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## Pseudohermaphroditism in *Ilyanassa obsoleta* (Mollusca: Neogastropoda)

**Abstract.** *Field studies, a rearing and maintenance program, and a transfer experiment have shown that anomalous male traits found in some female Ilyanassa obsoleta are an expression of environmentally controlled pseudohermaphroditism. The particular pattern of pseudohermaphroditism discovered in this species has not been reported previously from any other group, but may be characteristic of the Neogastropoda.*

Females with a penis have been found in a number of apparently dioecious neogastropod species in which most individuals are clearly male or female (1-12). The female's penis is located on the head behind the right cephalic tentacle, just as the male's is; it is sometimes "vestigial," but in other cases resembles the male's organ in both size and shape (1-9). In some females a "sperm duct" leads up to the penis, and the oviduct is convoluted, resembling the male's vas deferens (1-4). Although they possess male sexual traits, these penis-bearing females are not simultaneous hermaphrodites. Dissections, gonadal smears, and histology have shown that they have a complete female system and produce ova, but their male system is incomplete, the testis being absent (1-6).

Several hypotheses have been proposed to explain this phenomenon. The

most notable is that the snails are protandric hermaphrodites that first function as males, then change into females, losing the testis but keeping male secondary traits (4, 12, 13). But sex ratio and size frequency analyses done on populations of two species having some females with male structures have failed to provide any evidence of sex change (6, 11), and the hypothesis has not been tested experimentally.

The study of anomalous sex traits in neogastropods has been hampered by the similar coloration of the male and female systems in most species, which makes the distinction between males and penis-bearing females difficult (6, 13, 14). The mudsnail *Ilyanassa obsoleta*, a neogastropod in which some females have a penis (3), differs in this regard; males have a bright red-orange testis, whereas the females have a white ovary and a

white egg capsule gland with red lips. It is thus simple to tell whether a dissected *I. obsoleta* with a penis is actually a male, or a female with male traits. This made *I. obsoleta* well suited for a study of anomalous sex traits.

I examined over 10,000 *I. obsoleta* from more than 70 populations during a 10-year period. Each snail was cracked and removed from its shell; its sex and any anomalous sexual traits were recorded. To assure the accuracy of sexual identifications, I examined the entire reproductive system, not just a single diagnostic feature.

Female *I. obsoleta* with male traits were found to be common; however, the percentage of females having a penial organ varied greatly between sites. Although a complete range of population types was found, at many sites no females with a penial structure were ever collected; in many other locations nearly all the females examined had a penis of some kind (Table 1). The two extreme population types showed no geographical pattern. They did not appear related to sex ratio, and males were not scarce at the sites where most females had a penis (15). Repeated samples revealed that male traits were present year round at the sites where females had them. The female's penis was lost at the end of the reproductive season, just as the male's was, but in both sexes a penial bump remained from which a new penis later developed.

Most populations, including some followed for 10 years, stayed the same year after year. At those sites where females originally lacked male traits (for example, Newport River Causeway, North Carolina), they continued to lack them during the survey period. Where females originally had a penis (for example, Bogue Sound, North Carolina), females had penial organs throughout the study. This was true even where populations of the two extreme types were located within the same sound or estuary and only a few kilometers apart. This consistency cannot be explained either by genetic isolation or by low recruitment rates, since the larvae of *I. obsoleta* have a long planktonic existence (16), and immature forms comprise a large portion of the populations each year.

Males with a female character, the eggcase-molding foot gland, were also found; this anomaly has not been reported before from any neogastropod. Such males were very rare, and their occurrence did not appear related to the presence of penes in females.

Immature *I. obsoleta* form distinct

year classes that can be recognized readily (17). Examination of these individuals disclosed that although they lack a gonad and sex ducts until about 2½ years after metamorphosis, some have a bump or other penial structure in the same location as the adult's penis; this appears as early as 3 weeks after metamorphosis. There are no previous published reports of such sexual traits occurring in immature neogastropods. Immature forms having a penial structure and those lacking it were not distributed randomly among the populations sampled (Table 1). In those populations where the adult females lacked male traits, immatures with a penial bump and those without it were present in an approximate 1:1 ratio. In populations where most adult females had a penis, all the immatures had a penial structure also. This pattern suggested that *I. obsoleta*, like some other molluscs (18), has alternative patterns of sexuality; at some locations it acts as a dioecious species, with those immatures having a penis developing into males, those without developing into females; at other sites the snails function as pro-

tandric hermaphrodites, with all immatures developing first into males, then some or all changing into females later, retaining part of the male system from their previous sexual phase.

To test this hypothesis of alternative sexuality, I collected *I. obsoleta* from two populations that displayed the two extreme sexual patterns. The secondary sex traits of live uncracked snails were then ascertained by holding each snail in a bowl of seawater set on a microscope stage, until the animal fully extended its head and foot. Immatures were examined for a penial bump, adults for a penial organ and a pedal eggcase-molding gland. The snails were marked to indicate their source and sexual characteristics, then placed in identical outdoor tanks at the University of North Carolina's Institute of Marine Sciences. The tanks were close together, but there was no communication between them, and they were covered with wire mesh to prevent the snails from escaping.

Table 2 shows the design and expected results of the four experimental groups. In tank 1 were placed 200 immatures

from Folly Creek, Virginia, a "dioecious" population. Of these, 100 had a penial bump; the other 100 lacked such a structure. It was hypothesized that those with a penial organ would become males, those without would become females. In tank 2 were placed 200 immatures from Lewes, Delaware, a "protandric" population. All these possessed a penial organ, and it was hypothesized that all would become males. In the third tank, 500 males from Lewes were maintained in isolation from females, while in tank 4 were placed 250 males and 250 females from the same site. The snails in tank 4 were not marked to indicate their sex. It was hypothesized that if the Lewes population were protandric, some of the 500 isolated males would become females, and the ratio of females to males in the last tank would increase.

The snails were put in the tanks in May, at the end of the reproductive season, and kept there through the summer nonreproductive period. When the animals entered reproductive condition in the fall, they were removed from the tanks, cracked, and examined. The immatures from Folly Creek developed as expected (Table 2). All with a penial bump became males; all without became females. But the immatures from Lewes did not all develop into males. Approximately half of these immatures with a penial bump developed directly into penis-bearing females without passing through a male phase. The data did not depart significantly from results expected if potential females and potential males were present among the Lewes immatures in a 50:50 ratio ( $\chi^2 = .364$ ;  $P > .5$ ). Furthermore, none of the isolated males became females, and there was no significant change in the ratio of males to females in tank 4 ( $\chi^2 = .05$ ;  $P > .75$ ).

The basic sexuality of the two *I. obsoleta* populations is thus the same, despite the extreme differences between them in the occurrence of male secondary traits in immature and adult females. In both kinds of populations, potential males and potential females are present among the immatures in an approximate 50:50 ratio. And, in both, each immature becomes either a male or female when it reaches sexual maturity, then retains that sexual identity.

Thus *I. obsoleta* is a dioecious species in which anomalous sex traits occur in some individuals; this type of sexuality is called pseudohermaphroditism (19). The terms male and female pseudohermaphrodite have been used to describe the two forms of this sexual state,

Table 1. Occurrence of penial structures in female and immature *I. obsoleta*. Data from nine representative sample sites, showing the number of females without a penial structure (♀ ♀), females with a penial structure (♀ ♂ ♀ ♂), immatures without a penial structure (imm<sup>0</sup>), and immatures with a penial structure (imm<sup>♂</sup>) collected.

Sample site	Type of female		Type of immature	
	♀ ♀	♀ ♂ ♀ ♂	imm <sup>0</sup>	imm <sup>♂</sup>
Bird Shoals, Beaufort, North Carolina	59	0		
School Marsh, Wrightsville Beach, North Carolina	59	0		
Radio Island, Beaufort, North Carolina	103	0	13	13
Folly Creek, Virginia	78	0	179	167
Newport River Causeway, Beaufort, North Carolina	467	0	153	118
Bridgeport, Connecticut	2	56	0	96
Banks Channel, Wrightsville Beach, North Carolina	3	98	0	123
Bogue Sound, Morehead City, North Carolina	29	651	2	124
Lewes, Delaware	15	1188	0	1404

Table 2. Rearing and maintenance experiment. Symbols: ♂ ♂ = males; ♀ ♀ = females without a penial organ; ♀ ♂ ♀ ♂ = females with a penial organ; imm<sup>0</sup> = immatures without a penial organ; imm<sup>♂</sup> = immatures with a penial organ. Numbers in parentheses indicate the numbers of individuals recovered.

Sample site	Population type	Initial groups	Expected results	Actual results
Folly Creek	Dioecious	100 imm <sup>♂</sup>	Tank 1	
		100 imm <sup>0</sup>	100 percent ♂ ♂	100 percent ♂ ♂ (72)
			100 percent ♀ ♀	100 percent ♀ ♀ (81)
Lewes	Protandric	200 imm <sup>♂</sup>	Tank 2	
			100 percent ♂ ♂	48 percent ♂ ♂ (84) plus 52 percent ♀ ♂ ♀ ♂ (92)
Lewes	Protandric	500 ♂ ♂	Tank 3	
			Some ♀ ♂ ♀ ♂	100 percent ♂ ♂ (400)
Lewes	Protandric	250 ♂ ♂ plus 250 ♀ ♂ ♀ ♂	Tank 4	
			An increase in percentage of females	50 percent ♂ ♂ (241) plus 50 percent ♀ ♂ ♀ ♂ (246)

but these suffer from ambiguity and awkwardness. I propose that a male with false female traits be called a pseudogynous male and be symbolized  $\delta^g$ , and that a female with false male attributes be termed a pseudandric female and be symbolized  $\phi^d$ .

The pseudohermaphroditism of *I. obsoleta* appears to be the first demonstrated instance in the molluscs of this type of sexuality, which is known primarily from vertebrates (19). The kind of pseudohermaphroditism displayed by *I. obsoleta* is very unusual. The marked differences between populations, the correlation between the degree of pseudandry in adult females and the prevalence of penial organs in immatures, and the maintenance of population character despite apparent genetic exchange, together form a pattern that has not been reported in any other group of pseudohermaphrodites. Furthermore, this pattern suggests that in *I. obsoleta* pseudohermaphroditism is environmentally controlled. This hypothesis is supported by the results of a transfer experiment I conducted between a pseudandric and nonpseudandric population, using 400 *I. obsoleta*. Normal males transferred between the sites showed no change. But every pseudandric female moved to the nonpseudandric location either lost all male traits or kept only a small penial bump, while all their controls at the home site retained large penes. Similarly, some nonpseudandric females moved to the pseudandric location developed male attributes, while all their controls remained free of any male characteristics (20).

I also examined other neogastropods to determine how common anomalous sex traits are within the order. I found such traits in almost every species I collected, thereby increasing from 18 to 34 the number of species and subspecies in which they are known; these include members of all four superfamilies (21). Also, although they are not as extensive, my field data on *Urosalpinx cinerea*, *Nucella emarginata*, *Eupleura caudata*, *Nassarius vibex*, *Fasciolaria lilium hunteria*, *Terebra dislocata*, and others bear a striking resemblance to those on *I. obsoleta*. Populations differ in the prevalence of penial structures in adult females; the occurrence of these traits is related to the proportion of immatures bearing a penial organ; and populations maintain their character even when near different populations of the same species (20). These strong parallels indicate that environmentally controlled pseudohermaphroditism may be a major sexual

phenomenon in the Neogastropoda and, indeed, may be the basic sexual pattern in the order.

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15. For example, at those sites in Table 1 where females lacked male traits, the total percentage of females found in the samples ranged from 43 percent at Radio Island to 69 percent at Bird Shoals; at those sites where the females had a penial organ, the total percentage of females in the samples ranged from 46 percent at Bogue Sound to 63 percent at Banks Channel. These figures do not suggest that the occurrence of male traits in females is related to a scarcity of males in a population. However, determining the true sex ratio of an *I. obsoleta* population is difficult. These are social animals with complex behavior that varies with the season and depends on the sex of the individual and its state of

maturity, so samples taken from the same population within a short time may yield widely different sex ratios. Therefore, the question of whether there is any relation between sex ratio and the prevalence of anomalous male traits in females deserves more investigation.

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21. Prior to this study, anomalous male characters had been reported in females of 18 neogastropod species or subspecies (1–12). I have found such traits in the females of 12 other kinds of neogastropods. I have also discovered the ventral pedal gland, a female structure, in males of six neogastropod species and have seen penial organs in immatures of 12 neogastropod species. Anomalous sexual attributes of at least one sort are now known in *Murex florifer dilectus*, *M. pomum*, *Urosalpinx cinerea*, *U. cinerea follyensis*, *U. perrugata*, *U. tampaensis*, *Thais haemastoma floridana*, *T. haemastoma canaliculata*, *Nucella lapillus*, *N. emarginata*, *N. lima*, *Ocenebra erinacea*, *Eupleura caudata*, *E. caudata etterae*, *E. sulcidentata*, *Calatrophon ostrearum*, *Anachis avara*, *Mitrella lunata*, *Pisania tinctus*, *Melongena corona*, *Buscycon carica*, *B. contrarium*, *Nassarius vibex*, *N. trivittatus*, *Ilyanassa obsoleta*, *Fasciolaria lilium hunteria*, *Pleuroploca gigantea*, *Olivella biplicata*, *Marginella apicina*, *Conus mediterraneus*, *Terebra dislocata*, *Terebra protecta*, *Kurtziella cerina*, and *Propebela turricula*.
22. This work was done in partial fulfillment of Ph.D. requirements in Zoology at the University of North Carolina at Chapel Hill. I thank the faculty of the UNC Department of Zoology and the UNC Institute of Marine Sciences, Morehead City, N.C., for their assistance with this research, and give special thanks to C. E. Jenner for his guidance and support.

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## Substance P: Evidence for Diverse Roles in Neuronal Function from Cultured Mouse Spinal Neurons

**Abstract.** *Mouse spinal neurons grown in tissue culture were used to examine the membrane mechanisms of action of the peptide substance P. Two functionally distinct actions were observed, one being a rapidly desensitizing excitation, and the other being a dose-dependent, reversible depression of excitatory responses to the putative amino acid neurotransmitter glutamate. These effects on excitability suggest that substance P may play more than one role in intercellular communication in the nervous system.*

The presence of the peptide called substance P (SP) in specific neurons and pathways in the central nervous system (CNS) (1) has prompted many investigations into its role in neuronal function (2). Discrete pharmacological applications of SP onto central neurons in vivo and in situ alters their excitability (3–10), suggesting that the peptide mediates specific synaptic transmissions in the same way as a neurotransmitter (3, 7–9). The relatively slow time course of some SP responses (4, 6, 7, 10) and the finding that SP depresses responses to other putative neurotransmitters (11, 12) have led to the suggestion that the peptide may not function as a neurotransmit-

ter (4, 6, 7, 10, 11, 13). We have used dissociated mouse spinal neurons grown in cell cultures as a model system to study the membrane mechanisms of action of SP, and we report here that the peptide clearly has at least two operationally distinct actions on membrane properties, including a rapid excitatory effect.

Spinal neurons were dissociated from 13-day-old mouse embryos and grown in tissue culture for 2 to 3 months according to methods described (14, 15). The neurons, growing as a monolayer in a plastic petri dish, were then brought to the modified stage of an inverted phase microscope and viewed at  $\times 250$  magnification