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THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF ADULT GENERATIONS OF ENTOMOPATHOGENIC NEMATODE *STEINERNEMA ARENARIUM* ISOLATE CH

Ye. B. Yakovlev, V. A. Kharchenko

Schmalhausen Institute of Zoology NAS of Ukraine,
vul. B. Khmelnytskogo, 15, Kyiv, 01030 Ukraine
E-mail: nadfh2@gmail.com

The Effect of Temperature on the Development of Adult Generations of Entomopathogenic Nematode *Steinernema arenarium* Isolate CH. Yakovlev, Ye. B., Kharchenko, V. A. — *Steinernema arenarium* isolate CH was prepared at 22 °C and used as a control in laboratory experiments on rearing in *Galleria mellonella* larvae at different temperatures (18 and 28 °C). Host dead bodies were examined every two days. All reared adult nematodes were fixed in alcohol and mounted on permanent slides with glycerin solution in distilled water. The basic morphometric parameters (L, W, ES, ABD (CBD), T, V) were measured, and statistical analysis was performed. Morphometric data in males and females of both generations were shown to significantly change depending on speed of growth and nutrients concentration. In both experimental groups, pygmy forms of adults were found.

K e y w o r d s: *Steinernema arenarium*; pygmy; morphometry; variability; temperature.

Влияние температуры на развитие взрослых стадий энтомопатогенной нематоды *Steinernema arenarium* изолят CH. Яковлев Е. Б., Харченко В. А. — Изолят CH *Steinernema arenarium* был выделен при 22 °C и выбран в качестве контроля и в лабораторных условиях выращен на личинках *Galleria mellonella* при различной температуре (18 и 28 °C). Хозяев вскрывали каждые два дня. Все выращенные взрослые нематоды были зафиксированы в этиловом спирте и смонтированы в постоянные препараты в растворе глицерина в дистиллированной воде. Были измерены базовые морфометрические параметры (L, W, ES, ABD (CBD), T, V) и сделана статистическая обработка данных. Выявлено, что у самцов и самок обеих стадий существенно изменяются морфометрические параметры в соответствии со скоростью роста и концентрацией питательных веществ. В обеих экспериментальных группах были найдены пигмейные формы взрослых особей.

К л ю ч е в ы е с л о в а: *Steinernema arenarium*, пигмеи, морфометрия, изменчивость, температура.

Introduction

Entomopathogenic nematodes (EPNs) are important biological control agents of insects causing lethal processes in more than 1,000 insect species. The EPNs range includes almost all biogeographical zones excluding extremely cold ones.

Some peculiarities of EPNs' biology (host finding, infecting of poikilotermic animals, killing of hosts) considerably rise the effect of abiotic environmental factors (temperature, moisture) on nematode populations affecting their survival in extreme conditions (Cagnolo, Campos, 2008; Laznik, Trdan, 2014; Lewis, Shapiro-Ilan, 2002), persistence in soil (Půža, Mráček, 2007), geographical distribution and infectivity (Molyneux, 1986), generations development (Yu et al., 2008), etc.

Recent studies described the effect of temperature on the development of infective juveniles, their morphometry, changing of host-finding strategies, attraction, virulence and infectivity for different hosts (Hirao, Ehlers, 2009; Khlibsuwan et al., 1992; Koppenhöfer et al., 2013; Spiridonov et al., 2004; Trdan et al., 2009). Such changes, however, were not observed in adults.

Due to the high importance of EPNs as biocontrol agents, we have to cumulate our knowledge of the influence of abiotic factors on the development of all generations in their life cycles and efficacy of nutrients transformation.

Material and methods

Entomopathogenic nematode culture

Entomopathogenic nematodes *Steinernema arenarium* (Artyukhovsky, 1967) Wouts, Mráček, Gerdin et Bedding, 1982 isolate CH (Yakovlev, 2014; Yakovlev et al., in prep.) were obtained from the Exclusion zone of Chornobyl Nuclear Power Plant of Ukraine from the territory of periodical radioactivity control by the live baiting method (coordinates: 51°11.178′ N; 30°02.974′ E; height above the sea level 100 m). Isolate was cultivated in *Galleria mellonella* L. larvae on White traps at room temperature (22 °C) (White, 1927).

Nematode rearing

Larvae of *G. mellonella* were anesthetized with ethyl ether for 30 seconds, washed in 30 % alcohol, dried on sterile filter paper and used for the experimental infection. Prepared moth larvae were sprayed with the suspension of dauer juveniles of entomopathogenic nematodes at concentration 100 IJ/insect (Veremchuk, 1972). To prevent the shock of infective juveniles, treated test insects were placed into individual sterile Petri dishes with filter paper and kept for 15 min at room temperature, then divided into two groups, one of which was cultivated at 18 °C, and another at 28 °C.

Every day the Petri dishes were observed for drying and to prevent it filter paper in dishes was moistened with drops of medical sterile saline.

Every two days after the fourth day of experimental infection, one moth larva was dissected. The internal content of insects with nematodes was washed with the sterile saline and centrifuged at 1,000 rpm for 1 min with two times removing of supernatant. Sediments were observed in individual Petri dishes, hard parts of insects were removed, nematodes were killed and fixed with 70 % ethanol heated to 50–60 °C (Nguyen, Hunt, 2007).

Light microscopy

After fixation nematodes were moved to the Seinhorst solution for clearing of glycerin from ethanol (Seinhorst, 1959), and mounted in permanent slides with glycerin solution in distilled water (1 : 1). Permanent slides were studied under the light microscope at magnifications ×100–400. Main morphometric parameters, L (body length), W (body diameter), ES (esophagus length), ABD (CBD) (anal/cloacal body diameter), T (tail length), V (distance from anterior end to vulva), were measured, and the related coefficients D % = EP/ES*100 %; V % = V/L*100 %; a = L/W; b = L/ES; c = L/T were calculated (De Man, 1880; De Man, 1876; Weiser, 1955).

On the basis of calculated nominal morphometric parameters (L, W, ES, ABD (CBD), T, V), canonical analysis was performed with building of scatterplot of canonical scores in STATISTICA v. 10.0 (StatSoft, 2011).

Results

Life cycles of both populations were 16 days at 18 °C with number of emerged IJ ≈ 15,300, and 14 days at 28 °C with number of emerged IJ ≈ 9,400. Number of adults in groups 18 °C and 28 °C by days of experiment is shown in table 1.

The changes in morphometric data of adult nematodes depending on temperature of EPNs cultivation were observed and shown in tables 2–4.

Entomopathogenic nematodes were found to have morphological changes depending on the temperature. These changes were distinct, growing significantly at higher temperatures (fig. 1–2). Dispersion of the characters in both sexes increased with the higher temperature.

Morphometric changes were more visible in females (fig. 1). In the first generation, they have obvious distinctions as compared to group cultivated at 28 °C. In the second

Table 1. Number of adults in both generations of entomopathogenic nematodes at 18 °C and 28 °C

Days	♂ 18 °C	♀ 18 °C	♂ 28 °C	♀ 28 °C
6	36	47	20	22
8	109	127	25	88
10	133	147	17	38
12	111	120	17	29
16	0	2	0	0

Table 2. Morphometry of adults from EPN control group, µm; Mean ± SD (min–max)

Parameter	♂I 22 °C	♀I 22 °C	♂II 22 °C	♀II 22 °C
n	10	10	10	10
L	1088 ± 78 (970–1198)	2911 ± 189 (2653–3297)	1234 ± 124 (1129–1564)	2116 ± 193 (1931–2564)
W	75 ± 4 (68–80)	172 ± 7 (165–185)	79 ± 7 (73–98)	115 ± 9 (105–138)
ES	140 ± 11 (125–153)	184 ± 11 (160–195)	165 ± 11 (140–175)	203 ± 11 (190–220)
T	29 ± 3 (25–35)	47 ± 7 (35–60)	33 ± 4 (28–40)	62 ± 5 (55–73)
ABD (♀, J2d); CBD (♂)	42 ± 2 (40–45)	66 ± 8 (50–80)	41 ± 4 (35–45)	42 ± 3 (38–48)
V	–	1564 ± 104 (1436–1752)	–	1153 ± 128 (1059–1406)
V, %	–	54 ± 1 (51–55)	–	56 ± 2 (54–61)
a	15 ± 1 (40–45)	17 ± 1 (16–18)	16 ± 1 (15–17)	19 ± 2 (14–23)
b	8 ± 1 (7–9)	16 ± 1 (14–18)	8 ± 1 (7–11)	10 ± 1 (9–12)
c	37 ± 4 (34–47)	63 ± 10 (48–81)	38 ± 4 (31–45)	34 ± 2 (31–37)

Table 3. Morphometry of adults from EPN group reared at 18 °C, µm; Mean ± SD (min–max)

Parameter	♂I 18 °C	♀I 18 °C	♂II 18 °C	♀II 18 °C
n	10	10	10	8
L	1323 ± 114 (1195–1545)	4008 ± 408 (3185–4725)	1188 ± 76 (1050–1285)	2511 ± 437 (1910–3040)
W	83 ± 9 (70–100)	197 ± 26 (160–235)	67 ± 3 (60–70)	128 ± 22 (100–170)
ES	137 ± 10 (125–155)	183 ± 10 (170–200)	126 ± 16 (110–155)	173 ± 10 (155–190)
T	36 ± 4 (30–40)	47 ± 5 (40–55)	31 ± 2 (28–35)	47 ± 6 (40–55)
ABD (♀, J2d); CBD (♂)	44 ± 5 (35–50)	69 ± 8 (60–80)	42 ± 3 (35–45)	49 ± 6 (40–55)
V	–	2104 ± 201 (1750–2475)	–	1357 ± 192 (1065–1605)
V, %	–	52 ± 2 (48–55)	–	54 ± 2 (49–56)
a	16 ± 1 (14–18)	20 ± 2 (18–24)	18 ± 1 (16–20)	20 ± 3 (18–26)
b	10 ± 1 (8–11)	22 ± 2 (18–24)	10 ± 1 (7–11)	15 ± 2 (11–18)
c	38 ± 4 (34–47)	87 ± 15 (70–118)	39 ± 3 (33–43)	54 ± 6 (44–62)

Table 4. Morphometry of adults from EPN group reared at 28 °C, μm; Mean ± SD (min–max)

Parameter	♂I 28 °C	♀I 28 °C	♂II 28 °C	♀II 28 °C
n	10	10	9	10
L	1364 ± 208 (1155–1735)	6398 ± 1009 (4683–8390)	1264 ± 186 (950–1540)	3795 ± 789 (2760–5292)
W	92 ± 12 (70–105)	293 ± 24 (267–327)	87 ± 14 (65–110)	156 ± 24 (125+196)
ES	141 ± 15 (110–155)	203 ± 15 (183–228)	139 ± 15 (105–155)	168 ± 16 (152–200)
T	33 ± 4 (30–40)	55 ± 8 (43–70)	32 ± 3 (25–35)	65 ± 14 (45–97)
ABD (♀, J2d); CBD (♂)	46 ± 3 (40–50)	84 ± 9 (68–93)	44 ± 5 (35–50)	63 ± 12 (48–85)
V	–	3384 ± 415 (2574–3980)	–	1976 ± 383 (1482–2759)
V, %	–	53 ± 3 (47–57)	–	52±1 (50–54)
a	15 ± 2 (13–17)	22 ± 4 (17–28)	15 ± 2 (13–17)	24 ± 2 (22–27)
b	9 ± 2 (6–12)	32 ± 6 (24–46)	9 ± 1 (7–10)	23 ± 5 (18–32)
c	42 ± 8 (29–58)	117 ± 20 (83–153)	39 ± 5 (34–50)	60 ± 11 (40–80)

generation, such proportions in groups of 18 and 28 °C are higher compared to 22 °C group.

Females had stable coefficient V, % within 52–56 %.

In both groups pygmy forms of nematodes were found: at 28 °C there were 5.0 % of I generation males and 18.2 % of I generation females, 15.8 % of II generation females; at 18 °C there were 12.0 % of I generation females and 0.8 % of II generation females. At 22 °C in this experiment pygmies were not found.

Pygmy forms were fertile; the first generation females of both extreme groups showed *endotokia matricida*, females of the second generation layed eggs. In 28 °C group pygmy forms had noticeable morphological anomalies: deformations in vulval region and apical pole, wide cuticular "trails".

Discussion

The effect of temperature on morphometric parameters of adult entomopathogenic nematodes characterizes the speed of response of the nematode populations to stress caused by abiotic factors. At lower temperatures the life cycle is longer; nematodes grow on media for a longer time with great number of adults and higher individual masses. This correlates with the similar results on the effect of low temperature on measurements of infective juveniles from Hazir et al. (2001). On the contrary, rearing at higher temperatures causes too rapid growth of individual masses of adult nematodes, lack of nutrition, increased concentration of decay products, less number of adults, and reduced life cycle (table 1).

Pygmy forms of adults and infective juveniles of Steinernemadae and Heterorhbditidae were observed by some researchers who explained their presence by the adaptation to size and nutrition capability of host (Yu et al., 2008). Pygmies of *S. arenarium* were found for the first time in the present study. According to our observations, they

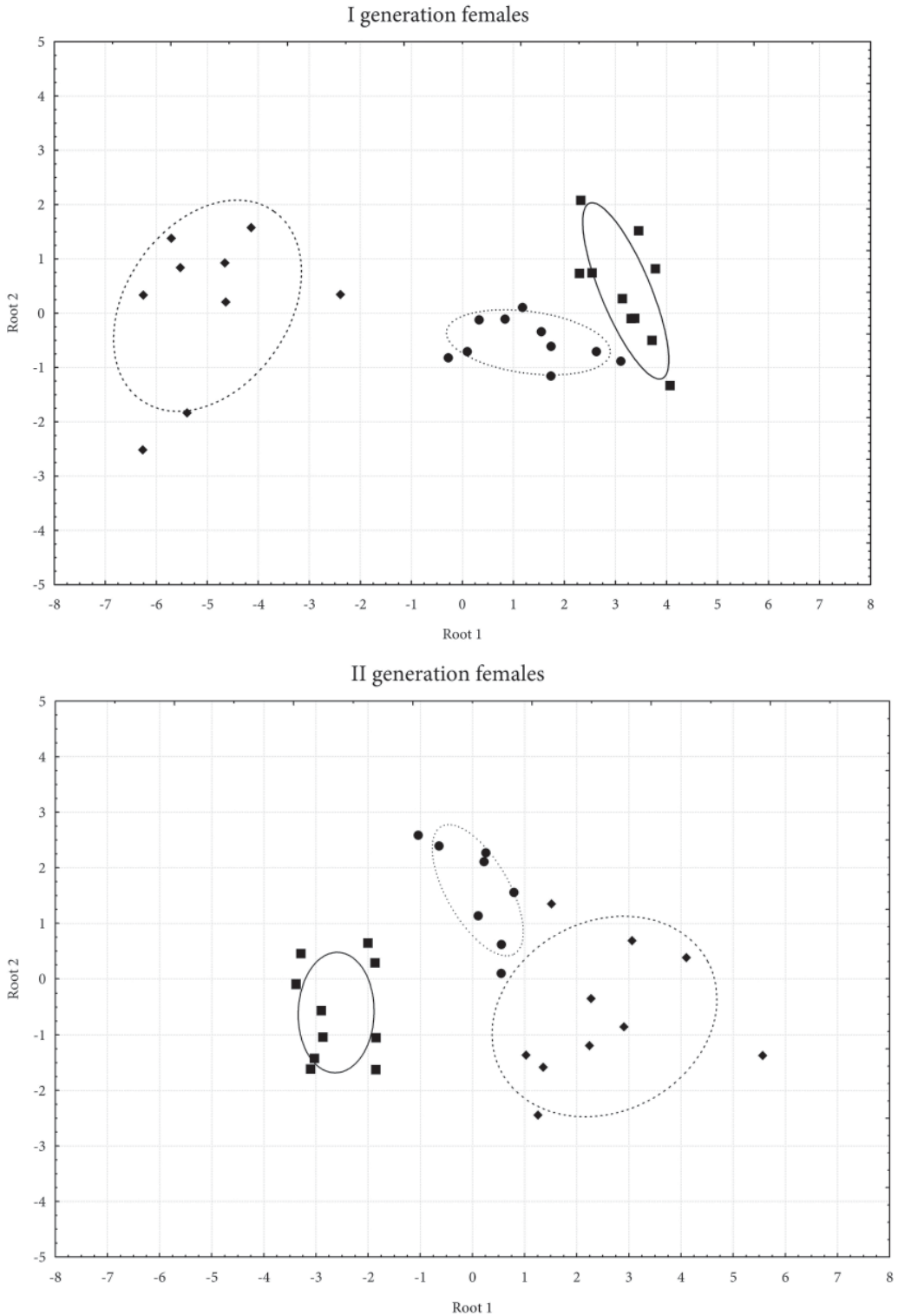

Fig. 1. Scatterplots of canonical scores for females of both generations. Legend: dots — 18 °C; squares — 22 °C; rhombs — 28 °C. Ranges of groups are ellipsed with coefficient 0.95.

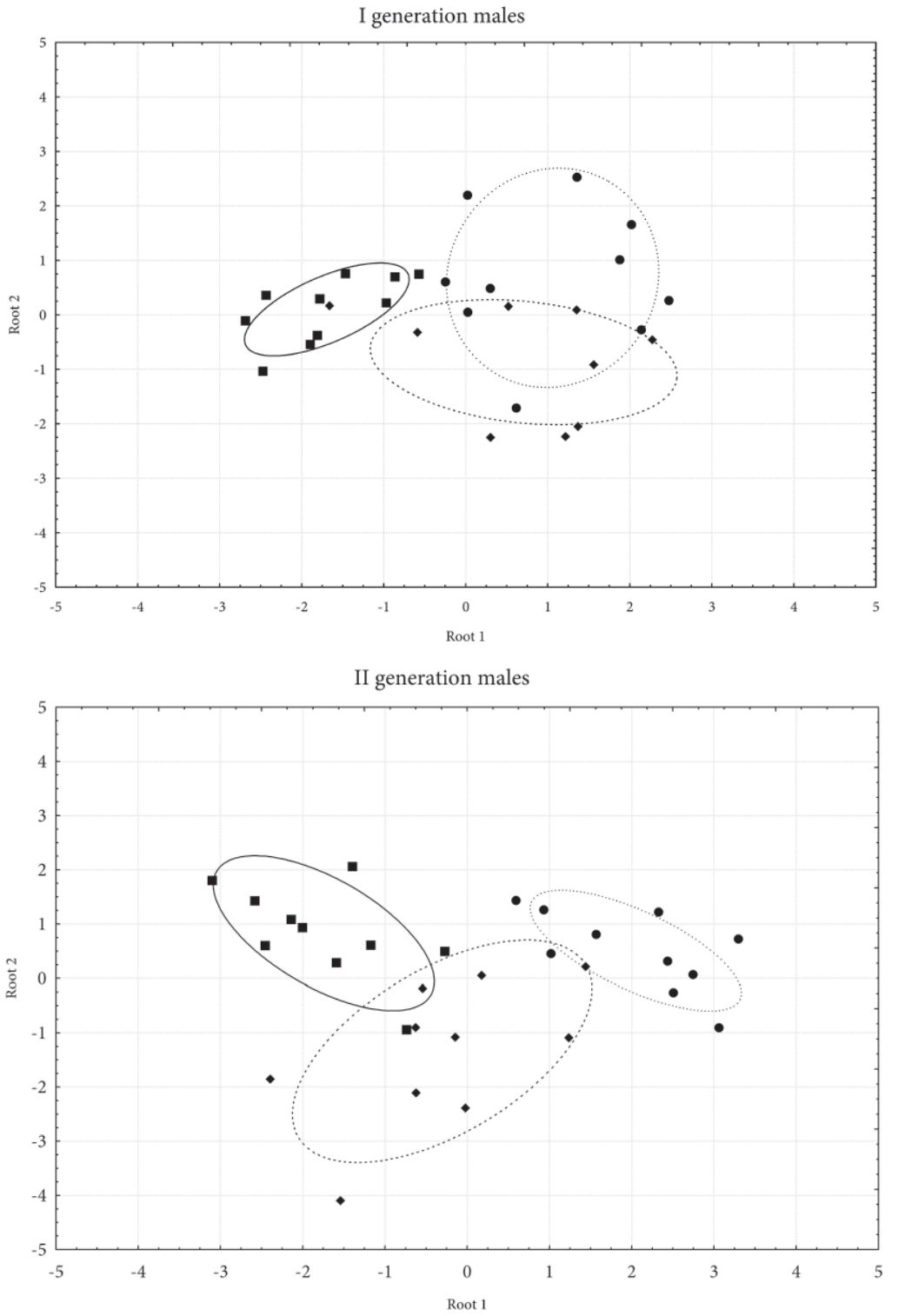

Fig. 2. Scatterplots of canonical scores for males of both generations. Legend: dots — 18 °C; squares — 22 °C; rhombs — 28 °C. Ranges of groups are ellipsed with coefficient 0.95.

are fertile in spite of critical morphological anomalies. This study shows that appearance of pygmy forms may be a result of the stress response in nematode populations, adaptation to temperature changes and lack of nutrition for different reasons: at lower temperature — due to the higher number of nematodes feeding on bacterial products; and at higher temperature — due to the rapid growth of adult nematodes. The nature of anomalies in 28 °C group give the evidence that cuticular and hypodermal structures have greater rates of development compared to other internal structures developed in lack of nutrition.

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