibly augmenting the probability of a mate encounter, and males appear more aggressive, competing with other males for the access of a female. Copulation is soon followed by spawning in autumn and eggs are carried through the winter and the subsequent spring. Hatching occurs in April and May, but the young still live attached to the female pleopods throughout two developmental stages, some 25 days after hatching (HOLDICH, 1992; GHERARDI *et al*., 1997).

Following the rationale that informations from behavioural studies help define management protocols and recovery plans for endangered species, our objectives here were (1) to explore the role exerted by *A. italicus* mate choice and male-male competition in inducing mating and (2) to investigate sperm competition and male control over multiple matings.

To these ends, we simulated in the laboratory mating interactions as observed in nature, where aggregations composed of two-four males were seen combating around a female (F. Gherardi, pers. observ.). Premises of this study were the results of a preliminary choice experiment in which a sex was free to choose between tethered individuals of the other sex with opposing phenotypic traits (VILLANELLI & GHERARDI, 1998). Females were found to select larger mates with intact chelipeds, while males mated indiscriminately without regard to the body size of the offered female.

MATERIAL AND METHODS

Animal collection and maintenance

Adult *A. italicus* were hand-collected in September 2002 from Torrente Resco in Pian di Scò (Arezzo). Crayfish were kept isolated under a natural light-dark cycle in plastic aquaria (bottom area: 30 x 16 cm) containing 3 litres of aerated water and a 10-cm long piece of an opaque PVC tube (diameter: 5 cm) as a refuge. They were fed twice a week on live earthworms. Individuals were measured using a vernier caliper for their cephalothorax length (CL). From their size, males were arbitrarily subdivided into three categories, small (S, CL < 29 mm), medium (M, CL 29-36 mm), and large (L, CL > 36 mm).

Experimental protocol

Before our observations commenced, we formed 17 groups composed of one receptive female (mean CL: 32 mm) and three differently-sized, receptive males (one S, one M, and L male), all with intact chelae. Females were defined receptive if they showed a spermatophoric plate on the seventh and eighth thoracic segments of sternites (F. Gherardi, unpubl. observ.), while male receptivity was assessed from their behaviour towards a receptive female. Every group was placed in a 40-cm diameter aquarium and was observed for two hours. We tape-recorded interactions occurring in every group and then analysed from these records:

1. Number of interactions. Within the female-male interactions, we distinguished "Mating Attempts" and "Successful Matings" (i.e. mating attempts ending with spermatophore deposition). Male-male interactions exclusively consisted of "Fights".
2. Sex of both the approaching and the retreating individual and, if male, its size category.
3. Duration of every interaction.

Crayfish were kept in the experimental aquarium for three subsequent days and examined three times per day for spermatophore deposition by males and for spawning by females.

Statistical analysis

Since the assumptions of normality of data and homogeneity of variance were not always met and some data were measured on an ordinal scale, we used nonparametrical statistical techniques (SIEGEL and CASTELLAN, 1988). Figures give median values and interquartile ranges (first-third quartiles). The level of significance under which the null hypothesis was rejected is $\alpha = 0.05$.

RESULTS

Female-male interactions

Females initiated interactions more often than the males (Wilcoxon signed ranks test: z=2.82, df=1, P<0.01) (Fig. 1). The number of both female and male approaches significantly increased with the male size

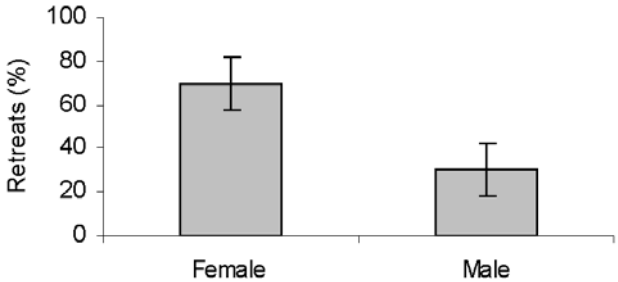

Fig. 1. Comparisons between sexes in the frequencies of approaches to the other sex (medians and interquartile ranges). Females: N= 17; males: N= 51.

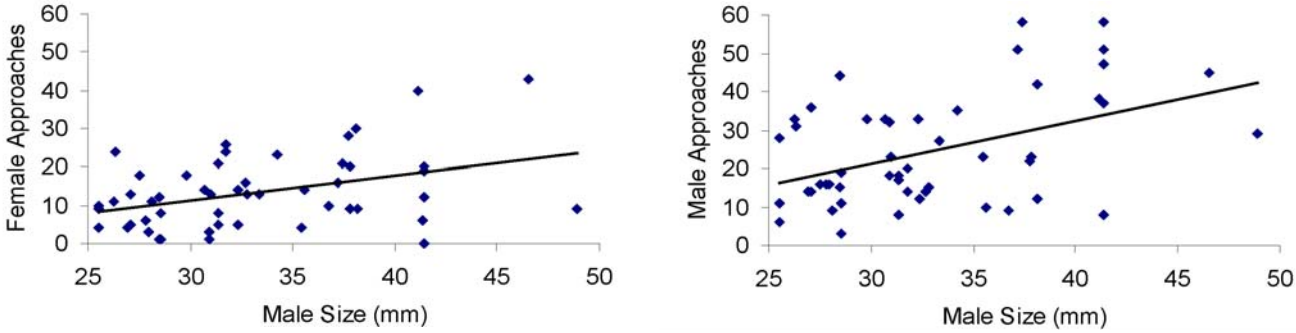

Fig. 2. The size of males plotted against (A) the number of female approaches to them and (B) the number of male approaches (B) to a female. N = 51

(Spearman rank-order correlation coefficient: r_s=0.416, df=49, P<0.01 and r_s=0.36, df=49, P<0.01) (Fig. 2).

Male-male interactions

Large males were the initiators of male-male interactions more often than the other size categories (Kruskal-Wallis: H=27.15, df=2, P<0.001) (Fig. 3). M-L pairs were engaged in more numerous fights (H=35.50, df=2, P<0.001) (Fig. 4) and combated on average longer (H=6.20, df=2, P<0.05) than S-M and S-L pairs. Most often, fights ended with the retreat of the smaller individual (G-test: G=97.95, df=2, P<0.001).

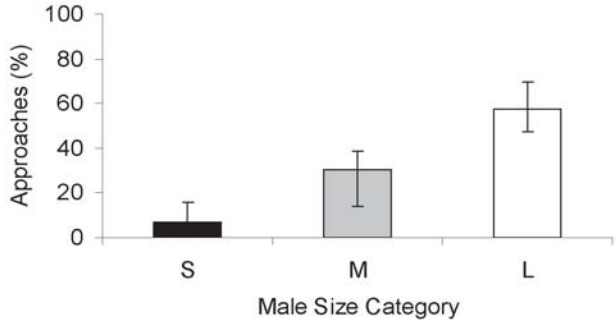

Fig. 3. Comparison among male size categories for the frequencies of their approaches in male-male interactions (medians and interquartile ranges). N= 51.

Mating attempts and successful mating

L males were engaged in more numerous and on average longer mating attempts than the other size categories (Friedman two-way analysis of variance by ranks: Fr=13.34, df=2, P<0.05 and Fr=6, df=2, P<0.05) (Fig. 5) and arrived more often to spermatophore deposition (G=6.094, df=2, P<0.05). However, successful matings were relatively rare. They occurred only in 16 instances out of 224 mating attempts (7.14 %), this low number being possibly the effect of the refusal by the female. In fact, mating attempts were often interrupted by the female retreats (z=-3.20, df=1, P<0.01) (Fig. 6). Three females were subjected to the deposition of more than one spermatophore by the

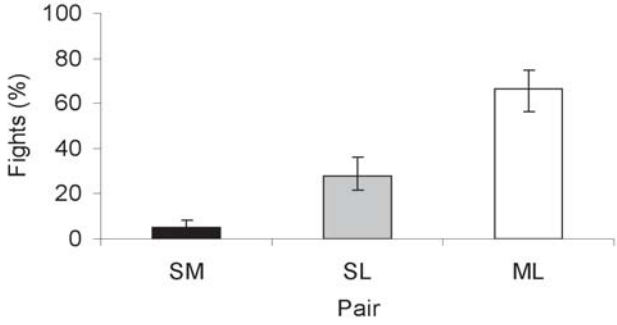

Fig. 4. Comparisons among pairs composed of differently-sized males for the frequency of fights (medians and interquartile ranges). SM= small-medium pair, SG= small-large pair and MG= medium-large pair. N= 51.

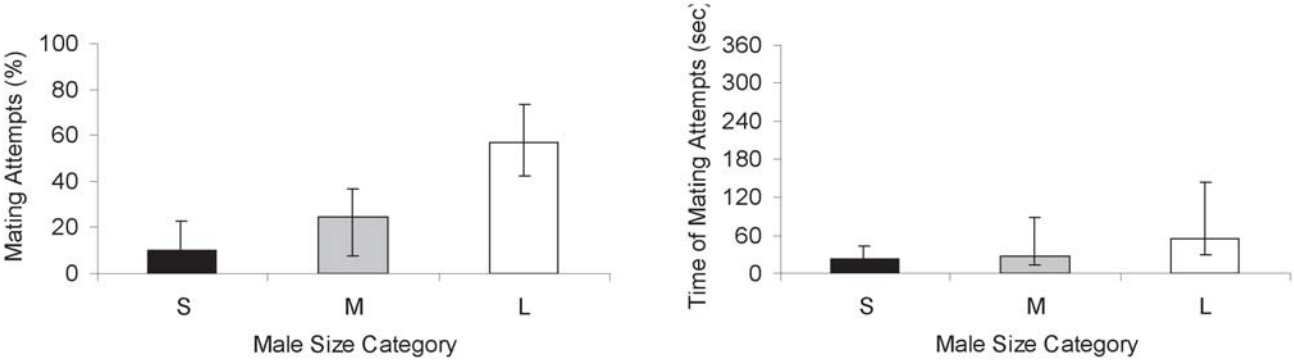

Fig. 5. Comparison among male size categories for the frequency (A) and mean duration (B) of mating attempts (medians and interquartile ranges). N= 51.

same or by a different male. Before attaching the new spermatophore, the male always removed the old one by feeding on it.

DISCUSSION

Our study clearly indicates that *A. italicus* females are both "reluctant" and "choosy" in their mating behaviour. On the one hand, they often interrupted mating attempts and also showed a form of "passive" resistance to the males by keeping their abdomen curled and therefore by impeding spermatophore deposition by males (BERRILL & ARSENAULT, 1984). On the other, they seemed to prefer males of bigger size. In fact, we found that females most often approached large, rather than medium or small, males. A similar female preference for large males was observed in other crustacean decapods studied so far (e.g. crabs, GREENSPAN, 1980; shrimp, RAHMAN *et al.*, 2002; crayfish, VILLANELLI & GHERARDI, 1998; FURRER, 2004). Reluctance and choosiness of *A. italicus* females can be justified by their large investment into offspring (see, also, *A. pallipes*, WOODLOCK & REYNOLDS, 1988 and the grapsid crab *Gaetice depressus*, FUKUI, 1995).

On the contrary, males seem to be undiscriminating in their choice of mates, probably because they have a little chance of encountering receptive females. In fact, (1) mating in *A. italicus* takes place over a short period of time, (2) females are a rare resource in space and time (BREWIS & BOWLER, 1985), and (3) the operational sex ratio (i.e. the ratio of one sex to the other among individuals ready to mate, ANDERSSON, 1994) becomes heavily skewed towards males as the mating season progresses (SNEDDEN, 1990).

The effects of mate choice by females on *A. italicus* mating are difficult to isolate from the direct influence of intrasexual competition. As expected in a species like *A. italicus* where receptive females are limited in space and time, male-male competition is particularly intense and is dominated by large males. Male-male combats may passively lead females to mate with a successfully competing male, which is usually the bigger individual in arthropods (BERRILL & ARSENAULT, 1984; WOODLOCK & REYNOLDS, 1988; GHERARDI, 2001). We found that large males were engaged in more numerous and longer mating attempts than the smaller size categories and more likely arrived at depositing spermatophores than the other males, notwithstanding that this event was relatively rare in the laboratory and in the taxon of study (see also FURRER, 2004).

In *A. italicus*, where any form of mate guarding is absent and male-male conflict for access to females is combined with external fertilisation, there is a particularly good opportunity for sperm competition (i.e. "the competition between ejaculates of two or more males for the fertilisation of the eggs of a single female"; PARKER, 1970). Indeed, the occurrence of sperm competition leading to the last-male advantage is confirmed by this and previous studies in *A. italicus* (VILLANELLI & GHERARDI, 1998) and other Astacidae (*Astacus astacus* and *A. leptodactylus*; FURRER, 2004). Males feed on the previously deposited spermatophore before copulating, being however unable to recognise their own spermatophores. This apparently primitive form of sperm competition was previously found in pseudoscorpions (WEYGOLDT, 1969), millipedes, and collembolans (SCHALLER, 1971), in which, however, spermathophores are deposited on the substratum and not attached onto the female abdomen.

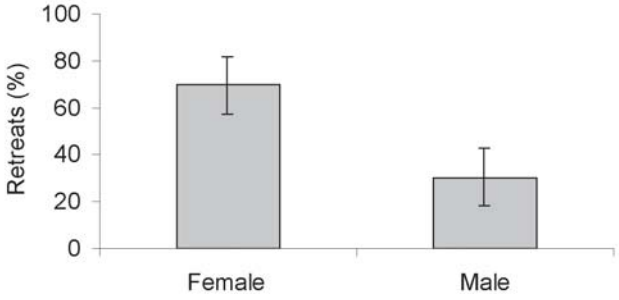

Fig. 6. Comparisons between sexes in the frequencies of their retreats from the other sex (medians and interquartile ranges). Females: N = 17, males: N = 51.

CONCLUSIONS

Mating in *A. italicus* is best explained by the large-male size advantage resulting from an active mate choice of females for large partners. The considerable investment of energy in offspring justifies female choosiness, whereas the restricted mating period and the low number of receptive females make, first, males relatively undiscriminating in their mate choice and, second, intrasexual competition over females (also in the form of sperm competition) particularly intense. In our study, we did not find an ultimate explanation for female preference of large males. Indeed, male size is a good predictor of the outcome of intrasexual competition for the access of mates. If males have genes that enable them to attain the larger size, females mating with big males might produce big sons and therefore gain better reproductive success through their sons' progeny.

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