Development of a Penis from the Vestigial Penis in the Female Apple Snail, *Pomacea canaliculata*

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In the apple snail (*Pomacea canaliculata*), females have an undifferentiated mass of tissue near the anus. Although this mass is called the vestigial penis, there are no signs of a hermaphroditic gonad or any structure that represents a transition from one sex to the other. Based on considerations of the steroid hormone theory of reproduction and in view of disruption of endocrine systems in molluscs by organotins, a study was made of the effects of tributyltin on female snails. Exposure to tributyltin resulted in the so-called imposex phenomenon, and both a penis and a penis sheath were newly generated from the so-called vestigial penis. The same phenomenon was also induced by testosterone. Thus the vestigial penis, named more than one hundred years ago, has been demonstrated for the first time to be a rudiment of the penis itself.

The Ampullariidae (Gastropoda, Prosobranchia, Architaenioglossa) is a family of freshwater prosobranchs that are widely distributed in Asia, Africa, and South America (1). It is well known that some molluscs exhibit unusual sexual diversity and hermaphroditism (2, 3). One of the most interesting features of members of the Ampullariidae is that females in 5 of the 10 genera—namely, *Pila* (4, 5, 6, 7, 8, 9, 10, 11, 12), *Pomacea* (13, 14, 15, 16), *Lanistes* (13, 17, 18), *Afropomus* (19), and *Turbinicola* (13)—have a so-called vestigial penis in addition to the normal reproductive system (Fig. 1). The vestigial penis was first described by Bouvier (4, 5), a skilled neuroanatomist, in *Ampullaria (Pila) polita* more than 100 years ago. Since then, many molluscan researchers have used that term for this tissue without further explanation. The question examined here is this: Is the organ historically described as a vestigial penis a remnant of a penis—a degenerate structure that lacks the capacity to develop further—or is it a rudiment, or precursor—an incipient structure that, under the proper conditions, could develop into a penis?

The vestigial penis is a tongue-like structure lying inside the elevated mantle skirt, near the anus (Fig. 2A). Histologically, it consists of connective tissue, and no differentiation of the structure is apparent during the life cycle of the female (Fig. 2B).

To my knowledge, no experimental evidence has been presented to justify the designation of the elevated tissue near the anus in some female snails as a “vestigial penis” (4, 5). No clear evidence of hermaphroditism has yet been shown in any extant species of Ampullariidae. In *Pomacea canaliculata*, the apple snail, the positions of the gonads are basically different: the testis is located at the tip of the spiral, and the ovary is spread over the surface of the hepatopancreas at a location similar to that of the testis. It has also been confirmed that there is no apparent precursor or vestige of a hermaphroditic condition in any part of the reproductive system throughout the life history. As Andrews (13) stated, the copulatory apparatus appears to develop at the same rate in both sexes until the gonad becomes active, when its growth is arrested in the female. Andrews hypothesized that the gonad might produce a hormone responsible for the cessation of growth, but in the early 1960s, when this work was published, the chemical nature of reproductive hormones in molluscs had not yet been established. In 1991, Berthold (20) proposed that the so-called vestigial organ be designated an “oriment”—a term implying that the tissue is a precursor with the potential to develop into an adult organ. The question then arose as to whether such a designation might be appropriate. As a basis for such a designation, at the very least, some experiments involving implantation of testes into females should be performed to determine whether a true penis might develop from the tissue mass.
As an approach to this problem, I examined the effects of an endocrine disruptor that appears to be associated with yet unresolved environmental problems. Organotins, and in particular tributyltin, which is a component of some anti-fouling paints, induce a condition known as “imposex” in prosobranch gastropods. Imposex, in which a penis and vas deferens develop in females (21), has been widely observed in marine snails that belong to the Caenogastropoda; _Nucella_ and _Littorina_ are common examples (22). Females of these species lack a vestigial penis, but the capacity exists for induction of a penis and vas deferens develop in females (21), has been widely observed in marine snails that belong to the Caenogastropoda; _Nucella_ and _Littorina_ are common examples (22). Females of these species lack a vestigial penis, but the capacity exists for induction of a penis and vas deferens. _Pomacea canaliculata_ has been proposed as a potential bioindicator for tributyltin (22), which is used as a biocidal agent against molluscs, in fungicides (23) and in anti-fouling paints in freshwater environments. Anticipating possible endocrine disruption by tributyltin, I examined its effects to see whether the vestigial penis in this species might develop further after female snails were exposed to this compound and, if such a penis did develop, how would it differentiate?

Female specimens of _P. canaliculata_ were reared in water that contained 30 ng/l tributyltin. About 3 months after the start of treatment, the outside tissue of the vestigial penis began to form a long process that resembled a penis, and its interior developed as a thick mass. These structures grew gradually and reached a maximum size after about 6 months of treatment with tributyltin (Fig. 3A). Histological staining revealed that the inside tissue mass contained a penis; the cross section of a penis was also found within the tissue mass (Fig. 3B). Thus, the outside structure appeared to be a penis sheath. Within about one further month, a complete penis had developed from the tissue of the vestigial penis (Fig. 4).

In _P. canaliculata_, the copulatory system of the male consists of a stout penis sheath and a long, slender penis.
within it (24). The penis and the penis sheath are located together to the left of the extreme right margin of the mantle cavity. In treated females, the arrangement of these male copulatory organs was similar but differed in the distance between the penis sheath and the penis: the penis sheath in females was located at the edge of the ctenidium at the mantle skirt, at a distance from the penis.

It has been suggested that tributyltin inhibits cytochrome P450 aromatase, which converts testosterone to estradiol in females (25, 26). Inhibition of aromatase activity thus increases levels of testosterone which induces imposex, with the development of male copulatory organs. Development of the imposex phenomenon in *P. canaliculata* was also confirmed by direct treatment with testosterone. Female snails reared in water that contained 500 ng/l testosterone exhibited changes similar to those induced by tributyltin, including the development of a penis sheath and a penis. Therefore, these observations support the proposed mechanism of action of tributyltin.

It is difficult to explain the unusual phenomenon of a rudimentary penis in females; however, I propose the following hypothesis. In the early stages of development, both sexual rudiments develop as an undifferentiated tissue mass. Once the sex of the snail is determined genetically (27), however, these rudiments differentiate in response to the secretion of specific sex steroid hormones. The undifferentiated tissue mass that develops into a penis in males is left as an arrested rudiment in females. The vestigial penis develops into a complete copulatory organ only if the anlage of the gonad becomes a testis.

**Figure 2.** (A) General appearance of the vestigial penis in a female apple snail (*Pomacea canaliculata: control*) reared by artificial mass culture. Scale bar represents 1 mm. (B) Histological appearance of the vestigial penis in A. The regions containing a vestigial penis were fixed in Bouin’s fluid and embedded in paraffin wax by the standard method. Sections were stained with hematoxylin and eosin. Scale bar represents 200 μm. A, anus; C, ctenidium; OD, oviduct; VP, vestigial penis.

**Figure 3.** (A) Morphology of the imposex induced by tributyltin in a female apple snail (*Pomacea canaliculata*). Female snails (n = 100) for experiments were reared in a freshwater tank that contained tributyltin (Tokyo Kasei, Co. Ltd., Tokyo, Japan) at 30 ng/l for about 6 months. The state of imposex was checked at weekly intervals. Scale bar represents 1 mm. Similar results were also obtained in female snails (n = 100) reared with testosterone (Wako, Co. Ltd., Osaka, Japan) at 500 ng/l for about 7 months. (B) Histological appearance of the vestigial penis in A. The arrowhead indicates the cross section of a penis. Hematoxylin and eosin stain. See legend to Fig. 2B for methods. Scale bar represents 200 μm. P, penis; PS, penis sheath; VD, vas deferens (see legend to Fig. 2 for other abbreviations).
The “steroid hormone theory,” which I proposed previously (28, 29) for the reproduction of terrestrial pulmonates, apparently also applies to prosobranch snails. This theory states basically that the development of accessory sex organs is controlled by steroid hormones secreted by the gonad. This concept of the effects of hormones on snail reproduction, together with the effects of endocrine disruption in molluscs, allowed me to demonstrate, for the first time, that the so-called “vestigial penis,” named more than one hundred years ago (4, 5), is a rudiment of the penis itself.

Literature Cited


Figure 4. (A) A penis that arose from the vestigial penis of a female apple snail (Pomacea canaliculata) after treatment with tributyltin for about 7 months. The same phenomenon was also seen in female snails reared with testosterone for about 8 months. See legend to Fig. 3A for methods. (B) The extirpated penis from the vestigial penis. See legends to Fig. 2 and 3 for other abbreviations. Scale represents 1 mm.


