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BILATERAL GYNANDROMORPH OF THE FRESH-WATER
CRAB *POTAMON FLUVIATILE* HERBST
(DECAPODA: BRACHYURA)

Fiorenza Micheli

A B S T R A C T

The morphology and gonad history of a bilaterally gynandromorphic fresh-water crab (*Potamon fluviatile* Herbst) is described. The abdomen and gonopods exhibit male characteristics on the left side of the body, female ones on the right, with the exception of a partial masculinization of the first right pleopod. The right side has a normal female gonopore, the left one a penis. Internally, testes and vas deferens are present on both sides, though the right vas deferens is incomplete and does not communicate with the outside. On the right, a seminal receptacle full of sperm indicates that the animal has mated as a female. An isolated portion of gonad, also showing testicular activity, is annexed to the seminal receptacle. Mature sperms are visible throughout the gonad and vas deferens. No ovarian tissue was detected in the entire reproductive apparatus of this specimen. The haploid chromosome number does not differ significantly between the male and female sides and averages 68 ± 1.3 . The role of the androgenic hormone on sex differentiation of Brachyura is discussed.

Though bilateral gynandromorphism among normally dioecious decapod crustaceans has already been reported (Chace and Moore, 1959; Ito, 1965; Farmer, 1972; Otto, 1979; Cargo, 1980; Johnson and Otto, 1981; Manning and Holthuis, 1981; Taylor, 1986), few works describe the gonad histology (Farmer, 1972; Cargo, 1980; Johnson and Otto, 1981). It is evident from the literature on this topic that various degrees of sexual differentiation have been encountered on the two sides of such specimens, ranging from definite bilaterality in both the primary and secondary sexual characters (the lobster *Homarus americanus*, Chace and Moore, 1959; the fresh-water prawn *Nephrops norvegicus*, Farmer, 1972), to either incomplete development (the blue crab *Callinectes sapidus*, Cargo, 1980) or partial masculinization of the gonad on the female side (*Callinectes sapidus*, Johnson and Otto, 1981).

Such abnormal sexual differentiations are of interest for what they may contribute to understanding the mechanisms of sexual determination, well known in some crustacean groups such as Isopoda and Amphipoda (Charniaux-Cotton and Payen, 1988), but still debated within Decapoda. In particular, while the role of the androgenic gland (AG) in determining the differentiation of primary and secondary sexual characters has been proven in the former two groups and in the Decapoda Natantia,

its role is uncertain in the Reptantia. In decapods, the AG is thought not to initiate sex differentiation, though it probably initiates puberty (Charniaux-Cotton, 1975). In several cases, implantation of the AG into prepubertal and pubertal decapod females did not masculinize the ovaries and seldom masculinized the external sexual characteristics (Charniaux-Cotton and Payen, 1988). In contrast with these results, the sex reversal of nonhermaphroditic female prawns (both mature and immature) implanted with the AG seems to indicate that this gland has a similar function in Amphipoda, Isopoda, and Decapoda (Nagamine *et al.*, 1980a, b).

A population of *Potamon fluviatile* inhabiting a hill stream near Florence, Italy (see references below for a description of the study site), has been the object of extensive ethological and ecological studies (Gherardi, 1987), including reproductive biology and growth patterns (Micheli *et al.*, 1990). Of the approximately 2,000 crabs sampled or observed in the course of 10 years' research, only one sexually abnormal individual was encountered. This paper reports the morphology and histology of this specimen.

MATERIALS AND METHODS

The gynandromorph was collected in August 1988, taken to the laboratory, and killed with chloroform. All its internal organs were normal in size and appearance except for the gonads, which were removed, fixed in Carnoy's fluid, and preserved in 75% ethanol. No evidence of a pathologic condition or of parasitic

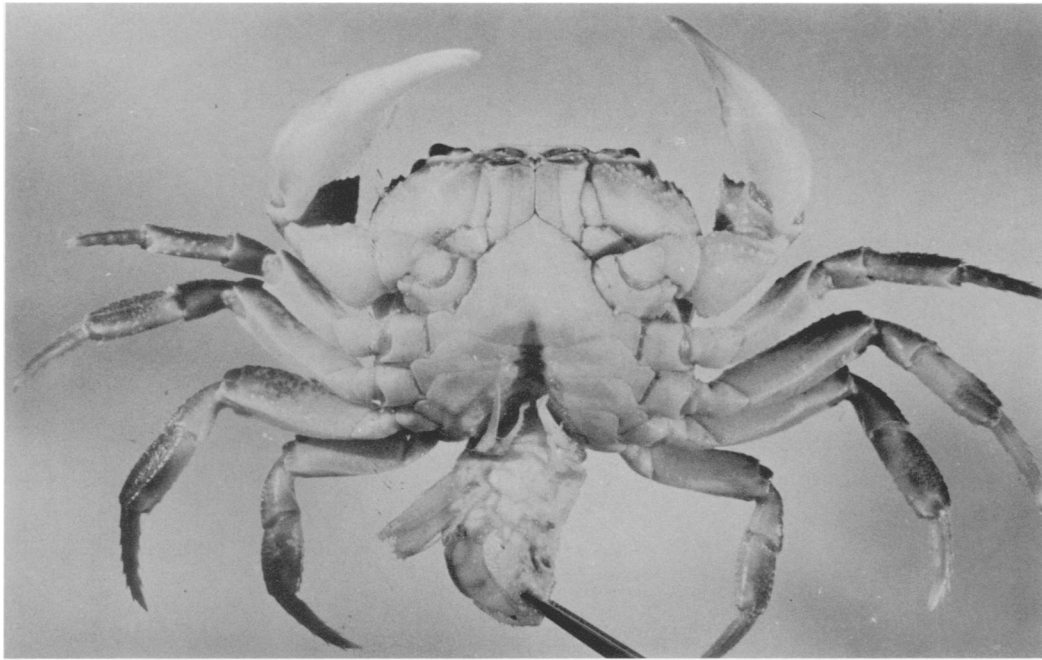


Fig. 1 Ventral view of a bilateral gynandromorph of *Potamon fluviatile*, showing the anomalous abdomen and gonopods. Male characteristics are evident on the left side of the body, female ones on the right side.

infestation was detected. The whole gonad was divided into subsamples and, along with the seminal receptacle, embedded in polystyrene resin, serially sectioned (7 μm), and stained with hemalum-eosin.

Meiotic chromosomes were prepared following a technique suggested by I. Lazzaretto and A. Libertini (personal communication). Immediately after dissection, tissue samples taken from both the left and right sides of the gonad were kept in bidistilled water for 10 min, then fixed in 3:1 ethanol/acetic acid and preserved at 4°C. Tissue subsamples were placed in 60% acetic acid for 3–5 min, then broken up by pipetting and ejecting the liquid and tissue several times. One drop of this cellular suspension was placed on a slide and stained in Giemsa diluted with a phosphate buffer 0.15 M (1:20).

RESULTS

Anatomy

The crab had a carapace length (CL) of 36.8 mm. It was, therefore, an adult, since, in this species, the puberty molt occurs around 35 mm CL (Micheli *et al.*, 1990). The histological examination confirmed that this specimen had mature gonads and therefore had undergone the puberty molt (see below). The crab had a hard exoskeleton, no sign of scars or mutilation, and was right-handed.

Externally, abnormality was evident only in the shape of the abdomen and pleopods

(Fig. 1). The abdomen was asymmetrical, broad and rounded on the right (female) side, narrow and straight on the left. The two typical male pleopods were present on the left side. On the right, the second, third, and fourth pleopods were biramous, with setae, as in normal females, while the first was also biramous, but shorter and thicker and with no setae, thus slightly masculinized. One female gonopore was present on the ventral right side of the sixth thoracic segment, one penis on the coxa of the left fifth pereopod.

The gonads appeared normal on the male side; testis and vas deferens were complete and well developed as in a mature male. The dorsal portion of the gonad on the female side was similar to a testis, being white and slender instead of thick and orange as in normal females of the same size. This testis was slightly smaller than the left one. A transverse commissure, leading to the gonad on the other side, was present.

A vas deferens had developed on the right side as well, but did not lead to the right genital opening. A small, unbranched portion of gonad, white like the rest of the reproductive apparatus, was present on the right ventral side. This part of the gonad

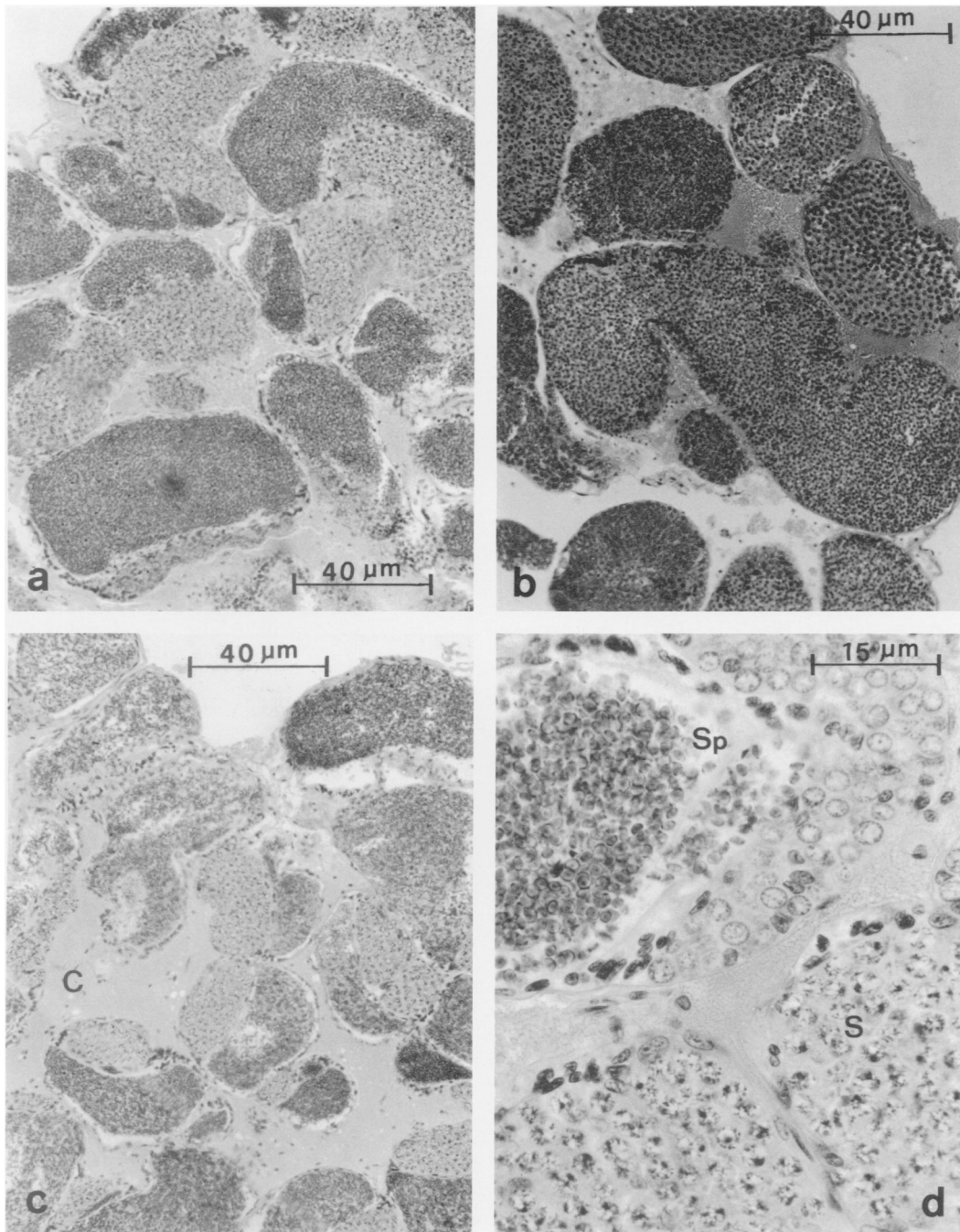


Fig. 2. Histological sections of the gonads of a bilateral gynandromorph of *Potamon fluviatile*. Hemalum-eosin. C = connective tissue. S = spermatocytes. Sp = sperms. a, Testicular lobules on the male side. b, Testicular lobules in the dorsal portion of the gonad on the female side. c, Testicular lobules in the portion of the gonad on the female side annexed to the seminal receptacle. d, Magnification of testicular lobules on the female side (as in b). Connective tissue separates lobules containing cells in the same maturational stage.

was connected to an apparently normal seminal receptacle and to the female gonopore.

No connection was observed between the ventral and dorsal gonads on the right side.

Histology

All the gonad portions were masculinized. Testicular lobules constituted the dorsal portions of both the right and left gonads (Fig. 2a, b, d), and also the ventral portion of the right gonad, annexed to the seminal receptacle (Fig. 2c). In the isolated ventral portion of the right gonad, abundant, poorly structured connective tissue filled the interlobular spaces (Fig. 2c).

All stages of spermatogenesis, mature sperms included, were detected in the gonad. Cells in the same developmental stage seemed to aggregate within the same lobules (Fig. 2d).

The vas deferens of both sides had a cubic epithelium wall and a lumen always containing sperms (Fig. 3a). In this species, spermatozoans are not included in spermatophores, and, therefore, the aspect of the sperm masses was similar throughout the whole length of the vas deferens.

The seminal receptacle resembled normal ones, previously sectioned (Micheli *et al.*, 1990). The wall consisted of a monolayered, tall columnar epithelium resting on a connective tissue sheath. Sperm masses were visible in the lumen (Fig. 3b), indicating that the animal had mated as a female.

Several meiotic divisions were observed on slides prepared for chromosome examination. Pachytene stages prevailed. Unfortunately only a few prometaphase plates, characterized by short, condensed chromosomes (Fig. 4), proved suitable for chromosome counts. In all other stages, chromosomes overlapped extensively and could not be individually distinguished. In the few plates selected, chromosomes were so variable in length and shape that individual chromosomes were not recognizable and a karyotype could not be made.

Haploid chromosome numbers, determined for the right (average = 68.4, SE = 1.1, $N = 9$) and left (average = 67.1, SE = 2.2, $N = 13$) portions of the gonad, were not significantly different ($t = 0.46$, $d.f. = 20$). In the 22 meiotic plates examined, haploid chromosome numbers ranged from 49–75, with an average of 68 ± 1.3 .

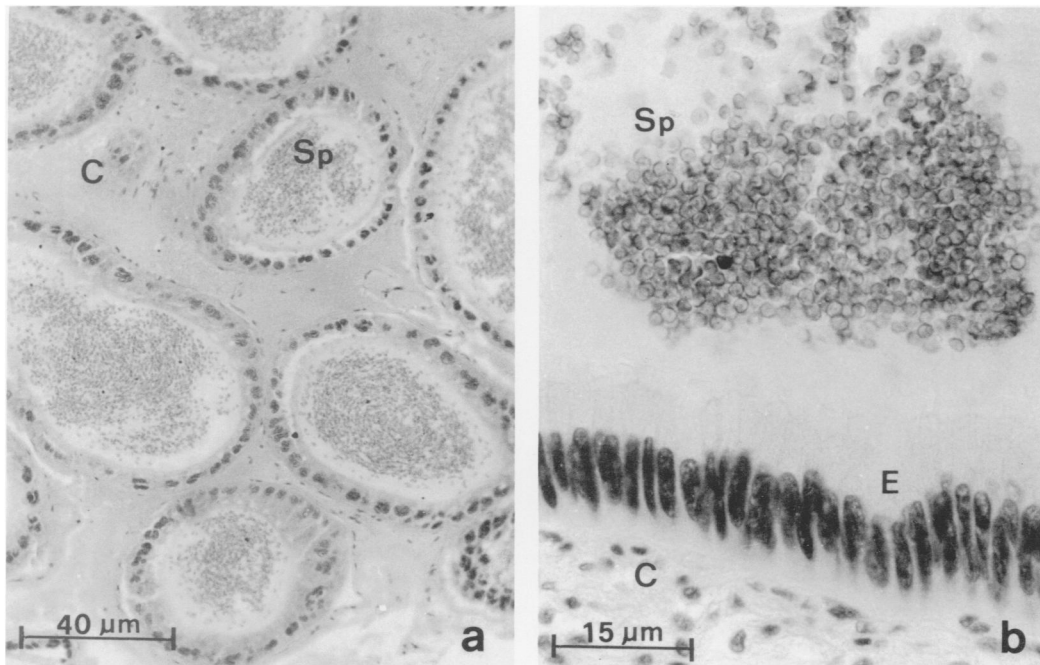


Fig. 3. Bilateral gynandromorph of *Potamon fluviatile*. a, Section of vas deferens. Hemalum-eosin. Symbols as in Fig. 2. b, Section of seminal receptacle, showing connective and epithelial layers of the wall, and a sperm mass in the lumen. Hemalum-eosin. E = epithelium, other symbols as in Fig. 2.

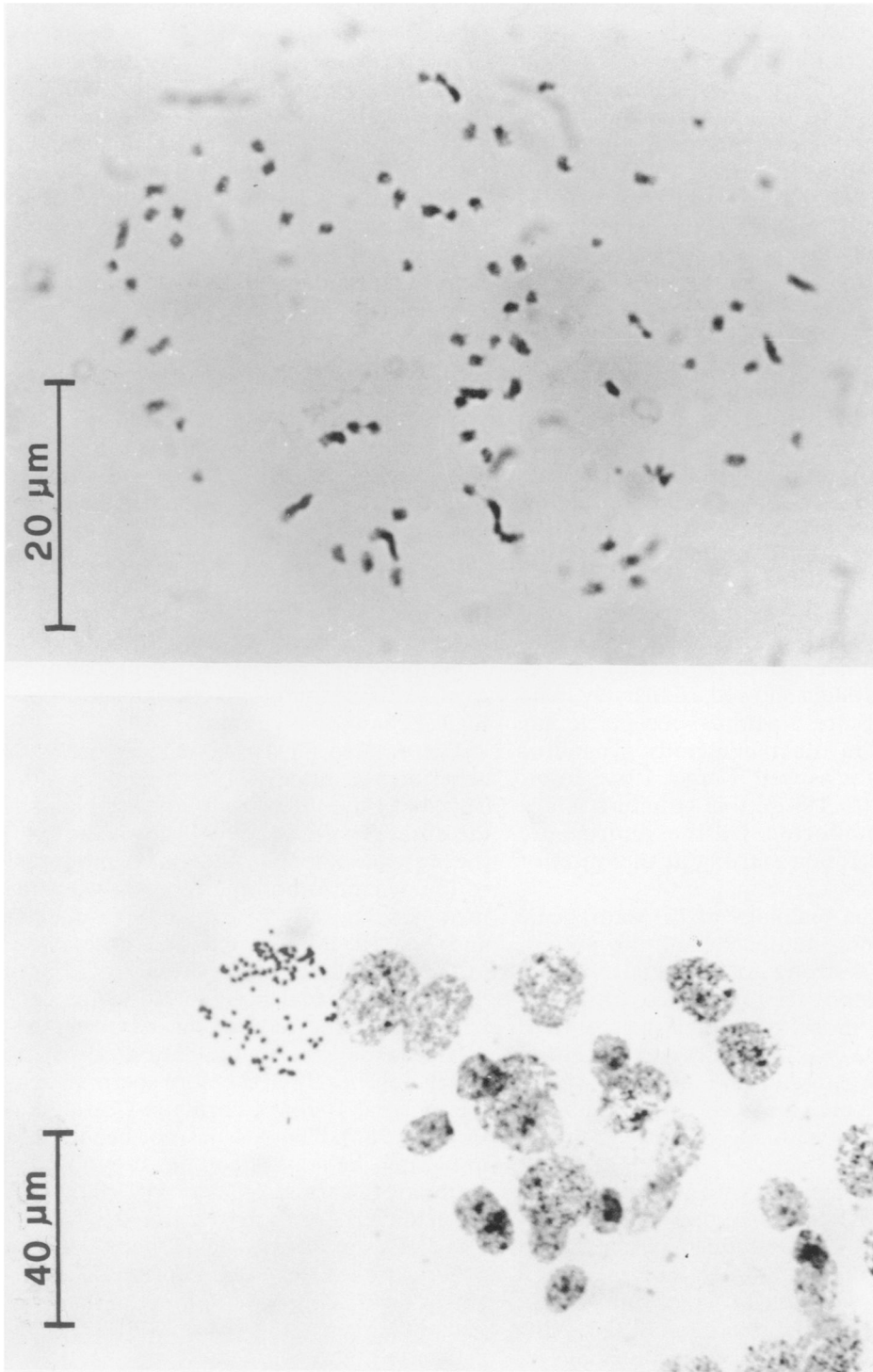


Fig. 4. Prometaphase plates prepared from testicular lobules of a gynandromorph of *Potamon fluviatile*. Giemsa.

DISCUSSION

In other bilaterally gynandromorphic *Brachyura* examined histologically, there was less expression of female than of male characteristics (Cargo, 1980; Johnson and Otto, 1981). This is the first example of a bilateral gynandromorph with an exclusively spermatogenic gonad.

A normal seminal receptacle was present and, in addition, the seminal receptacle was full of sperms. In *Potamon fluviatile*, mating usually occurs between large males and small, immature females. Prepubertal females (i.e., smaller than 35 mm CL) have completely developed seminal receptacles, some already containing sperm (Micheli *et al.*, 1990). The presence of a spermatheca containing sperm in the gynandromorph gives circumstantial evidence that the animal behaved as a female in its immature stages, and may indicate that a hormonal female milieu was prevailing prior to the onset of puberty.

In this sexually mature specimen, the seminal receptacle was identical to that of a normal female, while the rest of the reproductive system showed exclusively male characters. Since a seminal receptacle has been found in other bilaterally gynandromorphic crabs as well (Cargo, 1980; Johnson and Otto, 1981), this structure is apparently unaffected by the androgenic hormone secretion starting at the onset of sexual maturity.

Though the histology of the androgenic gland was not studied during the present work, there is strong experimental evidence that this gland is the only source of the androgenic hormone (AH) in all the groups of Malacostraca examined (Charniaux-Cotton *et al.*, 1966; Adiyodi and Adiyodi, 1970). Thus, all the changes occurring in the male in coincidence with the puberty molt depend on the AG activity.

The complete masculinization of the gynandromorphic gonads in this specimen, together with the successful masculinization of females of *Macrobrachium rosenbergii* implanted with AG (Nagamine *et al.*, 1980b), suggests that secretion of the AG is able to induce sex reversal in bisexual Decapoda. Moreover, ablation of the AG in immature males of *Macrobrachium rosenbergii* caused their complete feminization,

reversible with AG reimplantation, while andrectomized mature males exhibited an atrophy of testis and vas deferens (though meiosis was still occurring) and regeneration of immature pleopods after amputation (Nagamine *et al.*, 1980a). In the naturally occurring absence of the AG, the vas deferens did not differentiate, the male pleopods were immature, and, though the female secondary sexual characters developed, no ovarian tissue was found and spermatogenesis was not inhibited (in *Quadrrella coronata* (Trapeziidae), Galil and Tom, 1990). In other documented cases, spermatogenic activity, once initiated, continued in the absence of AH (in *Carcinus maenas*, Payen, 1974b), but more commonly spermatogenesis requires the presence of circulating AH (Charniaux-Cotton and Payen, 1988).

As a whole, the above observations give the following picture of the androgenic hormone's effect on *Brachyura*: (a) it initiates and stimulates spermatogenesis, though it is not always necessary for its maintenance, (b) it induces the formation of the vas deferens and is necessary for its maintenance, (c) it inhibits vitellogenesis, (d) it masculinizes ovarian tissue, and (e) it regulates morphological changes of gonopods.

The data so far available, together with experimental manipulation, seem to indicate that these modifications take place in the order reported above. If this is the case, the presence of female portions in the gonad of the gynandromorphic blue crabs examined histologically (Cargo, 1980; Johnson and Otto, 1981) might reflect an earlier phase in the masculinization process.

In crabs, sexual differentiation first appears in juveniles as external characters, later as gonads (Charniaux-Cotton, 1975), in which oogenesis starts before spermatogenesis (Payen, 1974a; Charniaux-Cotton and Payen, 1985). The AG has not been found until after the male gonad has begun to differentiate (Charniaux-Cotton, 1975), so that when the AH begins to be secreted, sex characteristics are already developed. Thus, in gonochoristic Decapoda, a hormonal mechanism controlling development of the male sexual characteristics prevails after the puberty molt, and counteracts the genetic determinism operating in the earlier stages.

In this specimen, in contrast with the complete masculinization of the gonads on

both sides of the body, female gonopods were present on the right side, even though a male morphology was recognizable on the first of these gonopods. A partial masculinization of the first pleopods was also observed in females of *Carcinus maenas* and *Rhithropanopeus harrisi* (see Payen, 1969, 1975) and *Procambarus clarkii* (see Nagamine and Knight, 1987) after implantation of the AG.

Insofar as the second, third, and fourth pleopods are concerned, the target territories may have lost their ability to respond to the AH, once they developed a complete female morphology, or the titer of circulating AH in this specimen may not have been high enough to induce a complete male morphology of the gonopods (Charniaux-Cotton and Payen, 1988). In addition, it can be expected that modification of external organs occurs more slowly than for internal ones, since shape changes can intervene only in coincidence with a molt, which is a rare event, especially after puberty (see discussion in Micheli *et al.*, 1990).

In conclusion, the role of the AG in sex determination seems similar in Isopoda, Amphipoda, and Decapoda as suggested by Nagamine *et al.* (1980a, b), but its effects, in the last group, are detectable only after maturity and probably need a longer time to become evident, particularly the secondary sex characters which are already well differentiated by the time puberty intervenes.

Karyotype analyses have demonstrated the existence of sex chromosomes in several crustacean species (Hedgecock *et al.*, 1982). In particular, an XO mechanism of the sex chromosomes has been found among Branchiopoda, Ostracoda, Copepoda, Rhizocephala, Isopoda, and Decapoda (Ginsburger-Vogel and Charniaux-Cotton, 1982). Therefore, even though an XY or an X_nY mechanism seems more common, differences between male and female chromosome numbers are often found among crustaceans. This is probably not the case for *P. fluviatile*, where an attempt to describe differences between the male and female genotypes, in this specimen, on the basis of chromosome number failed, since the number was variable and did not differ significantly on the two sides of the animal. Chromosome numbers vary greatly in other

decapod species (in *Homarus* sp., Roberts, 1969, Hughes, 1982; in *Nephrops norvegicus*, Farmer, 1974). Furthermore, the determination of chromosome number and cellular DNA content in four species of the genus *Penaeus* revealed a significant difference among species, but not between sexes (Chow *et al.* 1990).

In *P. fluviatile*, the variability found (numbers varied in a range 49–75) may be too high to allow detection of eventual small differences in chromosome numbers. Alternatively, sex determination may be linked to factors other than chromosome number (see review by Ginsburger-Vogel and Charniaux-Cotton, 1982).

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