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THE RELATION BETWEEN STYLET AND BODY LENGTH IN APHELENCHUS AVENAE WITH OBSERVATIONS ON PARATYLENCHUS NANUS

BY

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The body length and breadth of adult *Aphelenchus avenae* increased or decreased with food supply but stylet length remained unchanged. Correlations between stylet and body length were slight except in newly-moulted specimens and depended on the extent to which body length had changed after moulting. Initial body length of adults depended partly on the duration of feeding in the fourth-stage larvae. Correlations between body length and breadth were greater and varied less with changes in size of the body than did correlations between stylet length and body length (or breadth). In *Paratylenchus nanus*, the relation between stylet and body length in adult females was similar to that in *Aphelenchus avenae* so that there was little dependence of stylet length on body length. Therefore any classification of *Paratylenchus*, which relies on stylet length and body length being correlated would be unsatisfactory as it is partly based on invalid criteria.

Recently taxonomists have used mathematical relations increasingly to decrease variation in the characters used to separate species and have searched for new relations that may be helpful. Geraert (1965) based a classification of the genus *Paratylenchus* on the relation between stylet and body length in females, in which he assumed that longer nematodes had longer stylets so variation in stylet length could be diminished by considering body length as well. Geraert (1968) produced a graph relating stylet and body length in *Rotylenchus goodeyi* but suggested that while there was little relation between these two characters in *R. goodeyi* the relation was still valid in *Paratylenchus*. Yuen (1965) also thought that the "stylet length of L_4 (of *Helicotylenchus vulgaris*) increased with body length".

Yuen's measurements for H. vulgaris suggest that the relation between stylet and body length can be examined in two ways. (1) If growth in length of stylet and body of all stages is considered, then a relation between stylet and body length may be expected and the interruption of growth during moulting and the variation from changes in body length within a stage may be too small to upset the general relation. (2) If individuals within a stage are considered then no such relation between stylet and body length can be expected. Measurements of *Trichodorus christiei* (cf. Bird & Mai, 1968) also suggest separation into two different relations is valid. Stylets of *Paratylenchus projectus* (cf. Rhoades & Lin-

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ford, 1961) and *Xiphinema* sp. (Coomans & De Coninck, 1963) do not lengthen within a stage so that they cannot vary with changes in body length.

This paper presents information on the relation between stylet and body length in adult females of *Aphelenchus avenae* Bastian, 1865 and *Paratylenchus nanus* Cobb, 1923.

MATERIALS AND METHODS

Aphelenchus avenae was cultured on Rhizoctonia solani Kühn, on 1/10 dilution of Czapek-Dox plus yeast extract agar (Ainsworth & Bisby, 1954). Measurements were made on about 20 specimens killed in hot acetic acid (Seinhorst, 1962) and mounted in F.A. 4:10 (Goodey, 1963), from populations treated as follows:

- 1) Unfed during 4th stage at 23°C, transferred to sterile water and killed immediately after 4th moult (Data on the stimulus for moulting will be described elsewhere).
- 2) Fed during 4th stage at 23°C for 12 or 22 hrs, transferred to water and killed immediately after 4th moult.
- 3) Allowed to feed throughout 4th stage, then killed immediately after 4th moult.
- 4) As 3, then fed for 1, 3 or 5 days before killing.

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- 5) As 3, then fed for 5 days and starved in sterile water for 2, 5 or 15 days before killing.
- 6) As 3, but starved in sterile water for 3 or 20 days before killing.

Correlation coefficients (r) were calculated for populations of *Paratylenchus* nanus previously examined (Fisher, 1965).

RESULTS

Changes in length of stylet and body in Aphelenchus avenae

Effect of duration of feeding in the fourth stage on length and breadth of newlymoulted adults

The longer the nematodes fed in the fourth stage, the longer and broader were the bodies of newly-moulted adults and the longer their stylets. Significant values of r were obtained between body length, breadth and stylet length (Table I).

TABLE I

Effect of duration of feeding in the fourth stage on size of newly moulted adults of A. avenae

	Duration of feeding (hrs)			
	0	12	22	
Length (μ)	600 (544-616)	730 (649-774)	780 (734-813)	
Width (µ)	25 (23-30)	32 (26-36)	38 (33-43)	
Stylet (µ)	14 (13-15)	15 (14-17)	15 (14-17)	

S.E. Length \pm 5.23 Width \pm 0.47, Stylet \pm 0.17 Correlation Coefficients:

Length/stylet 0.69***, Length/width 0.92***, Width/stylet 0.64***

Changes in length and breadth of females with and without food

Stylet length did not change during the feeding programme but body length and breadth increased while feeding and decreased while starving (Table II). Body length increased by almost 50% (300μ) of initial adult length during feeding and decreased by almost 30% (200μ) during starvation. Increases and decreases in breadth amounted to 50% of initial adult breadth (Fig. 1). The values of r for stylet length and body length or breadth were small but significant but r for body length and breadth was large.



Fig. 1. Changes in length, breadth and stylets of females of Aphelenchus avenae with and without food.

Changes in length and breadth of females unfed after moulting

Stylet length did not change on starvation but body length and breadth decreased (Table III). Values of r for stylet length and body length or breadth were not significant whereas r for body length and breadth was small but significant.

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TABLE II

Changes in size of females of A. avenae with and without food

	Fed for (days)			Fed 5 days then starved for (days)			
	0	1	3	5	2	5	15
Length (µ) Width (µ) Stylet (µ)	709(669-767) 32(26-33) 15(13-16)	854(754-905) 41(39-46) 15(13-17)	959(885-1010) 47(43-53) 15(13-17)	994(964-1030) 48(46-53) 15(13-17)	907(859-964) 43(36-53) 15(13-17)	857(826-892) 35(30-39) 15(14-17)	804(767-839) 32(26-39) 15(14-17)
S.E. Length	\pm 6.03, Width	\pm 0.66, Stylet	± 0.21.				
Correlation	Coefficient :						

Length/stylet 0.24*, Length/width 0.85***, Width/stylet 0.20*

TABLE III

Changes in size of females of A. avenae after moulting and without feeding

•	Starved for (days)			
	0	3	20	
Length (µ)	715 (662-754)	729 (708-767)	683 (656-741)	
Width (µ)	32 (26-36)	30 (26-33)	26 (23-30)	
Stylet (μ)	15 (14-16)	15 (14-16)	15 (14-16)	

S.E. Length \pm 4.37, Width \pm 0.45, Stylet \pm 0.15.

Correlation Coefficient:

Length/stylet 0.19 N.S., Length/width 0.45***, Width/stylet 0.13 N.S.

TABLE IV

Stylet and body length in populations of Paratylenchus nanus (data from Fisher, 1965)

Environmental	Number	Mean	Mean length	
conditions in soil	measured	Body (µ)	Stylet (μ)	Coefficient
at 15°C	20	376	29.8	0.385 N.S.
at 20°C	20	388	29.1	-0.232 N.S.
at 22°/19°C	20	398	29.2	0.165 N.S.
at 25°C	20	364	26.7	0.327 N.S.
on apricot roots	20	413	30.2	0.280 N.S.
at population density	of			
37,000 per 500 g soil	20	412	30.3	0.416 N.S.
In presence of				
excess males	20	387	29.1	0.450 *

Correlation coefficients of all populations

The values of the correlation coefficient for stylet length and body length $(r = 0.32^{**})$ or breadth $(r = 0.29^{**})$ of all individuals were small but signi-



Fig. 2. Scatter diagram of stylet length and body length of females of populations of Aphelenchus avenae measured immediately after the moult.



Fig. 3. Scatter diagram of stylet length and body length of females of all populations of *Aphelenchus avenae* measured.

ficant whereas r for body length and breadth was large (0.90^{***}) . A comparison of the scatter diagrams of stylet length and body length for all adults measured immediately after the moult (Fig. 2) and for adults from all the different populations measured (Fig. 3), emphasises the effect that post-moult growth has in reducing the correlation between body length and stylet length in adults.

Correlation between stylet length and body length in Paratylenchus nanus

The only significant value of r between stylet length and body length occurred in the population containing excess males (Table IV).

DISCUSSION

The stylet of A. avenae did not change in length after the last moult, whereas body length and breath changed with changes in amount of food eaten. Immediately after the moult, body length (and breadth) were strongly correlated with stylet length but as they changed the correlation became weaker and sometimes statistically insignificant. When all thirteen populations were considered together (which is necessary to determine the relation in a species) a positive correlation was obtained, but this was too small to suggest that it would be of help in defining variation in stylet length.

The large value of r immediately after moulting suggests that body length and stylet length are related during re-organisation that accompanies the shedding of the cuticle. Thereafter changes in body length (while stylet length remains unchanged) destroys this relationship and there is little correlation between stylet length and body length. A small significant positive correlation was obtained when all populations were considered together because the relationship is unduly influenced by extreme values. A strong positive correlation indicates either a limited assessment of variation or that the nematodes were drawn from a uniform environment.

The standard error for stylet length for the populations fed for different lengths of time during the fourth stage, suggests that stylet length is partly determined by the duration of feeding. However, the accuracy with which stylet length can be measured by the methods used is about 1μ and as this was the largest difference recorded (Table 1) it is doubtful whether variation in stylet length could be assessed accurately enough to justify its use in a mathematical relation. The large value of r (for stylet and body length) substantiates the idea that stylet length changed with duration of feeding, but this needs confirmation.

Body length of *A. avenae* increased during feeding by almost 50% and decreased during starvation by 30% and the corresponding changes in breadth were 50% each. Neither change was taken to completion so larger ones could be expected. During the first 2 days when eggs were being laid, starved individuals shortened greatly, but after egg-laying stopped on the third day, shortening was slower (Fig. 1). Towards the end of the starvation period (10 to 15 days), breadth decreased erratically so the nematodes developed constrictions and appeared bulbous. This was visible in live specimens and was not an artefact of fixation. Therefore changes in length and breadth partly reflected changes in stored food and stored food was used preferentially from some body regions.

It is sometimes difficult to relate observations in one genus to those in another. *P. nanus* and *A. avenae* had similar values of r, and Rhoades & Linford (1961) showed that the stylet of adult *Paratylenchus projectus* did not lengthen whereas body length increased considerably, as in *A. avenae*; whether increase in body length is reversible in *Paratylenchus* is unknown. It seems likely that the relationship between body length and stylet length is similar in *Aphelenchus* and *Paratylenchus*. Geraert's (1965) revision of the genus *Paratylenchus* is probably invalid because it is based on the dependance of stylet length on body length within the adult stage. He claimed a strong relation but did not calculate r hence the validity of his argument cannot be assessed. When variation in body length is restricted, a population of *A. avenae* may have a large value for r, so if Geraert's populations had a large value, his estimation of variation was probably too limited for accurate species determination. The limits of his "species" need to be re-examined.

In determining the usefulness of relative measurements for taxonomy, it is necessary to understand how each character develops and the effect of environment on each. Growth in one uniform environment may differ greatly from that in another. To decide whether a relation to define a species is valid, it is necessary to study variations in populations reared under different conditions.

ZUSAMMENFASSUNG

Die Beziehungen zwischen Mundstachellänge und Körperlänge bei Aphelenchus avenae mit Beobachtungen an Paratylenchus nanus

Körperlänge und -breite von adulten Aphelenchus avenae vergrößerten oder verringerten sich in Abhängigkeit von der Nahrungsversorgung, während die Mundstachellänge unverändert blieb. Mit Ausnahme der frischgehäuteten Tieren waren die Beziehungen zwischen Mundstachel- und Körperlänge nur schwach ausgeprägt und hingen davon ab, wie stark sich die Körperlänge durch die Häutung veränderte. Die Anfangsgröße erwachsener Tiere hing zum Teil von der Dauer der Nahrungsaufnahme während des vierten Larvenstadiums ab. Die Beziehungen zwischen Körperlänge und -breite waren enger und variierten weniger mit Veränderungen der Körpergröße als die Beziehungen zwischen Mundstachellänge und Körperlänge oder -breite.

Bei Paratylenchus nanus war die Beziehung zwischen Mundstachellänge und Körperlänge von erwachsenen Weibchen ähnlich wie bei Aphelenchus avenae, so daß nur eine geringe Abhängigkeit der Mundstachellänge von der Körperlänge bestand. Daher ist jede Klassifizierung von Paratylenchus, die von der Annahme ausgeht, daß Mundstachellänge und Körperlänge miteinander gekoppelt sind, unbefriedigend, da sie zum Teil auf ungültigen Kriterien beruht.

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