

# Temperature Dependent Reproduction and Survival of the Soil Nematode *Pristionchus maupasi* In Vitro

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**Abstract:** In the present study, the reproduction potential and survival capability of the soil nematode *Pristionchus maupasi* was followed in 10 cm<sup>2</sup> Petri dishes at different constant temperatures: 5, 10, 15, 20, 25 and 30 °C. The experiment started by placing nematode eggs on each dish. The minimum number of days from eggs, at start, to adult egg producing nematodes, decreased from 7 days at 10 °C to 3 days at 25 °C. At 5 °C no eggs hatched, and at 30 °C the released larvae did not develop into egg producing adults. After 7 days all eggs had hatched in the interval between 15 and 30 °C and 87 % at 10 °C. From hatched eggs the 1<sup>st</sup> generation nematodes evolved.

At day 4, the number of 1<sup>st</sup> generation nematodes was almost similar at temperatures ranging from 10 to 25 °C, while the number at 30 °C was significantly lower. The 1<sup>st</sup> generation adult nematodes gave rise to a 2<sup>nd</sup> generation, which did not develop to fertility, but became arrested 3<sup>rd</sup> stage “dauerlarvae”, maybe due to reduced food supplies and increased concentration of wastes. From 100 ‘eggs at start’, 10000 to 14000 2<sup>nd</sup> generations “dauerlarvae” developed at temperatures between 10 and 20 °C. In the interval from 10 to 25 °C, a substantial number of these survived more than 2 months.

**Keywords:** Soil nematode, *Pristionchus maupasi*, Reproductive capacity, Survival, Temperature.

## INTRODUCTION

The top soil nematode community participates in the decomposition of organic matter. We found that one square meter of our experimental field (Copenhagen, Denmark) harboured approximately four million nematodes in the upper 20 cm of soil in the summer. During winter numbers were reduced to approximately three millions per square meter.

One of the species in our field is *Pristionchus maupasi* Potts (*Diplogastridae*). It is a hermaphroditic nematode, which has been found in soil, seashore mud, decaying potatoes and meat, excrements from a snail, and in cattle dung [1]. Moreover, *P. maupasi* may be phoretically associated with beetles of the family *Hydrophilidae* [1] and scarab beetles of the genera *Melolontha* and *Cetonia* [2]. *Pristionchus maupasi* feed on bacteria, but the specialised buccal cavity possibly allows utilization of other food items, e.g. fungi and nematodes. Sohlenius [3] observed nematode predatory behaviour by this species.

The aim of the present *in vitro* study was to evaluate the reproduction potential and survival capability of *P. maupasi* at different constant temperatures, to enrich the knowledge base of soil nematodes.

## MATERIALS AND METHODOLOGY

### Stock Cultures of the Soil Nematode *P. maupasi*

*Pristionchus maupasi* was cultivated on half the concentration of Nigons agar (1:2) [4]. The medium was inoculated with the bacterium *Pseudomonas fluorescens* (Trevisan) Migula as feed for the nematodes. Nematode eggs were collected for the experiment as described below.

### Population Dynamics of *P. maupasi* at Different Constant Temperatures

For identification all experimental Petri dishes (10 cm<sup>2</sup>) were given an individual number at start. Technically it was not possible to place the same number of eggs on the experimental dishes. Therefore, known numbers (between 30 and 50) of *P. maupasi* eggs were picked up on small agar lumps (3 x 3 mm<sup>2</sup>) from stock cultures and carefully placed at the centre of the experimental Petri dishes containing modified Nigons medium (1:2). In this way individual dishes were inoculated with known numbers of eggs at start. The bacterium *P. fluorescens* spread from the inoculation lumps together with the migrating newborn 1<sup>st</sup> generation larvae and served as food for the growing *P. maupasi* population. Petridishes were placed in the dark in climatic chambers at the following constant temperatures: 5, 10, 15, 20, 25 and 30 °C. The 1<sup>st</sup> generation grew up and produced eggs, which resulted in a 2<sup>st</sup> generation of nematodes.

At intervals (1, 2, 3, 4, 7, 10, 14, 17, 21, 28, 35, 43, 56, 63, 73 and 80 days after experimental start) nematodes were

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counted on the entire surface of each Petri dish until the 2<sup>nd</sup> generation of *P. maupasi* became numerous. Then the nematodes were enumerated using 3 fixed small counting areas (1/4 cm<sup>2</sup>) on each dish. Based on these counting's numbers in each 10 cm<sup>2</sup> Petri dish were calculated. At each temperature the experiment was performed in triplicates.

Over time, the inspections of the Petri dishes made it possible to determine a) the percentage of *P. maupasi* eggs that hatched, b) the minimum number of days from eggs, at start, to adult egg-producing 1<sup>st</sup> generation nematodes and c) the number of nematodes (pre-adults and adults, not including eggs) of the 1<sup>st</sup> and 2<sup>nd</sup> generation of *P. maupasi* in each Petri dish. A flow chart of the experiment is shown in Fig. (1). As described above, the number of eggs present at the start of the experiment varied for each Petri dish. For comparison, the 'number of nematodes' in Fig. 3 and 4 was proportionally calculated as if 100 eggs had been placed on each Petri dish at the beginning. It

was done in the following way: ((Number of nematodes counted on the different days X 100)/ (Exact number of eggs placed on each dish at start)).

**Statistical Evaluation**

The number of 1<sup>st</sup> generation larvae (Fig. 3) was evaluated by a t-test. The tests were executed on Log (n + 1) transformed data under a generalised linear mode (PROC GLM) using the associated LSMEANS statement that provide paired t-test for given temperatures. Results are provided for observation day 1, 4, 7, 17 and 80, which adequately defines the statistical differences during the experiment.

**RESULTS**

The 'eggs at start' developed differently at various temperatures. At 5°C no eggs hatched, but at 10°C 50 % had hatched

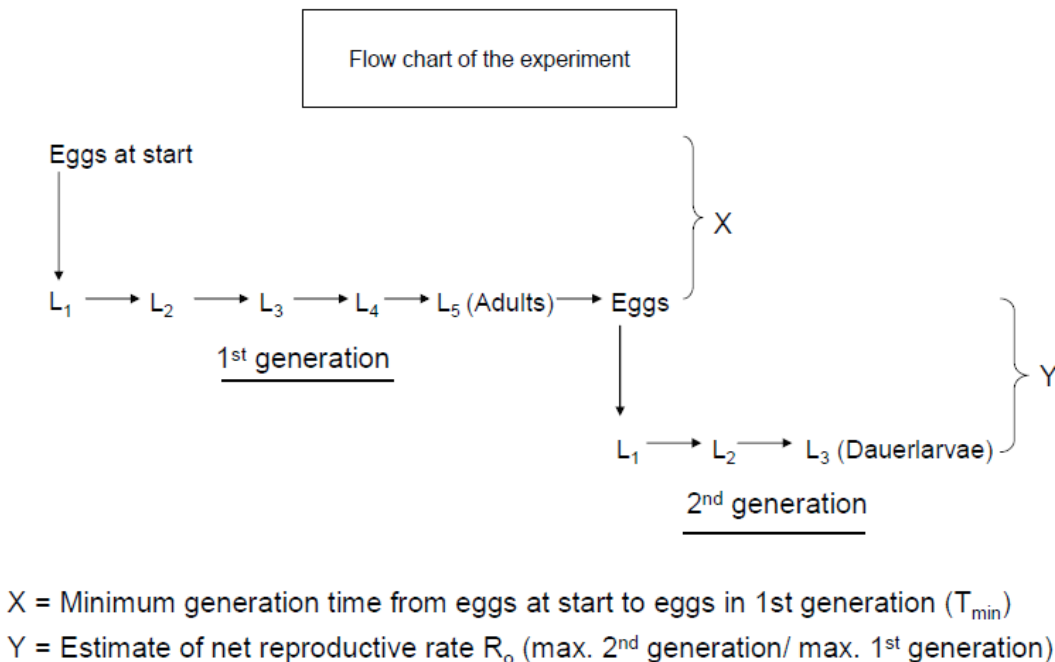


Fig. (1). Flow chart of the experiment, starting as eggs and ending as 2<sup>nd</sup> generation 'dauerlarvae'. L<sub>1</sub>, L<sub>2</sub>, L<sub>3</sub>, L<sub>4</sub> and L<sub>5</sub> indicate progressive larval stages. The fifth stage (L<sub>5</sub>) becomes egg laying adults.

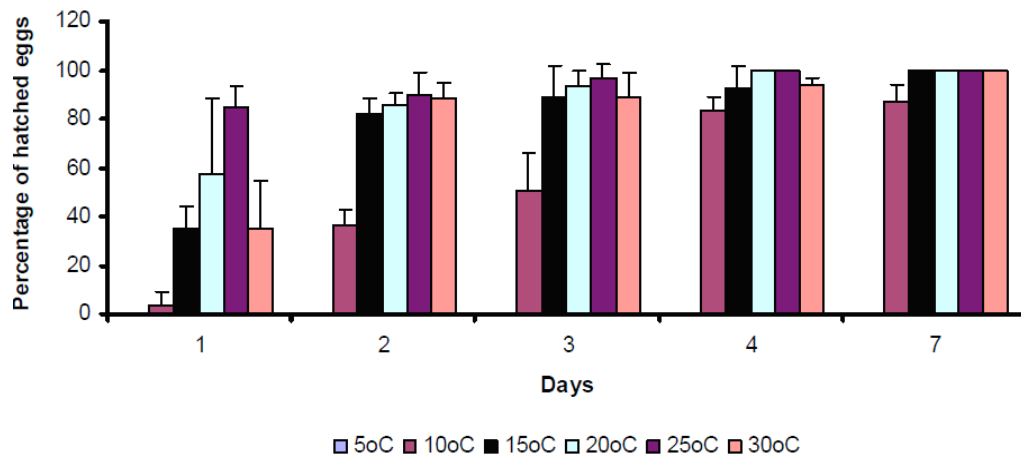


Fig. (2). The mean percentage of *P. maupasi* eggs that hatched over time at different constant temperatures (n=3). +S.D. is shown as vertical bars.

after just 3 days and 87 % had hatched on day 7. At the following temperatures 10, 15, 20, 25 and 30°C the hatching by day 2 was 36, 82, 86, 90 and 88 %, respectively. After 7 days all eggs had hatched in the interval between 15 and 30°C and 87 % at 10 °C (Fig. 2). From hatched eggs the 1<sup>st</sup> generation nematodes evolved.

On the first day of observation more 1<sup>st</sup> generation larvae were found at 15, 20 and 25 °C, than at 5, 10 and 30°C (t-test, P < 0.01), which divided them in a high and low temperature hatching group (Fig. 3). At day 4, the number of 1st generation nematodes was very similar over the temperature range from 10 to 25 °C, while the number at 30 °C was significantly lower (t-test, P < 0.002). The population sizes at 15, 20 and 25 °C were comparable for the rest of the experiment, except at day 7 when populations at 15 and 25 °C were significantly different (t-test, P=0.01) (Fig. 3). In the time interval from day 10 to day 35 the population level of nematodes at 10 °C continued to be larger than populations at 15, 20 and 25 °C (t-test, P< 0.001) (Fig. 3). At day 43 and onwards only few of the 1<sup>st</sup> generation nematodes were still living.

At 30°C the hatched nematodes did not develop to adult egg producing 1<sup>st</sup> generation individuals, and they died so quickly that the average number of survivors never exceeded 38 nematodes out of 100 eggs (Fig. 3). Between 10 and 25°C, 100 eggs

resulted in at least 80 nematodes, which became 1<sup>st</sup> generation adults. According to Fig. (3) the survival of the majority of the 1<sup>st</sup> generation was not longer than 43 days.

Taking all three replicates into account, the minimum number of days from hatching to egg producing 1<sup>nd</sup> generation adults (minimum generation time: T<sub>min</sub>) was observed to be 7, 5, 4, 3 at 10, 15, 20 and 25°C, respectively.

The 2<sup>nd</sup> generation of *P. maupasi* (Fig. 4) did not become fertile, but "dauerlarvae" (arrested development in the third larval stage). This made it possible to identify the 1<sup>st</sup> generation nematodes as they were the only adults in the Petri dishes. Temperatures between 10 and 20°C were acceptable for *P. maupasi* to build up a large 2<sup>nd</sup> generation of "dauerlarvae" (Fig. 4). Between 10 and 20°C a substantial number of "dauerlarvae" survived until the end of the experiment at day 80 (Fig. 4). At 10, 15 and 20°C the number of "dauerlarvae" that survived 73 days were 3574, 5930 and 8249, respectively. But at 25°C there were no survivors at 21 days (Fig. 4).

DISCUSSION

The present results indicate that the temperature-niche breadth for population increase of *P. maupasi* range from 10°C to 25°C. The highest number of 2<sup>nd</sup> generation *P. maupasi*

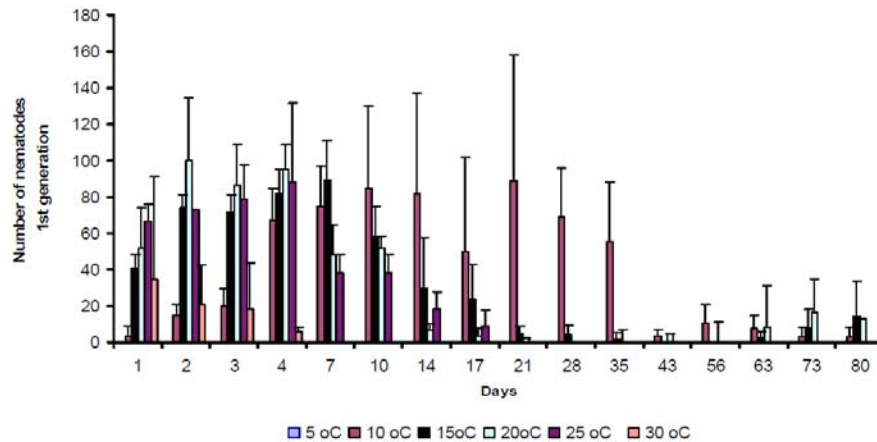


Fig. (3). The mean number of nematodes (adults and pre-adults) of the 1<sup>st</sup> generation of *P. maupasi* on 10 cm<sup>2</sup> Petri dishes (n=3). - The number of nematodes is calculated as if 100 nematode eggs had been placed on the agar at the start of the experiment. +S.D. is shown as vertical bars.

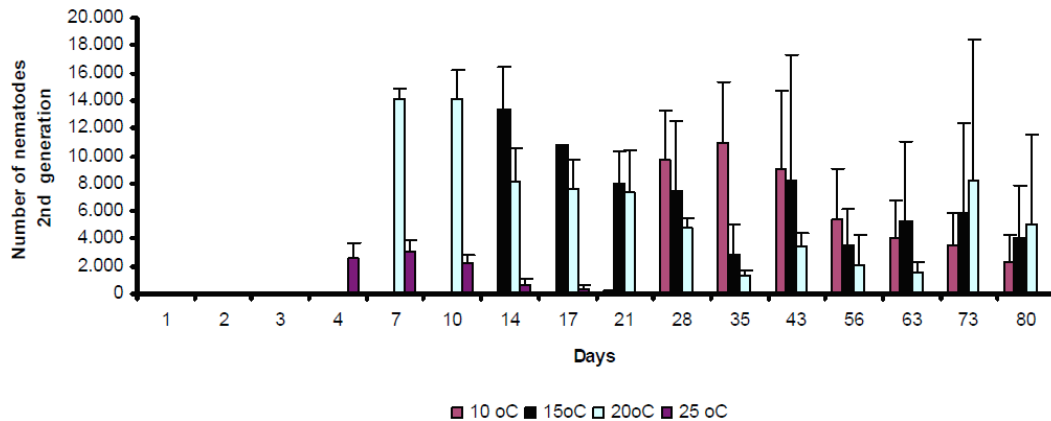


Fig. (4). The mean number of nematodes of the 2<sup>nd</sup> generation of *P. maupasi* on 10 cm<sup>2</sup> Petri dishes (n=3). The 2<sup>nd</sup> generation of *P. maupasi* did not develop to fertility, but became "dauerlarvae". A 2<sup>nd</sup> generation did not develop at 5°C and 30°C. - The number of nematodes is calculated as if 100 nematode eggs had been placed on the agar at the start of the experiment. +S.D. is shown as vertical bars.

nematodes (reflecting reproduction) was observed at 20 °C (Fig.4). In comparison, reproduction of the plant parasitic nematode *Heterodera cajani* was greatest at 25 °C [5].

Estimates of a generation time ( $T_{\min}$ ) for *P. maupasi* are of the same magnitude as for a range of other free-living nematodes, including its close relative *P. pacificus* [6]. In accordance with results on nematodes from sewage treatment plants [7], the life-span of *P. maupasi* decreased with increasing temperature, except when *P. maupasi* became 2<sup>nd</sup> generation “dauerlarvae”. In this arrested condition *P. maupasi* obviously survives unfavourable conditions longer, also at relatively high temperatures such as 20°C. Arrestment may be a result of crowding in combination with reduced food supplies and increased concentration of wastes. In nature such situations may occur when a food source is depleting. Surviving “dauerlarvae” was able to resume their development on fresh Nigon’s medium, comparable to renewal of a natural food base.

At a depth of 5 cm, Danish soil temperatures fluctuate between minus 1 and plus 9°C in the colder period (November to March) [8]. Temperatures about 5 °C may last for several days or weeks in this period. At such low temperatures *P. maupasi* is able to survive; otherwise this species would not be present in the experimental field. If temperatures approach 10°C for a week *P. maupasi* is able to complete its entire life cycle. During the warmer period (May to September), soil temperatures fluctuate between 10 and 20 °C [8], which allow *P. maupasi* populations to survive and reproduce if other important factors, such as soil moisture [9], are favourable.

**Table 1.** Estimation of ‘the Net Reproductive Rate’ ( $R_0$ ) and ‘the Intrinsic Rate of Natural Increase’ ( $r$ ) of *P. maupasi*

Temperature °C	$R_0$	$r$ (Days <sup>-1</sup> ) (1 Day=24 Hours)
5	0	-
10	104	0.7
15	150	1.0
20	129	1.2
25	35	1.2
30	0	-

The reproductive capacity may be expressed by ‘the intrinsic rate of natural increase’ ( $r$ ), which was estimated as described below. The number of eggs, produced by an average fertile nematode, are approximately (max. 2<sup>nd</sup> generation/ max. 1<sup>st</sup> generation). This is a rough estimate of ‘the net reproductive rate’ ( $R_0$ ) (Table 1). Furthermore, an estimate of the (minimum) generation time ( $T_{\min}$ , days) makes it possible to estimate  $r$  by the formula:  $r = (\log_e R_0) / T_{\min}$  (Table 1). ‘The intrinsic rate of natural increase’ is a sensitive coefficient in the logistic model for density-dependent population growth:  $dN/dt = rN ((K-N)/K)$  ( $K$  is the environmental carrying capacity and  $N$  is the actual population size).

The coefficient ( $r$ ) may be influenced by external factors such as temperature and chemicals. If ‘the intrinsic rate of natural increase’ is altered for a significant number of soil organisms it could ultimately result in changes in the rate of decomposition of organic matter in the soil ecosystem.

## CONCLUSION

*P. maupasi* has a large reproductive potential ( $r$ : 0.7 - 1.2 days<sup>-1</sup>) between 10 and 25°C, which means that *P. maupasi* has the ability to restore its population very fast in mild or warm periods of the year. Most likely population decline in such favourable periods is caused by lack of food, enemies or infections. Between 15 and 25°C  $r$  is remarkably stable between 1 and 1.2 days<sup>-1</sup> (Table 1). These  $r$ -values are of the same magnitude as those found for several bacterial feeding nematodes [9].

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## CONFLICT OF INTEREST

None declared.

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