GENERAL AND COMPARATIVE ENDOCRINOLOGY 77, 15–22 (1990)

Effect of Androgenic Gland Ablation on Morphotypic Differentiation and Sexual Characteristics of Male Freshwater Prawns, *Macrobrachium rosenbergii*

AMIR SAGI, DAN COHEN,[1] AND YORAM MILNER

Life Sciences Institute, The Hebrew University of Jerusalem, Jerusalem, Israel

Accepted March 3, 1989

Mature males of the freshwater prawn, *Macrobrachium rosenbergii* (de Man), may change from one to another morphotype, according to a set sequence. Small males may develop into orange-claw males and orange-claw males into dominant blue-claw males. Each of the three morphotypes demonstrates distinctive reproductive behavior and secondary sexual characteristics. The role of the androgenic gland in this morphotypic transformation was examined experimentally by bilateral androgenic gland ablation (andrectomy) of small males and orange-claw males. For andrectomy initiated in the small male morphotype, transformation to the next morphotype was permitted (orange-claw), but subsequent transformation to the blue-claw morphotype was blocked. Andrectomy of orange-claw males did not prevent transformation into the blue-claw. Andrectomy on both small and orange-claw males caused disappearance of the genital papillae and atrophy of the sperm ducts and testes. The growth rates of the andrectomized small and orange-claw males were significantly lower than those of the unoperated and sham-operated controls. We conclude that androgenic gland factors control not only the differentiation of male secondary sexual characteristics but also morphotypic differentiation. Bioassays based on the results of this study will be instrumental in the characterization of such a factor(s).

Three distinctive adult male morphotypes coexist in a *Macrobrachium rosenbergii* population. Each morphotype represents a different reproductive strategy. Small males, which employ a sneak-copulation strategy (Telecky, 1984), transform into orange-claw males. The orange-claw males are characterized by rapid somatic growth and do not exhibit courtship and mating behavior; the orange-claw males transform into dominant blue-claw males, which cease somatic growth and sequester, court, and mate with receptive females (Cohen *et al.*, 1981; Ra'anan and Cohen, 1985). Transformation from the small male to orange-claw morphotype is gradual through intermediate forms resulting in the distinctive strong orange-claw form which subsequently metamorphose into the blue-claw form (Kuris *et al.*, 1987). All males in the population are capable of transforming through all morphotypic stages, exhibiting changes in claw shape and coloration (Kuris *et al.*, 1987), growth rate, and reproductive behavior (Ra'anan and Sagi, 1985), as well as in the anatomy and physiology of the reproductive system and the midgut glands (Sagi and Ra'anan, 1988; Sagi *et al.*, 1988).

The crustacean androgenic gland was first described by Charniaux-Cotton (1954) in the amphipod, *Orchestia*. The glands are usually located on the subterminal portion of the sperm duct (Kleinholz and Keller, 1979). The androgenic glands of *M. rosenbergii* are strands of cells on or, as a pyramidal cellular cluster, loosely associated

[1] Present address: Aquaculture Production Technology (Israel) Ltd., P.O. Box 4330, Jerusalem 91042, Israel.

0016-6480/90 $1.50

with the posterior portion of the ejaculatory duct (Veith and Malecha, 1983).

In hermaphroditic crustaceans, the presence of the androgenic gland is necessary for the normal differentiation and regeneration of male secondary sexual characteristics (Charniaux-Cotton, 1957, 1959, 1960, 1961; Berreur-Bonnenfant and Charniaux-Cotton, 1965; Touir 1977a). Male secondary sexual characteristics are induced by the presence of the androgenic gland in nonhermaphroditic crustaceans as well (DeMeusy, 1970; Thampy and John, 1973; Nagamine *et al.*, 1980; Nagamine and Knight, 1987). Although Nagamine *et al.* (1980) demonstrated that the presence of the androgenic glands in *M. rosenbergii* is necessary for the development and regeneration of male secondary sexual characteristics, their work was performed before the existence of the morphotypes was recognized. It was necessary therefore to examine the effect of the androgenic gland on the process of morphotypic differentiation. We applied bilateral surgical ablation of the androgenic glands (andrectomy) on small males and orange-claw males and examined their transformation to the following stages.

MATERIALS AND METHODS

Source of animals. Orange-claw and small male prawns were obtained from a commercial fish pond. All individuals were of the same hatching batch and were reared under the same environmental conditions. The selection of 75 small males and 75 strong orange-claw males was performed according to the distinctive characteristics described by Kuris *et al.* (1987).

Experimental groups. The small males were divided into groups of 25. One group was subjected to bilateral andrectomy according to techniques described by Nagamine *et al.* (1980) and two groups served as controls. The same was done for the orange-claw males. The controls included a group of intact prawns and a group of sham-operated prawns. The vas deferens of the sham-operated controls was severed in a manner and location similar to those of the prawns actually undergoing the surgical removal procedure.

Prawns were placed in vertical, cylindrical net cages (30 cm diameter), within glass aquaria (100 × 37 × 39 cm). Three cages were placed in each aquarium, one containing an andrectomized prawn, one containing an intact prawn, and one containing a sham-operated prawn of the same initial morphotype. Each aquarium was equipped with a biofilter and a thermostat-controlled heating element which maintained a temperature of 25–27°. Each cage was equipped with a plastic pipe (10 cm in diameter and 20–25 cm long) which provided shelter for the prawn. All animals were fed daily *ad libitum* with live *Daphnia,* APT 85 artificial feed, and commercial fish pellets (25% protein). Uneaten food and other debris were siphoned from the aquaria once a week. Prawns were checked daily for molts and changes in morphotypic status.

One mature female was also included in each aquarium, outside of the net cages. The presence of mature females may increase the molting frequency of male prawns (A. Sagi, unpublished observations).

Data collection. At the beginning and at the end of the experiment, the carapace and propodus lengths of each prawn were measured (to 0.1 mm) with a caliper. Carapace length was defined as the distance from the posterior margin of the right orbit to the posterior margin of the carapace at the midline. The propodus, with the joint flexed, was measured along the lateral face from the proximal lateral condyle to the distal tip. The presence of the genital papillae was recorded.

At the end of the experiment, the prawns were weighed, the gonads were removed, and the wet weight of the gonads was recorded, using a precision balance (±1 mg). The gonadosomatic index (GSI) was calculated as

$$\text{GSI} = 100 \times (\text{gonad wt/body wt}).$$

During the dissection of the animals, the appearance of the sperm duct and ampullae was observed. The testes were clipped off the sperm duct and incubated in 2 ml *M. rosenbergii* saline (Nagamine *et al.*, 1980). The existence of sperm, released via the excised sperm duct opening, was recorded.

Growth rate (R) was calculated as

$$R = (1/T - t) \ln (W^T/W^t),$$

where T represents termination and t represents starting time in days. W^T and W^t represents weights at times T and t, respectively.

RESULTS

Effects of andrectomy on morphotypic differentiation. The effects of andrectomy on morphotypic differentiation are shown in Table 1. When the andrectomy was performed on small males, most of the males successfully transformed into orange-claw

males; however, none further transformed into the blue-claw morphotype. On the other hand, most of the andrectomized orange-claw males (76%) did transform to blue-claw males. In all of the control groups (intact and sham-operated), most of the prawns transformed into the blue-claw morphotype.

The relative propodus length of the andrectomized small males was smaller than that of either of the small male control groups. In Fig. 1, it can be seen that the claws of the sham-operated prawn grew larger with a relatively longer propodus than claws of andrectomized prawns. The claws of sham-operated males ultimately developed spination typical of the blue-claw male (Kuris *et al.*, 1987), whereas the claws of andrectomized prawns retained the spination characteristics of small males and orange-claw males (Kuris *et al.*, 1987).

Effects of andrectomy on the reproductive system. Genital papillae, a sexual secondary characteristic of males, were missing in all of the andrectomized small males and in 95% of the andrectomized strong orange-claw males (Table 2), resulting in a female-like appearance of the genital region of the andrectomized prawns (Fig. 2). Andrectomy also caused changes in the anatomical structure of the reproductive system (Table 2). The sperm ducts and testes were atrophied and the ampullae were missing. The sperm ducts of the andrectomized prawns were clearly shorter than those of the control prawns (Fig. 3a). In six cases, one of the two sperm ducts was completely missing. The testes of the andrectomized prawns were atrophied in comparison to those of the sham-operated prawns (Fig. 3a). The atrophy of the testes resulted in a lower gonadosomatic index of andrectomized prawns (Table 3). The ampullae, which were ablated in the course of the andrectomy, did not regenerate in any of the small males (Fig. 3b) nor in 66% of the strong orange-claw males. The scar of the sham operation can be clearly seen in Fig. 3b (arrow).

Effects of andrectomy on sperm production and growth rate. Mature sperm were present in the testes of almost all of the 112 prawns. In four andrectomized small males, no sperm were found. The growth rates of andrectomized prawns were significantly lower than those of the sham-operated and intact groups (Table 3).

DISCUSSION

When andrectomy is performed on male

TABLE 1

MORPHOTYPIC STATUS OF THE PRAWNS AT THE END OF THE EXPERIMENTAL PERIOD (5 MONTHS FOR EXPERIMENTS INITIATED WITH SMALL MALES, 3 MONTHS FOR EXPERIMENTS INITIATED WITH ORANGE-CLAW MALES)

Initial morphotype	Treatment	*N*	Transformed into orange-claw	Transformed into blue-claw	Undistinguishable[a]	Relative propodus length
Small male	Andrectomized	15	14 (93%) A	0 A	1 (7%)	1.33 ± .24 A
	Sham-operated	16	3 (19%) B	11 (69%) B	2 (12%)	1.69 ± .34 B
	Intact	16	4 (25%) B	10 (63%) B	2 (12%)	1.72 ± .35 B
			Remained orange-claw			
Orange-claw male	Andrectomized	21	5 (24%) B	16 (76%) B	0	1.84 ± .36 B
	Sham-operated	22	4 (18%) B	17 (77%) B	1 (5%)	1.79 ± .37 B
	Intact	22	2 (9%) B	20 (90%) B	0	1.97 ± .28 B

Note. Different letters represent significantly different values (χ^2 test and ANOVA, $P < 0.05$).

[a] Due to damaged or regenerated claws.

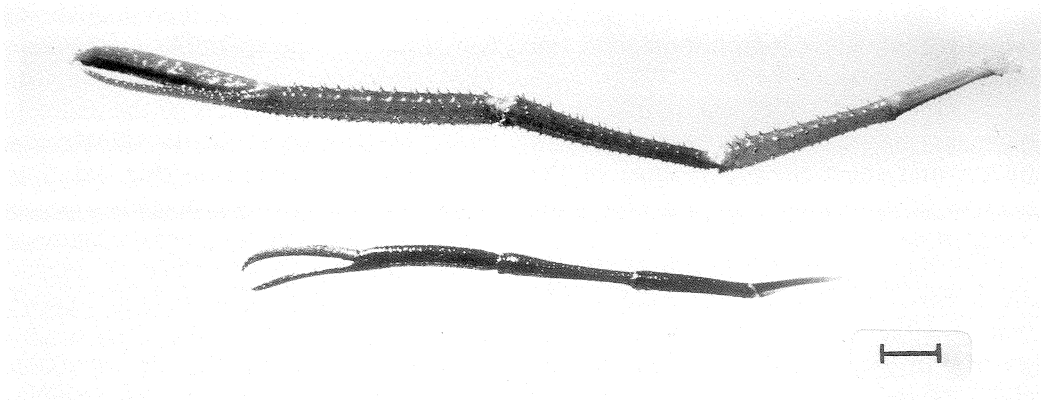

FIG. 1. Claws of an andrectomized (bottom) and a sham-operated (top) prawn at the end of the experimental period. Both prawns were small males at the beginning of the experiment and grew to nearly the same carapace length at the end of the experiment. Bar represents 0.5 cm.

crustaceans, prior to the differentiation of secondary sexual characteristics, such characteristics do not develop. This has been experimentally shown for amphipods (Charniaux-Cotton, 1957) and decapods (Touir, 1977b), including *M. rosenbergii* (Nagamine *et al.*, 1980). If males are andrectomized after secondary sexual characteristics were differentiated, these characteristics do not degenerate (Charniaux-Cotton, 1957; Touir, 1977b).

In experiments conducted by Nagamine *et al.* (1980) before the *M. rosenbergii* male morphotypes were recognized, two types of males with appendices masculina were andrectomized. "Stage II" were males with "immature claws" and "Stage III" were males with "mature chelipeds" (Nagamine *et al.*, 1980). Kuris *et al.* (1987) suggested that these two types of males were small males and orange-claw males. In Nagamine's experiment, the small males did not develop "mature claws" following the andrectomy while the orange-claw males did not lose their mature claws. However, after the classification of *M. rosenbergii* males into morphotypes (Ra'anan and Sagi, 1985) and the description of the morphotypic transformation process (Kuris *et al.*, 1987), it became important to clarify the effects of andrectomy on the morphotypic differentiation of *M. rosenbergii* males.

Figure 4 summarizes schematically the results of the surgical manipulation performed on different morphological stages during male differentiation. The typical differentiation process is described on line 1. When the andrectomy is performed on the orange-claw male (line 2), the morphotypic differentiation process can be successfully completed. Andrectomized small males (line 3) differentiate into orange-claw males, but further differentiation into blue-claw males is prevented. In earlier work

TABLE 2

THE EFFECT OF ANDROGENIC GLAND ABLATION ON MORPHOLOGICAL CHARACTERISTICS AND ANATOMICAL STRUCTURE OF THE REPRODUCTIVE SYSTEM OF THE PRAWNS (NORMAL SPERM DUCTS AND GENITAL PAPILLAE WERE OBSERVED IN ALL THE SHAM-OPERATED AND INTACT PRAWNS)

Initial morphotype	Genital papillae missing (see Fig. 2)	Atrophied sperm duct (see Fig. 3)	Ampullae missing (see Fig. 3b)
Small male	15 (100%)	14 (93%)	15 (100%)
Orange-claw male	20 (95%)	12 (57%)	14 (66%)

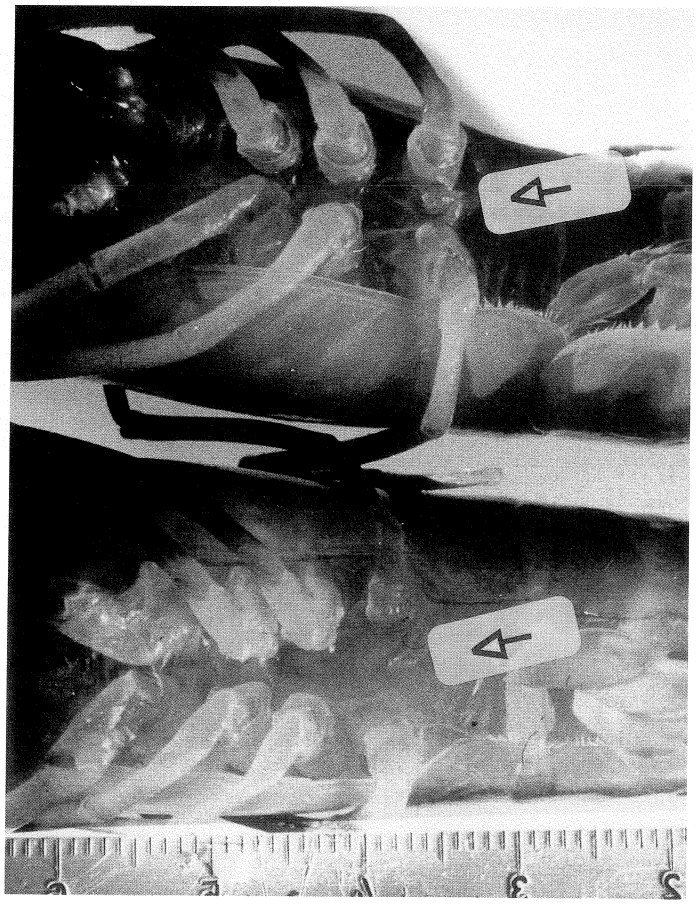

FIG. 2. A ventral view of an andrectomized (bottom) and a sham-operated (top) prawn at the end of the experimental period. The genital papillae (arrow) is missing in the former. Both prawns were small males at the beginning of the experiment and grew to nearly the same carapace length at the end of the experiment.

(Nagamine *et al.*, 1980), when andrectomy was performed on juvenile males (line 4), no further differentiation of claws occurred. In some juvenile males the reproductive tract and genitalia were feminized. Other male reproductive organs were retained. These animals resemble the small male morphotype.

It appears that the commitment to complete the differentiation process to the blue-claw morphotype is made during the strong orange-claw stage. During that stage, the transformation from orange-claw to blue-claw will take place even in the absence of the androgenic glands. Hence, the andrectomized male, regardless of the morphotypic stage, will transform to the next stage, despite the absence of the androgenic gland. However, further transformation is blocked. This suggests that the androgenic gland exerts control by releasing factors necessary for morphotypic transformation. But at the time of ablation, the androgenic gland has already initiated the processes responsible for transformation to the next stage. Analogous results are reported for the delayed affect of eyestalk ablation on lobster larval molting and development (Snyder and Chang, 1986).

Blocking of the morphotypic process was accompanied by atrophy of sperm ducts and testes. Atrophy occurs following abla-

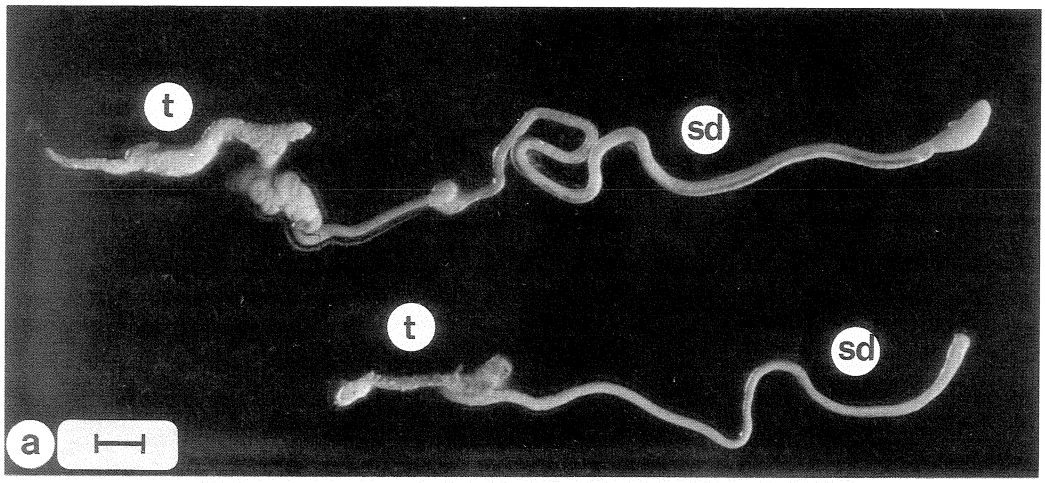

FIG. 3a. The sperm ducts of an andrectomized (bottom) and a sham-operated (top) prawn at the end of the experimental period. The testes (t) as well as the sperm duct (sd) of the andrectomized prawn are atrophied. Both prawns were small males at the beginning of the experiment and grew to nearly the same carapace length at the end of the experiment. Bar represents 0.5 cm.

tion in all stages and confirms previous work on *M. rosenbergii* (Nagamine *et al.*, 1980) as well as studies on other crustaceans (Charniaux-Cotton, 1954, 1957; Raimond and Juchault, 1983). The presence of sperm in the testes of almost all of the andrectomized prawns shows that ablation of the androgenic gland does not prevent spermatogenesis, although it may have an affect on the quantity of sperm produced or the rate of production. Exceptionally (four cases, all of them andrectomized small males), no sperm was found.

We have previously shown (Ra'anan and Cohen, 1985; Ra'anan and Sagi, 1985; Sagi and Ra'anan, 1988) that the growth rate of the orange-claw males was higher than that of the other male morphotypes. In the present work, we showed that the growth rate decreased after the andrectomy, regardless of the stage in which the surgical manipulation took place. It is possible that androgenic gland hormones not only control morphotypic differentiation but, as was suggested by Kuris *et al.* (1987), also affect somatic growth.

TABLE 3
THE GROWTH RATE, RELATIVE WEIGHT OF THE TESTES, AND EXISTENCE OF SPERM IN ANDRECTOMIZED, SHAM-OPERATED, AND INTACT PRAWNS

Initial morphotype	Treatment	Growth rate	Gonadosomatic index	Prawns in which sperm was present (%)
Small males	Andrectomized	0.54 ± .04 A	0.09 ± .02 A	73
	Sham-operated	0.68 ± .06 AB	0.21 ± .03 B	100
	Intact	0.72 ± .04 B	0.17 ± .03 B	100
Orange-claw males	Andrectomized	0.28 ± .03 A	0.14 ± .01 A	100
	Sham-operated	0.41 ± .06 B	0.19 ± .02 B	100
	Intact	0.48 ± .04 B	0.19 ± .03 B	100

Note. Different letters represent significantly different values (ANOVA, $P < 0.05$).

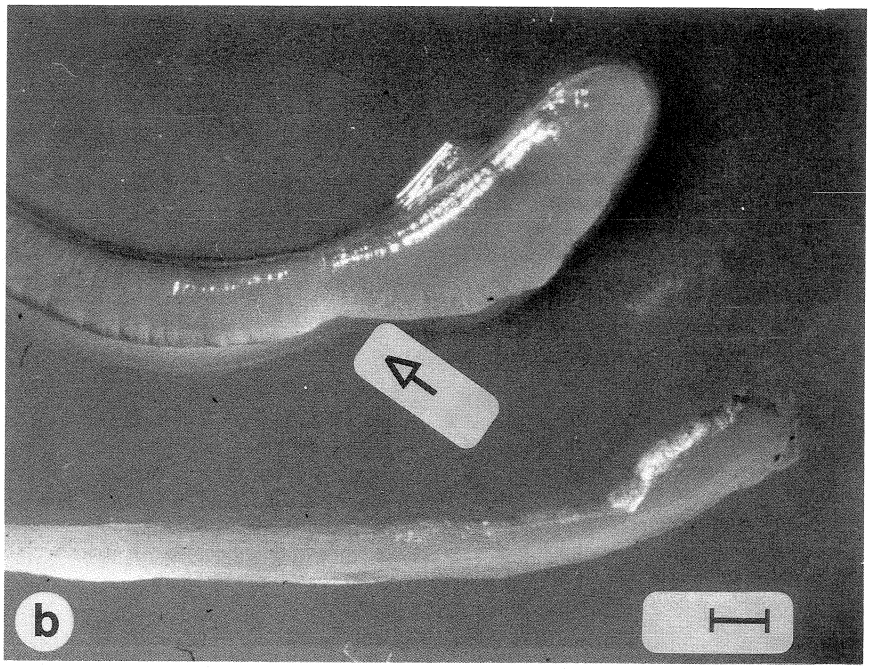

FIG. 3b. The proximal part of the sperm duct of an andrectomized (bottom) and a sham-operated (top) prawn at the end of the experimental period. The arrow points to the sham operation scar. Both prawns were small males at the beginning of the experiment and grew to nearly the same carapace length at the end of the experiment. Bar represents 0.1 cm.

In summary, we have demonstrated that (1) the presence of the androgenic gland is essential to the morphotypic differentiation process; (2) ablation of the androgenic gland has a delayed effect, after ablation, males transformed to the subsequent stage but further transformation did not occur; (3) spermatogensis can occur in the absence of the androgenic gland; and (4) absence of the androgenic gland causes a reduction of growth rates. These findings will be used to design bioassays that can be used in the isolation and characterization of the factor(s) in the androgenic glands controlling morphotypic differentiation and affecting growth rates.

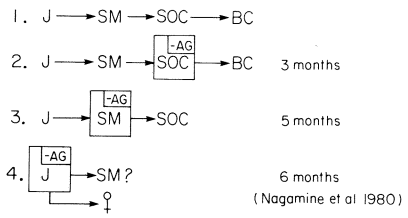

FIG. 4. The effect of andrectomy during different morphological stages of the male differentiation process. J, juvenile; SM, small male; SOC, strong orange-claw; BC, blue-claw male, -AG, androgenic glands ablation. The numbers of months between the andrectomy and the end of the experiment are indicated at the right.

ACKNOWLEDGMENTS

The authors thank Professor Armand Kuris and Professor Ernest Chang for helpful discussions. We thank them and Ms. Ellen Rosenberg for critically reviewing the manuscript. We also thank Aquaculture Production Technology (Israel) Ltd. for providing basic facilities and personnel. This study was supported by the University of California–Hebrew University reciprocity fellowship (to A.S.) and by U.S.–Israel Binational Agricultural Research and Development Fund Grant I-936-85.

REFERENCES

Berreur-Bonnenfant, J., and Charniaux-Cotton, H. (1965). Hermaphrodisme proterandrique et fonc-

tionnement de la zone germinative chez la Crevette *Pandalus borealis* Kroyer. *Bull. Soc. Zool. Fr.* **40,** 243–259.

Charniaux-Cotton, H. (1954). Decouverte chez un crustace amphipode (*Orchestia gammarella*) d'une gland endocrine responsable de la differenciation des caracteres sexuels primaires et secondaires males. *C.R. Hebd. Seances Acad. Sci. (Paris)* **239,** 780–782.

Charniaux-Cotton, H. (1957). Croissance, regeneration et determinisme endocrinein des caracteres sexuels d'*Orchestia gammarella* Pallas (Crustace Amphipode). *Ann. Sci. Nat. Zool. Biol. Anim.* **19,** 411–560.

Charniaux-Cotton, H. (1959). Masculinisation des femelles de la Crevette a hermaphrodisme proterandrique *Lysmata seticaudata,* par greffe de glandes androgenes. Interpretation de l'hermaphrodisme chez les Decapodes. Note preliminaire. *C.R. Hebd. Seances Acad. Sci. Ser. D.* **249,** 1580–1582.

Charniaux-Cotton, H. (1960). Physiologie de l'inversion sexuelle chez la Crevette a hermaphrodisme proterandrique fonctionnel, *Lysmata se ticaudata. C.R. Hebd. Seances Acad. Sci. Ser. D.* **250,** 4046–4048.

Charniaux-Cotton, H. (1961). Physiologie de la gonade de *Lysmata seticaudata* (Crevette a hermaphrodisme proterandrique) chez les individus normaus et les femelles masculinisees. *C.R. Hebd. Seances Acad. Sci. Ser. C.* **252,** 199–201.

Cohen, D., Ra'anan, Z., and Brody, T. (1981). Population profile development and morphotypic differentiation in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *J. World Maric. Soc.* **12**(2), 231–243.

DeMeusy, N. (1970). Chez les Decapodes Brachyoures gonochoriques Qualques aspects de la sexualite. *Bull. Soc. Zool. Fr.* **95,** 595–612.

Kleinholz, L. H., and Keller, R. (1979). Endocrine regulation in crustacea. *In* "Hormones and Evolution" (E. J. W. Barrington, Ed.), Vol 1, pp. 160–213. Academic Press, New York.

Kuris, M. A., Ra'anan, Z., Sagi, A., and Cohen, D. (1987). Morphotypic differentiation of male Malaysian giant prawn, *Macrobrachium rosenbergii. J. Crust. Biol.* 7(2), 219–237.

Nagamine, C., Knight, A. W., Maggenti, A., and Paxman, G. (1980). Masculinization of female *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae) by androgenic gland implantation. *Gen. Comp. Endocrinol.* **41,** 442–457.

Nagamine, C., and Knight, A. W. (1987). Masculinization of female crayfish. *Procambarus Clarki* (Giard). *Int. J. Invertebr. Reprod.* **11,** 77–87.

Ra'anan, Z., and Cohen, D. (1985). Ontogeny of social structure and population dynamics in the giant freshwater prawn *Macrobrachium rosenbergii* (de Man). In "Crustacean Issues 3: Crustacean Growth: Factors in Adult Growth," (Adrian Wenner, Ed.), pp. 277–311. A. A. Balkema Publishers.

Ra'anan, Z., and Sagi, A. (1985). Alternative mating strategies in male morphotypes of the freshwater prawn *Macrobrachium rosenbergii* (de Man). *Biol. Bull.* **169,** 592–601.

Raimond, R., and Juchault, P. (1983). Masculinisation des femelles prepuberes et puberes de *Sphaeroma serratum* Fabr., (Crustace Isopode Flabellifere) par implantation d'une gland androgene de male pubere. *Gen. Comp. Endocrinol,* **50,** 146–155.

Sagi, A., Milner, Y., and Cohen, D. (1988). Spermatogenesis and sperm storage in the testes of behaviourly distinctive male morphotypes of *Macrobrachium rosenbergii* (Decapoda, Palaemonidae). *Bio. Bull.* **174,** 330–336.

Sagi, A., and Ra'anan, Z. (1988). Morphotypic differentiation of the freshwater prawn *Macrobrachium rosenbergii* males: Changes in the midgut glands and the reproductive system. *J. Crustacean Biol.* **8**(1), 43–47.

Snyder, M. J., and Chang, E. S. (1986). Effects of eyestalk ablation on larval molting rates and morphological development of the American lobster, *Homarus americanus. Biol. Bull.* **170,** 232–243.

Telecky, J. M. (1984). Alternate male reproductive strategies in the giant Malaysian prawn *Macrobrachium rosenbergii. Pac. Sci.* **38,** 372–373.

Thampy, D. M., and John, P. A. (1973). Observations on variations in the male sex characters and their relation to the androgenic gland in the shrimp *Macrobrachium idae* (Heller). *Acta Zool. (Stockholm)* **54,** 193–200.

Touir, A. (1977). Donnees nouvelles concernant l'endocrinologie sexuelle des Crustaces Decapodes Natantia hermaphrodites et gonochoriques. I. Maintien des glandes androgenes et role de ces glandes dans de controle des gametogeneses et des caracteres sexuels externes males. *Bull. Soc. Zool. Fr.* **102,** 375–400.

Touir, A. (1977b). Donnees nouvelles concernant l'endocrinologie sexuelle des Crustaces Decapodes Natantia hermaphrodites et gonochoriques. III. Mise en evidence d'un controle neurohormonal du maintien de l'appareil genital male et des glandes androgenes exerce par le protocerebron median. *C.R. Acad. Sci. (Paris)* **285,** 539–542.

Veith, W. J., and Malecha, S. R. (1983). Histochemical study of the distribution of lipids, 3-alpha and 3-beta—hydroxysteroid dehydrogenase in the androgenic gland of the cultured prawn *Macrobrachium rosenbergii* (de Man) (Crustacea; Decapoda). *S. Afr. J. Sci.* **79,** 84–85.