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Sex determination in crayfish: are intersex *Cherax quadricarinatus* (Decapoda, Parastacidae) genetically females?

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Summary

In the Australian red-claw crayfish *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae), a gonochoristic species, seven different combinations of intersex individuals (with both male and female genital openings) have been described. However, to date, the genetic basis for this phenomenon has not been investigated. This study was designed to test a simple chromosome-based sex-determination model for *C. quadricarinatus* that assumes the male to be the homogametic (ZZ) sex. According to our model, intersex individuals that are functionally males are genetically females (WZ). Individual crosses were performed between intersex and female crayfish, with control crosses being performed between normal males and females. The control crosses yielded, in most cases, the expected 1 : 1 sex ratio in the F_1 progeny. Crosses between intersex individuals and females yielded a 1 : 3 (male : female) sex ratio in most crosses. According to our hypothesis, one-third of the females produced in a cross of a female with an intersex animal should be WW females. The hypothesis was tested by crossing normal males with F_1 females, which were progeny of intersex fathers. These crosses yielded almost 100% females, a finding that conforms to the above-suggested sex determination model for *C. quadricarinatus* and the female WZ genotype of intersex individuals.

1. Introduction

Among the Parastacidae, intersexuality has been reported for a number of genera, including the decapod genus *Cherax* (Rudolph, 1999). Anatomical, histological, physiological and molecular aspects of this phenomenon have already been well documented for the Australian red-claw crayfish *Cherax quadricarinatus* (von Martens) (Sagi *et al*., 1996, 2002). This crayfish is a large tropical freshwater crustacean that also grows and reproduces successfully in temperate climates and attains sexual maturity within 7–9 months (Rouse *et al*., 1991). The species is gonochoristic, with a bilaterally symmetrical reproductive system. In males, the reproductive system consists of pairs of testes, sperm ducts, androgenic glands and genital openings at the bases of the fifth pair of walking legs. Females have pairs of ovaries, oviducts and genital openings at the bases of the third pair of walking legs. There have been sporadic reports of intersex individuals with both male and female genital openings (Thorn & Fielder, 1991). In cultured populations of *C. quadricarinatus*, various types of intersex individuals, bearing both male and female openings, have been described, at frequencies from 2–4% (Brummett & Alon, 1994; Medley & Rouse, 1993; Thorn & Fielder, 1991) to as much as 17% (Medley & Rouse, 1993). The frequency in the population cultured in Israel has remained at a level of 1·3% (Karplus *et al*., 1995) for a number of years (Sagi *et al*., 1996).

In decapod crustaceans, studies of sex-determination mechanisms based on karyotype information were reported for nine species, all of them crabs (Brachyura) or hermit crabs (Anomura). These studies have shown the male to be the heterogametic sex, with variant formulae probably derived from an XY–XX scheme (see Niiyama and also Baffoni in the reviews of Ginsburger-Vogel & Charniaux-Cotton, 1982; Lecher *et al*., 1995). There is, however, no karyotype

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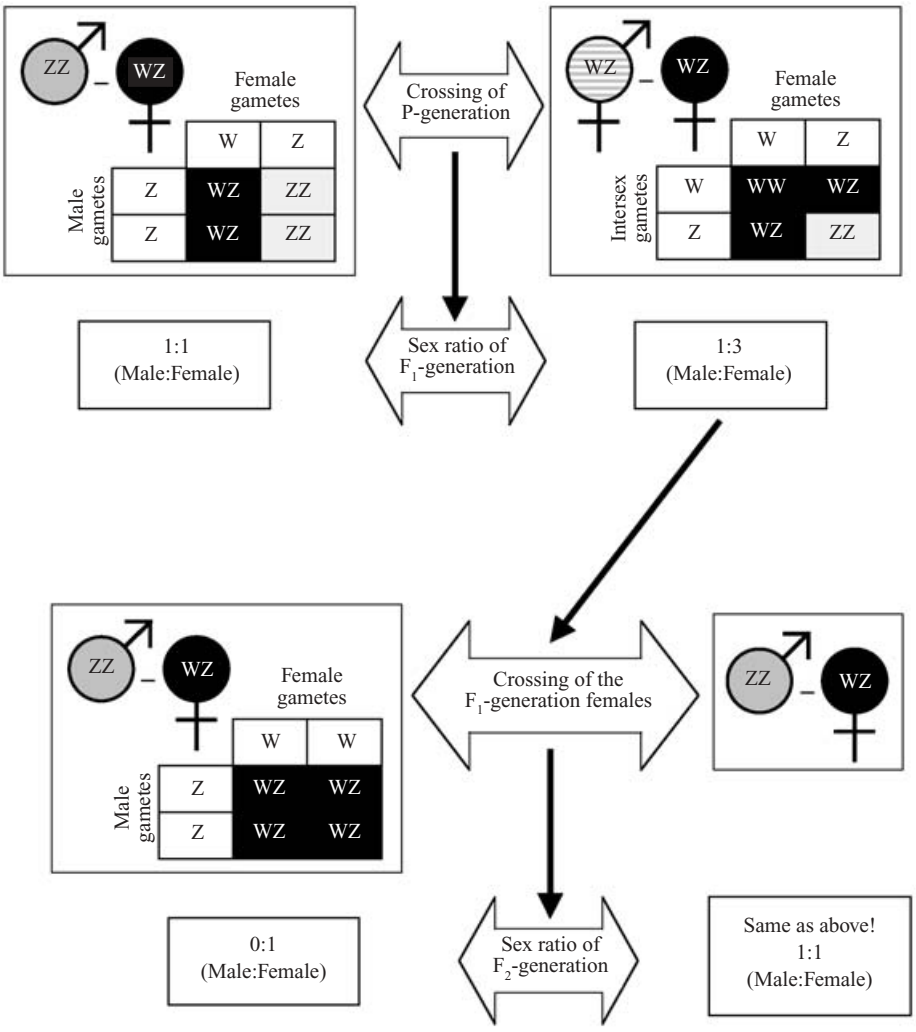

Fig. 1. The proposed sex heritability model for *C. quadricarinatus* normal males (left) and intersex animals (right). The entire process applied in this study for testing this model is represented.

information in the literature regarding sex chromosomes in Macrura (crayfish, lobsters, spiny lobsters, shrimps and prawns) (Legrand *et al*., 1987), except for a single report on *Macrobrachium scabriculum* (Lakra & Kumar, 1995). Indirect information about sex-determination mechanisms can be deduced from secondary and tertiary sex ratios in decapods, but such data are available only for a few macruran species. Studies providing this type of information usually involve controlled breeding and crossing of parents, which might be sexually inverted in some cases, followed by sexing of the F_1 progeny (Sagi & Cohen, 1990; Malecha *et al*., 1992; Austin & Meewan, 1999; Lawrence *et al*., 2000; Benzie *et al*., 2001). Most of the researchers performing the above-mentioned studies have suggested a WZ–ZZ scheme, with the female being the heterogametic sex. Our preliminary results, obtained from group crosses between *C. quadricarinatus* females and either normal or intersex males, suggested a WZ–ZZ scheme for this species as well. The working hypothesis tested in the present study was that intersex *C. quadricarinatus* individuals are phenotypically males, with intersexual genital openings, but genotypically females.

2. Materials and methods

(i) *Study design*

The study lasted from January 1997 (P-generation) to February 2003. The approach used to test the sex determination model in *C. quadricarinatus* is described in Fig. 1. The flow chart shows the crosses that were made during this study and the expected sex ratios of the progeny. The upper part of the figure gives the details of the two plausible crosses performed in the first phase of this study. The one on the left shows the presumed genotypes of a cross between a normal male and a normal female that will result in an offspring sex ratio of 1 : 1 if the working hypothesis is correct. The one on the right shows the possible outcome of a cross between an intersex individual (as the male parent) and a normal female. The working

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hypothesis predicts that a new genotype, WW, will be obtained. If this genotype is viable and phenotypically female then the sex ratio of the offspring will be 1 : 3 (male : female). However, if it is a lethal phenotype, then the sex ratio of the offspring will be 1 : 2 (male : female).

(ii) *P-generation animals*

C. quadricarinatus males, females and intersex animals were reared in our facility at Ben-Gurion University (BGU) of the Negev, Israel. The original broodstock was transferred to Israel in 1992 from Auburn (Alabama, USA), to where it had earlier been exported from Australia. Thus, the Israeli population had undergone at least two bottlenecks and potentially also a founder effect. This primary broodstock consisted of 80 animals (1 : 1 males : females) and these were the only *C. quadricarinatus* ever to be transferred to Israel (S. Harpaz and I. Karplus, pers. commun.). For this study, the experimental animals were kept in 100 l freshwater tanks, one pair of animals per tank (i.e. one intersex or male sire together with one female). The tanks, which were kept indoors at 27 ± 2 °C under a photoperiod of 14 hours of light and 10 hours of darkness (14L : 10D), were equipped with PVC piping as shelters for the animals. Water quality was assured by circulating the water through an immersed gravel biofilter via an airlift. The animals were fed three times a week with a mixture of wheat grains and fish pellets *ad libitum*. The tanks were cleaned by siphoning off the debris. Ammonia and nitrite levels were monitored on a weekly basis. The average weight of females used in the experiments was 51·4 ± 18 g (minimum, 21 g; maximum, 99 g) and the sires were matched to them by their size.

(iii) F_1 *generation*

Figure 2*A* shows a schematic representation of the ventral side of an intersex individual with a total of four genital openings: a male pair at the bases of the fifth pair of walking legs and a female pair at the bases of the third pair of walking legs. The simplified annotation shown in Fig. 2*B* is used in subsequent figures and tables. The seven intersex combinations that were investigated in this study are shown schematically in Fig. 2*CII*, *III*. Fig. 2*CII* shows intersex combinations that have only one male genital opening and one or two female openings. Intersex individuals of this type always posses a fully developed, functional male reproductive system on the side with the male opening and an arrested previtellogenic ovary on the opposite side (Sagi *et al*., 1996). Fig. 2*CIII* shows combinations with two male genital openings. Intersex individuals with two male openings and one or two female openings have a male reproductive system

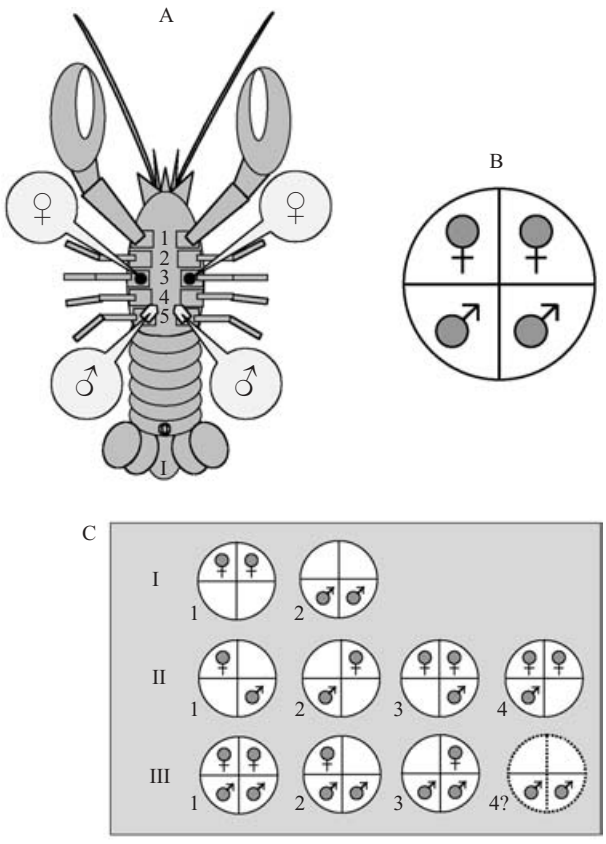

Fig. 2. (*A*) The ventral side of an intersex *C. quadricarinatus* with two female and two male genital openings (1–5 refer to the five pairs of walking legs). Female openings are present at the bases of the third pair of walking legs and male openings at the bases of the fifth pair of walking legs. (*B*) Simplification of the scheme in (*A*). (*C*) Summary of all the combinations of male and female openings investigated in this study. (*I*) Normal male and female. (*II*) Intersex individuals with only one male opening. (*III*) Intersex individuals with two male openings.

on both sides (Sagi *et al*., 1996). Intersex individuals could theoretically have only male genital openings and thus will be morphologically indistinguishable from normal males. Such an animal could be identified as an intersex only by the aberrant sex ratios of its progeny. Because it is possible that such animals do indeed exist, they have been included in group 2*CIII* (as 2*CIII4*) but with dashed scheme lines. Most of the intersex individuals found in the populations grown in Israel have one male and two female genital openings (Fig. 2*CII3*, *4*), and other combinations are very rare.

P-generation animals were paired on the basis of their genital openings. The crossed pairs, consisting of a male or an intersex and a female, were color tagged on their carapaces so that precise identification of all the crayfish in the study could be maintained over the long periods required for such an experiment. An animal that molted was re-tagged as soon as its cuticle hardened. Hatching and nursery procedures were performed as previously reported (Parnes & Sagi, 2002).

Table 1. *Sex ratios in progeny resulting from crossing* C. quadricarinatus *females with normal males at random*

Female	Progeny number	Males (%)	Females (%)	Intersex (%)	Female + intersex (%)
1	188	51·6	47·3	1·1	48·4
2	120	45·0	52·5	2·5	55·0
3	151	49·7	50·3	0·0	50·3
4	**145**	**31·0**	**46·9**	**22·1**	**69·0**
5	**49**	**34·7**	**61·2**	**4·1**	**65·3**
6	**183**	**42·6**	**56·8**	**0·5**	**57·4**
12	156	46·8	53·2	0·0	53·2
13	180	55·0	45·0	0·0	45·0
14	196	50·0	50·0	0·0	50·0
19b.1*	**233**	**51·1**	**29·2**	**19·7**	**48·9**
19b.2*	**41**	**46·3**	**43·9**	**9·8**	**53·7**
21	298	52·7	47·0	0·3	47·3
22	173	46·8	53·2	0·0	53·2
23	236	51·3	48·7	0·0	48·7
24	274	46·0	53·6	0·4	54·0
25	165	55·2	44·8	0·0	44·8
27	161	49·1	49·1	1·8	50·9
Mean ± SD	173 ± 66·9	47·3 ± 6·5	49·0 ± 6·8	3·7 ± 6·9	52·7 ± 6·5

* Replicate matings with a specific male.
Results in bold represent a significant deviation from the proposed model.

Juveniles were harvested, sexed with the aid of a dissecting microscope, separated into groups of males, females and intersex individuals, counted and weighed as a group. For each intersex individual, the combination of genital openings was recorded.

(iv) *F_2 generation*

Three P-generation females were mated with different intersex individuals as described above and the female offspring of those crosses (i.e. F_1-generation females, see chart in Fig. 1) were pooled. 40 of these females chosen at random were stocked individually in separate aquaria, either at BGU or at the Department of Aquaculture, Volcani Center, Bet Dagan, where they grew to maturity. Over 20 females from this group were mated with randomly chosen normal males and the offspring of those crosses (i.e. the F_2 generation) were reared and sexed according to the procedures described above.

(v) *Data analysis*

Data were analysed by means of the Basic Statistics software. Statistical analysis included χ^2 test for the observed sex ratios and one-way ANOVA followed by LSD test (where needed).

3. Results

Our hypothesis about the nature of the *C. quadricarinatus* sex-determination mechanism was formulated on the basis of preliminary results obtained from group crosses that were performed with either a normal male or an intersex. For female groups that were mated with an intersex crayfish, the total percentage of intersex plus female offspring was 75% of the progeny in any specific batch (data not shown). To verify these preliminary results, we performed more than 50 individual experimental crosses in the current study. For each cross, an average of 178 ± 73 (mean ± SD) juveniles were produced, with a mean weight on sexing day of 0·698 ± 0·060 g (mean ± SE). No significant differences were found between the weights of males, females and intersex animals.

17 crosses of females with normal males gave the expected 1 : 1 sex ratio, with four exceptions (Table 1, highlighted in bold): for female 6, the ratio deviated only slightly from 1 : 1 ($P = 0{\cdot}046$) and, for females 4 and 5, aberrant sex ratios were obtained ($P = 0{\cdot}000$ and 0·032, respectively). The progeny of females 4 and 5 might have been sired by intersex individuals of the type described in Fig. 2*CIII4*. The ratios obtained in cross 19b cannot be explained by the model alone, but the ratio of the sums of female and intersex offspring to that of the male agree with the model, being 1 : 1.

Table 2 shows that all 16 crosses between a female and an intersex animal having only one male genital opening (Fig. 2*CII*) gave the hypothetically predicted ratio of 1 : 3 (male : female), except for the crosses from females 3 and 6, which deviated from that ratio ($P = 0{\cdot}021$ and 0·036, respectively) but still gave a large surplus of females.

Table 3 shows that all crosses between a female and an intersex animal having two male and two female

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Table 2. *Sex ratios in progeny resulting from crossing* C. quadricarinatus *females with intersex individuals possessing only one male genital opening*

Female	progeny number	Males (%)	Females (%)	Intersex (%)	Female + intersex (%)
1	226	30·1	67·7	2·2	69·9
2	121	28·1	69·4	2·5	71·9
3	**168**	**17·3**	**79·2**	**3·6**	**82·7**
4	213	19·2	78·9	1·9	80·8
5	97	20·6	74·2	5·2	79·4
6	**56**	**12·7**	**87·3**	**0·0**	**87·3**
7	208	27·9	67·8	4·3	72·1
12a.1*	110	32·7	67·3	0·0	67·3
12a.2*	255	26·3	73·3	0·4	73·7
19c.1*	192	25·5	73·4	1·0	74·5
19c.2*	207	28·5	69·6	1·9	71·5
26	250	29·2	69·2	1·6	70·8
28	235	25·1	74·5	0·4	74·9
29	168	22·6	75·6	1·8	77·4
30	120	19·2	80·0	0·8	80·8
Mean ± SD	175 ± 61	24·3 ± 5·5	73·8 ± 5·7	1·8 ± 1·5	75·7 ± 5·5

* Replicate matings with a specific intersex individual (identified by a letter).
Results in bold represent a significant deviation from the proposed model.

Table 3. *Sex ratios in progeny resulting from crossing a female* C. quadricarinatus *with an intersex possessing two male genital openings*

Female	Progeny number	Males (%)	Females (%)	Intersex (%)	Female + intersex (%)
8	199	29·1	68·3	2·5	70·9
13d.1*	**226**	**31·4**	**63·3**	**5·3**	**68·6**
13d.2*	115	26·1	61·7	12·2	73·9
13d.3*	52	23·1	61·5	15·4	76·9
13e	215	25·1	72·1	2·8	74·9
14f.1*	279	22·6	77·4	0·0	77·4
14f.2*	119	25·2	74·8	0·0	74·8
15	371	23·7	53·6	22·6	76·3
16g.1*	215	24·7	70·2	5·1	75·3
16g.2*	208	27·9	67·3	4·8	72·1
16g.3*	54	22·2	57·4	20·4	77·8
17	291	21·3	78·7	0·0	78·7
18	183	30·1	50·3	19·7	69·9
20	174	23·0	55·2	21·8	77·0
Mean ± SD	193 ± 89	25·4 ± 3·1	65·1 ± 9·0	9·5 ± 8·8	74·6 ± 3·1

* Replicated matings with a specific intersex individual (identified by letter).
Results in bold represent a significant deviation from the proposed model.

genital openings (Fig. 2*A*, *B*, *CIII1*) conformed to a ratio of 1 : 3 (male : female), with the exception of the cross from female 13d.1, which deviated from that ratio but still gave a surplus of females ($P = 0{\cdot}026$). The percentage of intersex individuals in the progeny of these crosses was significantly higher ($P < 0{\cdot}001$) than that of the other two types of crosses described above: 9·5 ± 8·8% (mean ± SD) vs. 3·7 ± 6·9% (Table 1) and 1·8 ± 1·5% (Table 2).

The results of crosses of females with males and both intersex types are illustrated and summarized in Fig. 3. On average, the F_1 progeny from a normal sire (the left-hand pie chart) segregated into 47·3 ± 6·5% males, 49·0 ± 6·8% females and 3·7 ± 6·9% intersex, whereas the progeny from an intersex sire (the pie chart on the right) segregated into 24·9 ± 4·5% males, 69·6 ± 8·6% females and 5·5 ± 7·2% intersex. Summing the percentages for the female and intersex progeny gave 52·7 ± 6·5% for a normal sire and 75·2 ± 4·5% for an intersex parent.

To demonstrate the reproducibility of the results, reciprocal and repeated crosses with the same female

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Table 4. *Sex ratios in sets of crosses of a female* C. quadricarinatus *with both a normal male and an intersex* (*ISX*) *crayfish. Intersex type is shown as gender* (*female, F; male, M*) *at the four positions of the genital openings* (*left and right at the third and fifth pairs of walking legs*)

Intersex type	Females	Male type	Progeny number	Males (%)	Females (%)	Intersex (%)
F F	1	ISX_1 : 2	226	30·1	67·7	2·2
– M	1	Normal	188	51·6	47·3	1·1
or	2	ISX_1 : 2	121	28·1	69·4	2·5
F F	2	Normal	120	45·0	52·5	2·5
M –	3	ISX_1 : 2	168	17·3	79·2	3·6
	3	Normal	151	49·7	50·3	0·0
	4	ISX_1 : 2	213	19·2	78·9	1·9
	4	Normal	145	31·0	46·9	22·1
	5	ISX_1 : 2	97	20·6	74·2	5·2
	5	Normal	49	34·7	61·2	4·1
	6	ISX_1 : 2	56	12·5	85·7	0·0
	6	Normal	183	42·6	56·8	0·5
	19c.1*	ISX_1 : 2	192	25·5	73·4	1·0
	19c.2*	ISX_1 : 2	207	28·5	69·6	1·9
	19b.1*	Normal	233	51·1	29·2	19·7
	19b.2*	Normal	41	46·3	43·9	9·8
F F	13d.1*	ISX_2 : 2	226	31·4	63·3	5·3
M M	13d.2*	ISX_2 : 2	115	26·1	61·7	12·2
	13d.3*	ISX_2 : 2	52	23·1	61·5	15·4
	13	Normal	180	55·0	45·0	0·0
	13e	ISX_2 : 2	215	25·1	72·1	2·8
	14f.1*	ISX_2 : 2	279	22·6	77·4	0·0
	14f.2*	ISX_2 : 2	119	25·2	74·8	0·0
	14	Normal	196	50·0	50·0	0·0

* Replicate matings with a specific male (identified by a letter).
ISX_1 : 2, intersex with one male and two female openings; ISX_2 : 2, intersex with two male and two female openings.

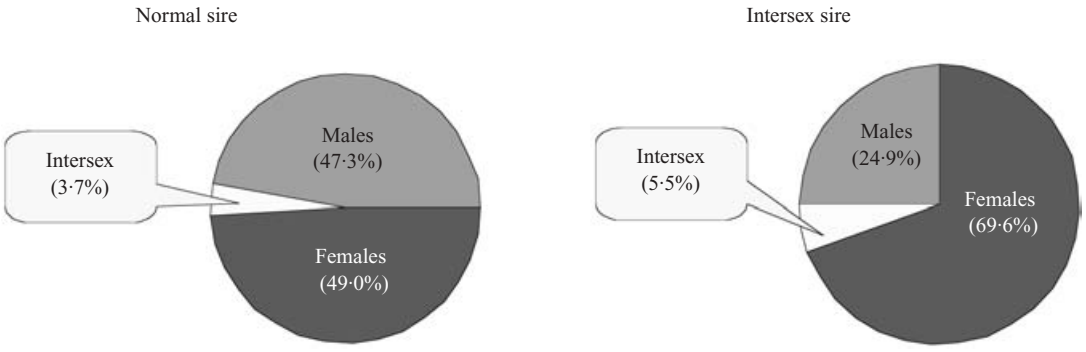

Fig. 3. A pie chart of the average percentages of male, female and intersex progeny from crosses of female *C. quadricarinatus* with normal male (left) and intersex (right).

are presented in Table 4. 24 crosses were performed in which the same female was mated once with a normal male and once with an intersex animal (1m : 2f or 2m : 2f genital openings as shown in Table 4). In some cases, the intersex cross was repeated two or three times (19c and 14f or 13d, respectively, Table 4) and, in one case, the same female was mated with two different intersex individuals (13d and 13e, Table 4). As can be seen from Table 4, almost all the sets of crosses (except for 5 and 4 with a normal male) gave progeny with the usual sex ratio of 1 : 1 for crosses with a normal male and 1 : 3 for crosses with an intersex animal.

Statistical analysis showed that only three of the 29 intersex crosses shown in Tables 2 and 3 deviated significantly from the 1 : 3 ratio (females 3, 6 and 13d.1) compared with 15 that could not be explained by a 1 : 2 model. An examination of the data for crosses

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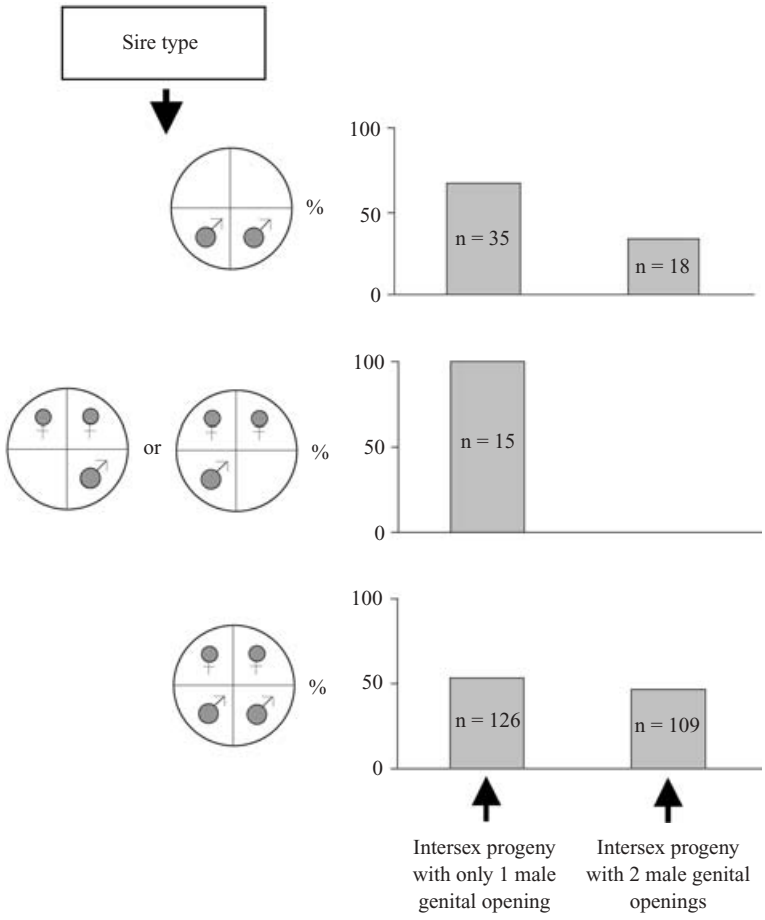

Fig. 4. Distribution of the combinations of genital opening in the F_1 intersex progeny from crosses of female *C. quadricarinatus* with different types of male and intersex individuals. The left-hand bar in each graph represents progeny with only one male opening (Fig. 2*CII*) and the right-hand bar represents progeny with two male openings and one or two female openings (Fig. 2*CIII*).

performed with the same sire but different females further validated the above-described results, in that crosses of the same normal male with different females gave a 1 : 1 sex ratio and those of the same intersex animal with different females gave a 1 : 3 ratio.

In 20 of the crosses, the genital opening combinations of all the intersex progeny were documented. Figure 4 shows that combinations having only one male opening clearly make up the majority of the intersex progeny, irrespective of the male genotype. This finding reflects the situation in free-breeding populations in Israel.

The F_2 sex ratio for crosses of normal males with F_1 females that are the progeny of an intersex sire are summarized in Table 5. According to the proposed inheritance model, the unique WW genotype composes one-third of the possible female offspring in such a cross (Fig. 1), the other two thirds being of the WZ genotype. Of the 13 crosses analysed, eight conformed to the 1 : 1 sex ratio (Table 5) and five gave an all-female/intersex result, except for cross 35. This finding fits the 1 : 2 expected ratio for WW : WZ female genotypes. The finding of all-female/intersex progeny confirms the existence, viability and fertility of the WW genotype. The single male that was produced in cross 35 could be an intersex individual with no female openings (Fig. 2*CIII4*; Table 1, crosses 4, 5).

4. Discussion

Information about secondary and tertiary sex ratios from reciprocal crosses contributes to establishing the sex-determination mechanism for a given species. For decapods, this type of information is a rare commodity: the small number of studies that have been reported were all performed on commercially important macruran species. On the basis of results from crosses of sex-reversed females (neomales) of the freshwater prawn *Macrobrachium rosenbergii* (Caridea), Malecha *et al.* (1992) concluded that the sex of this species is determined chromosomally, with females being heterogametic (ZW) and males homogametic (ZZ). Nevertheless, Malecha *et al.* stated that variation of the sex ratio among the individual crosses within mating types and evidence for environmental

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Table 5. *Sex ratio in F_2 progeny resulting from crossing a normal* C. quadricarinatus *male with F_1 females that are the daughters of an intersex sire*

Female	Progeny number	Males (%)	Females (%)	Intersex (%)	Female + intersex (%)
Progeny of females with a presumed WW genotype					
35	84	1·2	98·8	0·0	98·8
62*	312	0·0	95·5	4·5	100·0
61*	22	0·0	100·0	0·0	100·0
34	148	0·0	99·3	0·7	100·0
20	200	0·0	99·5	0·5	100·0
Mean ± SD	153 ± 111	0·2 ± 0·5	98·6 ± 1·8	1·1 ± 1·9	99·8 ± 0·5
Progeny of females with a presumed WZ genotype					
6	47	53·2	46·8	0·0	46·8
24	32	50·0	43·8	6·3	50·0
60	129	48·8	50·4	0·8	51·2
2	12	41·7	58·3	0·0	58·3
16	107	36·4	63·6	0·0	63·6
1	22	36·4	63·6	0·0	63·6
39	46	50·0	50·0	0·0	50·0
33	68	45·6	51·5	2·9	54·4
Mean ± SD	58 ± 41	45·3 ± 6·4	53·5 ± 7·5	1·2 ± 2·3	54·7 ± 6·4

* This cross was repeated and gave similar results.

Table 6. *Numbers of currently available studies on sex determining models in decapod crustaceans. Column 2 shows the number of cytological studies reporting the presence of heteromorphic chromosomes (probably the sex chromosomes) and associated references. Column 3 shows the number of studies (including the current study) reporting the experimental results from controlled crossing and breeding and associated references. Column 4 shows the heterogametic sex suggested by the authors*

Decapod group	Studies reporting chromosomes	Studies reporting crosses	Sex
Brachyura (crabs)	8 [Niiyama and also Baffoni in the reviews of Ginsburger-Vogel & Charniaux-Cotton (1982); Lecher *et al.* (1995)]	None	Male
Anomura (hermit crabs)	1 [Niiyama in the review of Lecher *et al.* (1995)]	None	Male
Macrura (lobsters, spiny lobsters, crayfish, shrimps and prawns)	1 [Lakra & Kumar (1995)]	5 [Sagi & Cohen (1990); Malecha *et al.* (1992); Austin & Meewan (1999); Benzie *et al.* (2001)]	Female/Male

effects implied that the complete explanation for sex-determination in this species is more complex. The study of Sagi & Cohen (1990), albeit on only two crosses of sex-reversed *M. rosenbergii* males (neo-females) with normal males, agreed with the model suggested by Malecha *et al.* (1992). Benzie *et al.* (2001) obtained a significantly skewed sex ratio in favor of males in hybrid juveniles from crosses between two closely related species of shrimp (Penaeidea), possibly suggesting that females are the heterogametic sex in penaeids. In their preliminary study, Austin & Meewan (1999) performed group crosses between two sub-populations of the Australian freshwater crayfish *Cherax destructor* but could not identify the mechanism of sex determination in that species. Their results did, however, suggest a chromosomal mechanism in which the female is the heterogametic sex. Lawrence *et al.* (2000) working on six Australian freshwater crayfish species, all belonging to the genus *Cherax*, have reported the production of all-male progeny in a cross performed between two of them that are geographically isolated (*Cherax rotundus* × *Cherax*

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albidus). However, these authors did not suggest a possible mechanism underlying this phenomenon.

To the best of our knowledge, no such crossbreeding studies have been carried out in brachyuran or anomuran species. Table 6 summarizes the numbers of currently available studies on sex-determining models in decapod crustaceans. Obviously, the data on the three large groups that compose the Decapoda are not the same; whereas there are karyological data for Brachyura and Anomura, there are no crossbreeding results for these groups. The same is true in reverse for the Macrura (i.e. crossbreeding data are available but karyological data are limited solely to chromosome numbers and morphology) (Morelli *et al*., 1998), except for a single report on two *Macrobrachium* species (Lakra & Kumar, 1995). Lakra & Kumar claimed to have found a pair of heteromorphic chromosomes in the males of only one of the species under investigation (*Macrobrachium scabriculum*) but not in the other (*Macrobrachium idella*).

The present study is the first time in decapod crustaceans that a genetic sex determination model has been so heavily laden with results from individual crosses. The results summarized below clearly support a model for sex determination in *C. quadricarinatus* in which the female is the heterogametic sex.

- The proportion of intersex individuals, irrespective of the actual numbers, always complements that of the females, whereas the proportion of male progeny remains constant.
- A cross of a normal male with a female yields an F_1 offspring ratio of 1 : 1 (male : female) versus a 1 : 3 ratio obtained when the sire is an intersex.
- Crossing the F_1 female progeny of an intersex sire with a normal male reveals that those F_1 females can be divided into two groups: two-thirds of these females yielded the usual 1 : 1 offspring ratio, whereas one-third produced only female/intersex progeny. The latter findings support the hypothesized existence of 25% WW genotypes among all possible zygotes in a cross between an intersex and a female (Fig. 1).

Our results and the above-described studies of other macruran species support the concept of a chromosome-based sex-determination mechanism with the female as the heterogametic sex.

Although the present study has shown that the suggested model does indeed have a firm foundation, we still lack the ultimate direct proof for the proposed sex-determination mechanism. This proof could come from a cross of a normal male and an intersex individual functioning as a female, resulting in a 1 : 1 sex ratio. In order to perform such a cross, attempts were made in our laboratory to cause functional sex inversion in a *C. quadricarinatus* intersex individual by ablating the single androgenic gland. Our results confirmed that andrectomized *C. quadricarinatus* intersex individuals do indeed go through a physiological sex inversion (Sagi *et al*., 2002), but successful egg laying and brood holding have not yet been observed. In addition to the above-described challenge, we have still to clarify the mechanism that controls the proportion of intersex individuals in the progeny of a given cross.

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References

Austin, C. M. & Meewan, M. (1999). A preliminary study of primary sex ratios in the freshwater crayfish, *Cherax destructor* Clark. *Aquaculture* **174**, 43–50.

Benzie, J. A. H., Kenway, M. & Ballment, E. (2001). Growth of *Penaeus monodon* × *Penaeus esculentus* tiger prawn hybrids relative to the parental species. *Aquaculture* **193**, 227–237.

Brummett, R. E. & Alon, N. C. (1994). Polyculture of Nile tilapia (*Oreochromis niloticus*) and Australian red claw crayfish (*Cherax quadricarinatus*) in earthen ponds. *Aquaculture* **122**, 47–54.

Ginsburger-Vogel, T. & Charniaux-Cotton, H. (1982). Sex determination. In *The Biology of Crustacea: Embryology, Morphology, and Genetics*, vol. 2 (ed. L. G. Abele), pp. 257–281. New York: Academic Press.

Karplus, I., Barki, A., Cohen, S. & Hulata G. (1995). Culture of the Australian red-claw crayfish (*Cherax quadricarinatus*) in Israel. I. Polyculture with fish in earthen ponds. *Israeli Journal of Aquaculture – Bamidgeh* **47**, 6–16.

Lakra, W. S. & Kumar, P. (1995). Studies on the chromosomes of two freshwater prawns, *Macrobrachium idella* and *M. scabriculum* (Crustacea, Decapoda, Palaemonidae). *Cytobios* **84**, 147–156.

Lawrence, C. S., Morrissy, N. M., Vercoe, P. E. & Williams, I. H. (2000). Hybridization in Australian freshwater crayfish – production of all-male progeny. *Journal of the World Aquaculture Society* **31**, 651–658.

Lecher, P., Defaye, D. & Noel, P. (1995). Chromosomes and nuclear DNA of crustacea. *Invertebrate Reproduction and Development* **27**, 85–114.

Legrand, J. J., Legrand-Hamelin E. & Juchault, P. (1987). Sex determination in crustacea. *Biological Reviews of the Cambridge Philosophical Society* **62**, 439–470.

Malecha, S. R., Nevin, P. A., Ha, P., Barck, L. E., Lamadrid-Rose, Y., Masuno, S. & Hedgecock, D. (1992). Sex-ratios and sex-determination in progeny from crosses of surgically sex-reversed freshwater prawns, *Macrobrachium rosenbergii*. *Aquaculture* **105**, 201–218.

Medley, P. & Rouse, D. B. (1993). Intersex Australian red claw crayfish (*Cherax quadricarinatus*). *Journal of Shellfish Research* **12**, 93–94.

Morelli, M., Le Dean, L., Vonau, V. & Diter, A. (1998). Karyotype of the marine shrimp *Penaeus indicus* (Crustacea, Decapoda) established by using an image analysis system. *Ophelia* **49**, 83–95.

Parnes, S. & Sagi, A. (2002). Intensification of redclaw crayfish *Cherax quadricarinatus* culture. I. Hatchery and nursery system. *Aquacultural Engineering* **26**, 251–262.

Rouse, D. B., Austin, C. M. & Medley, P. B. (1991). Progress toward profits? Information on the Australian crayfish. *Aquaculture Magazine* **17**, 46–56.

Rudolph, E. H. (1999). Intersexuality in the freshwater crayfish *Samastacus spinifrons* (Philippi, 1882) (Decapoda, Parastacidae). *Crustaceana* **72**, 325–337.

Sagi, A. & Cohen, D. (1990). Growth, maturation and progeny of sex-reversed *Macrobrachium rosenbergii* males. *World Aquaculture Report* **21**, 87–90.

Sagi, A., Khalaila, I., Barki, A., Hulata, G. & Karplus, I. (1996). Intersex red claw crayfish, *Cherax quadricarinatus* (von Martens): functional males with pre-vitellogenic ovaries. *Biological Bulletin* **190**, 16–23.

Sagi, A., Manor, R., Segall, C., Davis, C. & Khalaila, I. (2002). On intersexuality in the crayfish *Cherax quadricarinatus*: an inducible sexual plasticity model. *Invertebrate Reproduction and Development* **41**, 27–33.

Thorn, M. J. & Fielder, D. R. (1991). The red cuticle on the claw of male *Cherax quadricarinatus* (Decapoda: Parastacidae). *Memoirs of the Queensland Museum* **31**, 277.