Parthenogenesis in the Two Races of *Radopholus similis* from Florida[1]

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Abstract: Single larval inoculations of both the citrus and banana races of *Radopholus similis* from Florida indicate parthenogenetic reproduction is possible. These races normally reproduce amphimictically. Both races produced all-female populations from single larva inoculations after 80 d and male-female populations after 180 d. The all-female populations produced female progeny with viable eggs, but no spermatozoa were present in the spermatheca, indicating tychoparthenogenesis occurred. Uninseminated females in the latter study produced both male and female progeny. Approximately 95% of the female progeny of the male-female populations were inseminated, indicating that cross-fertilization had occurred. The proportion of males to females was as expected in a normal bisexual population. No intersex males were observed. The mechanism of sex determination is not fully understood but assumed to be environmentally induced. *Key words:* burrowing nematode, environmental sex determination, reproduction.

Radopholus similis (Cobb) Thorne is generally considered to be an amphimictic species (8), and both males and females have been described in the literature (5,6). Investigations of the ability of this nematode to reproduce by parthenogenesis have led to conflicting conclusions. Loos (5) was not able to produce progeny from single larva of the banana race of *R. similis*. Brooks and Perry (1), however, were able to produce up to three generations of the citrus race of this nematode from single egg inoculations.

The purpose of the following experiments was to clarify the role of parthenogenesis in the reproduction of the banana and citrus races of *R. similis.*

MATERIALS AND METHODS

The nematode populations used in this study were maintained on either banana (*Musa* spp.), citrus spp. (rough lemon or sour orange), or nightshade (*Solanum nigrum* L.) roots in a greenhouse at 22–25 C. Nematodes were recovered from washed roots which were aerated for approximately 48 h.

Okra (*Hibiscus esculentus* L.) seedlings, approximately 5 cm in height, were transferred to 200 individual 10-cm pots containing sterile Astatula fine sand. Each of 100 plants was inoculated with one larva of the banana race or one larva of the citrus race of *R. similis*. Second- and third-stage larvae were selected for the inoculation on the basis of size and the absence of a visible genital primordium.

Two experimental periods were used in this study. Eighty plants from each treatment were analyzed 80–125 d after inoculation. The remaining 20 plants were transferred to 20-cm pots 80 d after inoculation and grown an additional 14 wk before analysis. After the incubation period, the roots were removed from each plant and washed, chopped, and aerated for 48 h.

Nematodes recovered were stained *in toto* in a modified orcein propionic acid (3) and observed with a compound microscope. The sex of each larva was determined by examining the position of the genital primordium (3,8). Females were examined for possible mating by checking for the presence of spermatozoa in the spermatheca. In addition, the number of chromosomes present in females of both races were determined (4). The percentage of males in the latter study was compared to published estimates of sex ratios of both races of *R. similis* through chi-square analysis.

RESULTS

Reproduction from single nematodes occurred in both races of *R. similis*. All-female populations were produced in the 80–125-d incubation periods. Of the initial 80 plants inoculated with the citrus race of *R. similis,* only 60 plants survived and nematode development or reproduction occurred on only 19 plants (Table 1). The largest number of

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Table 1. Number of *Radopholus similis* recovered from okra roots 80-125 d after inoculation with a single larva of either the citrus or banana race.*

No. day after inoculation	Citrus race			Total no. in family	Banana race			Total no. in family
	L_2,L_3	L_4	♀		L_2,L_3	L_4	♀	
80			1	1			1	1
80							1	1
85							1	1
88	1		1	2				
90			1	1				
93	2		1	3				
104	2			2		3		3
110		2	1	3			1	1
120		2		2		4	1	5
120	4			4	5			5
120	4			4	7	5	2	14
120	2		3	5	3	2	1	6
120			4	4	8	7	3	18
120	1	2	1	4	2	5		7
120			1	1				
120	4		5	9				
120	3	2		5				
125	2	4	2	8				
125			3	3				
125	1	2	5	8				
125	7	2	6	15				

*A total of 19 plants out of 60 and 11 plants out of 55 were positive for the citrus and banana race, respectively.

progeny recovered from any one plant was 15 nematodes in various stages of development. Of the initial 80 plants inoculated with the banana race of *R. similis,* only 55 plants survived and nematode development or reproduction occurred on only 11 plants (Table 1). The largest number of progeny recovered from the "all-female families" was 18 individuals, none of which had spermatozoa in the spermatheca. Position of the genital primordia indicated all L_4 larvae were developing into females.

Both races of *R. similis* produced "families" with male and female progeny after 180–190 d (Table 2). The females from both races were stained *in toto,* and about 95% of the females were found to be inseminated. The remaining 5% of the females had ova in the uteri, but no sperm were observed in the spermatheca. The number of males observed in a "family" was similar to that expected in a normal amphimictic population. The males appeared normal in size and development, and the reproductive systems each contained one testis. No intersexes were observed in either sex. One fe-

Table 2. Number of progeny of either the citrus or banana races of *Radopholus similis* recovered from single larva inoculations of okra plants after 180-190 d.*

Citrus race				Banana race			
Female	Male	Larvae	Total no. in family	Female	Male	Larvae	Total no. in family
33	3	0	36	233	99	190	522
185	29	123	337	162	53	193	408
290	116	238	644	362	82	217	661
474	127	264	865				
258	83	227	568				

*A total of 5 out of 20 plants and 3 out of 20 plants were positive for the citrus and banana race, respectively.

male of the citrus race possessed an abnormal tail.

DISCUSSION

The observation of parthenogenetic reproduction confirms the work of Brooks and Perry (1). It further indicates that a more complicated mechanism is involved in sex determination of *R. similis* than had been reported previously. The ability of this normally bisexual nematode to reproduce parthenogenetically is not unusual. Many organisms are able to undergo tycho-parthenogenesis, accidental or occasional parthenogenesis (9). This species was able to produce all-female populations for a given time before shifting to the production of male-female progeny.

These results indicate a time lag is involved in the shift from amphimixis to parthenogenesis in *R. similis.* Loos (5) assumed that parthenogenetic reproduction in *R. similis* was not possible since progeny were not produced in the normal time span expected in an amphimictic population. DuCharme and Price (2) reported the life cycle of *R. similis* to be about 20 d at 24–27 C. Brooks and Perry (1), however, did not find egg production until 35 d, and the second generation did not begin to produce progeny for another 30 d. In the present study, progeny were not observed until 80 d after inoculation with single larvae. The number of progeny increased with time. Not only was there a time lag in parthenogenetic reproduction compared to amphimictic populations of this nematode, but the male-female progeny were not produced until the first and second generations were well established. The males, however, could have been produced by the founding female or later by second generation females.

Chromosome determinations were made on many females from the male-female populations produced by parthenogenesis. Oogenesis in these specimens appeared to be identical to all stages of oogenesis found in the normal amphimictic females of both races (4). Several stages of meiosis were observed, and the chromosome numbers were the same as in amphimictic populations (4). The mechanism of reestablishment of the diploid chromosome number in the ovum is not known.

Factors such as plant stress could be involved in parthenogenetic reproduction of *R. similis.* The okra plants received regular care and did not appear to have visible stress symptoms, but they were obviously stunted because of a constricted root system caused by the small pots in which they were grown. The 180–190-d-old plants exhibited abnormal stem development and stunting, yet the root systems appeared healthy with no evidence of root disease caused by other pathogens. Plant stress has been demonstrated as a contributing factor to sex alternations, and this possibility cannot be ruled out in this study.

The classification of *R. similis* as a strictly bisexual species requiring cross-fertilization to produce viable progeny can no longer be maintained (1,7). The ability to reproduce without fertilization may be of evolutionary importance in the species. White (9) suggests that a shift from bisexual reproduction to tychoparthenogenetic reproduction may be a precursor of speciation.

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