

Endocrine Balance Between Male and Female Components of the Reproductive System in Intersex *Cherax quadricarinatus* (Decapoda: Parastacidae)

ISAM KHALAILA, SIMY WEIL, AND AMIR SAGI*

Department of Life Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

ABSTRACT Intersex individuals of the crayfish *Cherax quadricarinatus* are functional males that also possess arrested ovaries. To study the role of eyestalk vs. androgenic gland factors in regulating the functional balance between the different components of the intersex reproductive system, andrectomy and/or unilateral destalking were performed. Andrectomized intersex specimens had atrophied testes and sperm ducts and showed a reduction in the number of testicular spermatogenic lobules. Large oocytes developed in their ovarian lobes, and the Gonadosomatic Index reached 1.60 ± 0.36 compared with 0.21 ± 0.03 for the control. In the polypeptide profiles of the ovarian lobes from andrectomized individuals, the 177-, 150-, and 106-kDa polypeptides predominated, resembling the profile of the secondary vitellogenic ovary. The andrectomized individuals neither lost their male external characteristics, such as the red patch on the propodus, nor developed ovigerous setae on their male-like pleopods. Unilateral eyestalk ablation did not cause significant differences in the male or the female components of the reproductive system compared with the control group. The maturation of the permanently arrested ovary and the arrest of the testis in andrectomized intersex individuals illustrated the central role of the androgenic gland in maintaining the endocrine balance in intersex *C. quadricarinatus*. *J. Exp. Zool.* 283:286–294, 1999.

© 1999 Wiley-Liss, Inc.

In populations of the crayfish *Cherax quadricarinatus*, intersex individuals, having both male and female openings, have been observed (Medley and Rouse, '93; Brummett and Alon, '94). Intersex individuals that possess a male opening on one of the fifth walking legs and a female opening on the opposite third walking leg also possess an androgenic gland and an active testis on the side of the male opening and an ovary on the opposite side. These intersex individuals have male secondary sexual characteristics and function as males, and their ovarian component is always previtellogenic. Thus, in *C. quadricarinatus*, intersexuality constitutes an aspect of nonfunctional hermaphroditism (Sagi et al., '96a), unlike species such as the burrowing crayfish, *Parastacus nicoleti*, in which intersexuality is a manifestation of functional hermaphroditism (Rudolph, '95).

Although the chemical nature and structure of the androgenic hormone have not been determined in decapods, the role of the androgenic gland in sex differentiation and gonad regulation in many malacostraca crustaceans is well known (Charniaux-Cotton, '54, '55, '57): male differentiation of the genital apparatus and secondary sexual char-

acteristics are controlled by a hormone synthesized by the androgenic gland, which is separated from the gonads (Charniaux-Cotton and Payen, '88). The androgenic gland also regulates spermatogenic activity in the testes (Payen, '73; Taketomi et al., '96) and inhibits ovarian maturation (Meusy and Payen, '88; Taketomi and Nishikawa, '96). Ablation of the androgenic gland (andrectomy) results in female differentiation in isopods, amphipods, and decapods (Charniaux-Cotton, '64; Nagamine et al., '80b; Sagi et al., '90).

In protandric hermaphrodites, the androgenic gland degenerates to allow the ovary and secondary sexual characteristics of the female to develop. Delayed appearance of the androgenic gland in protogonic hermaphrodites primarily allows female differentiation (Charniaux-Cotton, '75).

Reproduction in crustaceans is neuroregulated by hormones from the X organ–sinus gland (a com-

Grant sponsor: U.S.-Israel Binational Science Foundation; Grant number: 93-231.

*Correspondence to: Amir Sagi, Department of Life Sciences, Ben-Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel.

Received 23 February 1998; Accepted 7 July 1998.

plex located in the eyestalk). Gonad-inhibiting hormone, produced in this gland, apparently acts directly on the ovaries, whereas its action on the testes is thought to be indirect, via a direct effect on the androgenic gland (Fingerman, '95; Sagi et al., '97a). Eyestalk ablation also increased RNA synthesis in the androgenic gland (Brockenbrough-Foulks and Hoffman, '74). The negative regulation of gonad-inhibiting hormone on the ovaries is illustrated by the induction of vitellogenesis after bilateral eyestalk ablation (destalking) (Wilder et al., '94). Therefore, the role of the eyestalk in the endocrine balance between male and female components of the reproductive system in intersex individuals was addressed by our group.

In this study, we andrectomized and/or unilaterally destalked intersex individuals of the crayfish *C. quadricarinatus* and examined the effect of these endocrine manipulations on male and female primary and secondary sexual characteristics. This way, the role of androgenic gland and eyestalk-borne hormones in the endocrine balance between male and female components of the reproductive system could be investigated.

MATERIALS AND METHODS

Animals

Intersex *C. quadricarinatus* individuals, whose reproductive systems were composed of a female component (including an ovary and oviduct) on one side and a male component (including testis, sperm duct, and androgenic gland) on the other side (Sagi et al., '96b), were collected at the Aquaculture Research Station Dor, Israel, approximately 9 months after hatching. The intersex individuals were held in our facility at the Ben-Gurion University of the Negev for 1 month for acclimation prior to the experiment. Normal immature and vitellogenic females were used as references for the ovarian polypeptide profile.

Sixty-four intersex individuals, carapace length 30 to 50 mm, were sorted by size into 16 groups, four crayfish in each group. Each animal was subjected to one of the following treatments: sham operation (control), unilateral destalking, andrectomy, or both andrectomy and unilateral destalking. Sham operation comprised electrical cauterization of the coxa of the fifth walking leg on the side opposite that of the male opening. Unilateral eyestalk ablation was performed because such treatment had previously caused gonad maturation in females with an ovarian stage similar to that of intersex individuals (Sagi et al., '97b), whereas bilateral eyestalk

ablation resulted in uncontrolled molting and mortality. Andrectomy was performed by cauterization through the articular membrane above the coxa of the fifth walking leg, which had a male opening (Huxley, 1880). Destalking was performed with a pair of scissors, followed by cauterization to prevent bleeding. Each group was kept in a glass aquarium (40 × 50 × 40 cm) with polycarbonate partitions that formed four identical compartments, one for each individual. The temperature was maintained at 27 ± 2°C, and a photoperiod of 14L:10D was applied. Water quality was assured by circulating the water through a gravel biofilter. Food was supplied ad libitum.

Morphological and anatomical observations

Fifty days after endocrine manipulation, each individual was weighed and examined externally for endopod morphology (Sagi et al., '96a), carapace length, and the presence of the red patch. For anatomical studies, the animals were anesthetized in ice-cold water and dissected on ice. The weight of each ovarian lobe was recorded and the Gonadosomatic Index (GSI) was calculated ($\frac{\text{Ovarian lobe Wt.} \times 2}{\text{Body Wt.}} \times 100$). The weights of each testis and sperm duct were recorded, and the relative weights of these organs were calculated ($\frac{\text{Organ Wt.} \times 2}{\text{Body Wt.}} \times 100$). Intersex individuals possess only half of the reproductive system of each sex; thus, both the relative weight of male components and the GSI of ovarian components were calculated as if the organs were paired. This allows for comparison with normal individuals. The diameters of 15 fresh oocytes were measured under a light microscope. The colors and shapes of the male and female components of the reproductive system were recorded.

Polypeptide profiles

Ovarian lobes were homogenized individually on ice with 0.05 M Tris-HCl buffer, pH 7.4, supplemented with the following antiproteases: 0.8 mM benzamidine, 0.1 mM PMSF, 1 µg/mL leupeptin, 1 µg/mL aprotinin, and 1 mM EDTA. The samples were centrifuged at 10,800g for 15 min at 4°C. The pellet was resuspended in the above-mentioned buffer supplemented with 1% C₁₂E₉ (polyoxyethylene 9-lauryl ether polidocanol) for 30 min on ice and recentrifuged. Total protein in the supernatant was determined (Bradford, '76). The polypeptide profiles of samples (approximately 35 µg of protein per lane) were characterized by 7% mini-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

PAGE) (Laemmli, '70) and were stained either with Coomassie blue or with the cationic carbocyanine dye "Stains all" (King and Morrison, '76).

Histology

Ovarian and testicular tissues were fixed in Bouin solution for 24 hr, embedded in Paraplast, cross-sectioned at 5 μm , and stained with hematoxylin and eosin. Lobules in different spermatogenic stages were counted in three randomly chosen areas of 1 mm^2 /section. Sections were taken from two andrectomized and two sham-operated individuals.

Statistical analysis

Changes in weight, GSI, oocyte diameter, and abundance of testicular lobules were analyzed using ANOVA, followed by LSD test. Survival rate, maturing ovary, and molting were analyzed by the chi-square test. Probabilities below 0.05 and 0.001 were considered significant and highly significant, respectively.

RESULTS

Secondary sexual characteristics, such as the red patch on the propodus and plumose setae on the pleopods, remained unchanged throughout the experiment in all individuals. The survival rate was not significantly different among treatment groups: 88% for the sham-operated group, 81% for the destalked and the andrectomized groups, and 63% for the group that underwent both andrectomy and eyestalk ablation (Table 1).

All the surviving sham-operated individuals had arrested ovaries with small white-yellowish oocytes. Their sperm ducts were milky white and full of spermatophores (Fig. 1A). Twelve of the destalked individuals had arrested ovaries and milky white sperm ducts. Only one destalked animal had a maturing ovary with big creamy-green oocytes. The testis of this individual had degenerated, and the sperm duct was thin and transparent. Eleven andrectomized individuals (85%) and

seven animals from the group that underwent both andrectomy and destalking (70%) had maturing ovaries, with big creamy-green oocytes. Their sperm ducts were thin and transparent (Table 1, Fig. 1B). The remainder of the crayfish from these groups had arrested ovaries (Table 1). A significant difference was found between the number of maturing ovaries in the two andrectomized groups and in the other two groups (Table 1).

Andrectomy resulted in a significant decrease in molting rate compared with the sham-operated and destalked groups (Table 1).

The relative weights of the male components of the reproductive systems (testis + sperm duct) at the end of the experiment were similar to those of the sham-operated (1.21 ± 0.06) and destalked (1.11 ± 0.08) groups (Fig. 2). A similarity was also found between the andrectomized group (0.57 ± 0.06) and the group that underwent both andrectomy and destalking (0.64 ± 0.07). Highly significant differences ($P \leq 0.001$) in the relative weights of the male reproductive system were found between the two andrectomized groups and the other two groups (Fig. 2). Destalked individuals showed a significant decrease ($P \leq 0.05$) in the relative weight of the testis (0.27 ± 0.04) compared with the sham-operated individuals (0.37 ± 0.03). Yet, both groups were not significantly different from the andrectomized individuals for this parameter (Fig. 2).

The relative weight of the ovarian component in the sham-operated group was the smallest, but it was not significantly different from that in the destalked group, 0.21 ± 0.03 and 0.37 ± 0.15 , respectively. The relative weight of the ovarian component in the group subjected to both andrectomy and destalking was slightly higher (not significantly) than that in the andrectomized group, 1.78 ± 0.43 and 1.60 ± 0.36 , respectively. There were highly significant differences ($P \leq 0.001$) between the relative weights of the ovarian component of the two andrectomized groups and the other two groups (Fig. 3).

TABLE 1. Effect of the various endocrine interventions on molt, survival, and gonad maturation in intersex *C. quadricarinatus*¹

Treatment	Sham operated (%)	Destalked (%)	Andrectomized (%)	Destalked and andrectomized (%)
Molting	28.6 ^a	30.8 ^a	0 ^b	10 ^{ab}
Maturing ovary	0 ^c	7.7 ^c	84.6 ^d	70 ^d
Arrested ovary	100 ^e	92.3 ^e	15.4 ^f	30 ^f
Mortality	12.5	18.8	18.8	37.5

¹Different superscript letters represent significant differences between treatments (chi-square $df = 1$, $P \leq 0.05$).

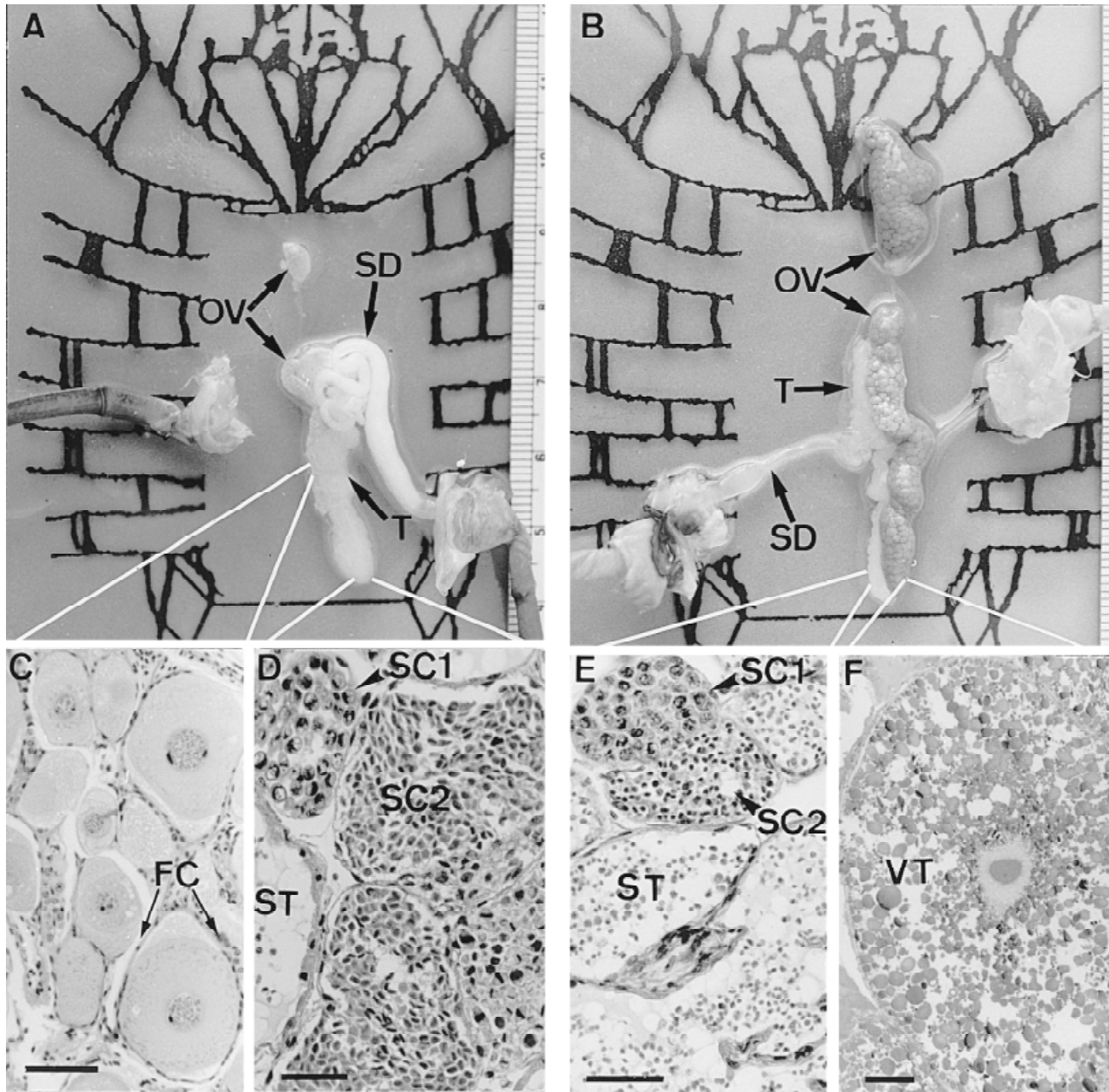


Fig. 1. Reproductive systems of sham-operated (A) vs. andrectomized (B) intersex *C. quadricarinatus* individuals and transverse sections of an ovary (C) and testes (D) from a sham-operated intersex individual and from an andrectomized intersex individual (F and E). OV indicates ovary; T, testes;

SD, sperm duct; FC, follicular cell; VT, vitellin (yolk granules); SC1, primary spermatocytes; SC2, secondary spermatocytes; and ST, spermatids. Bars represent 0.1 mm in C and F, and 0.05 mm in D and E.

At the end of the experiment, the populations of large oocytes of the two andrectomized groups had reached mean diameters of $1135 \pm 92 \mu\text{m}$ and $1308 \pm 139 \mu\text{m}$, respectively. These diameters were significantly higher than those of the sham-operated and the destalked groups, $528 \pm 44 \mu\text{m}$ and $670 \pm 87 \mu\text{m}$, respectively. The

mean diameter of the small oocyte population did not differ significantly among all four groups (Fig. 4).

Maturing ovarian lobes of andrectomized individuals and arrested ovaries of sham-operated individuals were investigated histologically because these groups represented significantly different

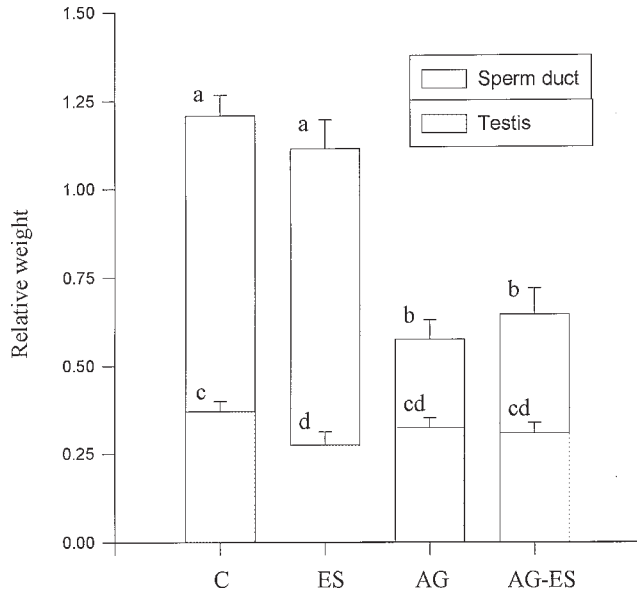


Fig. 2. Effect of various endocrine interventions on relative weights of the male components of the reproductive system in intersex *C. quadricarinatus* individuals. C indicates control (sham operation); ES, eyestalk ablation; AG, androgenic gland ablation; and AG-ES, androgenic gland and eyestalk ablation. The letters a and b represent significant differences in whole reproductive system relative weight and the relative weight of the sperm duct. Letters c and d represent significant difference in the relative weight of the testis alone (ANOVA followed by LSD test, $P \leq 0.05$).

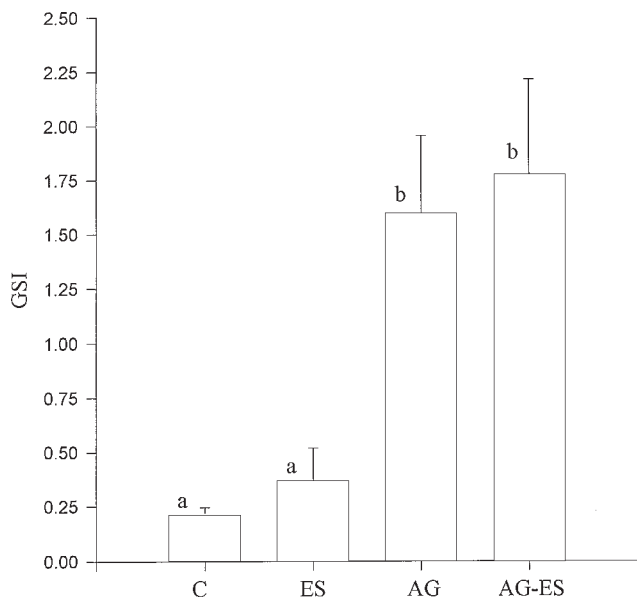


Fig. 3. Effect of various endocrine interventions on the ovarian relative weight (GSI) of intersex *C. quadricarinatus* individuals. C indicates control (sham operation); ES, eyestalk ablation; AG, androgenic gland ablation; and AG-ES, androgenic gland and eyestalk ablation. Error bars represent SE; bars labeled with different letters are significantly different (ANOVA followed by LSD test, $P \leq 0.05$).

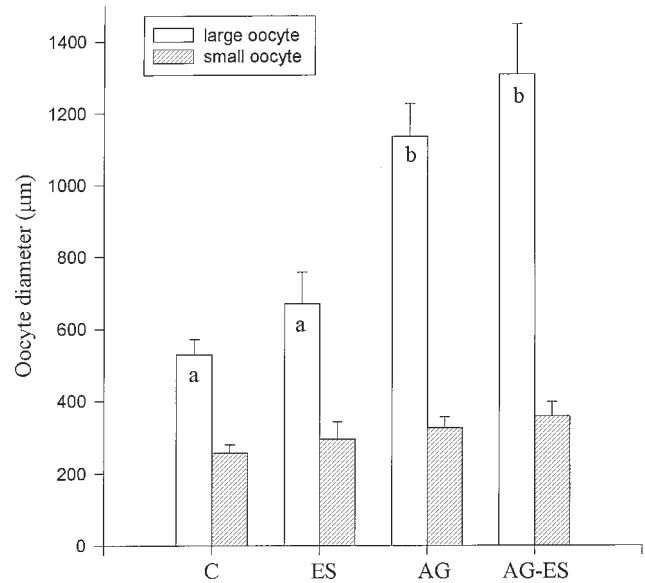


Fig. 4. Effect of various endocrine interventions on the oocyte diameters (μm) of the two different oocyte populations in the ovarian component of intersex *C. quadricarinatus* individuals. C indicates control (sham operation); ES, destalked; AG, andrectomized; and AG-ES, androgenic gland and eyestalk ablation. Error bars represent SE; bars labeled with different letters are significantly different (ANOVA followed by LSD test, $P \leq 0.05$).

ovaries with respect to GSI and oocyte diameter (Figs. 3 and 4, respectively). The oocytes were surrounded by follicular cells and contained lipoprotein vesicles, resembling the early-maturation-stage oocyte of an ovary in secondary vitellogenesis (Fig. 1F). Sections of ovarian lobes from a sham-operated intersex individual contained smaller oocytes with no yolk globules, i.e., oocytes resembling late-perinuclear to lipid-stage oocytes of a primary vitellogenic ovary (Fig. 1C).

The polypeptide profile of the ovarian lobe of an andrectomized intersex animal (with an oocyte diameter of $1362 \pm 44 \mu\text{m}$) showed three prominent polypeptides, with high molecular weights of approximately 177, 150, and 106 kDa, in a Coomassie blue-stained SDS-PAGE (Fig. 5, lane C). These three polypeptides were less prominent in the polypeptide profile of a sham-operated intersex ovarian lobe ($548 \pm 110 \mu\text{m}$) (Fig. 5, lane B). A significant similarity was evident between the polypeptide profile of the ovary of an immature female ($380 \pm 55 \mu\text{m}$) (Fig. 5, lane A) and of that of a sham-operated intersex individual (Fig. 5, lane B). The polypeptide profile of the andrectomized individual was the same as that of the ovary from mature female ($1350 \pm 123 \mu\text{m}$) (Fig.

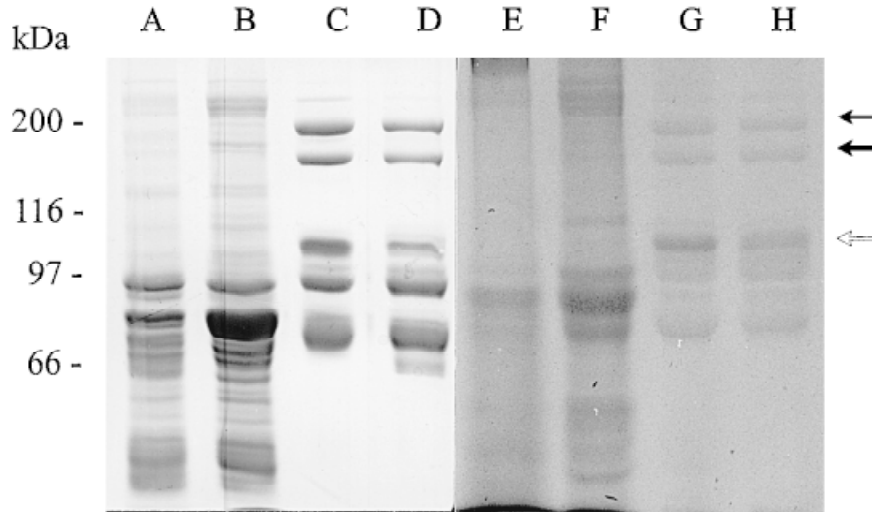


Fig. 5. SDS-PAGE of ovarian polypeptides from an immature female (A and E), a control intersex individual (B and F), an androrectomized intersex individual (C and G), and a vitellogenic female (D and H). A–D were stained with

Coomassie blue and E–H were stained with carbocyanine dye (“Stains all”). Arrows indicate major yolk polypeptides. White arrow indicates “Stains-all” positive polypeptide.

5, lanes C and D, respectively). The change in the polypeptide profile after androrectomy was best illustrated by the presence of the “Stains all,” negatively charged, 106-kDa polypeptide only in the maturing ovarian lobes of androrectomized intersex individuals and vitellogenic females (Fig. 5, lanes G and H, respectively).

Histological sections of the testis revealed a reduction in the relative abundance of spermatogenic lobules in androrectomized intersex individuals compared with the testes of sham-operated individuals (Fig. 1D and E, and Table 2). Lobules in metaphase and those containing spermatogonia and primary and secondary spermatocytes were significantly less abundant in the testes of the androrectomized individuals and were more frequently observed in the testes of sham-operated intersex individuals (Fig. 1E and D, and Table 2). Lobules containing spermatids were significantly more abundant in the testes of androrectomized individuals.

DISCUSSION

Androrectomy in *C. quadricarinatus* intersex individuals allowed secondary vitellogenesis to take place, as seen by the change in the diameter of the oocytes (Fig. 4), the GSI (Fig. 3), and the polypeptide profile of the cytosolic component of the oocyte (Fig. 5). This finding is in keeping with the report of Charniaux-Cotton and Payen ('85) that in protandric hermaphrodites, the androgenic gland inhibits secondary vitellogenesis in the oocytes, but primary vitellogenesis is permitted in the presence of the androgenic gland.

When crustacean yolk protein is subjected to SDS-PAGE, the protein is denatured into a number of polypeptide subunits, from 2 to 8, as in *Macrobrachium rosenbergii* (Derelle et al., '86) and *Penaeus monodon* (Chang et al., '93), respectively. In our study, three prominent polypeptides—177, 150, and 106 kDa—that are predominant in ovaries of vitellogenic females were found in the polypeptide profile of the androrectomized intersex

TABLE 2. Effect of androgenic gland ablation on the relative abundance of testicular lobules containing germ cells at different spermatogenic stages of intersex *C. quadricarinatus*¹

Treatment	Content of lobules		
	Spermatogonia and primary spermatocytes (%)	Secondary spermatocytes (%)	Spermatids (%)
Androrectomized	11.8 ± 3.3 ^a	15.6 ± 3.2 ^a	72.6 ± 6.2 ^c
Sham operated	25.9 ± 4.0 ^b	30.1 ± 5.4 ^{bd}	44.0 ± 6.5 ^d

¹Superscript letters represent significant differences between treatments (ANOVA followed by LSD, *P* ≤ 0.05).

ovary (Fig. 5C and D). These three polypeptides are most probably major yolk polypeptides because they are less prominent in the ovaries of both immature females and sham-operated intersex individuals. Moreover, the 106-kDa polypeptide, a "Stains all"-positive polypeptide (Fig. 5G and H), which is specific to secondary vitellogenesis (U. Abdu, personal communication), was present in the ovary of an andrectomized intersex individual. Yet, this polypeptide was totally undetectable in immature and sham-operated intersex ovaries. Thus, its presence in the ovaries of andrectomized intersex individuals clearly indicates the occurrence of secondary vitellogenesis. Our results are also consistent with the results of a recent study in which implantation of androgenic glands in immature females of the crayfish *Procambarus clarkii* inhibited vitellogenesis (Taketomi and Nishikawa, '96).

Our histological observations of the testes showed that andrectomy in intersex *C. quadricarinatus* individuals caused a decrease in the relative abundance of lobules containing primary and secondary spermatocytes, the majority of lobules containing spermatids. This decrease could be due to an arrest of new cycles of spermatogenesis, i.e., an arrest of mitotic activity after removal of the androgenic gland. It seems that the presence of the androgenic gland does not affect the conversion of primary spermatocytes to secondary spermatocytes and spermatids but rather the rate of sperm release from the testis into the sperm duct. It is also possible that in our study the sperm duct was blocked or partially destroyed because andrectomy was done by cauterization. Either possibility—the physical blockage of the sperm duct, as shown by the accumulation of spermatids, or a role of the sperm duct in the regulation of spermatogenesis (Nakamura, '92; Nagasawa et al., '95)—could account for our findings. On the other hand, our results agree with previous reports that the androgenic gland modulates spermatogenesis in the amphipod *Orchestia gammarella* and in the American crayfish *Cambarus bartonii bartonii* (Charniaux-Cotton, '60; Puckett, '64). Our results are also supported by recent circumstantial evidence showing that spermatogenic activity is related to the development of the androgenic gland throughout the juvenile development in male *P. clarkii* (Taketomi et al., '96). The presence of a few primary and secondary spermatocytes in the testes of andrectomized intersex individuals at the end of the experiment may be due to the relatively short experimental period (50 days) or the possibility that

the androgenic gland does indeed regulate the intensity of spermatogenesis (Touir, '77).

When the external male sexual characteristics are formed in the gonochorectic shrimp, the androgenic gland is not needed for their maintenance (Touir, '77). In our experiment, the external sexual characteristics of the andrectomized intersex crayfish remained unchanged, including the red patch on the propodus and the morphology of the pleopods. In this respect, the experimental period (50 days) could have been too short for the degeneration of the red patch or for the development of female ovigerous setae on the pleopods. The intervention could also have been too late to cause such external changes. Previous experiments in the prawn *M. rosenbergii* showed the development of female secondary sexual characters after andrectomy (Nagamine et al., '80b; Sagi et al., '90). The development of male secondary sexual characters after implantation of the androgenic gland has been reported in *M. rosenbergii* and in *P. clarkii* (Nagamine et al., '80a; Taketomi and Nishikawa, '96). Unlike our experiment, these latter experiments lasted for a longer period (more than 6 months) and were conducted on juvenile individuals.

It is well known that eyestalk-borne hormones have a moderating effect on the reproductive system of decapod crustaceans (Fingerman, '95; Sagi et al., '97a). Eyestalk neuropeptides apparently act directly on the female ovaries (Charniaux-Cotton and Payen, '88; Quackenbush, '91; Fingerman, '95), whereas in males their action on the testes was suggested to be indirect via a direct effect on the androgenic gland (Adiyodi, '84; Gupta et al., '89; Hasegawa et al., '93); for example, spawning activity in young *C. quadricarinatus* females increased after unilateral destalking (Sagi et al., '97b). Surprisingly, in *C. quadricarinatus* intersex individuals, unilateral destalking (including both unilateral destalking and andrectomy) had no significant effect on the GSI and oocyte diameter in the ovary. Despite the fact that the eyestalk has a proven moderating effect on the reproductive system of *C. quadricarinatus*, both male and female (Sagi et al., '97b; unpublished data), the present study showed that the androgenic gland plays the dominant role in permanently inhibiting vitellogenesis while stimulating spermatogenesis. However, a role of eyestalk-borne hormones could not be entirely ruled out because bilateral eyestalk ablation had not been feasible. This study strongly supports the notion that androgenic gland action keeps intersex individuals

functioning as males and gives rise to nonfunctional hermaphroditism. The central regulatory role of the androgenic gland was confirmed by the decrease in molting frequency of andrectomized intersex individuals, which have shifted energy into female reproduction.

Although the androgenic hormone has not been identified in any species of decapod, the androgenic hormone of an isopod has recently been identified (Okuno et al., '97). The central role of the androgenic gland in regulating intersexuality of *C. quadricarinatus* might serve as an instrumental model in the current effort to identify the androgenic hormone of decapod Crustacea.

ACKNOWLEDGMENTS

We thank Mr. Yoav Eran and Mr. Dan Joseph, Aquaculture Research Station Dor, for the supply of crayfish, and Ms. Inez Mureinik for her editorial review. This study was supported by a fellowship from the Israeli Ministry of Science to I.K. A.S. is the incumbent of the Judith and Murray Shusterman Chair for Career Development in Physiology.

LITERATURE CITED

- Adiyodi RG. 1984. Seasonal changes and the role of eyestalks in the activity of the androgenic gland of the crab, *Paratelphusa hydrodromous* (Herbst). *Comp Physiol Ecol* 9:427-431.
- Bradford M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 72:248-254.
- Brockenbrough-Foulks N, Hoffman DL. 1974. The effects of eyestalk ablation and β -ecdysone on RNA synthesis in the androgenic gland of the protandric shrimp, *Pandalus platyceros* Brandt. *Gen Comp Endocrinol* 22:439-447.
- Brummett RE, Alon NC. 1994. Polyculture of Nile tilapia (*Oreochromis niloticus*) and Australian red claw crayfish (*Cherax quadricarinatus*) in earthen ponds. *Aquaculture* 122:47-54.
- Chang FC, Lee FY, Huang YS. 1993. Purification and characterization of vitellin from the mature ovaries of prawn, *Penaeus monodon*. *Comp Biochem Physiol* 105:409-414.
- Charniaux-Cotton H. 1954. Découverte chez un Crustacé Amphipode (*Orchestia gammarella*) d'une glande endocrine responsable de la différenciation des caractères sexuels primaires et secondaires mâles. *CR Acad Sci Paris* 239:780-782.
- Charniaux-Cotton H. 1955. Le déterminisme hormonal des caractères sexuels d'*Orchestia gammarella* (Crustacé Amphipode). *CR Acad Sci Paris* 240:1487-1489.
- Charniaux-Cotton H. 1957. Croissance, régénération et déterminisme endocrinien des caractères sexuels d'*Orchestia gammarella* (Pallas) (Crustacé Amphipode). *Ann Sci Nat* 19:411-559.
- Charniaux-Cotton H. 1960. Sex determination. In: Waterman TH, ed. *The physiology of Crustacea*, vol 1. New York: Academic Press. p 411-447.
- Charniaux-Cotton H. 1964. Endocrinologie et génétique du sexe chez les crustacés supérieurs. *Ann Endocrinol* 25:36-42.
- Charniaux-Cotton H. 1975. Hermaphroditism and gynandromorphism in Malacostracan Crustacea. In: Reinboth R, editor. *Intersexuality in the animal kingdom*. Berlin: Springer-Verlag. p 91-105.
- Charniaux-Cotton H, Payen G. 1985. Sexual differentiation. In: Bliss DE, Mantel LH, editors. *The biology of Crustacea*. New York: Academic Press. p 217-299.
- Charniaux-Cotton H, Payen G. 1988. Crustacean reproduction. In: Laufer H, Downer RGH, editors. *Endocrinology of selected invertebrate types*. New York: Alan R. Liss. p 279-303.
- Derelle E, Grosclaude J, Meusy JJ, Junera H, Martin M. 1986. ELISA titration of vitellogenin and vitellin in the fresh water prawn *Macrobrachium rosenbergii*, with monoclonal antibody. *Comp Biochem Physiol* 85:1-4.
- Fingerman M. 1995. Endocrine mechanisms in crayfish, with emphasis on reproduction and neurotransmitter regulation of hormone release. *Am Zool* 35:68-78.
- Gupta NVS, Kurup KNP, Adiyodi RG, Adiyodi KG. 1989. The antagonism between somatic growth and testicular activity during different phases in intermolt (stage C4) in sexually mature freshwater crab, *Paratelphusa hydrodromus*. *Invert Reprod Dev* 16:195-204.
- Hasegawa Y, Hirose E, Katakura Y. 1993. Hormonal control of sexual differentiation and reproduction in Crustacea. *Am Zool* 33:403-411.
- Huxley TH. 1880. *The crayfish: an introduction to the study of zoology*. New York: D Appleton and Co.
- King LE, Morrison M. 1976. The visualization of human erythrocyte membrane proteins and glycoproteins in SDS polyacrylamide gels employing a single staining procedure. *Anal Biochem* 71:223.
- Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.
- Medley P, Rouse DB. 1993. Intersex Australian red claw crayfish (*Cherax quadricarinatus*). *J Shellfish Res* 12:93-94.
- Meusy JJ, Payen GG. 1988. Female reproduction in malacostracan crustacea. *Zool Sci* 5:217-265.
- Nagamine C, Knight AW, Maggenti A, Paxman G. 1980a. Masculinization of female *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae) by androgenic gland implantation. *Gen Comp Endocrinol* 41:442-457.
- Nagamine C, Knight AW, Maggenti A, Paxman G. 1980b. Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn *Macrobrachium rosenbergii* (de Man) with first evidence of induced feminization in a non-hermaphroditic decapod. *Gen Comp Endocrinol* 41:423-441.
- Nagasawa H, Hasegawa Y, Haino-Fukushima K, Hatayama H, Yanagisawa T, Katakura Y. 1995. Isolation and structural determination of seminal vesicle-specific peptides of the terrestrial isopod, *Armadillidium vulgare*. *Biosci Biotech Biochem* 59:1246-1250.
- Nakamura K. 1992. Differentiation of genital organs and androgenic gland in the kuruma prawn *Penaeus japonicus*. *Mem Fac Fish Kagoshima Univ* 41:87-94.
- Okuno A, Hasegawa Y, Nagasawa H. 1997. Purification and properties of androgenic gland hormone from the terrestrial isopod *Armadillidium vulgare*. *Zool Sci* 14:837-842.
- Payen GG. 1973. Etude descriptive des principales étapes de la morphogénèse sexuelle chez un crustacé décapode à développement condensé, l'Écrevisse *Pontastacus leptodactylus leptodactylus* (Eschscholtz, 1823). *Ann Embryol Morphol* 6:179-206.

- Puckett DH. 1964. Experimental studies on the crayfish androgenic gland in relation to testicular function. PhD dissertation. University of Virginia.
- Quackenbush LS. 1991. Regulation of vitellogenesis in penaeid shrimp. In: Deloach PF, Dongherty WJ, Davidson MA, editors. *Frontiers in shrimp research*. Amsterdam: Elsevier. p 125–140.
- Rudolph EH. 1995. Partial protandric hermaphroditism in the burrowing crayfish *Parastacus nicoleti* (Philippi, 1882) (Decapoda: Parastacidae). *J Crust Biol* 15:720–732.
- Sagi A, Cohen D, Milner Y. 1990. Effect of androgenic gland ablation on morphotypic differentiation and sexual characteristics of male freshwater prawns, *Macrobrachium rosenbergii*. *Gen Comp. Endocrinol* 77:15–22.
- Sagi A, Khalaila I, Barki A, Hulata G, Karplus I. 1996a. Intersex red claw crayfish, *Cherax quadricarinatus* (von Martens): functional males with pre-vitellogenic ovaries. *Biol Bull* 190:16–23.
- Sagi A, Shoukrun R, Khalaila I, Rise M. 1996b. Gonad maturation, morphological and physiological changes during the first reproductive cycle of the crayfish *Cherax quadricarinatus* female. *Invert Reprod Dev* 29:235–242.
- Sagi A, Snir E, Khalaila I. 1997a. Sexual differentiation in decapod crustaceans: role of the androgenic gland. *Invert Reprod Dev* 31:55–61.
- Sagi A, Shoukrun R, Levy T, Barki A, Hulata G, Karplus I. 1997b. Reproduction and molt in previously spawned and first-time spawning red-claw crayfish *Cherax quadricarinatus* females following eyestalk ablation during the winter reproductive-arrest period. *Aquaculture* 156:101–111.
- Taketomi Y, Nishikawa S. 1996. Implantation of androgenic glands into immature female crayfish, *Procambarus clarkii*, with masculinization of sexual characteristics. *J Crust Biol* 16:232–239.
- Taketomi Y, Nishikawa S, Koga S. 1996. Testis and androgenic gland during development of external sexual characteristics of the crayfish *Procambarus clarkii*. *J Crust Biol* 16:24–34.
- Touir A. 1977. Données nouvelles concernant l'endocrinologie sexuelle des Crustacés Décapodes Natantia hermaphrodites et gonochoriques. I. Maintien des glandes androgènes et rôle de ces glandes dans le contrôle des gamétogenèses et des caractères sexuels externes mâles. *Bull Soc Zool Fr* 102:375–400.
- Wilder MN, Okumura T, Suzuki Y, Fusetani N, Aida K. 1994. Vitellogenin production induced by eyestalk ablation in juvenile giant freshwater prawn *Macrobrachium rosenbergii* and trial methyl farnesoate administration. *Zool Sci* 11:45–53.