Sperm in "Parthenogenetic" Freshwater Gastrotrichs

Abstract. *Freshwater members of the phylum Gastrotricha have been considered obligate parthenogens. In* Lepidodermella squammata, *the species for which there is most evidence for parthenogenesis, sperm have been discovered. This finding will necessitate reexamination of the nature of sexuality and life cycles and of the concept of "species" in freshwater gastrotrichs.*

The Gastrotricha are a phylum of microscopic animals, traditionally included in the invertebrate assemblage known as "aschelminths." About half of the known gastrotrich species inhabit fresh water (*1*), and these are widely distributed within the same habitats as rotifers and ciliated protozoans, for which they are frequently mistaken. The body of freshwater gastrotrichs, often only 70 to 200 μm long, includes a straight gut with mouth and anus, a brain, protonephridia, gonads, muscle cells, a highly ornamented cuticle, and ventral locomotory cilia. In addition, the freshwater gastrotrichs show certain features of general interest, including the largest ratio of egg size to adult size in the animal kingdom (*2*), cell constancy (*3*), and an ostensibly parthenogenetic mode of reproduction.

Of the two orders (*4*) into which the phylum is divided, the Macrodasyida are exclusively marine or brackish-water forms, while the Chaetonotida are found mostly in fresh water. Although all well-studied macrodasyids and some marine chaetonotids are known to be hermaphroditic (*5*), the freshwater chaetonotids have been widely considered to be obligate parthenogens (*6*). In no freshwater gastrotrich has convincing evidence appeared for the existence of male gametes (*7*). Thus the usual biological definition of species, which entails interbreeding or potential interbreeding, could not be expected to apply to this group composed of perpetually independent clones. Studies of one common species, *Lepidodermella squammata* (*8–12*), have provided the strongest evidence for a parthenogenetic life cycle. We report here the unexpected discovery of sperm in individuals of this species (*13*).

Specimens from mass culture (*14*) were studied alive under Zeiss planapochromatic optics (numerical aperture 1.4) equipped for Nomarski differential interference contrast. The strong optical transparency of the organisms permits internal structures to be readily observed in living specimens. Additional observations on sperm nuclei were made from Feulgen preparations of animals fixed in a buffered mixture of paraformaldehyde and glutaraldehyde (*15*). Some Feulgen-stained specimens were mounted in euparal, whose relatively low refractive index permits clear visualization of the sperm's unstained cytoplasm with Nomarski optics.

The sperm occur grouped in packets situated lateral to the intestine near the ventral surface of the organism, about two-thirds of the distance from the anterior end (Fig. 1A). The packets agree in position with, and clearly correspond to, the testes or "sperm sacs" known in some marine chaetonotids (*16, 17*). No associated limiting membrane or ducts are discernible with our methods. Sperm-bearing individuals possess either one bilateral pair of packets (Fig. 1, A and E) or a single unilateral packet (Fig. 1, B and C). Although additional aggregations, such as might be expected within a storage duct or vesicle, have not been found elsewhere in the body, Feulgen preparations reveal that individual

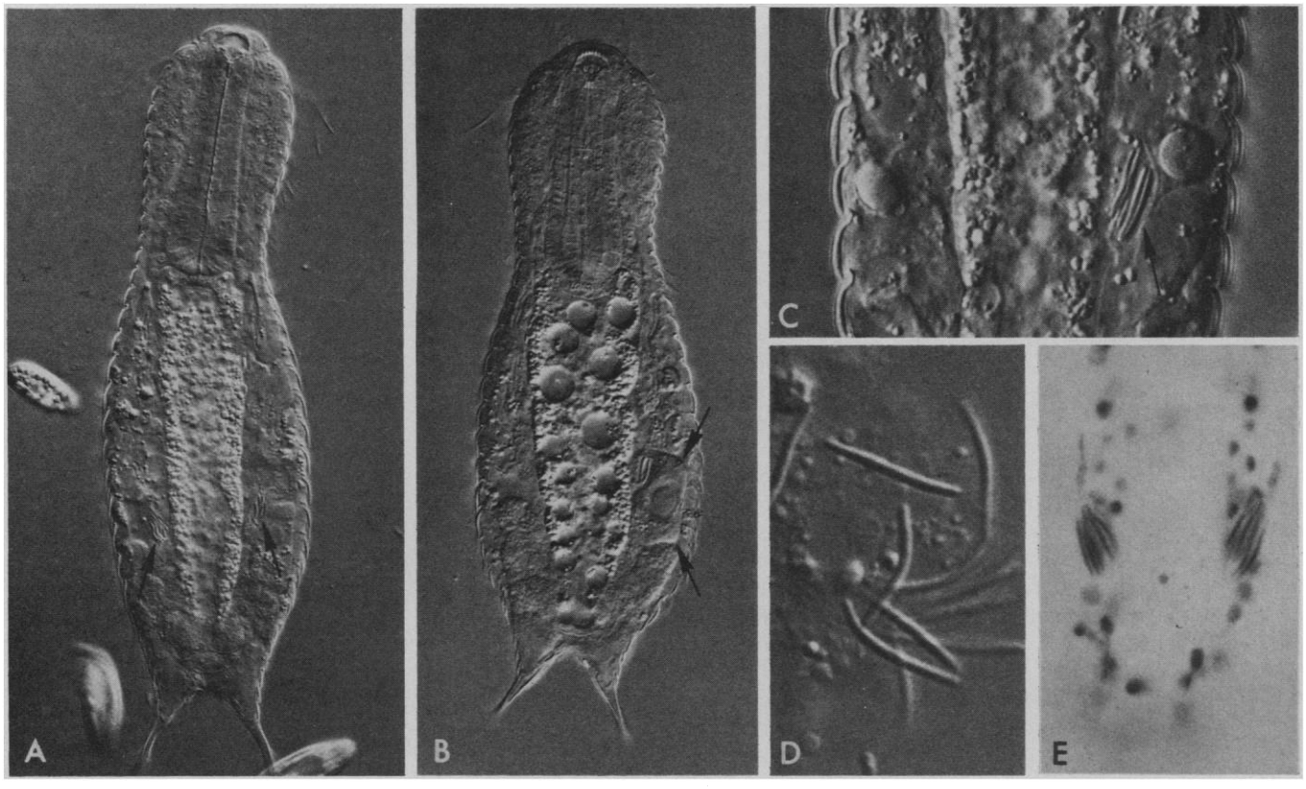

Fig. 1. (A to C) Photomicrographs of living animals (Nomarski optics). (A) Fully grown animal with bilateral sperm packets (arrows) (×610). (B) Young animal with a unilateral sperm packet. Two sperm in this packet are visible adjacent to a maturing ovum (delimited by arrows), which exhibits a large nucleus. Other sperm lie outside the focal plane (×610). (C) High-magnification view of several sperm (arrow) within a unilateral packet (×1310). (D) I ıving sperm artificially freed from the body (Nomarski optics) (×2300). (E) Posterior portion of an animal showing Feulgen-stained bilateral sperm packet (bright field) (×1640).

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sperm may occur far removed from the packets, usually anteriorly. These isolated sperm are variously oriented and probably lie in body spaces.

Within a packet the sperm cells are often closely packed and typically lie parallel or at a slight angle to the longitudinal axis of the body (Fig. 1C). Although occasionally the sperm are tightly curled, they usually resemble slightly bowed, elongated rods, 8 to 9 μm in length and about 0.8 μm in diameter, with no obvious differentiation into anterior and posterior ends (Fig. 1D). Critical Nomarski observations of living sperm have failed to reveal flagella or internal structures. The size and shape of the nuclei as seen in Feulgen preparations (Fig. 1E) suggest that each sperm consists of a highly elongated nucleus surrounded by a thin layer of cytoplasm. The number of sperm per packet, though variable, is difficult to determine accurately because of their close packing. At least 13 can occur in one packet, and the maximum may approximate 16. The latter number, if correct, would correspond to the meiotic products of exactly four spermatocytes. No signs of motility have been observed in living sperm.

In culture, most fully grown and some younger animals exhibit ova in various stages of development, with no evidence of sperm. The highest proportion of sperm-bearing animals found, determined from an examination of 1800 Feulgen-stained specimens randomly collected from a culture fixed in toto, was only 8 percent; of these, 70 percent possessed bilateral and 30 percent possessed unilateral packets. In two living and 18 stained animals with uni- or bilateral sperm packets, a highly enlarged ovum was clearly present. Moreover, both living and Feulgen-stained animals with sperm packets commonly exhibit smaller ova (Fig. 1B). Thus, individuals with sperm packets are not males but hermaphrodites.

Sharp changes in the proportion of sperm-bearing animals in cultures have been observed over short periods of time. Hermaphroditism is therefore apparently facultative; parthenogenesis is probably the usual condition. Like the males of some rotifers, hermaphrodites may appear only infrequently in nature, which would help explain their having been overlooked. At present the factors responsible for their production remain speculative. In addition, the question remains whether hermaphrodites and parthenogens differ in genotype or only in phenotypic expression.

A variety of sperm types occurs within the Gastrotricha, in both macrodasyids (*3*, *18*) and chaetonotids (*3*, *19*, *20*). Differences in fertilization biology are believed to be pronounced within this phylum (*21*). As in other animals (*22*), the structural diversity of sperm is probably closely tied to these differences. The simplified, rodlike sperm of *Lepidodermella squammata* represent a hitherto undescribed type in Gastrotricha, although the short but still undescribed sperm of the brackish-water chaetonotid *Heterolepidoderma hermaphroditum* (*17*) may be similar.

The small number of sperm in *Lepidodermella squammata* and their lack of flagella indicate that fertilization, assuming that it occurs, is internal and does not involve free discharge into the water. Maturing ova lie freely in the body space with no sign of a limiting epithelium, and sperm packets in living specimens have been observed directly contacting ova (Fig. 1B). Therefore the possibility of self-fertilization, which has already been raised for some macrodasyids (*20*), must be seriously considered. Isolated sperm studied in Feulgen preparations have also been observed in direct contact with maturing ova, but the possibility that these sperm are of external origin cannot be excluded.

The discovery of sperm and thus the likelihood of sexuality (*23*) in *Lepidodermella squammata* is of particular significance because it is this species, the most frequently studied freshwater gastrotrich, in which the evidence for obligate parthenogenesis has been strongest (*8*–*12*). Consequently, in the many other, less well-known freshwater species, sexuality may be more widespread than has been assumed (*24*). The interrelated questions of sexuality, life cycles, and the nature of "species" in the freshwater Gastrotricha need to be reexamined.

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References and Notes

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2. A. Remane, in *Handbuch der Zoologie*, W. Kükenthal and T. Krumbach, Eds. (de Gruyter, Berlin, 1929), Band 2, Teil 4, p. 121.
3. A. Remane, in *Klassen und Ordnungen des Tierreichs*, H. G. Bronn, Ed. (Akademische Verlagsgesellschaft, Leipzig, 1936), Band 4, Abt. 2, Buch 1, Teil 2, pp. 1–385.
4. The phylum is subdivided into orders rather than classes. G. C. Rao [*Cah. Biol. Mar.* **11**, 109 (1970)] has emended the ordinal endings from "-oidea," which should be restricted to superfamilies, to "-ida."
5. For examples of hermaphroditic marine chaetonotids, see H. Schrom, *Helgol. Wiss. Meeresunters.* **23**, 286 (1972); W. D. Hummon (*16*).
6. This common belief is expressed by major reviewers [L. H. Hyman, *The Invertebrates: Acanthocephala, Aschelminthes, and Entoprocta* (McGraw-Hill, New York, 1951), vol. 3; P. de Beauchamp, in *Traité de Zoologie*, P.-P. Grassé, Ed. (Masson, Paris, 1965), Tome 4, Fasc. 3, p. 1381; R. W. Pennak, *Fresh-Water Invertebrates of the United States* (Wiley, New York, ed. 2, 1978)]. Nevertheless, some specialists who have worked with freshwater gastrotrichs [C. E. Packard (*8*); R. B. Brunson (*9*); W. D. Hummon (*1*)] have remained open to the possible existence of nonparthenogenetic freshwater species.
7. A. Remane (*3*) reports the presence in the freshwater species *Polymerurus oligotrichus* of two small bodies which he considers highly rudimentary testes, but he does not suggest that they have retained reproductive capacity. Another species described by Remane, *Chaetonotus hermaphroditus*, possesses well-developed sperm, but its habitat is unstated. In a recent abstract, M. R. Hummon [*Ohio J. Sci.* **78**, 11 (1978)], on the basis of preliminary analysis of electron micrographs, reports "the possible presence of sperm heads in post-reproductive" specimens of the freshwater species *Lepidodermella squammata*.
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13. Some of our findings have recently appeared in abstract form [M. J. Weiss and D. P. Levy, *Am. Zool.* **18**, 631 (1978); D. P. Levy and M. J. Weiss, *ibid.*, p. 631].
14. Cultures labeled "*Lepidodermella*" were obtained during June and July 1978, from Connecticut Valley Biological Supply Co., Inc., P.O. Box 326, Southampton, Mass. 01073. We are informed that the same stock has been maintained in laboratory culture for many years without addition. We have confirmed W. D. Hummon's (*12*) earlier identification of specimens from this source as *Lepidodermella squammata* (Dujardin, 1841).
15. Free aldehydes were blocked by sodium borohydride according to the procedure of F. H. Kasten and R. Lala [*Stain Technol.* **50**, 197 (1975)]; D. P. Levy and M. J. Weiss, in preparation.
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23. Although direct evidence for fertilization in *L. squammata* is lacking, the presence of differentiated sperm cells composed largely of nuclear material, and the widespread occurrence of sexuality in marine gastrotrichs [W. D. Hummon (*1*)], suggest that sperm in this species participate in pronuclear fusion.
24. Some previous findings are suggestive of the possible existence of two sexual phenotypes within the largest freshwater genus, *Chaetonotus*. Like many other freshwater invertebrates, some gastrotrichs have been shown to produce two kinds of eggs, resting and nonresting [R. B. Brunson (*10*)]. Nevertheless, three egg types have been reported in *C. spinifer* from nature [A. C. Stokes, *The Microscope* **7**, 33 (1887)] and in *C. tachyneusticus* from laboratory culture [R. B. Brunson (*9*, *10*)]. In the latter species, two egg types were found to be nonresting, and the organism would reproduce successfully only when two or more individuals were initially present.
25. We thank L. F. Gardiner and N. H. Hart for commenting on the manuscript. This work was supported in part by grant BNS 76-09645 from the National Science Foundation and by grants from the Busch Fund and Research Council of Rutgers University.

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