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Temperature-induced morphometrical variability in an isolate of *Pratylenchus vulnus* Allen & Jensen, 1951 (Nematoda: Tylenchida)

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Summary – The influence of temperature on morphometrical variability of females and males of an isolate of *Pratylenchus vulnus* was studied. Nematodes were reared monoxenically on carrot disk cultures and incubated for 4 months at 16, 21, 25 and 28°C. The morphometrics of several characters were significantly affected depending on sex and temperature. It is concluded that temperature can significantly influence several morphometrical characters of *P. vulnus*.

Keywords – intraspecific variation, morphometry, root-lesion nematode.

The root-lesion nematode, *Pratylenchus vulnus* Allen & Jensen, 1951 has been considered a well characterised species (Loof, 1978). However, many populations and isolates have shown considerable variability for most morphometrical characters, as well as for a few morphological features (Roman & Hirschmann, 1969; Mizukubo, 1990; Doucet *et al.*, 1996, 1997, 1998; Doucet & Lax, 1997).

Environmental factors such as soil type, host, and climate can influence the variability of morphometrical characters in different nematode species (Fortuner & Quénehervé, 1980; Brown & Boag, 1988). Temperature has a substantial effect on different aspects of the life cycle and behaviour of some plant parasitic nematodes (de Guiran & Ritter, 1979; Ferris & Van Gundy, 1979; Fagbenle *et al.*, 1987, 1989; Alston & Schmitt, 1988; De Waele & Wilken, 1990; Fallas & Sarah, 1995; Feil *et al.*, 1997). Temperature effects on reproductive potential and mobility have been studied for some species of the genus *Pratylenchus*

(Dickerson, 1979; Towson & Lear, 1982; Pinochet *et al.*, 1995; Mizukubo & Adachi, 1997). However, information is not available on how temperature might affect the morphometry of these nematodes.

The objective of this work was to study the influence of four different temperatures on the morphometry of females and males of an isolate of *P. vulnus*.

Materials and methods

A single, gravid *P. vulnus* female obtained from a population associated with rose (*Rosa multiflora* L.) growing at Cabriils, Barcelona, Spain, was placed on a carrot (*Daucus carota* L.) disk in monoxenic culture (Moody *et al.*, 1973) and incubated at 23–24°C for four to five generations (4 months). To establish new cultures at different temperature regimes, nematode inoculum was recovered from the original single female culture, surface sterilised with 2000 ppm streptomycin sulphate for 3 h, and

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Table 1. Measurements (mean \pm standard deviation, range and coefficient of variation) of females and males of an isolate of *Pratylenchus vulnus* ($n = 20$). (All measurements in μm , except L (in mm). The measurements are given as: arithmetic mean \pm standard deviation; (minimum-maximum) and coefficient of variation.)

	Temperature ($^{\circ}\text{C}$)							
	16		21		25		28	
	females	males	females	males	females	males	females	males
L	0.63 \pm 0.04 (0.57-0.71)	0.55 \pm 0.03 (0.47-0.60)	0.64 \pm 0.07 (0.52-0.76)	0.54 \pm 0.02 (0.50-0.59)	0.61 \pm 0.02 (0.56-0.65)	0.54 \pm 0.02 (0.48-0.59)	0.61 \pm 0.03 (0.56-0.65)	0.56 \pm 0.03 (0.49-0.62)
	6.35	5.45	10.94	3.70	3.28	3.70	4.92	5.36
a	27.58 \pm 2.21 (24.83-34.83)	30.87 \pm 1.70 (28.35-34.25)	30.39 \pm 3.68 (24.14-36.61)	31.19 \pm 2.43 (25.17-35.63)	27.94 \pm 1.81 (25.09-31.72)	30.49 \pm 1.46 (27.93-34.48)	27.46 \pm 1.78 (24.28-31.55)	31.30 \pm 2.28 (25.95-35.27)
	8.01	5.51	12.11	7.79	6.48	4.79	6.48	7.28
b	6.61 \pm 0.52 (5.44-7.72)	5.84 \pm 0.36 (5.23-6.74)	6.61 \pm 0.66 (5.14-7.82)	5.77 \pm 0.30 (5.25-6.40)	6.35 \pm 0.26 (5.77-6.90)	6.03 \pm 0.29 (5.60-6.65)	6.16 \pm 0.25 (5.72-6.61)	6.07 \pm 0.34 (5.29-6.75)
	7.87	6.16	9.98	5.20	4.09	4.81	4.06	5.60
b'	4.82 \pm 0.51 (3.51-6.00)	4.45 \pm 0.35 (3.69-5.04)	4.88 \pm 0.56 (3.73-5.93)	4.56 \pm 0.27 (4.03-4.86)	4.78 \pm 0.26 (4.22-5.17)	4.69 \pm 0.26 (4.22-5.18)	4.86 \pm 0.25 (4.39-5.42)	4.81 \pm 0.27 (4.29-5.29)
	10.58	7.86	11.47	5.92	5.44	5.54	5.14	5.61
c	19.72 \pm 2.06 (16.03-23.82)	20.81 \pm 1.58 (19.12-26.27)	20.01 \pm 1.69 (17.35-24.70)	20.22 \pm 1.28 (18.16-22.81)	20.13 \pm 1.40 (17.75-22.78)	20.37 \pm 1.48 (18.30-22.84)	21.23 \pm 1.54 (18.10-24.40)	21.92 \pm 1.52 (19.33-24.28)
	10.45	7.59	8.45	6.33	6.95	7.27	7.25	6.93
c'	2.27 \pm 0.24 (1.79-2.63)	1.97 \pm 0.20 (1.50-2.33)	2.49 \pm 0.31 (2.07-3.17)	2.09 \pm 0.20 (1.73-2.50)	2.27 \pm 0.23 (1.93-2.83)	2.09 \pm 0.18 (1.79-2.50)	2.11 \pm 0.16 (1.73-2.46)	1.96 \pm 0.16 (1.76-2.33)
	10.57	10.15	12.45	9.57	10.13	8.61	7.58	8.16
V	79.83 \pm 1.87 (75.90-83.75)	-	80.67 \pm 1.81 (77.54-84.34)	-	79.99 \pm 1.46 (76.67-82.51)	-	80.67 \pm 4.89 (65.09-88.96)	-
	2.34	-	2.24	-	1.82	-	6.06	-
Distance from ant. end to pharyngo-intestinal valve	96.00 \pm 6.61 (87.00-109.00)	94.00 \pm 5.74 (87.00-111.00)	96.00 \pm 4.94 (89.00-106.00)	94.00 \pm 3.55 (87.00-100.00)	96.00 \pm 4.23 (85.00-101.00)	90.00 \pm 3.61 (84.00-96.00)	98.00 \pm 5.19 (90.00-111.00)	92.00 \pm 3.71 (87.00-101.00)
	6.88	6.11	5.14	3.78	4.41	4.01	5.29	4.03
Distance from ant. end to nerve ring	73.00 \pm 4.54 (67.00-84.00)	71.00 \pm 4.95 (62.00-84.00)	76.00 \pm 2.22 (72.00-80.00)	72.00 \pm 2.35 (68.00-77.00)	74.00 \pm 3.04 (65.00-78.00)	71.00 \pm 3.18 (65.00-76.00)	75.00 \pm 2.54 (72.00-80.00)	73.00 \pm 2.12 (70.00-77.00)
	6.22	6.97	2.92	3.26	4.11	4.48	3.39	2.90
Distance from ant. end to valve of median bulb	62.00 \pm 4.87 (54.00-72.00)	60.00 \pm 5.45 (55.00-76.00)	63.00 \pm 2.63 (58.00-69.00)	61.00 \pm 2.22 (57.00-67.00)	61.00 \pm 2.82 (55.00-65.00)	58.00 \pm 3.52 (51.00-62.50)	61.00 \pm 2.50 (56.00-66.00)	59.00 \pm 1.64 (56.00-61.50)
	7.85	9.08	4.17	3.64	4.62	6.07	4.09	2.78
Distance from ant. end to excretory pore	88.00 \pm 5.55 (80.00-101.00)	83.00 \pm 4.22 (75.00-90.00)	86.00 \pm 7.94 (73.00-100.00)	85.00 \pm 4.79 (74.00-93.00)	92.00 \pm 4.05 (82.50-97.00)	84.00 \pm 3.84 (77.00-91.00)	94.00 \pm 4.90 (85.00-101.00)	88.00 \pm 3.68 (80.00-95.00)
	6.31	5.08	9.23	5.63	4.40	4.57	5.21	4.18
MB	47.26 \pm 2.65 (40.83-52.94)	48.69 \pm 2.05 (44.58-52.56)	48.20 \pm 3.02 (41.28-54.55)	50.95 \pm 2.49 (46.15-56.36)	47.88 \pm 1.92 (44.44-51.20)	49.87 \pm 1.41 (47.33-52.54)	49.14 \pm 2.94 (43.48-56.36)	50.66 \pm 1.99 (47.72-55.05)
	5.61	4.21	6.26	4.89	4.01	2.83	5.98	3.93
Pharynx length	132.00 \pm 13.26 (113.00-169.00)	123.00 \pm 10.61 (107.50-154.00)	131.00 \pm 8.68 (110.00-149.00)	119.00 \pm 5.62 (110.00-130.00)	128.00 \pm 5.81 (115.00-138.00)	116.00 \pm 6.13 (106.00-127.50)	125.00 \pm 6.80 (110.00-138.00)	116.00 \pm 5.12 (107.00-124.00)
	10.04	8.63	6.63	4.72	4.54	5.28	5.44	4.41
Stylet	15.35 \pm 0.46 (15.00-16.00)	15.03 \pm 0.30 (14.00-15.50)	15.10 \pm 0.26 (15.00-16.00)	15.08 \pm 0.24 (15.00-16.00)	15.05 \pm 0.15 (15.00-15.50)	14.90 \pm 0.31 (14.00-15.00)	15.00 \pm 0.00 (15.00)	14.90 \pm 0.31 (14.00-15.00)
	3.07	2.00	1.73	1.60	1.00	2.07	0.00	2.07
Conus	7.00 \pm 0.49 (7.00-8.50)	7.00 \pm 0.41 (7.00-8.00)	7.00 \pm 0.49 (7.00-8.50)	7.00 \pm 0.38 (7.00-8.00)	7.50 \pm 0.41 (7.00-8.00)	8.00 \pm 0.43 (7.00-8.00)	7.50 \pm 0.43 (7.00-8.00)	8.00 \pm 0.37 (7.00-8.00)
	7.00	5.86	7.00	5.43	5.47	5.37	5.73	4.62
m	48.37 \pm 2.81 (43.75-53.33)	49.44 \pm 2.85 (45.16-53.33)	49.17 \pm 3.08 (46.67-56.67)	49.59 \pm 2.51 (46.67-53.33)	49.99 \pm 2.59 (46.67-53.33)	51.19 \pm 2.97 (46.67-57.14)	49.67 \pm 2.84 (46.67-53.33)	51.50 \pm 2.02 (46.67-53.33)
	5.81	5.76	6.26	5.06	5.18	5.80	5.72	3.92
o	18.27 \pm 3.20 (12.50-26.67)	16.48 \pm 3.18 (12.90-23.33)	18.52 \pm 3.95 (13.33-26.67)	17.09 \pm 3.32 (12.50-23.33)	18.60 \pm 2.43 (13.33-23.33)	18.76 \pm 3.51 (13.33-23.33)	18.00 \pm 3.65 (13.33-26.67)	20.48 \pm 3.61 (16.67-26.67)
	17.51	19.30	21.33	19.43	13.06	18.71	20.28	17.63
Post vulvar sac	48.00 \pm 4.76 (36.50-58.00)	-	40.00 \pm 4.01 (34.50-50.00)	-	44.00 \pm 7.10 (32.00-58.00)	-	37.63 \pm 6.31 (30.00-52.00)	-
	9.92	-	10.02	-	16.14	-	16.77	-
Dist. from vulva to anus	95.00 \pm 12.37 (68.50-114.00)	-	91.00 \pm 11.82 (75.00-124.00)	-	91.00 \pm 10.02 (79.00-117.00)	-	88.00 \pm 27.60 (35.00-176.00)	-
	13.02	-	12.99	-	11.01	-	31.36	-
Tail	32.00 \pm 2.89 (25.00-36.00)	26.00 \pm 2.25 (21.00-30.00)	32.00 \pm 3.05 (28.00-38.00)	27.00 \pm 1.26 (25.00-30.00)	30.00 \pm 1.86 (28.00-34.00)	27.00 \pm 2.07 (24.00-30.00)	29.00 \pm 1.90 (26.00-32.00)	26.00 \pm 1.91 (22.00-29.00)
	9.03	8.65	9.53	4.67	6.20	7.67	6.55	7.35
Tail annules	26.00 \pm 2.49 (23.00-32.00)	-	28.00 \pm 3.63 (22.00-35.00)	-	28.00 \pm 3.44 (23.00-33.00)	-	26.00 \pm 2.92 (21.00-33.00)	-
	9.58	-	12.96	-	12.29	-	11.23	-

Table 1. (Continued).

	Temperature (°C)							
	16		21		25		28	
	females	males	females	males	females	males	females	males
Body diam.	23.00 ± 1.57 (20.00-26.00) 6.83	18.00 ± 1.27 (15.00-19.00) 7.05	21.00 ± 1.92 (18.50-25.00) 9.14	18.00 ± 1.47 (15.00-20.00) 8.17	22.00 ± 1.13 (20.00-24.00) 5.14	18.00 ± 0.95 (16.00-20.00) 5.28	22.00 ± 1.54 (20.00-25.00) 7.00	18.00 ± 0.65 (17.00-19.00) 3.61
Body diam. at anus	14.00 ± 0.92 (12.50-16.00) 6.57	13.00 ± 1.36 (12.00-18.00) 10.46	13.00 ± 1.25 (11.00-15.00) 9.61	13.00 ± 0.95 (12.00-15.00) 7.31	13.00 ± 1.20 (11.00-15.00) 9.23	13.00 ± 0.92 (11.50-15.00) 7.08	14.00 ± 0.65 (12.00-15.00) 4.64	13.00 ± 0.62 (12.00-14.00) 4.77
Spicules	-	18.00 ± 1.95 (15.00-23.00) 10.83	-	18.00 ± 1.08 (16.00-20.00) 6.00	-	19.00 ± 1.70 (15.00-22.00) 8.95	-	18.00 ± 1.63 (15.00-20.00) 9.06
Gubernaculum	-	6.00 ± 0.62 (5.00-7.00) 10.33	-	6.00 ± 0.51 (5.00-7.00) 8.50	-	5.00 ± 0.59 (4.50-6.00) 11.80	-	5.00 ± 0.89 (4.50-7.00) 17.80

rinsed three times in sterile water prior to inoculation of carrot disks cv. Nantesa. Single carrot disks (2.5 g) were placed in 5 cm-diam. Petri plates and five gravid females, individually collected with a micropipette, were delivered to the surface of the carrot disk in 0.1 ml water suspension. Eight cultures per temperature were prepared and kept in four incubators at 16, 21, 25 and 28°C for 110 days. Nematodes were recovered by washing the Petri plate and passing the resultant suspension through a 150 µm-pore sieve and twice through a 25 µm sieve. Debris from carrot tissue collected on the 150 µm sieve was discarded and nematodes were recovered from the 25 µm sieve.

All specimens collected were fixed (Netscher & Seinhorst, 1969), transferred to anhydrous glycerine, and mounted individually on glass slides (Seinhorst, 1962). Morphometrical characters considered to describe *P. vulnus* (Allen & Jensen, 1951) were measured using a compound microscope with a fitted ocular micrometer. The morphometrical data for each character tabulated were: mean value, standard deviation, minimum and maximum values (range) and coefficient of variation (CV). Differences among values of morphometrical characters obtained for female and male specimens at each temperature were evaluated through analysis of variance (ANOVA). In the special case of stylet length, the effect of temperature was investigated throughout the analysis of frequency distribution for the few different values that could be clearly identified.

In order to describe the effect of temperature over the complete set of morphometrical characters, a linear discriminant analysis (Johnson & Wichern, 1992) was performed for females and males with temperature as the grouping variable. Characters included in this procedure

corresponded to those with P -values ≤ 0.10 in the univariate tests for comparison of means. This procedure was also used to understand the relationships among characters and their dependence on temperature effect by the inspection of discriminant coefficients.

In order to identify groups of morphometrical characters having a similar response to temperature, the set of standardised mean profiles of responses to temperatures of each morphometrical character was submitted to cluster analysis, applying average linkage method to the Euclidean distances between profiles (Johnson & Wichern, 1992).

Results

Measurements and statistics for the morphometrical characters of females and males of the isolate of *P. vulnus* studied are presented in Table 1. A summary of the result of the analysis of variance for the effect of temperature is shown in Table 2. About 50% of the characters analysed showed significant differences ($P \leq 0.05$) between means for the different temperature conditions of growth. For stylet length, because of the few different values observed, the data were treated as categorical and the P -value reported corresponds to the chi-square statistic for the null hypothesis of equal proportions (of the different values under the four temperature conditions). The frequency distribution for females is shown in Table 3.

Considering correlation coefficients between original characters and canonical score, it is shown that, in the case of females, canonical axis 1 was mainly correlated with ratios a , c and c' ; axes 2 and 3 were mainly correlated with body length, tail length and also with ratio c . In male

Table 2. Common within variance standardized discriminant coefficients and P-values for the univariate comparison of females and males of *Pratylenchus vulnus* grown at different temperatures.

	Females				Males			
	ANOVA	Discriminant coefficients			ANOVA	Discriminant coefficients		
	P-value	axis 1	axis 2	axis 3	P-value	axis 1	axis 2	axis 3
L	0.059	-0.476	-2.715°	3.971°	0.280	-	-	-
Body diam.	0.004*	-1.446	-1.149	-1.545	0.795	-	-	-
a	0.001*	-1.703°	-1.860	-1.296	0.583	-	-	-
Dist. from ant. end to pharyngo-intestinal valve	0.400	-	-	-	0.009*	0.256	3.402	1.509
b	0.005*	0.182	-0.056	1.098	0.009*	0.644	3.893°	1.493
Dist. from ant. end to nerve ring	0.079	-0.342	-0.643	-0.178	0.133	-	-	-
Dist. from ant. end to valve of median bulb	0.215	-	-	-	0.051*	-2.677°	0.497	-14.042°
MB	0.168	-	-	-	0.003*	1.767°	0.350	9.674°
Dist. from ant. end to excretory pore	0.000*	-0.554	0.806	0.848	0.001*	0.667	0.265	0.058
Pharynx	0.051	0.700	0.217	0.509	0.004*	1.498	-4.928°	12.825°
b'	0.867	-	-	-	0.001*	-0.664	-4.692°	-1.868
Stylet	0.001*	0.433	0.319	-0.217	0.144	-	-	-
Conus	0.887	-	-	-	0.154	-	-	-
m	0.300	-	-	-	0.024*	0.268	0.195	-0.279
o	0.942	-	-	-	0.002*	1.797°	-2.738	-0.654
Tail	0.000*	1.436	3.343°	-6.380°	0.103	-	-	-
c	0.034*	2.363°	4.401°	-4.619°	0.002*	0.661	0.042	0.557
Body diam. at anus	0.001*	1.321	0.979	1.349	0.196	-	-	-
c'	0.000*	1.610°	0.859	2.242	0.028*	0.572	0.557	-0.142
V	0.698	-	-	-	-	-	-	-
Post vulvar sac	0.000*	0.576	0.255	0.665	-	-	-	-
Dist. from vulva to anus	0.676	-	-	-	-	-	-	-
Tail annules	0.224	-	-	-	-	-	-	-
Spicules	-	-	-	-	0.324	-	-	-
Gubernaculum	-	-	-	-	0.000*	-0.485	0.418	0.052

* Significant mean differences ($P \leq 0.05$).

° Indicate the three highest absolute values of the common within variance standardized discriminant coefficients.

specimens, the characters 'distance from anterior end to valve of median bulb', ratios MB and o were associated with canonical axis 1; axis 2 was correlated with ratios b and b', and pharynx length; axis 3 showed high correlation with 'distance from anterior end to valve of median bulb', oesophageal length and ratio MB (Table 2).

The observations for the different temperatures were represented graphically on the basis of the first two canonical axes (Fig. 1). The results showed that the first canonical axis clearly separates the extreme temperatures (16 and 28°C) for both females and males, while the second canonical axis mainly separates specimens grown at 21°C from the rest, and this was more evident in the case of females.

Table 3. Stylet length (μm) frequencies (%) of females in relation to temperature.

Stylet length	Temperature (°C)			
	16	21	25	28
15	60	85	90	100
15.5	10	10	10	-
16	30	5	-	-

It was possible to define three groups of characters through the cluster analysis of different temperatures. Their profiles are shown in Fig. 2 and the allocation to the different groups is presented in Table 4.

Discussion

The ranges of the measurements taken from specimens reared at four temperature regimes were usually well within previously recorded ranges for this species (Corbett, 1974; Frederick & Tarjan, 1989; Doucet *et al.*, 1997). The ratio V for specimens reared at 28°C was an exception being more variable than previously reported.

The morphometrical characters of *P. vulnus* showed variability among the different temperatures. This variability differed according to sex and temperature. For most of the characters of both sexes, the lowest CV values were observed at 25 and 28°C, whereas the highest were found at 16 and 21°C.

In most cases, CV values for males were lower than for females; this is consistent with previous observations (Doucet *et al.*, 1997), except at 25°C when males presented higher values. In addition, CV values in males did not exceed 11%, except for ratio o which reached values up to 17%. In general, CV values for females did not exceed 13%, except for ratio o, distance from vulva to anus and post-vulvar sac length. CV values ranged from 13 to 31%. Stylet length showed the lowest CV values (1-3%) among the common characters for females and males. The CV values for the different characters corresponded with

values equivalent to those of other cases studied previously (Doucet *et al.*, 1996, 1997; Doucet & Lax, 1997). However, a discrepancy was found for the ratio V, CV values ranging from 1 to 3%, the value being 6% in the case of the isolate reared at 28°C.

The analysis of variance revealed significant differences for about half of the morphometrical characters evaluated for both males and females. These differences were found even for stylet length in females. Indeed, stylet length decreased with increasing incubation temperature. The rest of the characters were influenced differently in relation to temperature.

From a multivariate point of view, specimens reared at different temperatures were aligned along the first canonical axis. In females the first axis was mainly associated with ratios a, c, and c' denoting changes in size. The same axis in males was strongly determined by 'distance from anterior end to valve of median bulb', and ratios MB and o, showing changes in length on certain segments of the body.

Second axis separated individuals of both sexes growing at 21°C from the rest. It is very difficult to prove the behaviour of the morphometrical characters relevant to this axis because of the correlation among them and the correlation between characters and ratios.

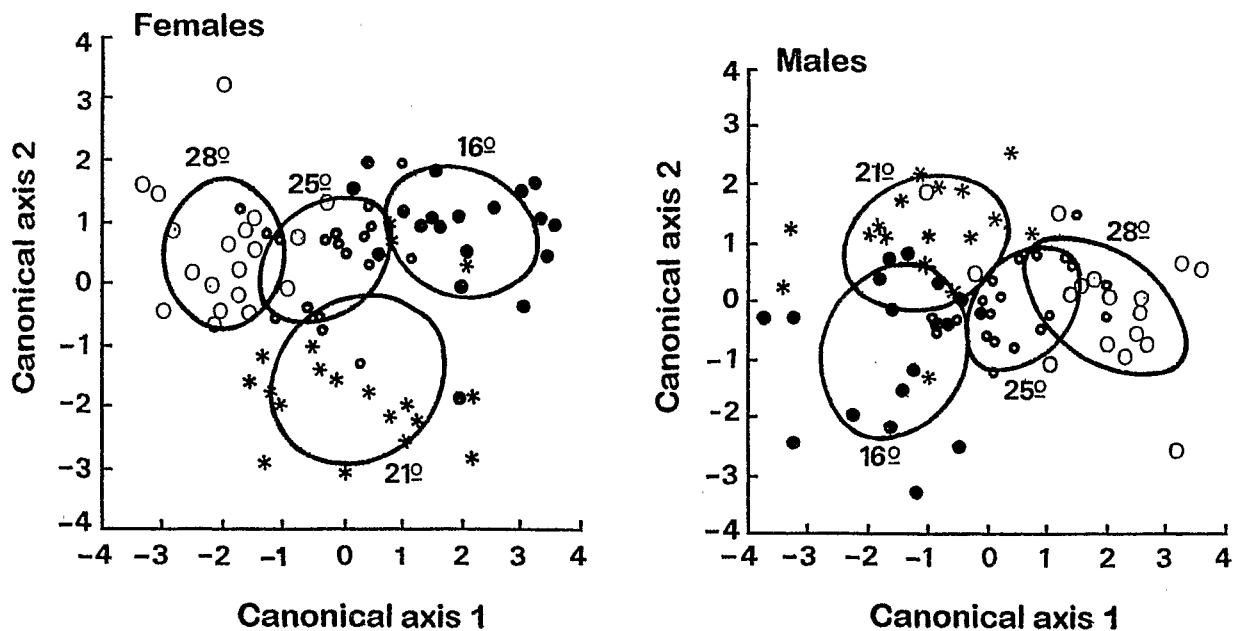


Fig. 1. Scatter plot and ellipsoids delimiting 50% prediction region in the discriminant space for females and males of an isolate of *Pratylenchus vulnus* growing at four different temperatures.

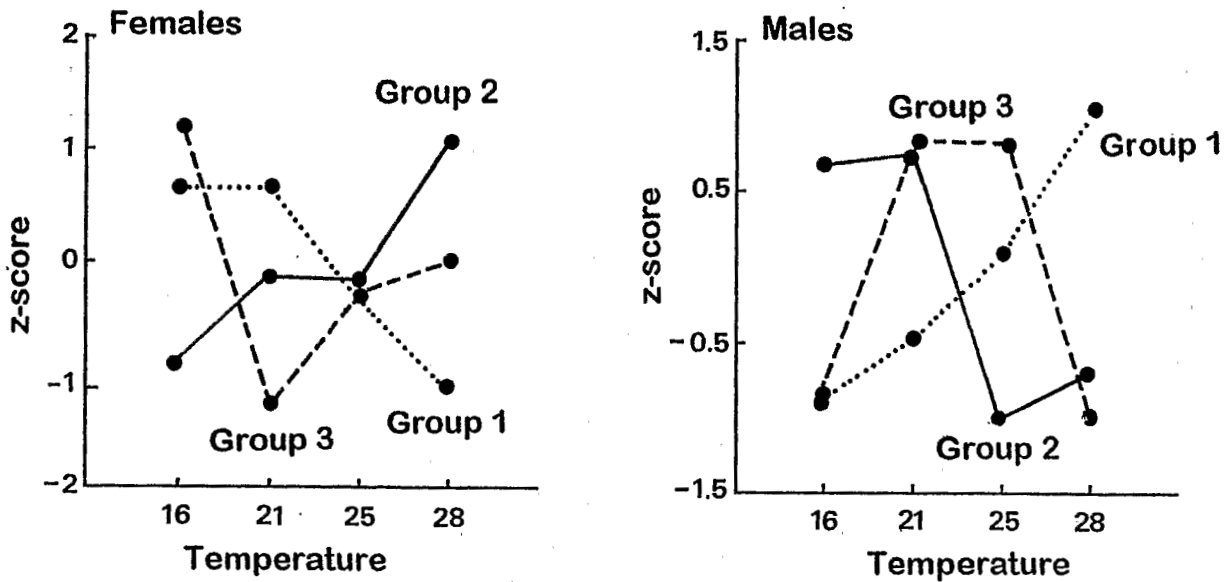


Fig. 2. Response of groups of morphometrical characters in relation to temperature.

Table 4. Groups of morphometrical characters with similar response to the different temperatures.

	Group 1		Group 2		Group 3	
	Females	Males	Females	Males	Females	Males
L	x	-	-	-	-	-
Body diam.	-	-	-	-	x	-
a	x	-	-	-	-	-
Dist. from ant. end to pharyngeal-intestinal valve	-	-	-	x	-	-
b	x	x	-	-	-	-
Dist. from ant. end to nerve ring	-	-	x	-	-	-
Dist. from ant. end to valve of median bulb	-	-	-	x	-	-
MB	-	x	-	-	-	-
Dist. from ant. end to excretory pore	-	x	x	-	-	-
Pharynx	x	-	-	x	-	-
b'	-	x	-	-	-	-
Stylet	x	-	-	-	-	-
m	-	x	-	-	-	-
o	-	x	-	-	-	-
Tail	x	-	-	-	-	-
c	-	x	x	-	-	-
Body diam. at anus	-	-	-	-	x	-
c'	x	-	-	-	-	x
Post vulvar sac	x	-	-	-	-	-
Gubernaculum	-	-	-	x	-	-

(x) indicate characters with the same response.

Analysis of groups of morphometrical characters with similar response to temperature changes showed groups with different behaviour patterns for males and females. Considering females, Group 1 involves morphometrical characters that decrease in their mean values when temperature is above 21°C. These characters are mainly longitudinal ones, and according to their behaviour females tend to be shorter when temperature increases. Group 2 increases almost linearly with temperature and is also related to other longitudinal segments. Meanwhile, Group 3 includes body diam. and body diam. at anus, and shows a clear fall at 21°C. As a conclusion, females growing at 21°C are larger and thinner.

Morphometrical characters in males presented a different way of grouping. Group 1 increases almost linearly with temperature and mainly involves ratios. Group 2 is similar to Group 1 for females, and involves some characters that decrease with temperature, and finally the ratio c' differentiates intermediate temperatures from extreme ones.

This study revealed that different temperatures can influence variability of several morphometrical characters in the isolate of *P. vulnus* analysed, resulting in significant differences among the measured values. It is also shown that response to temperature depends on sex and that there are groups of morphometrical characters responding in some special patterns that also depend on sex. In spite of the disparity in responses to temperature according to sex, when responses are considered from a multivariate point of view, specimens growing at different temperatures arrange in a very similar way independently of sex, revealing a deep and common strategy to temperature changes.

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