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Mutual mate choice in crayfish: large body size is selected by both sexes, virginity by males only

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Keywords

mate choice; body size; mating status; crayfish; *Procambarus clarkii*.

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Abstract

Mate choice is often assumed to be a prerogative of females because of their putatively larger reproductive investment than males. However, recent evidence suggests that spermatogenesis is far from being limitless and that males show a high selectivity towards their mates, thus maximizing their reproductive success. We investigated mutual mate choice in the crayfish *Procambarus clarkii* through two experiments. The first experiment explored the effects of body size, chelar size and chelar symmetry and social status of a potential partner. In the second experiment, we asked whether this species can discriminate between partners of the same body size but with different mating status. We used a binary choice test paradigm, in which two 'targets' with opposing characteristics were simultaneously presented to a test animal, the 'chooser'. The results showed that *P. clarkii* males are more selective than expected. Similar to the other sex, they were significantly attracted by targets with large body sizes, but not by individuals with larger and symmetric chelae or with a dominant status. An inter-sexual difference was found in the second experiment, in which only males seemed to select virgin potential mates. The several adaptive explanations for these preferences, still under debate, are finally discussed.

Introduction

Overwhelming evidence has been accumulated showing that animals select mates that allow them to maximize their reproductive success (Ridley, 1983; Andersson, 1994). Females, who invest in few costly gametes, are highly choosy, whereas males, who produce large numbers of cheap sperm, are usually limited in their reproductive output only by the frequency of mating (Trivers, 1972). Because of this inter-sexual difference in investment, selection gives rise to 'reluctant' females, on the one hand, and 'ardent' males with exaggerated morphological and/or behavioural traits on the other (Krebs & Davies, 1981). As a consequence, female mate choice and inter-male competition act as driving forces behind sexual dimorphism (Andersson, 1994).

These traditional ideas have been abundantly discussed in recent years. The direction of sexual selection depends on several factors, including the operational sex ratio, variation in mate quality and sex-specific parental investment (Kokko & Monaghan, 2001; Kokko & Johnstone, 2002). Sperm production often requires several days and males dispose of a restricted number of gametes at each mating attempt (Adiyodi, 1985). As a result, sperm availability may be limited and males are also expected to choose their mates (e.g. Dewsbury, 1982).

With a few exceptions (e.g. Ridley, 1983; Karnofsky & Price, 1989; Johnstone, Reynolds & Deutsch, 1996; Johnstone, 1997; Fawcett & Johnstone, 2003), the literature

abounds with studies investigating the characteristics selected by females during mate choice. In arthropods, the usual female preference for large mates has been explained by the increase with body dimension of male dominance (Bovbjerg, 1953) and spermatophore sizes (e.g. Gwynne, 1981). Large body size (BS), in turn, was found to be related to large chelae in most crustacean decapods (Gherardi, Barbaresi & Raddi, 1999a). At least in males, chelae are the weapons used during fights (O'Neill & Cobb, 1979) and large chelae are prerequisites for winning encounters (Rutherford, Dunham & Allison, 1995). They allow the bearer to acquire vital resources, such as sexual partners and shelters (Stein, 1976). As suggested, chelar asymmetry might reduce fighting ability, lower social status (O'Neill & Cobb, 1979) and possibly affect male ability to seize and turn the female over before copulation (Stein, 1976).

In arthropods with multiple matings, males may differ in their fertilization ability, owing to repeated copulations, and females risk mating with sperm-depleted males (see references in Wedell, Gage & Parker, 2002). Sperm competition is likely to occur in several species of insects, spiders and some crustaceans due to the female ability to store sperm and to mate repeatedly, so that males are also expected to be careful in choosing mates and in allocating their sperm (Wedell *et al.*, 2002). As a consequence, both sexes of some arthropods were found to assess the mating status (MS) of a potential partner, thereby reducing the risk of wasting time and energy (Sato *et al.*, 2005; Sato & Goshima, 2007).

In the present study, we tested hypotheses about mutual mate choice in arthropods, using the red swamp crayfish, *Procambarus clarkii*, as a model organism. We conducted two experiments, in which the effects of BS, chelar size/symmetry, social status (in Experiment 1) and MS (in Experiment 2) were investigated in both sexes. We followed a binary choice test paradigm, in which pairs of live crayfish, the ‘targets’, with opposing characteristics, were simultaneously offered to an experimental individual, the ‘chooser’. On the basis of previous studies in other crayfish species (Stein, 1976; Berrill & Arsenault, 1984; Snedden, 1990; Villanelli & Gherardi, 1998; Galeotti *et al.*, 2006; Gherardi *et al.*, 2006), we hypothesized that *P. clarkii* females would select dominant males, according to the perceived hierarchical status and/or to their large body and chelar size, whereas males would be attracted only by potentially more fecund females, i.e. females with a large BS. Because *P. clarkii* mates several times in the same reproductive season and females store sperm in the *annulus ventralis* before extruding their eggs, we assumed that sperm competition in this species is strong and we expected males to recognize the MS of the partner and to behave accordingly.

Methods

The study species

Procambarus clarkii, native to north-eastern Mexico and south-central USA, has been introduced since the 1960s into all continents, except Australia and Antarctica (Gherardi & Holdich, 1999). Notwithstanding the abundant literature focused on its invasive potential (e.g. Gherardi, 2006), little is known about its breeding system. It is an *r*-selected species with a lifespan ranging between 12 and 18 months under laboratory conditions (Huner, 2002) and, at least in southern Europe, reproduces from June to October (Gherardi *et al.*, 1999b; Scalici & Gherardi, 2007), when the inhabited water bodies are subject to high temperatures and persistent desiccation (Aquiloni, Ilhéu & Gherardi, 2005).

This species shows a promiscuous breeding system, with both sexes copulating repeatedly. Fertilization is external, but in *P. clarkii* and in the other cambarids the spermatozoa are deposited into a spermatheca, the so-called ‘*annulus ventralis*’, at the posterior end of the female seventh thoracic sternite (Adiyodi, 1985). When the mature eggs are released through the gonopores, the spermatozoa are made free by spermatozoa dissolution; the eggs are fertilized and attached to the pleopods for brooding (Vogt, 2002).

Collection of animals and maintenance

In April 2005 and 2006, before the reproductive period, a total of 520 crayfish of both sexes were collected with baited traps from the Massaciuccoli Lake (Tuscany, Italy; water temperature: 14 °C). Once in the laboratory, they were kept, sexes apart, in plastic tanks (80 × 60 × 60 cm) containing

clay pots as refuges at a density of 15 m⁻². They were maintained for about 6 weeks in still water, in a natural light:dark cycle at room temperature (20 °C) and fed *ad libitum* with live *Calliphora* sp. larvae. Water was changed weekly.

We measured the length of the cephalothorax (from the tip of the rostrum to the posterior edge of the carapace) and of the chelae (from the tip of the dactyl to the back of the propodus) using vernier calipers (to the nearest 0.1 mm), and marked each crayfish individually on the cephalothorax using a waterproof paint. Only hard-shelled crayfish with all appendages intact were used. At the end of the study, crayfish were used in other laboratory experiments.

Experimental design and apparatus

We used a binary choice test paradigm, in which for 20 min a test crayfish, either a male or a female, the ‘chooser’, was offered a pair of potential mates, the ‘targets’. The pairs of targets were live receptive partners that differed in only one characteristic, the others being equal. The experimental apparatus (modified from Sato & Goshima, 2007; Fig. 1) consisted of an elliptical plastic aquarium (65 × 40 cm, water level: 10 cm). Two circles (diameter: 20 cm), 20 cm apart, were drawn on its bottom. The release point of the chooser was the centre of the ellipse, equidistant from the centre of the two circles. Twenty minutes before the trials started, each of the two targets was inserted into a 22 cm-high piece of a transparent plastic container (10 × 4 cm), finely drilled (3 mm holes, 4 cm⁻²), placed on the centre of each circle, hereafter called ‘target’s area’. The used containers precluded any physical contact between the targets and the chooser, but permitted the free circulation of the ‘odours’ possibly emitted by the experimental individuals. Each chooser was acclimatized to the experimental condition by keeping it for 5 min on the release point inside a cylindrical wire netting; the experiment started at the lift of the wire

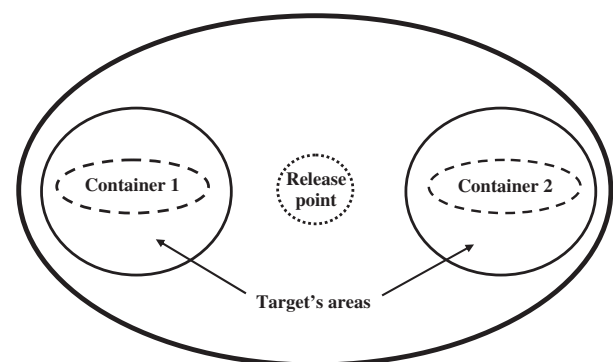


Figure 1 Experimental apparatus, consisting of an elliptical aquarium (65 × 40 cm; water level: 10 cm). Two circular areas (diameter: 20 cm) – the target’s areas – were drawn on the bottom on opposite sides, 20 cm from the release point. The targets were inserted into two transparent plastic containers (1 and 2) finely drilled (10 × 4 cm, height: 22 cm), placed in the centre of each target’s area.

netting. Between trials, the experimental apparatus was thoroughly washed with clean tap water.

Criteria for selecting choosers and targets and their subsequent isolation

We used only sexually responsive crayfish, that is individuals that, once paired, attempted to mate. Responsive individuals were removed before the copulation took place and held in isolation for at least a week before the start of the experiment. After being used, choosers and targets were again observed for their sexual responsiveness. If a potential partner was found to be non-responsive, the corresponding trial was excluded from the analysis. To avoid pseudo-replications, choosers were used only once and in a single treatment. Owing to the difficulty of finding pairs of crayfish that met the morphological criteria required by each treatment, the same pair of targets were used twice in Experiment 1 and a maximum of four times in Experiment 2, but always on different and not consecutive days. In any case, the crayfish used as targets were shown to be always sexually responsive when tested after the trials.

The choosers and targets used did not have any possibility to meet before the experiment, at least within the 6–8 weeks of maintenance in the laboratory, so that we can exclude any effect of previous social experience (Bergman & Moore, 2005). The cephalothorax length of the choosers was on average 49.5 ± 12.5 mm in females and 47.5 ± 17.5 mm in males. The morphometric measures of the crayfish used as targets are reported in Table 1.

Collection of data

Crayfish behaviour was video-taped using a Sony digital camera (DCR-TRV33E; Sony Corporation of America, New York, NY USA) activated at a distance to avoid disturbance to the experimental animals. Video-tapes were subsequently analysed to record:

- (1) the first target visited and the number of visits per target. 'Visits' were defined as the instances in which the chooser entered the target's area, excluding cases in which the chooser entered the area by swimming or walking backwards, and
- (2) the total and mean duration of visits in seconds, which gives information about the chooser's persistence in his/her choice. The duration of each single visit was computed between the time the rostrum of the chooser entered a target's area and the time its telson exited it.

Experiment 1: morphological characteristics and hierarchical status

In Experiment 1, we asked whether males and females have a preference for specific morphological characteristics and/or for the dominance status. In four treatments, we analysed the attractive value of: (BS: large size vs. small size), chelar length (CL: long chelae vs. short chelae), chelar symmetry (CS: symmetric chelae vs. asymmetric chelae) and hierarchical status (HS: dominant vs. subordinate crayfish). The behaviour of the chooser was also observed in the absence of targets (control, C). The experiment was conducted between 25 July and 12 August 2005 from 08:00 to 14:00 h. The

Table 1 Morphometric data (means \pm SE) of males and females used as targets in binary choice tests in Experiment 1 (treatments: body size, BS; chelar length, CL; chelar symmetry, CS; hierarchical status, HS) and Experiment 2 (treatment: mating status, MS)

Treatments	Targets	Cephalothorax length (mm)		CL (%)		CS (%)	
		Males	Females	Males	Females	Males	Females
BS	Small body size ($n=10$)	35.24 ± 1.07	35.37 ± 0.78	69.56 ± 3.98	54.12 ± 1.68	94.58 ± 2.22	94.59 ± 2.33
	Large body size ($n=10$)	49.87 ± 2.50	54.35 ± 1.53	86.55 ± 2.87	69.08 ± 1.84	96.47 ± 0.71	96.46 ± 0.75
	t (d.f. = 18)	-5.097	-10.446	-5.54	-5.69	-0.762	-0.762
	P	< 0.001	< 0.001	< 0.001	< 0.001	0.456	0.456
CL	Small chelar size ($n=10$)	42.80 ± 1.98	42.08 ± 2.25	58.92 ± 4.82	45.13 ± 2.85	94.74 ± 2.14	89.83 ± 5.11
	Large chelar size ($n=10$)	42.68 ± 1.81	42.55 ± 2.16	84.87 ± 4.25	62.95 ± 2.96	95.54 ± 1.18	96.90 ± 0.59
	t (d.f. = 18)	0.042	-0.150	4.038	4.332	0.326	1.373
	P	0.967	0.882	< 0.001	< 0.001	0.748	0.187
CS	Symmetric chelae ($n=10$)	44.04 ± 2.59	46.45 ± 1.56	81.87 ± 3.22	62.44 ± 1.77	96.64 ± 0.88	96.28 ± 3.16
	Asymmetric chelae ($n=10$)	44.21 ± 2.54	46.50 ± 1.65	74.78 ± 1.04	57.16 ± 1.90	68.18 ± 3.08	61.17 ± 1.28
	t (d.f. = 18)	-0.047	-0.022	2.090	2.025	8.418	20.114
	P	0.963	0.983	0.051	0.058	< 0.001	< 0.001
HS	Dominant ($n=10$)	41.11 ± 2.75	43.31 ± 1.79	78.23 ± 2.58	63.62 ± 1.61	96.55 ± 0.80	95.40 ± 0.92
	Subordinate ($n=10$)	41.01 ± 2.81	43.59 ± 2.31	75.22 ± 3.34	60.89 ± 1.03	93.47 ± 1.73	94.11 ± 1.39
	t (d.f. = 18)	0.025	-0.096	0.675	1.428	1.615	0.772
	P	0.980	0.925	0.508	0.17	0.124	0.45
MS	Mated ($n=6$)	46.23 ± 0.85	44.08 ± 0.97	90.81 ± 0.77	64.65 ± 0.55	97.62 ± 0.61	95.51 ± 1.07
	Virgin ($n=6$)	44.61 ± 0.64	44.15 ± 0.34	88.45 ± 1.92	64.32 ± 0.87	93.49 ± 1.66	96.88 ± 1.19
	t (d.f. = 10)	1.517	-0.065	1.140	0.323	1.706	-1.659
	P	0.16	0.95	0.281	0.754	0.119	0.128

For the comparison between targets, P values for Student's t -tests for independent samples (t) are given. The number of the different targets used per treatment and the degrees of freedom (d.f.) are given in parentheses. Significant values in bold.

treatments and the control were replicated 20 times in both sexes following random sequences, reaching, for each sex, a total of 100 different choosers and 10 different pairs. Targets of approximately the same body size (± 1 mm) were used in each treatment and for each trial, except obviously in BS.

Table 1 shows details of the morphometry of the targets used and the corresponding statistics. The effect of BS was assessed using pairs of large and small crayfish as targets that differed by an average of 25% in cephalothorax length for males and 35% for females. Obviously, a difference in the cephalothorax length was accompanied by a difference in the size of the chelae, due to their allometric growth. The pairs of crayfish with short and long chelae were composed of individuals of the same cephalothorax length (± 1 mm) but in which the chelae of the former were on average 15% shorter than the chelae of the latter. The effect of CS was assessed using crayfish with the same cephalothorax length (± 1 mm) but with one cheliped regenerated. An individual was classified as 'asymmetric' if its chelae differed in length by at least 30%, whereas it was 'symmetric' if the difference did not exceed 5%.

The HS of a pair was determined by keeping two crayfish together in a circular aquarium for 24 h in the absence of food and shelter. Crayfish were matched for cephalothorax length (± 1 mm) and chelar size and had symmetric chelae. Notwithstanding their body similarity, all the pairs established a clear dominance order, in accordance with Vye *et al.* (1997) and other subsequent studies (e.g. Gherardi & Daniels, 2003) showing that characteristics other than body and chelar size, such as plasma protein level, exoskeleton calcium concentration along with experience, might contribute in determining the dominance order of decapods.

Ten minutes before the start of the experiment, we recorded the number of fights won by each individual of the pair (i.e. the fights ended with the retreat of the opponent to a distance of at least 10 cm; Gherardi & Cioni, 2004). Following the protocol of previous studies (e.g. Gherardi & Cioni, 2004), a dominant HS (and a subordinate HS) was assigned to the individual winning more than 90% (and <10%) of the battles fought.

Experiment 2: MS

In Experiment 2, we asked whether *P. clarkii* males and females select their partner on the basis of its MS. Hence, the two targets (which had an equal cephalothorax length ± 2 mm) differed in their MS only (Table 1).

To obtain pairs of 'mated' and 'virgin' targets, each of six pairs of male and female receptive crayfish was inserted into one of two sets of $40 \times 25 \times 25$ cm aquaria, the 'mating' or the 'non-mating' aquaria. In the mating aquaria, males of each pair were changed daily to avoid the possible reduction with time of their ejaculate size as a consequence of repeated copulations with the same female, as found in fish (Marconato, Tessari & Marin, 1995). In the non-mating aquaria, males and females were separated by a plastic wall to avoid copulation. The wall was finely drilled to permit the free circulation of the 'odours' possibly emitted by the two individuals in the water.

Crayfish behaviour was video-taped using a Sony digital camera as above in the interval recording function (30 s every 5 min). Crayfish were deemed 'mated' (and those in the other aquarium 'virgin') after males ceased to copulate (after 7 days of cohabitation with a different female per day) and females had received at least 25 ejaculates by six different males. The number of ejaculates was assessed by recording tail flips and body movements by males in the copulating position, following Rubolini *et al.* (2006). These individuals were immediately used as targets in the experiment.

The experiment was conducted in August 2006 between 08:00 and 14:00 h. The behaviour of the choosers was also described in the absence of targets (control, C). The treatment and the control were replicated 20 times in both sexes following random sequences, reaching a total, for each sex, of 40 different choosers and six different pairs.

Statistical analyses

Data were first checked for normality and homogeneity of variance using the Kolmogorov–Smirnov and Levene tests, respectively, and then analysed by Student's *t*-tests (statistic: *t*) for independent samples (for morphometric data) and for paired data (for the other analyses). For frequencies, we used *G*-tests with William's correction (H_0 = uniform distribution) (statistic: *G*) (Sokal & Rohlf, 1969). The level of significance at which the null hypothesis was rejected is $\alpha = 0.05$.

Results

Experiment 1: morphological characteristics and hierarchical status

The mean values and statistical results are shown in Tables 2 and 3 for males and females, respectively. The choosers, independent of their sex, visited the larger targets more frequently than the smaller ones and spent significantly more time in their proximity. Moreover, it was the larger rather than the smaller target that was first visited by the chooser, if female. Conversely, larger and symmetric chelae and dominant hierarchical status did not apparently elicit any preference in either sex. The only case in which the *P* value was close to significance was the more frequent first visit by males as choosers of female targets with asymmetric chelae. As expected, no significant result was obtained in the controls.

Experiment 2: MS

The mean values and statistical results are shown in Table 4. A significant difference in the total and mean durations of visits, but not in the first target visited and in the number of visits, was found when the choosers were males. They seemed to discriminate and select virgin, rather than mated, females, spending more time in their proximity. Conversely,

Table 2 Experiment 1. Frequencies of the first target visited by male choosers, means \pm SE of the number of visits and of their total and mean duration after binary choice tests (top)

Male choosers	Control (n=20)		BS (n=20)		CL (n=20)		CS (n=20)		HS (n=20)	
	1	2	Small	Large	Small	Large	Symmetric	Asymmetric	Dominant	Subordinate
First target visited	9	11	10	10	10	10	6	14	8	12
Number of visits	9.65 \pm 1.02	9.55 \pm 1.03	7.00 \pm 0.67	8.95 \pm 0.86	8.65 \pm 1.37	8.35 \pm 1.24	8.30 \pm 0.88	7.75 \pm 0.83	8.60 \pm 0.96	8.95 \pm 1.02
Total duration of visits (s)	381.1 \pm 33.2	394.8 \pm 35.6	340.5 \pm 29.4	554.5 \pm 42.8	390.3 \pm 39.3	479.3 \pm 45.3	534.6 \pm 46.0	414.0 \pm 36.1	446.0 \pm 40.6	484.1 \pm 36.2
Mean duration of visits (s)	47.69 \pm 5.45	51.0 \pm 6.8	59.6 \pm 8.3	91.7 \pm 24.0	91.1 \pm 30.5	107.7 \pm 24.8	108.3 \pm 26.3	98.8 \pm 40.4	67.2 \pm 8.5	74.4 \pm 13.8
Statistical results	Control (n=20)		BS (n=20)		CL (n=20)		CS (n=20)		HS (n=20)	
	G/t	P	G/t	P	G/t	P	G/t	P	G/t	P
First target visited	0.195	0.412	0.000	0.588	0.000	0.588	3.211	0.057	0.786	0.252
Number of visits	0.166	0.87	3.428	0.003	0.578	0.570	-1.421	0.172	-0.751	0.462
Total duration of visits (s)	-0.212	0.834	3.239	0.004	-1.132	0.272	-1.583	0.129	-0.512	0.614
Mean duration of visits (s)	-0.516	0.611	1.655	0.114	-0.888	0.385	-0.345	0.734	-0.568	0.576

Comparisons between targets using a G test (G; d.f. = 1) for the first target visited and Student's t-tests (t; d.f. = 19) for paired data for the other parameters (bottom). Sample sizes are given in parentheses. See Table 1 for the meaning of BS, CL, CS, and HS. Significant values in bold.

Table 3 Experiment 1. Frequencies of the first target visited by female choosers, means \pm SE of the number of visits and of their total and mean duration after binary choice tests (top)

Female choosers	Control (n=20)		BS (n=20)		CL (n=20)		CS (n=20)		HS (n=20)	
	1	2	Small	Large	Small	Large	Symmetric	Asymmetric	Dominant	Subordinate
First target visited	9	11	2	18	7	13	12	8	9	11
Number of visits	8.70 \pm 0.85	8.75 \pm 0.86	8.25 \pm 0.77	9.60 \pm 0.98	9.45 \pm 1.03	9.70 \pm 0.96	9.00 \pm 0.46	9.25 \pm 0.61	10.10 \pm 0.87	10.75 \pm 1.07
Total duration of visits (s)	388.1 \pm 35.7	394.3 \pm 35.4	288.8 \pm 31.6	442.1 \pm 31.9	341.4 \pm 37.7	358.6 \pm 43.7	413.9 \pm 24.5	388.6 \pm 31.6	331.9 \pm 29.9	389.3 \pm 32.4
Mean duration of visits (s)	50.9 \pm 6.7	52.0 \pm 5.7	37.6 \pm 4.4	54.2 \pm 5.2	43.6 \pm 7.4	42.8 \pm 7.5	47.4 \pm 2.8	44.9 \pm 5.1	37.3 \pm 4.9	41.6 \pm 4.1
Statistical results	Control (n=20)		BS (n=20)		CL (n=20)		CS (n=20)		HS (n=20)	
	G/t	P	G/t	P	G/t	P	G/t	P	G/t	P
First target visited	0.195	0.412	14.363	0.0002	1.783	0.132	0.786	0.252	0.195	0.412
Number of visits	-0.103	0.919	2.321	0.032	-0.107	0.916	0.546	0.592	-1.006	0.327
Total duration of visits (s)	0.101	0.929	2.888	0.009	-0.241	0.812	-0.488	0.631	-1.138	0.269
Mean duration of visits (s)	-0.162	0.873	2.899	0.009	0.083	0.935	-0.417	0.682	-0.945	0.357

Comparisons between targets using a G test (G; d.f. = 1) for the first target visited and Student's t-tests (t; d.f. = 19) for paired data for the other parameters (bottom). Sample sizes are given in parentheses. See Table 1 for the meaning of BS, CL, CS and HS. Significant values in bold.

Table 4 Experiment 2: frequencies of the first target visited by male (top) and female (bottom) choosers, means \pm SE of the number of visits and of their total and mean duration and comparisons between targets using a G test (G ; d.f. = 1) for the first target visited and Student's t -tests for paired data (t ; d.f. = 19) for the other parameters

	Control (20)				MS (20)			
	1	2	G/t	P	Mated	Virgin	G/t	P
Male choosers								
First target visited	9	11	0.195	0.412	11	9	0.195	0.412
Number of visits	9.90 \pm 1.06	10.00 \pm 0.97	-0.23	0.706	6.15 \pm 0.61	6.90 \pm 0.66	1.543	0.139
Total duration of visits (sec)	378.1 \pm 30.7	388.3 \pm 33.2	0.175	0.863	358.0 \pm 33.7	518.3 \pm 40.8	-2.435	0.025
Mean duration of visits (sec)	47.6 \pm 5.4	51.0 \pm 6.8	-0.659	0.518	66.5 \pm 8.4	92.9 \pm 13.3	-2.217	0.039
Female choosers								
First target visited	9	11	0.195	0.412	12	8	0.786	0.252
Number of visits	8.85 \pm 0.89	9.00 \pm 0.89	-0.45	0.659	10.95 \pm 0.96	10.40 \pm 0.82	-0.735	0.471
Total duration of visits (sec)	390.2 \pm 35.0	389.0 \pm 31.2	-0.022	0.982	394.7 \pm 30.8	452.4 \pm 34.7	-0.967	0.204
Mean duration of visits (sec)	50.9 \pm 6.7	52.0 \pm 5.7	-0.162	0.873	41.5 \pm 4.7	55.7 \pm 12.6	-1.316	0.247

Sample sizes are given in parentheses. MS, mating status. Significant values in bold.

no significant results were obtained when the chooser was a female and, as expected, in the control.

Discussion

Our results are clear in suggesting that *P. clarkii* males are more selective in their mate choice than found previously. In fact, similar to the other sex, males are significantly attracted by potential mates with a large body size. They were also more attracted by unmated rather than mated females, whereas virginity seemed not to be selected (or detected) by females.

Mate choice by females

Females of a wide array of species select mates on the basis of their size (reviewed in Andersson, 1994). Female preference for larger males has been demonstrated in crustaceans (e.g. isopods Shuster, 1981; decapods, Greenspan, 1980), including crayfish (*Astacus astacus*, Furrer, 2004; *Austropotamobius pallipes* Villanelli & Gherardi, 1998; Gherardi *et al.*, 2006; and *Orconectes rusticus* Berrill & Arsenault, 1984).

In the majority of the species studied so far, large males are successful when competing with other males for access to females (Jennions & Blackwell, 1996). Large crayfish males are dominant over smaller ones (Berrill & Arsenault, 1984; Villanelli & Gherardi, 1998) and more easily force females to copulate (Snedden, 1990; Gherardi *et al.*, 2006). However, the advantage of large males outcompeting other males or coercing females does not necessarily imply a female preference for large males, as found in the present study and suggested for other crayfish species (*Austropotamobius italicus* Gherardi *et al.*, 2006; *Cherax quadricarinatus* Barki & Karplus, 1999). Therefore, the female choice of larger males might reflect the advantages that the female herself gains from mating with them.

Several hypotheses can be raised to explain such a female preference. First, females might select large-sized males

because they offer vital resources, such as burrows, of high quality (Christy, 1983). Indeed, during the reproductive period, sexual pairs of *P. clarkii* have been found to share the same burrow (Huner, 1992) that is actively defended against intruders (Figler, Blanck & Peeke, 2005). Larger males are more likely to win fights for acquiring or defending burrows and this might favour the selection by females of partners of larger sizes.

A second direct benefit that females gain by mating with large males might be their production of more abundant sperm, as observed by Uhl (1998) in spiders, so that females might choose them to reduce the risk that their eggs are not fertilized (Williams, 1992). A recent study on *Au. italicus* clashes with this idea, showing that the extent of ejaculates decreases in this species with increased male size (Rubolini *et al.*, 2006). However, if the risk of sperm limitation is so high in this species, we would expect the females to evolve the ability to assess male sperm storage and to choose a male who is not sperm-depleted (Sato & Goshima, 2007). Such an ability was not found in our study possibly because, due to the minimal risk of sperm limitation in a habitat crowded with receptive males, there is no selective advantage for females to evolve specific mechanisms to avoid it (Kendall & Wolcott, 1999).

The selection of large mates might offer indirect benefits to the female, such as those hypothesized by the 'good genes' model: a large size might be the expression of high-quality genes and females mating with a large male will transmit this quality to their offspring (Hunt, Brooks & Jennions, 2005). Alternatively, under the predictions of the 'runaway selection' model (Harvey & Arnold, 1982), a slight ancestral preference for large males might have led to both an increased frequency of hereditary 'large-body genes' throughout males and the female preference for large males (Weatherhead & Robertson, 1979).

We found that females do not discriminate between partners on the basis of their hierarchical status or of the size and symmetry of their chelae, or, at least, they do not

use these characteristics to select a mate. Galeotti *et al.* (2006) showed that *Au. italicus* females can adjust their reproductive efforts in relation to the size of the male chelae: the females who mate with small, large-clawed males lay fewer but larger eggs than the females who mate with large, small-clawed males. Indeed, large chelae have a major role in the ability of males to reproduce (Stein, 1976; Snedden, 1990) and their acquisition of voluminous chelae might be under the pressure of sexual selection (Stein, 1976; Berrill & Arsenault, 1984; Gherardi, Acquistapace & Barbaresi, 2000). This hypothesis seems to be questioned by our results.

A similar unexpected result of our study is that chelar asymmetry has apparently no effect on mate choice by females. It was previously observed in other crayfish species that asymmetric chelae decrease the ability of males to secure females for copulation (Woodlock & Reynolds, 1988; Rubolini *et al.*, 2006). On the contrary, in *P. clarkii* there is no evidence that asymmetry of chelae might compromise mating success.

The important role that the male hierarchical status might play in sexual selection is also controversial. Although it has been proven that *P. clarkii* can recognize the social status of conspecifics (Zulandt-Schneider & Moore, 2000), our results show that, different from *Homarus americanus* (Atema, 1986), females do not make a choice between equally sized dominant or subordinate individuals.

Mate choice by males

A novel result of our study is to have shown that males can choose their mate, selecting large and virgin females. The selection of females of a large size has been previously found in a wide number of insects (e.g. Gwynne, 1981), in spiders (Uhl, 1998) and in a few non-decapod crustaceans (gammarid amphipods, Elwood, Gibson & Neil, 1987; isopods, Ridley & Thompson, 1979), but, except in the spiny lobsters (MacDiarmid & Butler, 1999), it has never been demonstrated in decapods (see, e.g. Villanelli & Gherardi, 1998; Gherardi *et al.*, 2006). The only indirect evidence comes from Rubolini *et al.* (2006), who showed the ability by male *Au. italicus* to adjust the quantity of sperm allocated as a function of the female size.

In other crayfish species, males seem not to be choosy in their selection of a mate as a result of (1) the restricted mating period (Gherardi, Villanelli & Dardi, 1997), (2) the chronic low number of receptive females (Brewis & Bowler, 1985), (3) the operational sex ratio skewed towards males (Snedden, 1990) and (4) the elevated predation risk while mating (Trivers, 1972). Some of the above-listed factors are not met by *P. clarkii*, whose mating period lasts for a relatively long time and is not constrained by the low frequency of receptive females. Besides, males of this species invest heavily in the production of spermatophores. Taken together, these pieces of evidence might suggest that there is a strong pressure towards the selection by males of a fecund female, as honestly expressed by her large size (Savalli & Fox, 1998). Indeed, a positive correlation between egg number and body size is widespread in invertebrates, includ-

ing crayfish (e.g. *As. astacus*, Cukzerkis, 1988; *Astacus leptodactylus*, Köksal, 1988; *Au. pallipes* Rhodes & Holdich, 1982; *Au. italicus*, Rubolini *et al.*, 2006; and *P. clarkii* Nobblitt, Payne & DeLong, 1995).

Procambarus clarkii males also seem to be capable of detecting differences in the MS of females. In species in which sperm is stored before fertilization, as in several crayfish species, sperm competition may occur, decreasing the probability of paternity. When the spermatophores are deposited externally on the female abdomen, such as in *Au. italicus* and in other Astacidae, males feed on the spermatophores deposited by the previous mates (Villanelli & Gherardi, 1998). In *P. clarkii*, the spermatophores previously deposited by the rival are inaccessible for males, so that sperm competition may be avoided by either adjusting the length of copulation as a function of the female MS (for other species, see Andrés & Cordero-Rivera, 2000) or, as seems more plausible from our results, selecting virgin females (Arnaud & Haubruge, 1999), possibly recognized by chemical cues (Sato & Goshima, 2007).

In synthesis, *P. clarkii* females, when free to choose, select mates on the basis of their large body size and males may also contribute to sexual selection by choosing both large (i.e. more fecund) and virgin females. Further studies are obviously needed to understand the proximate and ultimate causes of such a choice and the relationship between the reproductive behaviour of this species and the environmental constraints faced in the diversified areas colonized.

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