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Morphological Variation in Pratylenchus penetrans

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Abstract: Variability of morphological characters used to separate *Pratylenchus penetrans* from other species of the genus was studied in a population originating from a single gravid female. Pronounced heteromorphism was observed and characterized. About 30% of females had a crenate-tail terminus. Several shapes of stylet knobs were characterized; 50% of them were anteriorly flattened to indented. The outer margin of the cephalic framework extended into the body from one-half to two annules. The shape of the spermatheca varied from round to oval. A fifth lateral line was observed in many specimens. Environmental factors, and particularly the host plant, influenced such morphometric characters as body length, width, esophagus length, stylet length, *V* value, *a* and *b'* ratios, as well as qualitative characters such as tail terminus, growth of ovary, and shape of median bulb. Nematodes reared on pea and cabbage had a higher percentage of females with a crenate-tail terminus than those from tomato, rye, beet, and alfalfa callus culture. Nematodes from peas were longer and wider; they often had gonads that extended to esophagi, but they had shorter esophagi and stylets than those from callus culture. Populations from different geographical locations also exhibited variability in morphological characters, as did the Cornell population. The validity of many characters used in species identification is discussed, and the possibility that other related *Pratylenchus* species are conspecific with *P. penetrans* is suggested. *Key Words:* taxonomy, crenate tail, smooth tail, host effect, selective response.

Knowledge of intraspecific variability of many morphological characters used in taxonomy of plant-parasitic nematodes is often lacking. As a result, the taxonomic value of many such characters is doubtful, and proper identification is made very difficult. Investigators concerned with morphological variation have conducted research mainly on quantitative characters. The magnitude of variations cannot be estimated unless detailed studies on morphology and frequency of variants, as they occur naturally within different populations and as they are affected by ecological factors, are conducted.

Naturally occurring, intraspecific variability in nematodes is considered in few taxonomic reports and reviews (4, 5, 6, 15, 23, 25, 31). Variation has been associated with some aspects of nematode behavior (2, 4, 7, 18, 22), as well as with changes occurring during nematode development (4, 7, 9, 19). Induced variability has been considered in relation to population density (10), method of killing and fixation (13), environmental factors (10), host species or growth medium (1, 10, 11, 12, 14), and host physiology (3). Differences related to geographical location have also been suggested in a few cases (17, 29).

The genus *Pratylenchus* contains approximately 40 described species, many of which differ only in small morphological features. Intraspecific variation has been suggested by Taylor and Jenkins (30) and by Loof (19), and it has been carefully studied by Roman and Hirschmann (25). No attempt has been made to study variability in living populations under a wide range of environments, or to determine the extent of variability in different geographical populations.

The present study was undertaken to determine the extent of morphological variation in *Pratylenchus penetrans* (Cobb, 1917) Filip. & Schuurm.-Stekh., 1941, especially with respect to those diagnostic characters used at the species level. The effects of different environments and the geographic source of populations were studied.

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MATERIALS AND METHODS

A population of *P. penetrans* originating in 1963 from a single gravid female was propagated on alfalfa callus tissue growing on modified White's medium at approximately 23 C in the dark (24). Extraction for inoculation and microscopic observation was carried out by placing pieces of infected callus on a fiber filter supported by a screen in a shallow layer of water in a pie-pan. Cultures used were not necessarily of the same age nor were they inoculated at the same time in the various experiments.

Additional fixed specimens of *P. penetrans* were obtained for study from: The Netherlands, Japan, Canada, Trinidad, England, and from the states of Maryland, New York, North Carolina, Florida, Kentucky, and Washington of the U.S.A.

In experiments designed to determine the influence of environmental factors on quantitative and qualitative characters, seeds of Wando peas, *Pisum sativum* L., were germinated in sterile sand and the seedlings transplanted 3 days later to 5-cm clay pots which contained a sandy loam orchard soil (pH 6.7) with a low nitrogen and phosphorus content. *Pratylenchus penetrans* was then added by placing a suspension of 500-1,000 nematodes close to the root tip of each seedling. One week after inoculation, intact soil balls were transferred to 20-cm clay pots where they remained for the duration of the experiment. Unless indicated otherwise, peas were replanted twice during an experiment. No fertilizer was applied except when the effect of nutrition was tested in a particular treatment.

At harvest, nematodes were extracted by cutting the roots in pieces 1/2 to 1 cm long, blending them with water for 60 sec, pouring the suspension on a paper filter, and collecting the nematodes after 48 h. When nematodes were extracted from soil, 100 ml of soil were spread evenly over a fiber filter supported by a screen in a shallow layer of water and the nematodes collected after 24 h.

Adult females picked at random were used in morphological observations. They were killed by being immersed in hot water (65 C) for 2 min and fixed and mounted in 2.5% formalin.

Variation of 8 quantitative and 10 qualitative and meristic characters, most of them commonly used in species differentiation, was studied. Quantitative data were analyzed by the analysis of variance and by Tukey's honestly significant-difference procedure (THSD) at the 5% probability level. Coefficients of variability were also calculated.

RESULTS

CHARACTERIZATION OF VARIATION IN THE CORNELL POPULATION OF *P. penetrans:* The diagnostic characters of *P. penetrans* (28) consist of three lip annules, broadly rounded stylet knobs, lack of striations around the female-tail tip, round spermatheca, short post-uterine branch, ovary not extending to esophagus, four lateral lines, and outer margins of sclerotized labial framework extending posteriorly into the body about one body annule. Microscopic observations of more than 500 females obtained from alfalfa callus tissue, however, revealed extensive heteromorphism in most characters.

Stylet knobs varied in shape from sloping anteriorly to being indented (Fig. 1), with about 50% being anteriorly flattened to indented.

Female tails were usually rounded at the tip, but truncated, pointed, or somewhat clavate tails were also found. The tail terminus varied from smooth to distinctly crenate. About 30% of the nematodes possessed coarsely annulated or crenate-tail tips in which one or more indentations, forming annules usually wider than the body annules, occurred at any point around the terminus but mostly toward the dorsal side (Fig. 2). Coarsely annulated tail tips, independent of number and location of indentations, were classified as crenate. Crenate-tail tips, with one and two indentations oriented toward the dorsal side, were most common. The remaining 70% of the nematodes had typically smooth tails. Although, in some cases annulation continued posteriorly on one side, it was never coarse, and the tail tip was smooth.

The outer margin of the sclerotized cephalic framework occasionally extended from one-half to two annules posteriorly on the body, but in most specimens, it

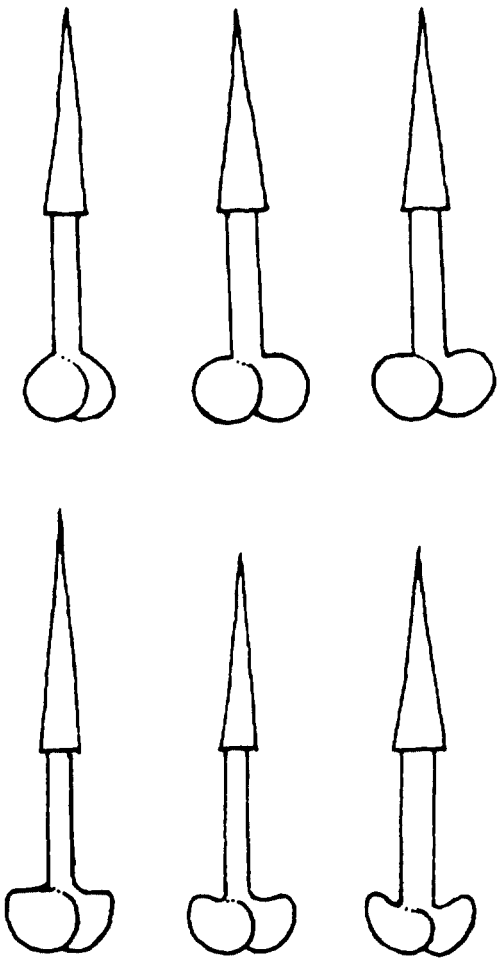

FIG. 1. Shapes of stylet knobs found in the Cornell University population of *Pratylenchus penetrans* cultured on alfalfa callus tissue. Top left to right: sloping anteriorly, broadly rounded, irregularly rounded. Bottom left to right: flattened anteriorly, slightly indented, indented.

extended about one body annule. The spermatheca varied from round to oval and in some cases was flattened. A fifth lateral line, present in the middle of the lateral field of some specimens, usually disappeared at the level of the vulva. No deviations from the normal, 3-annule lip pattern were found. The median bulb was oval in most specimens, although in a few cases it was nearly round. The female gonad did not extend anteriorly to the region of the esophageal glands. The vulva region varied from nonelevated to elevated with most females having slightly elevated vulval lips.

INDUCED VARIATION IN MORPHOLOGY AND MORPHOMETRICS: *Effects of host.*—Ten randomized replicates of five host plants: 'Wando' pea (*Pisum sativum* L.), rye (*Secale cerale* L.), 'Rutgers tomato' (*Lycopersicon esculentum* Mill.), 'Round-up' cabbage (*Brassica oleracea capitata* L.), and 'Ruby Queen' beet (*Beta vulgaris* L.) were inoculated with a suspension of *P. penetrans* and kept for 6 months in the greenhouse at an average temperature of 21 C. After completion, 50 randomly picked females from each treatment were measured and studied. Treatments were compared with each other and with 50 females previously preserved from the initial inoculum. Differences among treatments were found in body length, width, esophagus length, stylet length, and ratios *a* and *b'* (Table 1). The longest and widest nematodes were recovered from peas, and the next longest (in descending order) were from the initial inoculum, rye, tomato, cabbage, and beet, respectively. The stylets in nematodes from all host plants were slightly shorter than the stylets in nematodes from the initial inoculum. Although esophagus length was about the same in nematodes reared on tomato and pea, the *b'* ratio differed because of differences in body length of the nematode in the two treatments. No differences were found in *V.* value.

Observations of certain qualitative characters revealed that, in 30% of females obtained from peas, the gonad extended anteriorly to the esophageal glands, whereas the same occurred in only 4% of those from tomato, and in none from the remaining treatments. A higher number of individuals recovered from peas also had an almost round median bulb, in comparison with the oval median bulb from other treatments. The percentage of crenate-tail females increased to 42% in peas and 59% in cabbage. Many nematodes with stylet knobs flattened anteriorly or indented were observed in all treatments.

Effects of feeding vs. storing without food.—A suspension containing 100 females and 100 males was added to each of five replicates of: *(i)* Wando peas in 5-cm clay pots; *(ii)* sterilized soil in 5-cm clay pots; and *(iii)* tap water in counting dishes. Treatments *i* and *ii* were maintained in a growth chamber at 21 C with a light intensity of 21,520 lux (2,000 ft-c.) and a

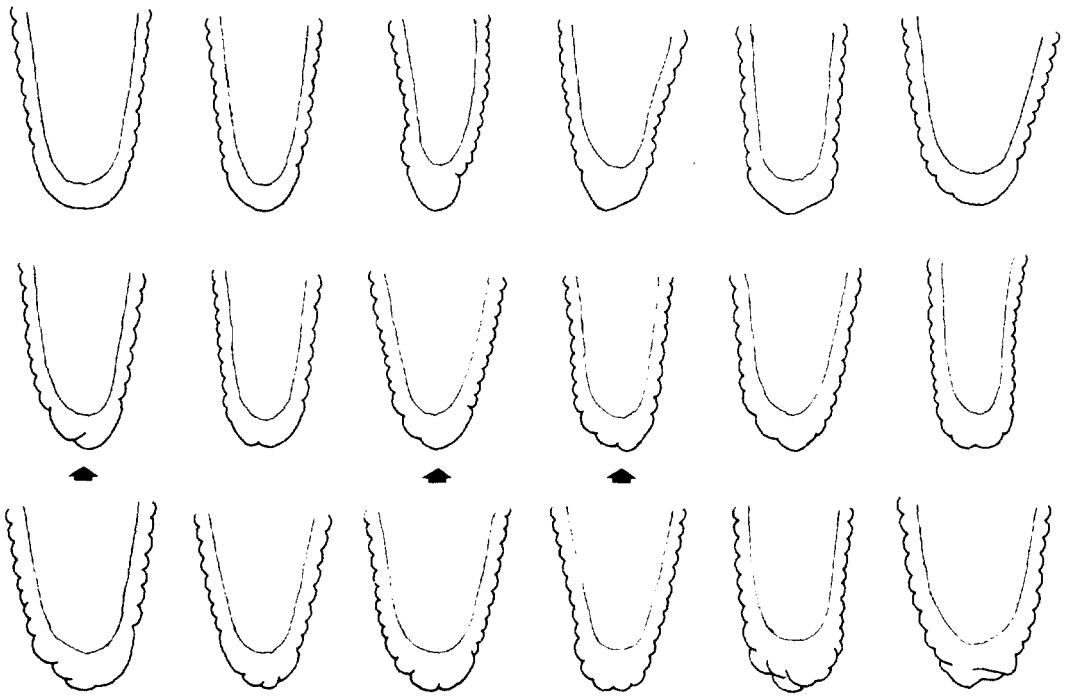

FIG. 2. Types of tail tips found in the Cornell University population of *Pratylenchus penetrans*. Top row: Types of smooth-tail tips. Middle and bottom rows: Types of crenate-tail tips. Arrows indicate the three most common types of crenate tails. All tails are oriented laterally with the dorsal side to the left.

photoperiod of 12 h. Treatment *iii* was kept in darkness in an incubator at 15 C. Three weeks after inoculation, the nematodes were extracted from roots in treatment *i* and soil in treatment *ii*, and 50 females were picked at random from each treatment and compared with the original inoculum. Differences among treatments were found in body length and width; esophagus length; stylet length; and *a*, *b'*, and *c ratios* (Table 3). Nematodes recovered from pea roots were longest and widest; the next (in descending order) were from alfalfa callus, water, and soil, respectively. In comparison with the stylets of nematodes from inoculum, stylets of nematodes from peas were shorter. Because of longer body length of nematodes feeding on pea roots, their *b'* ratio was different from that of the nematodes from initial inoculum. The *c* ratio was higher in nematodes feeding on peas than in other treatments.

TABLE 1. Effects of host plant on morphometric characters of females of *Pratylenchus penetrans*.[z]

Treatment	L (μm)	Width (μm)	Esophagus (μm)	Stylet (μm)	V %	a' (ratio)	b' (ratio)	c (ratio)
Alfalfa callus	578.2 a	22.3 b	131.5 a	17.3 a	79.8 a	26.0 a	4.4 b	18.6 ab
Peas	589.3 a	26.8 a	126.4 ab	17.0 ab	79.6 a	21.1 c	4.7 a	19.4 a
Rye	522.0 b	21.3 bc	120.4 b	17.0 ab	80.1 a	24.7 ab	4.3 bc	18.8 ab
Tomato	508.7 bc	20.9 c	125.6 ab	16.8 b	80.1 a	24.5 b	4.0 d	18.7 ab
Cabbage	507.1 bc	20.3 c	121.6 b	16.9 ab	80.2 a	25.0 ab	4.1 cd	18.3 b
Beet	489.0 c	20.6 c	122.4 ab	16.8 b	80.0 a	23.9 b	4.0 d	18.0 b
Coefficient of variability %	7.16	10.23	12.81	4.55	1.83	9.75	8.66	9.86

[z]Treatment means with a letter in common do not differ, using Tukey's Honesty Significant Differences ($P = 0.05$).

TABLE 2. Effects of host plant on qualitative morphological characters of *Pratylenchus penetrans.*

Character	% females					
	Alfalfa callus	Peas	Rye	Tomato	Cabbage	Beet
Stylet (Lateral View)						
Sloping	4	18.2	4.3	11.4	4.2	3.3
Rounded	50	25.0	39.1	31.8	47.9	38.7
Flat. or indented	46	56.8	56.6	56.8	47.9	58.0
Median Bulb						
Oval	82	64.3	83.7	95.4	88.6	86.2
Almost round	18	35.7	16.3	4.6	11.4	13.8
Gonad						
Extending	0	29.8	0	4.4	0	0
Not extending	100	70.2	100	95.6	100	100
Tail Terminus						
Smooth	65.4	58	68.2	65.4	40.8	67.7
Crenate	34.6	42	31.8	34.6	59.2	32.3

No differences were found in *V* value.

Qualitative characters of *P. penetrans* on peas were influenced in a manner similar to that observed in the preceding experiment (Table 4). In 26.1% of females recovered from peas, the gonad extended anteriorly to the region of the esophageal glands, but this was not observed in the remaining treatments. A very high percentage of nematodes recovered from peas also showed an almost round median bulb as compared with the normal oval median bulb occurring in most remaining treatments. In all treatments, the percentage of crenate tails remained about the same as that of the initial inoculum. The shape of stylet knobs also varied among treatments.

Effects of nitrogen and light intensity.—'Wando' pea plants in 10 randomized replicates of four nitrogen and light intensity levels (low N: low light, low N: high light, high N: low light, and high N: high light) were inoculated with 500-1,000 *P. penetrans.* The low-light-intensity treatments [3,870 lux (360 ft-c.)] were established by shading with cloth, as opposed to high-light-intensity treatments maintained at 21,520 lux (2,000 ft-c.). Nitrogen levels were obtained by fertilizing twice a week with 0.02 M ammonium nitrate (high N treatments), whereas fertilizer was not added to the nitrogen-deficient soil used. The plants were kept in a growth chamber at 21 C and a photoperiod of 12 h for 18 weeks. Nematodes were extracted and 60 randomly selected females from each treatment and 60

TABLE 3. Morphometric characters of *Pratylenchus penetrans* as influenced by feeding vs. storage without food.[a]

Treatment	L (μm)	Width (μm)	Esophagus (μm)	Stylet (μm)	V (%)	a (Ratio)	b' (Ratio)	c (Ratio)
Alfalfa callus	573.0 b	22.4 b	136.5 a	17.1 ab	79.6 a	25.8 b	4.2 b	18.4 b
On peas	596.6 a	26.6 a	131.5 ab	16.8 b	80.0 a	22.5 c	4.6 a	19.9 a
In soil	540.4 c	19.5 c	130.6 b	17.3 a	79.9 a	27.8 a	4.1 b	18.3 b
In water	556.9 bc	21.9 b	133.0 ab	17.3 a	79.5 a	25.7 b	4.2 b	18.4 b
Coefficient of variability (%)	7.64	9.65	8.05	4.91	1.85	9.48	9.48	11.97

[a]Treatment means with a letter in common do not differ, using Tukey's Honesty Significant Differences ($P = 0.05$).

TABLE 4. Morphological characters of *Pratylenchus penetrans* females as influenced by feeding vs. storage without food.

Character	% females			
	Alfalfa callus	Peas	Soil	Water
Stylet Knobs				
Sloping	21.6	16.6	5.5	14.0
Rounded	21.6	42.6	41.8	36.0
Flat. or indented	56.8	40.8	52.7	50.0
Median Bulb				
Oval	92.4	24.5	78.4	91.5
Almost round	7.6	75.5	21.6	8.5
Gonad				
Extending	0	26.1	0	0
Not extending	100	73.9	100	100
Tail Terminus				
Smooth	66.0	68.3	73.9	73.6
Crenate	34.0	31.7	26.1	26.4

from the initial inoculum were compared. No differences were found among the treatments in body length and width, esophagus length, and *b'* ratio. However, there was a difference in the ratio *a* between nematodes from the initial inoculum and those from all treatments. Nematodes were longer and wider, and had a shorter esophagus in these treatments. Stylets were shorter in the high N: high-light-intensity treatment than in the other treatments.

The female gonad extended anteriorly to the region of the esophageal glands in 20.3%, 15.4%, 21.1%, and 21.2% of the nematodes from the low N: low light, low N: high light, high N: low light, and high N: high-light treatments, respectively. This type of gonad was not observed in any of the nematodes from the initial inoculum. Compared to that of the inoculum, a higher percentage of females with an almost round median bulb occurred in the treatments, especially in the high-light treatments. The percentage of females with a crenate tail was 44.1%, 48.2%, 55.1%, and 47.2% for the low N: low light, low N: high light, high N: low light, and high N: high light treatments respectively, in comparison to 32% in the inoculum.

Comparison of P. penetrans from alfalfa callus and pea plants at 29 C.—A suspension of *P. penetrans* was added to each of 24 'Wando' pea seedlings. The plants were kept for 6 months in a growth chamber at 29 C. Numerous nematodes were extracted from roots of plants grown at 29 C, and 50 randomly selected females were studied and compared with 50 females from the initial inoculum.

Differences were found in total length of the nematode, width, esophagus length, stylet length, and *V value* (Table 5). Nematodes from peas were longer and wider, had a shorter esophagus and stylet, and the vulva was located in a more posterior position in comparison with those of nematodes from the initial inoculum. The female gonad extended anteriorly to the esophageal glands region in 17.6% of the nematodes from pea roots, but did not in any of the nematodes from the inoculum (Table 5). The percentage of females with crenate tails was 51.1 from pea roots in comparison with 32% of the females from the inoculum with crenate tails. The morphological response of the nematodes to feeding in pea roots in this experiment was for most characters identical to that of the preceding experiments. Only the V value changed.

Effects of culture media.—Nematodes cultured in Krusberg's (K) medium (16) and modified White's (W) medium (24) for 5 months were extracted, and 30 females from each culture medium were studied and compared. Fewer nematodes and a higher ratio of males to females were obtained with W than with K medium.

The bodies and stylets of females reared in K medium were longer than in those reared in modified W medium. No differences were found in width, esophagus length, and *V* value. The female gonad did not extend anteriorly to the region of the esophageal glands in either treatment. The percentage of females with crenate tails was higher in K medium (44.8%) than in W medium (27.5%).

VARIATION IN POPULATIONS FROM DIFFERENT GEOGRAPHICAL LOCATIONS: Preserved specimens from populations of *P. penetrans* from 14 geographical locations in Europe, Asia, North America, and the Caribbean were examined. In most, a variability similar to the Cornell population was noted. The female-tail terminus was observed for 466 specimens (2-20/population) and 33.3% 0-80%/population) had a crenate-tail tip (Table 6). The same types of tail

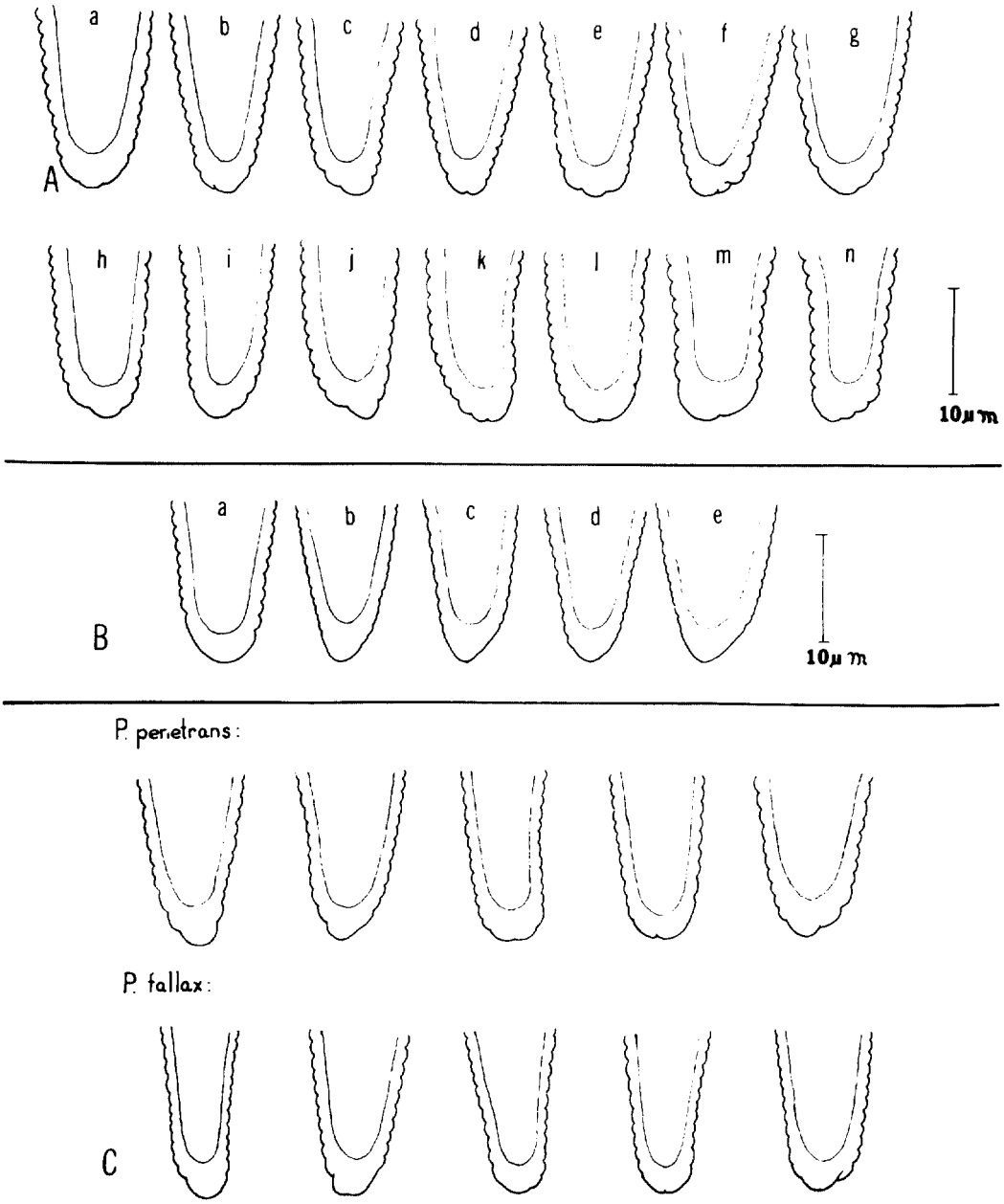

FIG. 3-(A-C). Crenate-tail types observed in *Pratylenchus penetrans* females from various geographical locations. a,b—Holland; c,d—Japan; e—Canada; f,g—Long Island, New York; h,i,j—College Park, Maryland; k,l—Raleigh, North Carolina; m,n—Florida. *B)* Female-tail tips of *Pratylenchus penetrans* from Trinidad (b-e) compared to a "normal", smooth-tail tip (a) from the Cornell university population. *C)* Comparisons of tail tips from *Pratylenchus penetrans* and *P. fallax* females.

crenation found in the Cornell University population were found in most of the geographical populations studied (Fig. 3-A, B). Morphological observations made for each population were recorded in Table 6.

DISCUSSION

The Cornell population and others from different geographical locations exhibited extensive morphological variation. Different

TABLE 5. Comparison of morphometric qualitative morphological characters of *Pratylenchus penetrans* from alfalfa callus and pea plants at 29 C.*

Treatment	L (μm)	Width (μm)	Esophagus (μm)	Stylet (μm)	V	Gonad extending	Tail terminus	
							Smooth	Crenate
Alfalfa callus	558 a	20 a	137 a	17.5 a	79.3 a	0	68	32
Peas	601 b	26 b	125 b	17.0 b	80.3 b	17.6	48.9	51.1
Coefficient of variability (%)	4.94	7.75	7.8	3.7	1.94	—	—	—

*Treatment means with a letter in common do not differ, using Tukey's Honesty Significant Differences ($P = 0.05$).

environments induce variation in morphometric and morphological characters. The validity of many characters used for species determination is, therefore, questioned. The value of many others will undoubtedly depend on results of careful studies of their variability. The shape of the stylet knobs is variable, and this variation supports Roman and Hirschmann's (25) statement that it is a questionable character for separating species of *Pratylenchus*. The outer margin of the cephalic framework is also variable in length, and in taxonomic studies, its extension posteriorly into the body annulation should be utilized with caution. Variation in the shape of spermatheca and vulval lips should be carefully determined. Both characters, as commonly used in species differentiation, are not very reliable because of their variability.

The presence of a fifth lateral line in specimens indicates that caution should be used when consideration is given to the number of lateral lines. Roman and Hirschmann (25) indicated that 4, 5, 6, or 8 incisures in addition to other modifications may occur in *P. penetrans,* and Sher and Allen (28) mentioned that in the genus *Pratylenchus,* sometimes 5 and 6 lines are seen in the region immediately anterior to the vulva.

In agreement with Roman and Hirschmann (25), no deviations from the normal, three-annule lip pattern were found in any of the populations of *P. penetrans* studied. Also, a change in number of lip annules was not induced by altering the environment. These data indicate that this is a reliable taxonomic character for *P. penetrans.*

Taylor and Jenkins (30) stated that some specimens of *P. penetrans* appeared to have striations around the tail tip. Dolliver (8) also mentioned that a few specimens in a New York population of *P. penetrans* from orchard grass (*Dactylis glomerata* L.) had annulated-tail tips. Female nematodes with a crenate tail occurred in most populations that we studied. The proportion of individuals having this character was changed by altering the environment. Of the factors tested, host plant was most effective in inducing changes in this qualitative character. The proportion of crenate-tail females was higher in individuals from cabbage and peas than from the other hosts or initial inoculum from alfalfa callus. The only instance in which the percentage of crenate tails remained as low as in the initial inoculum was in the short-term experiment testing the effect of feeding on peas, in which nematodes recovered 3 weeks after inoculation were probably the same nematodes used as inoculum. Nematodes cultured on K medium have a higher percentage of crenate tails as compared with those cultured in W medium.

The constant occurrence of 30% females with crenate tails in callus-culture populations on W medium, regardless of age of the culture, and the increase of this percentage when nematodes fed on pea roots for several generations, suggest that peas may modify the phenotypical expression of tail type not only as a direct influence of such environment but quite possibly through a selective force. Selection, as pointed out by Mayr (21), is the most important of the factors that induce evolutionary changes by affecting the frequency of genes in populations by a mechanism such as differential reproduction or differential survival. He stated that the selective

TABLE 6. Comparisons of populations of *Pratylenchus penetrans* from various hosts and geographical locations.

Geographic location	Host	No. specimens	No. with crenate-tail tip	Other observations
Canada, Ontario	Celery (*Apium graveolens* L.)	16	5	All specimens with a round spermatheca; lip region more flattened than normal.
England, Rothamsted	Alfalfa callused tissue (*Medicago sativa* L.)	16	2	Very similar to Cornell population.
Japan, Hokkaido	Japanese mint (*Mentha arvensis* v. *piperascens* Malinvaud)	16	4	Esophageal glands wider and dorsal gland longer than usual.
The Netherlands, Horst	Lettuce (*Lactuca sativa* L.)	17	2	One female with an oval spermatheca; one with a fifth lateral line.
Trinidad, Chaguanas	Tobacco (*Nicotiana tabacum* L.)	14	0	Annulation finer than most other populations.
United States				
Connecticut	Alfalfa (*Medicago sativa* L.)	3	0	One female with an oval spermatheca.
Florida No. 1	Leather leaf fern (*Acrostichum aureum* L.)	9	6	Most females with somewhat clavate tails and anteriorly flattened stylet knobs.
Florida No. 2	Leather leaf fern	18	13	Same as for Florida No. 1.
Kentucky	Vetch (*Vicia sativa* L.)	4	1	One female with an oval spermatheca.
New York	Potato (*Solanum tuberosum* L.)	12	5	Some specimens with finer annulation than usual; stylet knobs varied from sloping anteriorly to indented.
North Carolina	Cowpea (*Vigna sinensis* (Torner) Savi	20	4	Some stylet knobs flattened anteriorly. Some oval spermatheca noted.
Washington No. 1	Mint (*Mentha arvensis* L.)	2	0	Characters similar to generic description.
Washington No. 2	Apple (*Malus sylvestris* Mill).	5	4	Except for crenate tails characters similar to genetic description.

response is extraordinarily sensitive to slight environmental changes. Assuming that this is a genetically controlled character, as our unpublished data indicate, it is very likely that peas are selective for crenate tails. The possibility exists that, because of the relative adaptive capability of genotypes, some hosts will be even more selective than peas for females with crenate tails. The presence or absence of striations in the tail terminus should not be used as a main diagnostic character for separating species in the genus *Pratylenchus*.

Host plant also influenced the length of the female gonad and the shape of the median bulb. In 15.4% to 29.8% of the females feeding on pea roots, the gonad extended anteriorly into the region of the esophageal glands, whereas it did not extend that far in females from callus culture. An indication of a similar situation was reported by Loof (19) who observed that, in a population of *P. penetrans* from narcissus, the ovary usually extended past the esophageal glands and sometimes even past the median bulb. Ovary length varied considerably and it appears to be dependent on age and nutritional status of the individual (19, 25). In apparently older individuals, it extends up to the esophageal glands. The use of this character in species diagnosis is not advisable.

Nematodes from peas were longer and wider, whereas their stylets and esophagi were shorter in comparison to those in nematodes from the alfalfa callus inoculum. Esophageal length is independent of body length, and therefore, any ratio involving these two characters is of no taxonomic value. The fact that ratio *b'* has no meaning supports in part Geraert's (11) statement about the invalidity of its similar ratio *b*.

The *V* value, because it was not normally changed by the environment to a considerable extent and had the lowest coefficient of variability, is of good diagnostic value. Stylet length, although having a low coefficient of variability, was shorter in nematodes from peas and other host plants than in those from alfalfa callus tissue. A morphometric character showing a low coefficient of variability, however, can be modified by the environment and, if so, it should be carefully used in diagnosis.

Some morphometric characters extended beyond the range limits given by Sher and Allen (28) and Loof (19). *Pratylenchus subpenetrans* Taylor and Jenkins, 1957; *P. fallax* Seinhorst, 1968; *P. pseudopratensis* Seinhorst, 1968; *P. convallariae* Seinhorst, 1959; *P. vulnus* Sher and Allen, 1953; *P. andinus* Lordello et al., 1961; and *P. sudanensis* Loof and Yassin, 1970 are very similar in morphology to *P. penetrans* (19, 20, 25, 26, 27, 28, 29).

The variability of *P. penetrans* originating from a single gravid female was so extensive that a certain percentage of the individuals in any of the environments tested possessed all of the characters of another *Pratylenchus* species. Individuals possessing all characters of *P. fallax* Seinhorst, 1968; *P. convallariae* Seinhorst, 1959; and *P. pseudopratensis* Seinhorst, 1968 and most characters of *P. sub-penetrans* Taylor & Jenkins, 1957; *P. vulnus* Allen & Jensen, 1951; and *P. sudanensis* Loof & Yassin, 1970 were observed. *P. andinus* Lordello, Zamith & Boock, 1961 is reported to have no males and no spermatheca; yet under certain conditions, a low percentage of males may be present in *P. penetrans,* and because of the fixation technique, the spermatheca may appear to be absent. No indication of the number of specimens examined is given in the description of *P. andinus*. According to Seinhorst (27), *P. fallax* differs from *P. penetrans* only in having a crenate-tail tip. When specimens of *P. fallax* were studied, almost no differences were found in the female-tail tips when they were compared with the tail tips of similar *P. penetrans* females (Fig. 3-C).

It is highly probable that some of the previously mentioned species may be conspecific with *P. penetrans*. However, only a detailed study of the variation present, and possibly the performance of interbreeding tests with *P. penetrans,* will reveal their specific status.

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