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Germ cell cysts and simultaneous sperm and oocyte production in a hermaphroditic nematode

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ABSTRACT

Studies of gamete development in the self-fertile hermaphrodites of *Caenorhabditis elegans* have significantly contributed to our understanding of fundamental developmental mechanisms. However, evolutionary transitions from outcrossing males and females to self-fertile hermaphrodites have convergently evolved within multiple nematode sub-lineages, and whether the *C. elegans* pattern of self-fertile hermaphroditism and gamete development is representative remains largely unexplored. Here we describe a pattern of sperm production in the trioecious (male/female/hermaphrodite) nematode *Rhabditis* sp. SB347 (recently named *Auanema rhodensis*) that differs from *C. elegans* in two striking ways. First, while *C. elegans* hermaphrodites make a one-time switch from sperm to oocyte production, *R.* sp. SB347 hermaphrodites continuously produce both sperm and oocytes. Secondly, while *C. elegans* germ cell proliferation is limited to germline stem cells (GSCs), sperm production in *R.* sp. SB347 includes an additional population of mitotically dividing cells that are a developmental intermediate between GSCs and fully differentiated spermatocytes. These cells are present in males and hermaphrodites but not females, and exhibit key characteristics of spermatogonia — the mitotic progenitors of spermatocytes in flies and vertebrates. Specifically, they exist outside the stem cell niche, increase germ cell numbers by transit-amplifying divisions, and synchronously proliferate within germ cell cysts. We also discovered spermatogonia in other trioecious *Rhabditis* species, but not in the male/female species *Rhabditis axei* or the more distant hermaphroditic *Oscheius tipulae*. The discovery of simultaneous hermaphroditism and spermatogonia in a lab-cultivatable nematode suggests *R.* sp. SB347 as a richly informative species for comparative studies of gametogenesis.

1. Introduction

Most animal species are dioecious (male/female). However, when mates are scarce, selective pressures can favor the evolution of self-fertile hermaphrodites (Clark, 1978; Ghiselin, 1969; Jarne and Charlesworth, 1993). Within the phylum Nematoda, evolutionary transitions from outcrossing males and females to self-fertile hermaphrodites have occurred in multiple, separate lineages (Denver et al., 2011; Kiontke et al., 2004; Kiontke and Fitch, 2005). Across the phylum, hermaphroditic nematodes can be found in both parasitic and free-living species (Castro, 1996; Criscione et al., 2005; Kanzaki et al., 2013; Kiontke and Fitch, 2005).

In the widely studied model nematode *Caenorhabditis elegans,* studies of the developmental and molecular underpinnings of nematode gametogenesis have contributed significantly to our understanding of both germ cell biology and fundamental mechanisms of cell and developmental biology (Corsi et al., 2015). *C. elegans* hermaphrodites are somatic females which achieve self-fertility by producing sperm as larvae, storing their sperm in a pouch-like structure called the spermatheca, and then producing exclusively oocytes as adults (Fig. 1A). As individual oocytes mature, they pass one-by-one into the spermatheca, where they are fertilized by the stored sperm (Fig. 1A) (L'Hernault, 2006; Ward and Carrell, 1979).

This same pattern of "sperm first, then oocytes" gamete production has been observed in self-fertile hermaphrodites of the *Caenorhabditis* and *Pristionchus* genera (Rudel et al., 2005; Sommer, 2005). However, the molecular mechanisms by which self-fertile hermaphroditism is achieved can differ even within the same genus; *C. elegans* and *C. briggsae* hermaphrodites convergently evolved distinct molecular changes to support sperm production in an otherwise female body (Guo et al., 2009; Hill et al., 2006; Hill and Haag, 2009; Liu et al., 2012; Nayak et al., 2005). Alternative patterns for achieving hermaph-

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Table 1
Terms used.

Germline stem cells (GSCs)	Undifferentiated, self-renewing stem cells that are maintained by a stem cell niche (the distal tip cell, DTC).
Spermatogonia	A proliferative, developmental intermediate between GSCs and spermatoctyes. Exist in germ cell cysts.
Gametocytes	Germ cells that have committed to the meiotic program and are differentiating or have differentiated. Either spermatocytes or oocytes.
Cell cluster	An isolated group of cells physically associated together.
Germ cell cyst	A cluster of germ cells that are interconnected by cytoplasmic bridges.

rodite self-fertility have also been reported; for example, hermaphrodites in the parasitic species *Rhabdias ranae* simultaneously produce both oocytes and sperm within discrete zones of the ovatestis (Runey et al., 1978). However, current studies are too limited to assess whether the sequential hermaphroditism strategy adopted by *Caenorhabditis* and *Pristionchus* predominates within the larger phylum. We sought to expand our understanding of nematode reproductive strategies by exploring germ cell development of more diverse nematode species.

Rhabditis sp. SB347 is a free-living, trioecious (male/female/hermaphrodite) nematode from the same *Rhabditidae* family as *C. elegans* (Félix, 2004; Kiontke and Fitch, 2005), but its reproductive biology differs in several ways (Chaudhuri et al., 2015, 2011; Félix, 2004; Shakes et al., 2011). First, whereas *C. elegans* is androdioecious (male/ hermaphrodite) (Corsi et al., 2015; Maupas, 1901), *R.* sp. SB347 is trioecious, with XX animals developing into either females or hermaphrodites (Félix, 2004). In *R.* sp. SB347, hermaphroditism is specifically linked to passage through a stress-resistant and dispersive larval morph, referred to as “dauer” in free-living nematodes and “infective juvenile” in parasitic species (Chaudhuri et al., 2011; Félix, 2004). Additionally, the offspring of *R.* sp. SB347 exhibit non-Mendelian sex ratios with self-fertilizing hermaphrodites producing an unexpected high number of male offspring (10%) (M. Farrell, unpublished; Chaudhuri et al., 2015; Félix, 2004), and male/female outcrosses yielding exclusively XX feminine progeny due to a modified

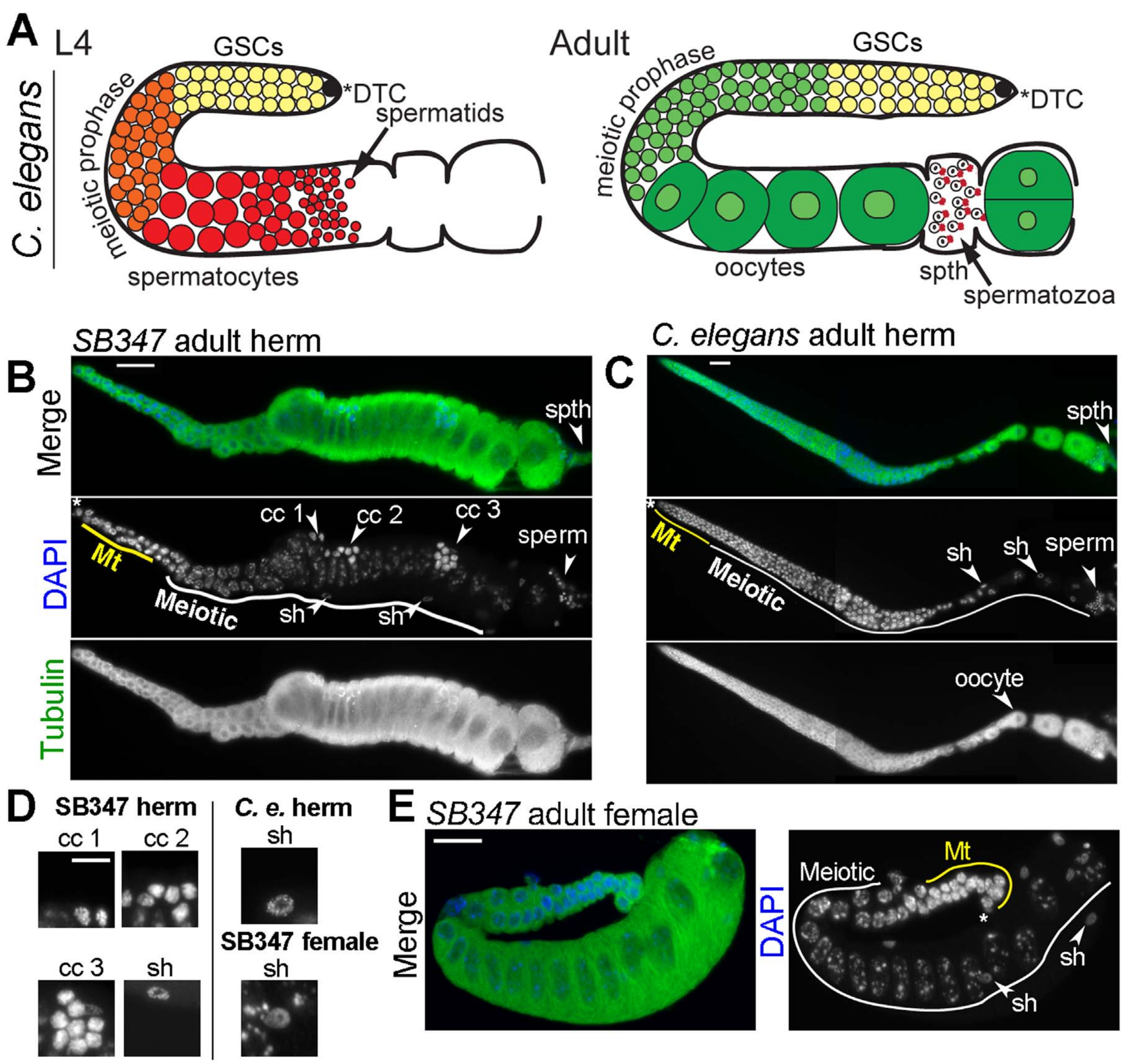

Fig. 1. *Organization of Caenorhabditis elegans and Rhabditis sp. SB347 oocyte-producing gonads.* (A) Schematic of germ cell development within the gonads of *C. elegans* hermaphrodites. Description in text. One half of whole gonad (one of the two gonad arms) is shown. (B, C, and E) Isolated gonad arms labeled with DAPI (blue) and anti-α-tubulin antibody (green). *= location of distal tip cell (DTC). (B) *R.* sp. SB347 adult hermaphrodite gonad. (C) *C. elegans* adult hermaphrodite gonad. (D) Full-size DAPI images of cell clusters from (B) and somatic sheath cell nuclei from (B), (C), and (E). E) *R.* sp. SB347 female gonad. Abbreviations: cc= cell cluster, DTC (distal tip cell), GSCs (germline stem cells), Mt= distal mitotic region, sh (somatic sheath cell), and Spth (spermatheca). All scale bars = 20 μm.