Polar Biol (1993) 13: 151 - 156

Polar Biology

Life cycle of the microbivorous Antarctic Dry Valley nematode *Scottnema lindsayae* (Timm 1971)

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Received: 3 January 1992/Accepted: 4 August 1992

Abstract. The life cycle of the Antarctic Dry Valley soil nematode, *Scottnema lindsayae* (Timm 1971) was studied in laboratory culture at two temperatures, 10°C and 15°C. Soil yeast and bacteria isolated with the nematodes were used as the food source. The species reproduced sexually. The higher temperature had a negative effect on the life cycle. The number of eggs per female and the number of juveniles developing per female were greater at 10°C than at 15°C. Juveniles developed faster at 10°C and four juvenile stages were observed outside of the egg at both temperatures. The unusually long life cycle (218 d at 10°C) suggests that more than one austral summer may be required for successful completion. An increase in Dry Valley soil temperatures associated with potential global environmental change may have detrimental effects on soil nematodes.

Introduction

Studies of Antarctic nematodes have generally been concentrated in the maritime region such as Signy Island (Spaull 1973; Maslen 1981; Pickup 1990), with much less emphasis on nematodes of the Dry Valleys of southern Victoria Land (77°S 170') and similar ice-free regions of the continent. The climate of the Dry Valleys is less favorable for soil organisms than the maritime regions. The Dry Valleys are more arid, colder and have a wider range of soils including highly saline and alkaline soils (Claridge and Campbell 1968). Maslen (1979) noted that the number of nematode species described from the continental Antarctic was fewer than in the maritime region, presumably due to these extreme climate conditions. However, favorable growth conditions for Antarctic soil organisms has been noted at 10°C (Vincent 1988) and higher (15°C), temperatures which are frequently recorded at the ground surface in the Dry Valleys during the austral summer (Claridge and Campbell 1968).

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Scottnema lindsayae (Timm 1971) (Fig. 1) was described from a sandy soil near the La Croix Glacier of Taylor Valley. Maslen (1979) regarded this monospecific genus *Scottnema* as endemic to the Dry Valleys. The type species, *S. lindsayae*, has a wide distribution, occurring in dry volcanic and stony soils, mossy soils, algal mats, and in small runoff streams and lakes of other nearby Dry Valleys (Timm 1971; Wharton and Brown 1989; Freckman and Virginia, in press). Freckman and Virginia (1990) in an extensive survey of the Dry Valleys found *S. lindsayae* to be the dominant species, representing approximately 80% of the soil nematode community (based on >250 soil samples across nine Dry Valleys). The nematode did not occur in high densities (mean = 358 kg^{-1} dry soil; range = 0–4000 kg^{-1} dry soil) in soils in the Dry Valleys (Freckman and Virginia 1990), making the extraction and isolation of specimens for experimental studies difficult. Morphology indicates the nematode would feed on microflora (actinomycetes, bacteria, yeast) (Banage 1963).

There have been no studies of the life cycle of nematodes from Antarctic Dry Valleys or similar polar desert environments. How long does it take these organisms to complete their life cycle? How does soil temperature affect their development and population structure? The objectives of this study were to provide the first description of the life cycle of an important nematode of the Dry Valleys and to determine how temperature influences its development and survival.

Materials and methods

Soil samples used in this study were collected from the upper 10 cm from locations in Taylor and Wright Valleys, frozen at −10°C and shipped to UC Riverside. Nematodes were extracted by the centrifugal flotation technique (Jenkins 1964) from soil samples acclimated to room temperature (22°C) (Freckman and Virginia, in press). Identification of *Scottnema lindsayae* was confirmed by E.M. Noffsinger, Univ. of California Davis.

To provide a food source on which to culture Antarctic nematodes, nematodes were extracted by the above method from a sandy soil collected in 1990 from Taylor Valley. The nematodes were handpicked from the water-extraction solution using sterile techniques

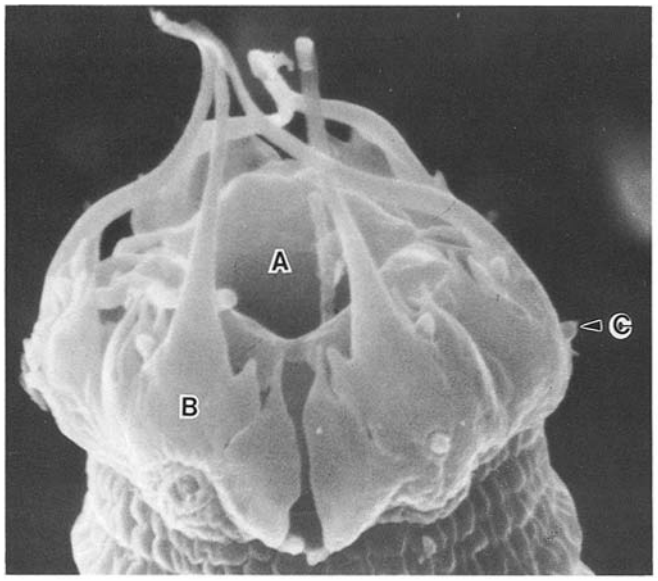

Fig. 1. En face view of *Scottnema lindsayae* (9705X) showing *A* stoma, *B* cephalic probolae, and *C* cephalic papillae. (SEM photograph by M. Mundo)

and placed on nutrient agar (Difco) petri plates. Prior to the experiment, nutrient agar, potato dextrose agar and corn meal agar (CMA) (all Difco) were tested for yeast growth, and for growth of several bacteria isolated from the same soil habitats. Yeast and bacterial growth on one-quarter strength CMA (4.25 g CMA + 12.75 g Bacto-Agar (Difco)/1) was sufficiently transparent for observation of the nematodes using an inverted microscope (Olympus CK2). Unhealthy and dying nematodes appear more transparent on agar petri plates, whereas healthy, well-fed, nematodes have a light-adsorbing granular-dense intestine. These preliminary experiments showed several of the bacterial isolates to be detrimental to the nematodes. In addition, single bacterial isolates (# 541, 542, 543) from the Dry Valleys (obtained through courtesy of E. I. Friedmann, Florida State University) were detrimental to the growth of *S. lindsayae*. For the experiment, small (60 mm diameter × 15 mm high) petri dishes were filled with 1/4 CMA to a 5 mm depth. A yeast strain isolated with the nematodes (B-TV 24) grew well across a wide range of temperatures (2°C to 26°C) and subsequently was used as the food source in all experiments. Plates were initially incubated with B-TV 24 for one week at a higher temperature of 26°C to increase the food source biomass prior to nematode transfer.

To obtain nematodes for the life cycle study, nematodes were extracted from soil samples collected from four sites in Taylor Valley (TV 31, a terminal rocky moraine; TV 48, a dry sandy slope above Mummy Pond; and TV 54 and 57, patterned ground (polygons) near Lake Fryxell), one sandy site in Garwood Valley (GV 25, in the vicinity of the Onyx River), and from one site in Wright Valley (WV 27, patterned ground (polygons) near Lake Brownsworth). In total, six different soils were used to provide experimental subjects. At the time of collection (January 1990), soil moistures at these sites ranged from 0.27% to 3.53% and soil temperatures at 0–5 cm depth ranged from 3.5°C to 18.8°C. The soil pH (1:2 soil/water) for the samples was: TV 31 = 7.5; TV 48 = 8.5; TV 54 = 9.8; TV 57 = 9.1; GV 25 = 9.9; WV 27 = 9.1. The nematodes were not surface-sterilized prior to the experiment. All *Scottnema* males and females extracted were transferred onto 12 plates with B-TV 24 under a laminar flow hood using sterile techniques. Each plate was considered a replicate. Because the reproduction of nematodes isolated from the 6 soil habitats might vary, nematodes from different sample sites were not placed together on a plate. Depending on the density of nematodes extracted from these 6 sites, the number of nematodes per plate varied between two and twenty. After three weeks observation to determine if any nematodes were damaged by transferring, six plates containing more than three females and two males were selected for the experiment and analysis.

Temperature experiment

Nematode life cycle parameters were observed at two temperatures, 10°C (±0.3°C) and 15°C (±0.5°C). These temperatures were selected to represent moderate and high Dry Valley soil temperatures, based on our field measurements (see Methods) and other Dry Valley reports (Claridge and Campbell 1968). There were three replicate plates per temperature: 10°C-TV 48, TV 31, and TV 57; 15°C-TV 48, TV 54, and WV 27. The petri plates were taped at the edges with laboratory film (Parafilm™) to prevent moisture loss and placed in the incubators. The nematodes were observed twice a week at room temperature, beginning 16 days after the setup of the experiment. The position of each nematode stage was marked on the lid of the petri dish with an identifying code, and because of the frequent observation, could be followed easily throughout the duration of the experiment.

The parameters measured were: numbers of living and dead females and males; numbers of living and dead juveniles grouped into four stages (based on body length of immobility prior to molting), (J1) = < 272.6 μm, (J2) = 272.6 μm to 385.7 μm, (J3) = 385.7 μm to 533.6 μm, (J4) = > 533.6 μm and not adult; and body width measurements for juveniles and adults. An immobile, but living, nematode was distinguished from a dead nematode by marking on the petri dish lid, the position of the nonmotile worm. If the nematode was found in this position after frequent observations, and it appeared to be decaying, it was considered dead. If the nematode had moved from the marked position, or if when present the valvular apparatus in the terminal bulb was pulsing, the nematode was considered immobile. To confirm the existence of four discrete stages based on body characteristics of immobile juveniles, body length and width (at the terminal bulb) of selected juveniles were measured when the new and old cuticle were visible (Sohlenius 1973a).

Selected eggs were followed individually throughout embryonic development to hatching to estimate time steps from one egg development stage into the next. Molting of juveniles could be followed by recording the numbers of juveniles at each individual time step. Estimates of the time for development of each juvenile stage was determined from data by accumulating time in each stage until transition to the next one.

For standardization and data analyses, data are expressed as number of life stages per female of initial inoculation per plate. Statistical analysis was performed by analysis of variance procedures using Statview 512$^+$ (ANOVA procedure).

Results

Life cycle stages

Eggs. Egg production had begun by the first observation 16 days after the setup of the experiment (Fig. 2). Egg production at 15°C was lower than at 10°C, but the differences were not significant. From about 60 to 100 d, egg production was constant at the lower temperature, but declined at the higher temperature until few eggs were observed after 100 days. There were no significant differences in egg production at the two temperatures until maximum fecundity of the females was reached at 10°C (86 days) ($p = 0.010$). Plates at the 15°C treatment were moved to 10°C at day 124 to determine if the cultures could eventually recover. Lowering the temperature had no