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ORIGINAL ARTICLE

An effective sperm competition avoidance strategy in crabs drives genetic monogamy despite evidence of polyandry

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Abstract For polyandrous species where females have sperm storage structures, males develop several strategies to avoid sperm competition and thus to maximize the number of eggs fertilized. On the other hand, females may receive several benefits from multiple paternity (indirect and directly), and a potential sexual conflict can arise. This research describes the mating systems of an exploited crab species (*Metacarcinus edwardsii*), integrating (1) the individual level by assessing the mating behavior in a scenario of potential polyandry, (2) the organ level by examining histological sections of seminal receptacles from localities with scenarios of contrasting sex ratios, and (3) the genetic level by measuring the number of parents involved in egg clutches. We found that females can mate with multiple males under experimental conditions. Further, in all localities, we found histological evidences that sperm receptacles stored ejaculates from more than one male. However, contrary to expectations, genetic analysis revealed high probability of single male paternity of all progeny in each egg clutch. In this mating system, males compete to be the single male that mates with a receptive female, investing energy in guarding behavior and foregoing opportunities to mate with other females, all in order to ensure their paternity. However, females benefit from multiple mating (or potential for it) by prolonged guarding behavior, protecting them from predation after molt (soft-shelled period). The mating system of *M. edwardsii* can be defined as polygamous (where both sexes can mate multiple times) with genetic monogamy.

Keywords Sperm competition · Mating behavior · Decapoda · Paternity · Polyandry

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Introduction

Understanding mating systems is the key to comprehending the biological traits of a species, from demographic distributions to the intensity of sexual selection (Emlen and Oring 1977). One of the most common mating systems in nature is polygamy, which occurs where males or females are able to access multiple mates (polygyny and polyandry, respectively). The principal consequences of polygamy are intense intrasex competition and multiple paternity.

In crabs, several mating systems have evolved based on the manner of competition among males for females (see Christy 1987). This diversity is associated with morpho-functional variations in one of the most remarkable features of Eubrachyura, the presence of seminal receptacles in the genital duct (McLay and López Greco 2011). This internal structure is specialized for postcopulatory sperm storage (Hartnoll 1968; Diesel 1991), which promotes both male–male competition and male strategies to increase the probability of paternity.

The presence of receptacles provides several evolutionary benefits but mainly allows for multiple paternity, which promotes genetic variability within a population and maintains effective population size (Moran and Garcia-Vazquez 1998;

Jennions and Petrie 2000). Furthermore, in cases where crab mating occurs only after a molt (soft females), two energetically costly processes, gonadal development and molting, can be asynchronous (Sainte-Marie 2007).

Sperm reserves are usually estimated based on receptacle weight, total ejaculate weight, and/or stored sperm counts from females. These measurements have been useful as a way to relate receptacle condition with particular female characteristics (i.e., size, precedence, reproductive condition) and to estimate the potential for sperm limitation in harvested species; nevertheless, they do not elucidate the presence of multiple ejaculates. Quantitative histological estimation of the ejaculate load in the female receptacle can describe the recent mating history of females and highlight the potential for multiple paternity.

In cancrid crabs, females may mate with more than one male (polyandry), storing ejaculates stratified in their seminal receptacles (Orensanz et al. 1995; Jensen et al. 1996; McKeown and Shaw 2008; Pardo et al. 2013). This fact suggests the possibility that progeny comes from several parents. On the other hand, all cancrid seminal receptacles described to date have been of the ventral type (sensu Diesel 1991; McLay and López Greco 2011). This receptacle design implies that more recently transferred ejaculates are deposited closer to the oviduct connection; therefore, the last male to mate increases his probability of fertilizing oocytes. This "last-male sperm precedence," which was proposed by Diesel (1991) has been largely accepted. However, few paternity studies have been performed (i.e., Sainte-Marie et al. 2008). Recently, Jensen and Bentzen (2012) demonstrated sperm precedence in a controlled mating experiment performed with *Metacarcinus magister*. They found that most females extruded embryos sired by only one male, and in the cases where multiple paternities were detected, these disproportionately favored one male. In another cancrid, *Cancer pagurus*, no evidence of multiple paternity was detected in 18 clutches of wild caught females described by McKeown and Shaw (2008). Similarly, in a preliminary study on *Metacarcinus edwardsii*, an exclusive mono-paternity pattern was found in five clutches from one locality in Southern Chile (Rojas-Hernandez et al. 2014).

Interspecific and intraspecific variation in prevalence of multiple paternity could be related to local sociosexual scenarios, which result in different frequencies of multiple paternity (as in other decapods, Gosselin et al. 2005). For example, the effectiveness of male behavior in preventing subsequent mating could change in the context of different sex ratios and with the length of premating and postmating guarding behavior.

This study aims to test if variation in sex ratios influences the number of ejaculates in seminal receptacles and whether this affects the prevalence of multiple paternity in female clutches. We tested this premise using the exploited crab *Metacarcinus edwardsii* as model species. This study was aided by previous descriptions of seminal receptacle morphology and sperm storage dynamics (Pardo et al. 2013). First, we performed a controlled experiment in order to describe the mating behavior of crabs under different sex ratio scenarios in a controlled environment. Then, we conducted a field study to compare the ejaculate load (i.e., number of ejaculates) and incidence of multiple paternity patterns in sites with different fishing intensity (i.e., a proxy for local variation in sex ratio) (Pardo et al. 2015). We expected, as a result of a male-biased harvest, that in localities with low fishery intensity, female receptacles would have a higher number of ejaculates and the prevalence of multiple paternity would increase in comparison to localities with high fishery intensity.

Methods

Individual level: male–male competition and mating behavior

In order to evaluate if one female can mate with more than one male during a mating season and test male behavior in differing scenarios of sex ratios, individuals of *M. edwadsii* of both sexes were collected from the locality of Los Molinos (39° 51′ 16.7″ S; 73° 23′ 40.3″ W), transported to Calfuco Marine Laboratory and maintained separated by sex in 500-l tanks with flowing seawater and air supply. Two males with bilaterally ablated gonopods were added to the female tanks in order to detect females' receptivity to mates (before molting), checking daily for guarding behavior. Once a couple was detected, the female was put in an experimental tank of 100 l in one of three experimental conditions: (1) a sex ratio 1♀:1♂, (2) a sex ratio 1♀:2♂, with males of similar size (<2 % of differences in carapace width), and (3) a sex ratio 2♀:1♂. All behavioral interactions were recorded continually in high definition mode by video cameras equipped with infrared light. In total, 23 trials were conducted and analyzed (seven in the 1:1 treatment; 11 in the 1:2, and five 2:1 treatment group). Three dependent behavior variables were record by trial: (1) total time of precopulatory guarding, (2) copulating time, and 3) total time of postcopulatory guarding. In the case of 1♀:2♂ and 2♀:1♂ sex ratios, the incidence of multiple mating (polyandry and polygyny) was recorded.

A one-way ANCOVA with the Welch procedure, which does not assume homogeneity of variance (Welch 1937; Zar 1999), was performed to test differences in time spent on each dependent behavior variable for each sex ratio. All tests were run using R software.

Histological level: field monitoring of females

In order to evaluate the prevalence of multiple ejaculates in the seminal receptacle in different crab populations, mature

females (Pardo et al. 2009) were caught in commercial traps from five localities in southern Chile: Los Molinos (39° 51′ 16.7″ S; 73° 23′ 40.3″ W), Calbuco (41° 45′ 47.1″ S; 73° 05′ 20.1″ W), Ancud (41° 50′ 59.8″ S; 73° 51′ 32.5″ W), Dalcahue (42° 22′ 46.3″ S; 73° 35′ 42.5″ W), and Quellón (43° 08′ 18.4″ S; 73° 36′ 43.4″ W). Previous studies have shown, through direct evaluation of crab population structure, that these localities have contrasting sex ratios due to large differences in the intensity of male-biased fishery management (Los Molinos and Calbuco, low fishery intensity and ♂ more abundant than ♀; Ancud, Dalcahue, and Quellon, high fishery intensity and ♂ similar abundant than ♀) (Pardo et al. 2015).

Female crabs were measured (carapace width, CW) and transported to laboratory. The histological condition of the seminal receptacle was determined by dissecting the right seminal receptacles and fixing them in Bouin solution for at least 2 days. Samples were then sequentially passed through 50–70–80–96–100 % ethanol solutions for 30 min, 100 % ethanol–buthylic alcohol (1:1 *v*/*v*) for 30 min, buthylic alcohol for 25 min (twice), and embedded in paraffin. These samples were cut sagittally to the vaginal constriction plane in serial sections of 6 μm and stained with hematoxylin-eosin. Each receptacle was categorized in terms of four different ejaculate conditions: (a) receptacles with few remaining sperm, where sperm were scarce and scattered in the receptacle, (b) receptacles with one ejaculate, (c) receptacles with two ejaculates, and (d) receptacles with three or more ejaculates (Fig. 1). Between ten and 15 females were sampled seasonally by locality during 2012–2013. Only nonovigerous females were used in this survey. In two localities (Calbuco and Quellon) during autumn (March–June 2013), females were not available from commercial catches.

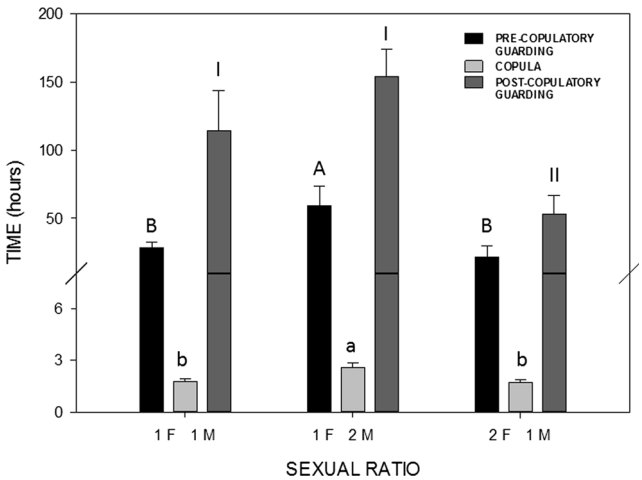

Fig. 1 Guarding and copulation times under different sex ratio treatments. Only receptive females were used in experimental trials. In the case of 2♀:1♂ treatment, guarding and copulation time were recorded based on the first female that a male embraced. *Different letters and numbers* indicate statistical differences ($p<0.05$)

In order to establish differences in the frequency of the seminal receptacle condition, a multinomial categorical model was constructed using the CAT MOD routine in the SAS statistical software (Stokes et al. 2001). This analysis uses categorical multinomial seminal receptacle condition (0, 1, 2, and 3 ejaculate load) as dependent variable and the localities (Dalcahue, Los Molinos, Ancud, Calbuco, and Quellon) nested in the intensity of fishery (high and low) as the independent variables. After this, a Fisher exact test was used to the statistical differences for pair-wise comparisons.

Genetic level: multiple paternity analyses

In order to test the multiple paternity hypothesis, two different analyses were performed to increase both the number of females and embryos analyzed. First, ovigerous females from each of sampling localities, Los Molinos ($n=6$), Calbuco ($n=6$), Dalcahue ($n=7$), Ancud ($n=6$), and Quellon ($n=6$), were collected and maintained individually in the laboratory until larval release. Each zoea ($n=10$ per female) was stored individually in a vial with ethanol 95 %, and a small sample of muscle from the pereiopod was obtained from each female. In order to obtain larvae representing the complete spectrum of offspring carried by each female and to increase probability of detecting multiple paternity, we sampled larvae from two extreme moments during the larvae release, which occur at night during the first and second week of release. The rationale behind this is that differences in larval release time reflect the position of embryos in the egg clutch, because the gradient in oxygen availability across the egg clutch produces asynchronous embryo development (Fernández et al. 2003). Therefore, sampling larvae at different moments of release increases the probability of obtaining larvae from different sections of egg clutch.

Thus, five zoeae were sampled during the first day of larval release and five during the last day (1 or 2 weeks after). Total genomic DNA was extracted using the Qiagen QIAamp (Mississauga, Canada), and the eight microsatellites identified for this species (Rojas-Hernandez et al. 2014) were amplified. The PCR products obtained were genotyped by Pontificia Universidad Católica de Chile, using the internal size standard LIZ 500 (Applied Biosystems). Alleles were then scored using GeneMarker software (SoftGenetics). In order to detect the number of fathers involved in each clutch, the genotype of each progeny and the female were used to estimate the likelihood of the minimal number of males involved in that clutch using the GERUD software (Jones 2001).

Second, a new set of ovigerous females from Los Molinos ($n=7$), Calbuco ($n=6$), Dalcahue ($n=3$), Ancud ($n=4$), and Quellon ($n=4$) were analyzed by pooling 20 larvae for the genetic analysis. Six microsatellites (Cedw15, CedCrab1,

CedCrab4, Cedw16, Cedw5, and Cedw12) were amplified for each female and their respective pool of larvae. The identification of more than four alleles in a heterozygote female would be indicative of multiple paternity in a clutch.

Finally, the likelihood of detecting multiple paternity was assessed for pairs of males using the software PrDM (Neff and Pitcher 2002). The simulation was performed using ten larvae (as the individual analysis) and 20 larvae (as the pooled analysis) for three different male levels of paternity contribution: 50:50, 90:10, and 80:20.

Results

Mating behavior

General description of mating In all cases, males performed a precopulatory embrace. Typically, males actively approached passive females. The male held the female under its body and remained in this position during the precopulatory embrace. If males changed position, they carried females gently using their chelae without scraping the female's body. In this way, females are protected during molt by males. After approximately an hour, both crabs adopt the sternum–sternum position, and males introduce their pleopods in the female genital duct. Copulating time ranged between 0.8 and 5.7 h, depending on sex ratio scenario (F=3.6, p=0.05). In the male-biased treatment, copulation was longer (mean 2.6 h) than in other 2♀:1♂ or 1♀:1♂ treatments (mean 1.7 and 1.8 h) (Fig. 1). After copulation, males embraced the female again for a variable time, also related to the experimental sex ratio. In general, mean time elapsed in both precopulatory and postcopulatory embrace (guarding) was always higher in the male-biased scenario (1♀:2♂) and the lowest in the female-biased scenario (2♀:1♂) (Fig. 1). For precopulatory guarding, the means were 22.2, 28.3, and 59.5 h (2♀:1♂=1♀:1♂<1♀:2♂) (F=3.2, p=0.07), while for postcopulatory guarding, the means were 53, 114, and 154 h (2♀:1♂<1♀:1♂=1♀:2♂) (F=6.2, p=0.01).

For all 1♀:2♂ sex ratio trials, agonistic interactions between males were frequently recorded, and sometimes during the postcopulatory, guarding the second male would gain access to the female and mate with her (see video in https://www.youtube.com/watch?v=WFf9S_qtG7Q). In total, 27 % of females were recorded as having mated with both males in the male-biased treatment. Despite that the two contenders were immobile during long periods, male–male agonistic interactions were recorded in all cases, involving chelipeds attacks, scrapes, and threats. For the male guarding the female, these interactions were dissuasive and defensive in nature during the precopulation and postcopulation periods (precopulatory dissuasive: range 1–18 interactions; precopulatory defensive: range 3–167 interactions; postcopulatory dissuasive: range 2–256 interactions; postcopulatory defensive: range 2–202 interactions). In the case of 2♀:1♂ sex ratio scenario, the 40 % of males mated with both females.

Histological analyses of seminal receptacles

All four categories of receptacle condition were recorded in all localities (Fig. 2), with the exceptions of Dalcahue, where no receptacles with little remaining sperm were detected (category=0) and Los Molinos where no receptacles with

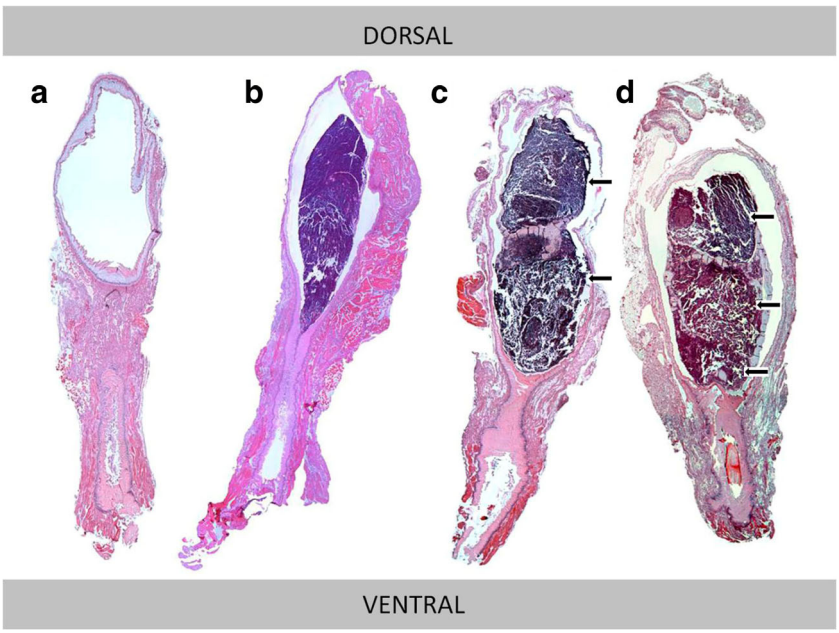

Fig. 2 Four categories of receptacle load condition from histological data. **a** No sperm or very few sperm detected, **b** one ejaculate present, **c** two ejaculates present in a stratified pattern, and **d** three or more ejaculates present in a stratified pattern. *Arrows* indicate different ejaculates

three and more ejaculates (category=3) were recorded (Table 1). The effect of fishery intensity showed a nonsignificant effect on the frequencies of the ejaculate loads ($p>0.05$); however, localities nested in fishery intensity did present a contrasting pattern, with Calbuco showing the highest frequency of three more ejaculates while Los Molinos without them (Table 2). The distribution of ejaculate load categories in Calbuco was uniform for one and two ejaculate categories compared to Los Molinos ($p<0.0001$), Ancud ($p=0.0533$), and Dalcahue ($p=0.0006$), where it was skews to one ejaculate category (Tables 1 and 2).

Paternity analysis

The microsatellites analyzed showed clear peaks in each female and their progeny.In the first analysis (i.e., by individual zoea), some microsatellites did not amplify in all zoea mainly due to the low amount of DNA obtained from the extraction. Overall, more than three microsatellites were amplified for most larvae. In this context, the power of three to four markers to detect multiple paternity for the number of larvae analyzed per female ranged from 65 to 100 % (Table 3). For all females analyzed ($n=31$), the multilocus paternity analysis performed with GERUD software showed the presence of only one male implicated in each clutch. The minimal number of alleles found in all progeny in a clutch was 3, of a maximum of 4, evidence of the presence of only two individuals involved in the production of these clutches (Table 3).

In the PrDM software developed by Neff and Pitcher (2002), the genotype of a known genetic parent can also be an input for individual broods, but when estimating PrDM for a population or set of independent broods, it may be omitted and instead randomly generates the analyses based on the population allele frequency data. In our case, the likelihood to detect broods in the population that contain genes from at least two males in a proportion 90:10 ranged from 0.13 (1–0.87) to 0.36 (1–0.64), when this was considered one female and ten larvae. However, to each locality, we used, at least, a total of 60 larvae, increasing the statistical power to detect multiple paternity to individual female to PrDM>0.99. Of course, if multiple paternity is present in *M. edwardsii*, this has low probability.

In the second analysis, pooled larvae showed between three and four alleles, also indicating that only one male was implicated in each clutch (Table 4). Interestingly, we obtained more clear peaks and scores with pooled larvae than from individual larva, pointing out the importance of the amount of DNA needed in this kind of analysis.

Table 1 Total number and percentage of seminal receptacles containing from 0 to 3 ejaculates from different localities with contrasting fishery intensity

Locality	Fishery	Number of ejaculates			
		0 (%)	1 (%)	2 (%)	3 (%)
Los Molinos	Low	2 (2.1)	72 (75.0)	22 (22.9)	0 (0.0)
Calbuco	Low	3 (8.8)	13 (38.2)	13 (38.2)	5 (14.7)
Ancud	High	4 (8.7)	31 (67.4)	8 (17.4)	3 (6.5)
Dalcahue	High	0 (0.0)	38 (79.2)	8 (16.7)	2 (4.2)
Quellón	High	3 (6.4)	26 (55.3)	16 (34.0)	2 (4.3)

Table 2 Maximum likelihood analysis of variance

Factor	*df*	χ^2	*p* value
Fishery	3	3.98	0.3324
Locality (fishery)	9	24.92	0.0031
Pair-wise comparison	n	Fisher Exact test *p* value	
Los Molinos–Calbuco	130	<0.0001	
Los Molinos–Ancud	142	0.0174	
Los Molinos–Dalcahue	144	0.1686	
Los Molinos–Quellón	143	0.0181	
Calbuco–Ancud	80	0.0533	
Calbuco–Dalcahue	82	0.0006	
Calbuco–Quellon	81	0.2762	
Ancud–Dalcahue	94	0.1973	
Ancud–Quellón	93	0.3155	
Dalcahue–Quellón	95	0.0308	

In this analysis, four ejaculate loads (from 0 to 3) were used as the categorical and dependent variable; localities (Los Molinos, Calbuco, Ancud, Dalcahue, and Quellon) nested within the fishery (high and low) were the independent variables

Discussion

Evidence of multiple mates during one mating season was recorded in the laboratory for *M. edwardsii*. This behavior is hard to record in the field; however, histological examination of seminal receptacles confirms that females frequently store multiple ejaculates (34 %). Due to the capacity of females to retain sperm after molt, the multiple ejaculates present in the receptacle may come from either the previous or current mating season. In both cases, rival sperm competes for fertilization.

In species where females are polyandrous and seminal receptacles store multiple ejaculates, it could be expected that more than one male could participate in the fertilization process of the abundant number of oocytes produced in the ovary. However, in this study, cases of multiple paternity were not detected in the embryos analyzed. This finding was consistent in all the localities studied, despite differences in sociosexual environment, specifically sex ratio and size differences

Table 3 Probability of detecting multiple paternity in *Metacarcinus edwardsii* with PrDM simulation

	Three microsatellites (cedw15, cedw12, and crab4)			Four microsatellites (cedw15, cedw12, crab4, and crab1)		
	50:50	80:20	90:10	50:50	80:20	90:10
10 larvae	0.993	0.879	0.636	0.997	0.885	0.647
20 larvae	0.999	0.983	0.866	1.000	0.988	0.872

Simulations were performed for 10 or 20 sampled larvae and for two males with equal contribution (50:50) or skewed distribution (90:10 and 80:20)

between sexes (Pardo et al. 2015). This supports the idea that only single paternity, or at most a very low incidence of multiple paternity, occurs in *M. edwardsii*. This genetic monogamy has been also detected in *Cancer pagurus* (McKeown and Shaw 2008), but other related species (*M. magister*) up to 40 % of the embryos mass of wild caught females were sired by a different male than the rest of the embryos (Jensen and Bentzen 2012). In fact, for decapods, multiple paternity is the norm rather than the exception (Table 5), regardless of the great diversity in mating strategies exhibited by the group (Hartnoll 1969). However, when genetic analyses are performed for species with internal sperm storage (Eubrachyura), paternity may be strongly biased in favor of one male (Table 5). Therefore, the genetic benefit of polyandry in terms of progeny (Jennions and Petrie 2000; Johnson and Brockmann 2013) could be low.

To females, the indirect benefits of multiple mating are unclear when there is no consequence in genetic compatibility or genetic diversity of the progeny (although male–male

Table 4 Microsatellite genotype obtained from the pooled larvae

Location	Female	Alleles offspring					
		Cedw15	CedCrab1	CedCrab4	Cedw16	Cedw5	Cedw12
Ancud	AN409	160[a],166,168[a]	213[a],219[a],239	190[a],198,236[a]	422[a],446,46[a]2,476	246[a],278[a],288	285,291[a],305,311[a]
	AN410	168,170[a],182[a]	203,215[a],221[a],229	184,196,202[a],256[a]	410[a],422,444,486[a]	268[a],278,292[a]	293,299[a],303,311[a]
	AN411	168[a]	221[a],229	184,198[a],202[a]	422[a],430,456	258[a],274[a],306	299,303[a],305,309[a]
	AN412	166[a],168[a],184	223[a],229	182[a],184[a],198,200	430[a],446[a],476	264[a],280,290[a]	–
Calbuco	CA435	–	217[a],235[a]	192,194[a],198	428[a],440[a],456,468	282,288[a],306[a]	279[a],293[a],305,311
	CA436	168[a],182[a]	199,221[a],229	180,196[a],202[a]	420[a],428,456[a],470	262[a],286,292[a]	283[a],285,291[a],293
	CA437	168[a],182	217,225[a],229	192,194[a],202[a]	412,430[a],456[a],468	264,272[a],278[a]	285,299[a],321,323[a]
	CA448	168[a],172	–	–	416,428[a],440	–	291,293[a],297[a]
	CA449	168[a],170,182[a]	223[a], 249	192[a],200[a]	434[a],456[a],470	248[a],266,286[a]	283[a],297[a],299
	CA450	160[a],168,172[a]	215[a],221[a],239	196,202[a],204[a]	406[a],428,434[a],468	248,258,276[a]	291,293[a],303,311[a]
Dalcahue	DA420	168[a]	213[a],221,229[a]	192,194,198[a]	420[a],436[a],446,480	272[a],280,298,306[a]	285,297,299[a],311[a]
	DA509	168[a],170	221,223,239[a]	182,188,194[a],210[a]	416[a],426[a],436,466	256[a],264,272,280[a]	279[a],301,317,323[a]
	DA513	166,168[a],172[a]	207[a],221[a],229	188[a],194,202,206[a]	434[a],480[a]	276[a]	–
Los Molinos	LM438	168[a],170	213,217[a],225[a],235	182[a],184,192[a]	432[a],456[a],470	262[a],268[a],272,292	285[a],289[a],299,317
	LM441	166[a],168,182[a]	203,217,225[a]	190[a],196,198[a],202	434,444, 480[a]	–	285[a],289,319,321[a]
	LM443	168[a]	223[a],225	184[a],196[a]	420[a],446	264[a],270,276[a]	293[a],297,309[a]
	LM444	168[a]	217[a],225[a],239	196[a],198[a],200,204	426[a],430[a],462,476	250,264[a],276[a],294	293,321[a],323[a]
	LM446	166,168[a]	213,221[a],225[a],239	188[a],194[a],202	418,430[a],450,456[a]	276[a],290	285,291[a],299[a],315
	LM487	166[a],168[a]	209[a],219,231[a]	184,188[a],202[a]	420[a],446,462	252[a],258	285[a],297[a],301
	LM490	160,168[a],170	215,229[a],235[a]	194,198[a],202[a]	430,440[a],456	–	285,301,305[a]
Quellón	QU498	168[a],170[a]	217[a],225,229[a],239	194[a],202	412[a],456[a],470	278[a],284,290	291[a],293[a],309
	QU500	–	207,213[a],225[a]	194[a],200	–	254[a],272[a],292	279[a],293,303,305[a]
	QU501	166,168[a],174[a]	209,217[a],223[a]	188[a],192,200,202[a]	422,434[a],468[a]	264[a],288,328[a]	293[a],299[a]
	QU504	166,168[a],170	217[a],223[a],229,233	180,190[a],194[a],202	418[a],436[a],446,458	250[a],258,264[a],274	293,299[a],303

[a] The alleles of the female

Table 5 Summary of paternity patterns for other crustacean species

Infraorden	Species	Name	Paternity (prevalence)	Contribution of single male	Reference
Brachyura	*Chinoecetes opilio*	Snow crab	Multiple (12 %)	90 to 60 %	(Sainte-Marie et al. 2008)
	Dissodactylus primitivus	Pea crab	Multiple (60 %)	92 %	(Jossart et al. 2014)
	Scopimera globosa	Sand-bubbler crab	Multiple (ND)	94 %	(Koga et al. 1993)
	Ucides cordatus	Mangrove land crab	Multiple (40 %)	ND	(Baggio et al. 2011)
	Uca mjoebergi	Fiddler crab	Multiple (56 %)	98 %	(Reaney et al. 2012)
	Metacarcinus magister	Dungeness crab	Multiple (40 %)	98 %	(Jensen and Bentzen 2012)
	Cancer pagurus	Brown crab	Single	100 %	(McKeown and Shaw 2008)
	Metacarcinus edwardsii	Brown crab	Single	100 %	This study
Anomura	*Munida rugosa*	Squat lobster	Multiple (86 %)	68 %	(Bailie et al. 2011)
	Munida sarsi	Squat lobster	Multiple (100 %)	50 %	(Bailie et al. 2011)
	Paralithodes camtschaticus	Red King crab	Single	100 %	(Vulstek et al. 2013)
	Petrolisthes cinctipes	Intertidal crab	Multiple (80 %)	ND	(Toonen 2004)
Caridea	*Caridina ensifera*	Freshwater shrimp	Multiple (100 %)	30 to 80 %	(Yue and Chang 2010)
	Rhynchocinetes typus	Marine rocky shrimp	Multiple (73 %)	50 to 90 %	(Bailie et al. 2014)
Palinurida	*Homarus americanus*	American lobster	Multiple (13 %)	70 %	(Gosselin et al. 2005)
	Nephrops norvegicus	Norway Lobster	Multiple (55 %)	50 %	(Streiff et al. 2004)
Astacida	*Orconectes placidus*	Crayfish	Multiple (40 %)	83 %	(Walker et al. 2002)

competition could provide "good genes" to females). However, direct benefits from male protection provide a clear advantage in survival (Jivoff 1997). As is demonstrated in this study, when males are exposed to treatments that increase the potential for sperm competition, male–male competition for receptive females increases, and the males extend their guarding time and thus increase the protection period for postmolt females (soft-shelled), which are highly vulnerable to predation or cannibalism. Evidence of females having multiples mates has been recorded in other cancrid crabs, namely *Cancer borealis* (Elner et al. 1985), *M. magister*, and *M. gracilis* (Orensanz and Gallucci 1988), all species that mate immediately after molt, supporting the idea that polyandry, as part of the mating system, can play an important role in female survival for these species.

Another direct benefit for females from multiple mates may be related to the seminal material delivered by extra males, which could have a nutritional or antimicrobial component (Subramoniam 1993) that could increase gamete survival inside of the seminal receptacle. On the other hand, the high amount of sperm storage by females is an assurance against sperm limitation in future reproductive cycles. Taking into account all these benefits, polyandry could be expected to have a positive impact on the reproductive success of females.

If multiple mating provides several benefits to females, why was it not more frequent (27 %) in the laboratory experiment using similar sized males? One plausible explanation is because multiple mating produces sexual conflict. For males, multiple mating is not necessarily associated with reproductive success, given that the morphological configuration of the seminal receptacle in *M. edwardsii* promotes the last-male sperm precedence (see below). Thus, males under risk of sperm competition increase the investment in a single female and spend more time guarding females (precopulation and postcopulation) to the detriment of opportunities to search for other mates. Also, they spend more time in the act of copulation, which could imply a higher volume of seminal material delivered and/or the deposition of a well-structured sperm plug. All these are investments to increase the probability of paternity (Dewsbury 1982; Jivoff 2003).

The mechanics explaining single paternity, despite polyandry and female capacity to store multiple ejaculates, can be associated with the functional morphology of the seminal receptacle and a male strategy to avoid sperm competition. The seminal receptacle in this species is a simple enlarged, undivided sac, which allows sperm stratification along the dorsal-ventral axis. This stratification was clear and consistent in histological sections; each ejaculate is clearly separated by basic sperm gel produced by subsequent mates. In *M. edwardsii*, the seminal receptacle is of the ventral type (Pardo et al. 2013); therefore, new ejaculates displace old ones toward the blind section of the receptacle, away from the oviduct-receptacle connection and vagina, ensuring last-male precedence during fertilization. In addition, males deposit a rigid sperm plug after copulation, occluding the vaginal opening to prevent a second mate (Pardo et al. 2013).

However, guarding behavior seems to be the most effective male strategy to avoid multiple mating. Males actively defend females from the agonistic attacks of other males and also perform dissuasive attacks. In the laboratory experiment, theoretical predictions (Parker 1974) were confirmed showing the plasticity of male guarding behavior given the different sex ratio scenarios. In the field, guarding time likely varies not only with operational sex ratio but also with environmental resource availability, especially in species where mating strategies are resource intensive (Sal Moyano et al. 2014). Effectiveness of guarding is also size dependent. Therefore, different results could be expected when males of different sizes compete for females. For example, Orensanz et al. (1995) found that in more than 90 % of cases, larger males will take away receptive females guarded by smaller males.

Observations in field and laboratory indicate that males of *M. edwardsii* display a polygamous mating system (females and males can mate multiple times) and a female-centered mating competition strategy (Emlen and Oring 1977; Christy 1987), which is based on the aggressive defense of receptive females (soft carapace), including precopulatory and postcopulatory guarding. This is similar to behavior observed in other cancrid crabs like *Cancer productus* and *Metacarcinus magister* (Orensanz and Gallucci 1988; Orensanz et al. 1995; Jensen et al. 1996). However, due to an effective sperm competition avoidance strategy, this species presents genetic monogamy.

Conclusion

Female brown crabs, *Metacarcinus edwardsii*, can mate with multiple males and frequently store multiple ejaculates in their seminal receptacles. This scenario should promote a pattern of multiple paternity within the abundant offspring carried by females. However, we found genetic monogamy in all cases analyzed. Moreover, single paternity was always found, regardless of the sociosexual context across five localities with large differences in local population structure. The presence of polyandry without genetic benefits to progeny may be an evolutionarily stable strategy because polyandry maximizes male–male competition to increase probabilities of single paternity, which in turn increases the time males dedicate to guarding females, improving female survival. This mating system may be common in species where females are vulnerable to predation during their receptive period, as is the case of crab species where females mate soft-shelled. For exploited species of crabs with this mating system, male-biased harvest may produce a relaxation of male–male competition in natural populations, which means that males would protect females for a shorter time during the vulnerable molt stage, increasing eventually female mortality. In this context, crab fishery policies should take into account the mating system of the exploited species to avoid indirect negative effects of management.

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