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Short Note

Parthenogenetic Reproduction by the Entomopathogenic Nematode, *Steinernema carpocapsae* (Nematoda: Steinernematidae), on Poultry Egg Yolk or *Galleria* Larva

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Wen-Feng Hsiao and Liang-Kuang Chu (1996) Parthenogenetic reproduction by the entomopathogenic nematode, *Steinernema carpocapsae* (Nematoda: Steinernematidae), on poultry egg yolk or *Galleria* larva. *Zoological Studies* **35**(3): 227-229. Three strains of *Steinernema carpocapsae* (strains 25, DD-136, and All) were used to investigate the possibility of parthenogenesis in this species of entomopathogenic nematode. A plaster plate with a depression (dia. of 1 cm) in the center was designed for this experiment. Heat-treated last instar *Galleria* larvae or an equivalent weight of autoclaved poultry egg yolk were used in separate experiment to fill up the hole. A single *S. carpocapsae* infective juvenile was inoculated onto each sample of medium. Each plaster plate was assigned as 1 replicate and each treatment consisted of 20 replicates. For both media plates without nematode inoculation were assigned as the control. Development was observed daily and the number of progeny produced per nematode was counted 7 days after inoculation. For the yolk medium, the ratios of replicates producing progeny were 45% for strains 25 and DD-136, and 55% for strain All. The number of progeny produced by strain 25 was below 100. The number of progeny produced by DD-136 ranged from 10^3 to 10^4 with a high proportion at 10^3. For strain All, the number of progeny produced ranged from 10^2 to 10^4 with a high proportion at 10^3. For the *Galleria* larva medium, the ratios of replicates producing progeny were 50% for strains 25 and All, and 55% for strain DD-136. The number of progeny produced by strain 25 was below 100. The number of progeny produced by DD-136 ranged from 10^3 to 10^4 with a high proportion at 10^3. For strain All, the number of progeny produced ranged from 10^2 to 10^4 with a high proportion at 10^3.

Key Words: Parthenogenesis, *Steinernema carpocapsae*, Progeny, Poultry egg yolk, *Galleria*.

Nearly 40 nematode families are associated with insects, yet very few of these nematodes cause insect mortality. Only 2 families, Steinernematidae and Heterorhabitidae, have a wide range of insect hosts, and they are thought to be prospective agents for the biological control of soil-inhabiting insect pests (Klein 1990, Georgis and Gaugler 1991).

The infective juveniles (IJs) of the entomopathogenic nematode, *Steinernema carpocapsae*, the free-living stage, play a key role in searching for hosts. Once they locate a host and enter through some natural opening, they then penetrate the midgut into the hemocoel. The associated bacterium *Xenorhabdus* rapidly multiplies and kills the host by septicemia. The immature nematodes ingest bacterial cells and host tissues and then develop into amphimictic females or males. Females mate with males and produce progeny for 2 to 3 generations inside the host (Woording and Kaya 1988, Poinar 1990).

One of the major differences between *Steinernema* and *Heterorhabditis* is in their development subsequent to the infective stage. In *Steinernema*, the infective juveniles develop into amphimictic females or males but never hermaphrodites (Poinar 1990). In *Heterorhabditis*, each infective juvenile develops into a hermaphroditic female and never an amphimictic female or male. However, the 2nd generation in both genera consists of amphimictic females and males, and in this generation mating occurs with amphimictic females (Poinar 1990).

In a previous culture medium study, we found that a single adult could produce a number of progeny. This arised the question of the possibility that Steinernematid infective juveniles are able to reproduce without mating. To resolve this question, we designed this experiment to determine whether or not parthenogenesis exists in Steinernematid.

Materials and Methods—Three different strains of *Steinernema carpocapsae*, e.g., strains All, DD-136, and 25 were used in this study. Strains All and DD-136 were originally obtained from the University of California at Davis and strain 25 was purchased from Biosys Co. (USA) and maintained with the methods of Dutky et. al. (1964).

Plaster and water agar plates were prepared, and a hole

Table 1. Parthenogenetic reproduction by *Steinernema carpocapsae* on poultry egg yolk or *Galleria* larva 7 days after inoculation

Strain	Culture medium	% of replicates with successful parthenogenesis (n = 20)	Categories of no. of progeny[a]		
			10	100	1 000
DD-136	egg yolk	45.0	0.0	35.0	10.0
	Galleria larvae	55.0	0.0	50.0	5.0
All	egg yolk	55.0	5.0	45.0	5.0
	Galleria larvae	50.0	3.3	40.1	6.7
Strain 25	egg yolk	45.0	45.0	0.0	0.0
	Galleria larvae	50.0	50.0	0.0	0.0

[a]The values represent the number of progeny present in the culture plates.

(dia. 1 cm) was made in the center of each test plate. Either egg yolk or heat-treated *Galleria* larvae were placed into this hole. The egg yolk of poultry was autoclaved (121 °C, 15 lb) for 20 min, while the *Galleria* larvae were sterilized by hypochloride and then placed in a water bath set at 50 °C for 5 seconds, and finally frozen until test. A single IJ3 was randomly selected from the nematode suspension under the microscope and then placed into this arena. After inoculation, the development of IJ_3 was observed microscopically and the numbers of progeny produced were counted daily. Each treatment used 3 replicates with 20 plates per treatment.

Results and Discussion—Some of the IJ_3 died immediately after inoculation. A low number of adult males were appeared in this in vivo bioassay. Table 1 shows the reproduction results of the Steinernematid. For the yolk medium, the ratios of replicates producing progeny were 45% for strains 25 and DD-136, and 55% for strain All. The number of progeny produced by strain 25 was below 100. The number of progeny produced by DD-136 ranged from 10^3 to 10^4 with a high proportion at 10^3. For strain All, the number of progeny produced ranged from 10^2 to 10^4 with a high proportion at 10^3. For the *Galleria* larva medium, the ratios of replicates producing progeny were 50% for strains 25 and All, and 55% for strain DD-136. The number of progeny produced by strain 25 was below 100. The number of progeny produced by strain DD-136 ranged from 10^3 to 10^4 with high proportion at 10^3. For strain All, the number of progeny produced ranged from 10^2 to 10^4 with a high proportion at 10^3.

Morphological characterization and hybridization are commonly used to identify entomopathogenic nematode species (Curran 1990). From the present results, it is possible that parthenogenesis is a strategy of reproduction in this genus. Thus, to identify such Steinernematid and Heterorhabditid species, it is necessary to use alternative methods, such the molecular methods suggested by Curran (1990).

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蟲生線蟲 *Steinernema carpocapsae* 在家禽蛋黃或臘蛾幼蟲培養基上之孤雌生殖

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本研究係在二個不同的實驗室內進行以觀察蟲生線蟲 *Steinernema carpocapsae* 不同品系的生殖方式。在所製備的直徑 9 公分之石膏培養皿中央挖直徑 1 公分的洞，並分別填滿經熱處理過的末齡蠟蛾幼蟲及等量殺過菌的蛋黃。爾後每皿再接入一隻致病性線蟲幼蟲，測試品系有 Strains 25, DD-136, 和 All 品系三種。並以不接種致病性線蟲幼蟲爲對照，接種 7 天後，每日觀察其發育情形並計數所產後代數。每皿視爲一重複，每品系每種培養基重複 20 次。結果顯示其中以卵黃爲培養基者，產生後代的比率，Strains 25 及 DD-136 皆爲 45%，All 品系爲 55%，所產後代數 Strain 25 相當低，都在 100 隻範圍內，而 DD-136 品系，後代數分布在 10^3-10^4 之間，以 10^3 比率最高。All 品系分布的等級分布在 10^2-10^4 之間，以 10^3 比率最高。以蠟蛾爲培養基者，產生後代的比率，Strains 25 及 All 皆爲 50%，DD-136 品系爲 55%，所產後代數 Strain 25 和蛋黃培養基所飼養者相同皆相當低，在 100 隻範圍內，而 DD-136 品系，後代數分布在 10^3-10^4 之間，以 10^3 比率最高。All 品系分布的等級分布在 10^2-10^4 之間，以 10^3 比率最高。實驗結果顯示蟲生線蟲 *Steinernema carpocapsae* 在特殊情況下亦能行孤雌生殖。

關鍵詞：孤雌生殖, *Steinernema carpocapsae*, 子代, 家禽蛋黃, *Galleria*。

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