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Morphometric Evaluation of Hypotriploid and Triploid Populations of *Meloidogyne arenaria*[1]

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Abstract: A morphometric comparison of seven hypotriploid populations with five pooled triploid populations of *Meloidogyne arenaria* was made using standard descriptive statistics, stepwise discriminant analysis (SDA), and cluster analysis. Six morphometric characters of females, 14 of second-stage juveniles (J2), and 18 of males were measured for each population. Useful differentiating characters included: body length in J2; stylet length in females and J2; stylet-knob dimensions in females and males; dorsal esophageal gland orifice distance in all three life stages; esophagus-length ratio in males and J2; excretory pore position in J2; and spicule length in males. SDA and cluster analysis showed that in each life stage, the hypotriploid populations were set off to varying degrees from the triploid populations. In addition, the relationships among populations differed when different life stages were compared. No consistent relationships could be detected among the populations, when morphometric data of the present study and morphological findings of the same populations in a parallel study were considered. Morphometric differences were not sufficient to propose any of the hypotriploid populations as new species.

Key words: cluster analysis, cytological race, enzyme phenotype, host race, hypotriploid, light microscopy, *Meloidogyne arenaria,* morphology, morphometrics, nematode, root-knot nematode, stepwise discriminant analysis (SDA), taxonomy, triploid, variation.

Morphometric variation within and between populations of nematode species has been investigated previously (1–3,5–8,10–16,19). Measurements have been especially helpful in species that are difficult to separate morphologically. In addition to standard descriptive statistics of important morphometric characters, various methods of multivariate analysis, including stepwise discriminant analysis (SDA), canonical variate analysis (CVA), and cluster analysis have been used to compare them and assist in taxonomic evaluation. Such quantitative analyses provide an objective means of assessing the relative differences and similarities among species and populations of species on the basis of a combination of selected morphometric variables and can be used to supplement morphological data.

A statistically based morphometric comparison of all life stages of two field populations each of host races 1 and 2 of *Meloidogyne arenaria* (Neal) Chitwood showed no significant differences between the two races in any of the characters measured (14). However, significant differences were found when means of various characters of females, males, J2, and eggs were compared among the four populations. In a morphometric comparison of two morphologically variant populations of *M. arenaria* with the typical *M. arenaria,* the values of most characters were also significantly different (2).

The present study was undertaken (i) to evaluate the morphometric variation in seven hypotriploid (2n = 40–48 chromosomes) and five pooled triploid (2n = 51–54 chromosomes) populations of *M. arenaria* using SDA and cluster analysis, in addition to standard descriptive statistics, and (ii) to determine if the morphometric data can be correlated with previous morphological findings (17).

MATERIALS AND METHODS

Morphometrics of seven hypotriploid populations previously used to evaluate

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morphology (17) were compared with each other and with five pooled populations, representing the typical triploid *M. arenaria* (2) (Table 1). The latter populations were pooled because they were very similar to each other.

The hypotriploid populations were maintained by periodic subculturing on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse at 22–28 C. Females and egg masses were hand picked from infected roots. Males and second-stage juveniles (J2) were obtained after incubation of infected roots or egg masses in moist chambers at room temperature. Females were fixed in 2% formalin, and their anterior portions, including the esophageal region, were severed with an eye knife and mounted in 2% formalin. Males and J2 were fixed in hot (70–80 C) TAF (7 ml 40% formaldehyde, 2 ml triethanolamine, 91 ml distilled water) and mounted on microscopic slides in the same fixative. Measurements were completed within 24–30 hours of slide preparation.

Six morphometric characters were selected for study in females, 14 in J2, and 18 in males (Tables 2–4). Twenty-five or 30 females, 30 J2, and 30 males were measured from each population. Standard descriptive statistics were calculated using the general linear models procedure with the Waller-Duncan k-ratio *t*-test, in SAS version 5 (18). Morphometric data for the pooled triploid populations were taken from previously obtained measurements (2) and were analyzed similarly.

The BMDP 2M cluster analysis of cases (BMDP Statistical Software, Los Angeles, CA) was employed to group females, J2, and males of all populations into clusters on the basis of their degree of similarity with respect to the characters used. Morphometric data of a total of 325 females, 360 J2, and 330 males were evaluated and illustrated in dendrograms. The BMDP 7M stepwise discriminant analysis (BMDP Statistical Software) also was performed to classify the females, J2, and males of all populations into groups on the basis of the same morphometric characters used for the cluster analysis. Two-dimensional canonical plots were drawn for each life stage recording the populations means and the distribution of all individuals of a given population (Figs. 1–3).

Results

Standard descriptive statistics

Females

The six morphometric characters evaluated can be of limited use in differentiating some of the populations (Table 2).

Table 1. Populations of the *Meloidogyne arenaria* species complex evaluated for morphometric analysis.

Population number	Population designation and origin	Chromosome number (2n)	Host race†	Enzyme phenotype‡	
				Est	Mdh
		Hypotriploid populations			
1	E255—Ecuador	40–42	1	A2	N1
2	E445—El Salvador	41–42	2	M3–F1	N1
3	E467—Korea	44–46	1	A2	N1
4	E551—Ivory Coast	42	1	S1–M1	N3
5	E553—Ivory Coast	40–44	1	A2	N1
6	E927—West Samoa	46–48	1	A2	N3
7	E1033—China	41	1	A2	N1
		Triploid populations			
	54—Virginia	51–53	1	A2	N3
	56—North Carolina	52–53	2	A2	N3
8§	256—Colombia	53	1	A2	N3
	413—Nigeria	53–54	1	A2	N3
	480—North Carolina	54	2	A2	N3

† 1 = Reproduces on peanut; 2 = does not reproduce on peanut.
‡ Phenotype designation as in reference 4; Est = esterase; Mdh = malate dehydrogenase.
§ Population 8 represents five triploid populations pooled.

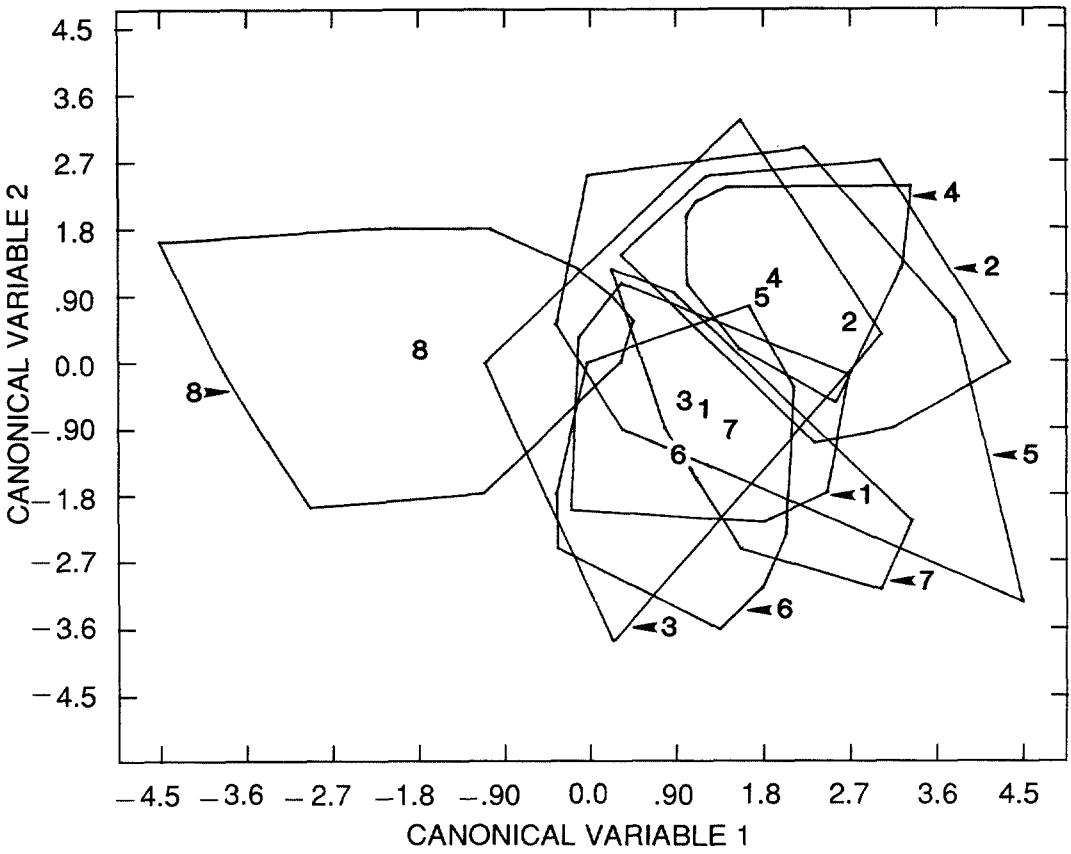

FIG. 1. Canonical plots of females of eight populations of *Meloidogyne arenaria,* illustrating morphometric limits for each population (number + arrow) by using maximum convex polygonals; location of population mean (canonical centroid) indicated by number within polygonal.

Stylet: Stylet length overlaps between several of the hypotriploid and the pooled triploid populations and is not useful in differentiating the populations. The hypotriploid populations 1 and 7 have the longest stylets, whereas populations 4, 5, and the triploid populations have the shortest. Stylet-knob size is a more characteristic feature. All hypotriploid populations can be distinguished from the triploids by their smaller stylet-knob height and consequently larger knob width/height ratio, whereas stylet knob width is similar to that of the triploid populations. The knobs of the hypotriploid populations are at least twice as wide than high, with population 5 having proportionally the widest knobs (largest width/height ratio).

Dorsal esophageal gland orifice (DGO): The DGO distance can be used to distinguish population 2 with the shortest distance from all other populations. Also, population 1 and the triploid populations can be separated from the rest by their longest DGO distance.

Excretory pore position: The distance of the excretory pore to the head end varies much within and among populations and is the least useful differentiating character for females. Population 6, on the average, has the most posteriorly situated excretory pore, whereas populations 2 and 5 have the pore closest to the head end. Populations 1 and 7 are intermediate with respect to excretory pore location and similar to the triploid populations.

Except for the slightly longer stylet and different stylet-knob dimensions, the hypotriploid populations are similar to the triploid populations. Among the hypotriploids, population 4 is characterized by the smallest stylet knobs and population 2 by

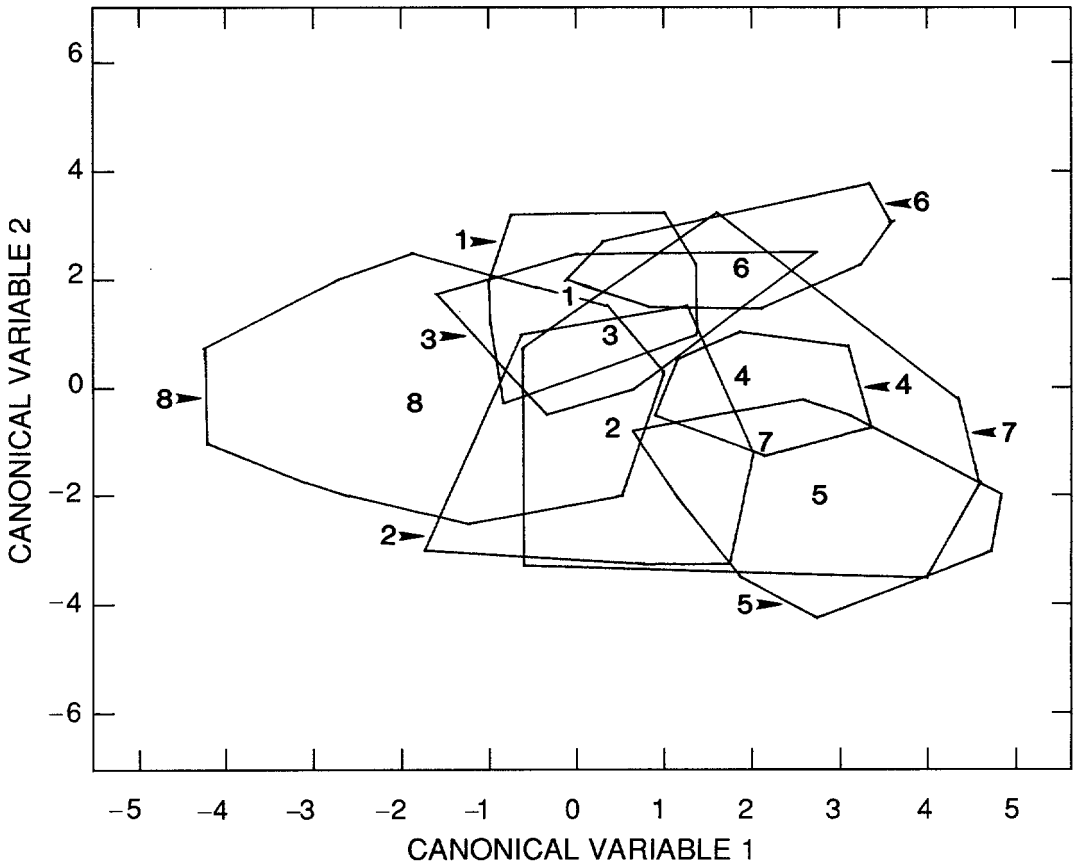

FIG. 2. Canonical plots of J2 of eight populations of *Meloidogyne arenaria*, illustrating morphometric limits for each population (number + arrow) by using maximum convex polygonals; location of population mean (canonical centroid) indicated by number within polygonal.

the shortest DGO and excretory pore distances.

Second-stage juveniles

Among 14 morphometric characters evaluated, only 10 were found to be useful in differentiating some of the populations (Table 3).

Body length: J2 body length overlaps among the hypotriploid and triploid populations and cannot be used as a reliable differentiating character. On the average, however, J2 of hypotriploid populations are shorter and stouter (i.e., with smaller a ratio) than those of the triploid populations. Populations 4, 5, and 7 with the smallest J2 can be distinguished from the triploid populations on the basis of body length.

Stylet: As in the females, J2 stylet length overlaps between the hypotriploid and triploid populations. The longest stylets are present in populations 1, 3, and 6 of the hypotriploids. The triploids have only slightly shorter stylets. Population 5 has the shortest stylets of all populations. Similar trends also are observed among the populations, when the distance between stylet base and head end is measured instead of actual stylet length. Stylet base to head end dimensions may be preferable, because they are easier to measure without errors.

Dorsal esophageal gland orifice (DGO): The DGO distance is similar in most hypotriploid and the triploid populations. Only population 4, with the shortest distance, and population 5, with the longest, can be distinguished by this character.

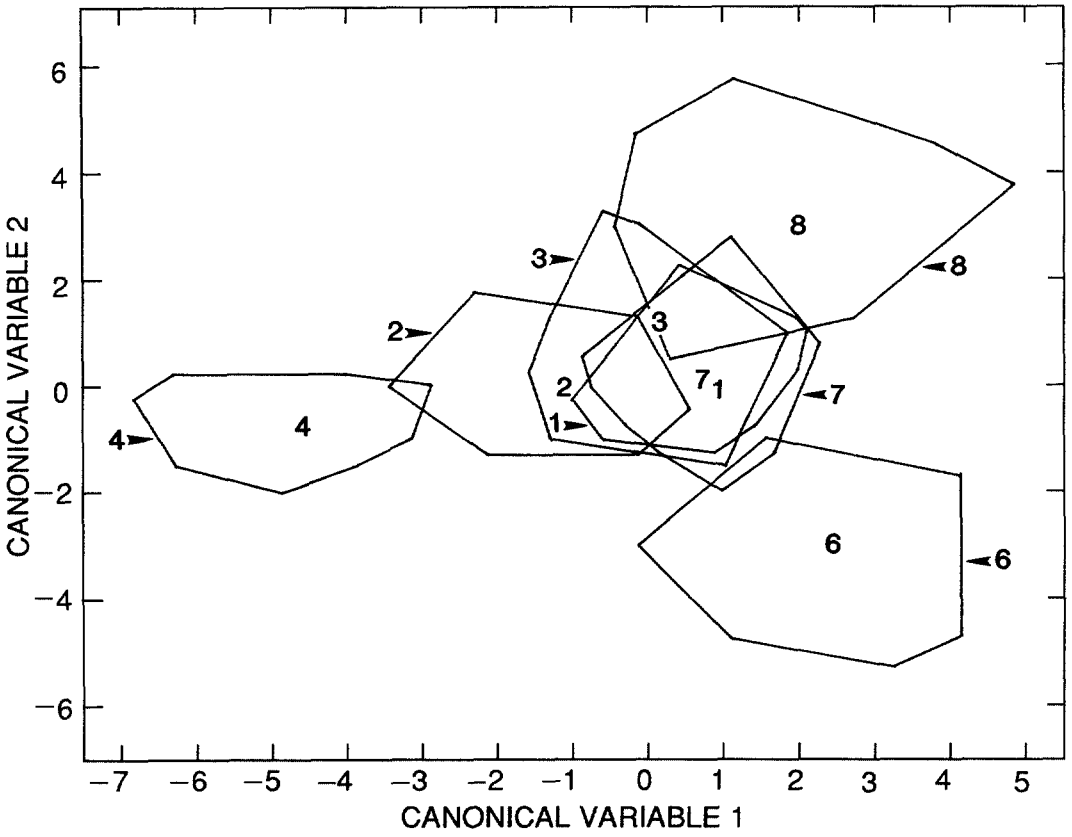

FIG. 3. Canonical plots of males of seven populations of *Meloidogyne arenaria,* illustrating morphometric limits for each population (number + arrow) by using maximum convex polygonals; location of population mean (canonical centroid) indicated by number within polygonal.

Body length/head end to metacorpus valve: The esophagus-length ratio is significantly different among all eight populations and is a useful distinguishing character. Population 6 has the lowest value, corresponding to the longest esophagus, whereas the triploids have the highest value (i.e., the shortest esophagus).

Excretory pore percentage: The excretory pore position is a good differentiating character in J2. The hypotriploid populations 4 and 5 have the excretory pore situated further posteriad than all other populations, including the triploids, which have the most anteriorly located excretory pore.

Tail: Tail length overlaps among all populations. The c ratio, in general, is similar and ranges from 8.0–9.0 among all populations, indicating a positive correlation between tail length and body length. The body width at the anus is smallest in populations 4, 5, 6, and 7, which have the shorter tails, whereas population 2 and the triploids have larger anal widths. The d ratio (tail length/body width at anus) is smallest in population 5.

In general, population 1 among the hypotriploids resembles most closely the triploid populations in the dimensions of the majority of the characters. It has the longest stylet among all populations. Populations 4, 5, and 7 that have J2 shorter than 400 μm have smaller values for six of the characters measured. Population 5, with the shortest J2, has overall the smallest mean values for seven characters and is characterized by the shortest stylet and tail and longest DGO distance. In contrast, the pooled triploid populations exhibit the

TABLE 2. Morphometric comparison of females of hypotriploid and triploid populations of *Meloidogyne arenaria*.

Character	Hypotriploid populations (N = 25)							Triploid populations (pooled; N = 150)
	1	2	3	4	5	6	7	8
Stylet length	16.3 ± 0.15 a (14.8–17.8)	15.8 ± 0.14 b (14.1–17.0)	15.9 ± 0.18 b (14.1–17.4)	15.2 ± 0.17 c (13.6–16.4)	15.2 ± 0.30 c (13.4–20.2)	16.1 ± 0.16 ab (14.8–17.1)	16.3 ± 0.13 a (14.8–17.8)	15.1 ± 0.05 c (13.4–16.7)
Stylet-knob height	2.3 ± 0.04 c (1.9–2.6)	2.1 ± 0.03 d (1.9–2.4)	2.3 ± 0.04 bc (1.9–2.6)	2.1 ± 0.04 d (1.7–2.5)	2.1 ± 0.03 d (1.8–2.5)	2.4 ± 0.03 b (2.1–2.6)	2.3 ± 0.04 c (1.7–2.5)	2.8 ± 0.02 a (2.1–3.8)
Stylet-knob width	4.5 ± 0.05 c (4.1–5.0)	4.5 ± 0.07 c (3.7–5.2)	4.6 ± 0.08 bc (4.1–5.5)	4.2 ± 0.08 d (3.3–4.8)	4.7 ± 0.07 bc (4.1–5.2)	4.9 ± 0.06 a (4.4–5.6)	4.8 ± 0.06 ab (4.0–5.3)	4.7 ± 0.03 bc (3.8–5.5)
DGO distance	4.8 ± 0.21 a (3.1–8.2)	3.1 ± 0.11 d (2.2–4.4)	4.4 ± 0.19 b (2.8–6.7)	3.9 ± 0.19 c (2.7–6.7)	3.9 ± 0.16 c (2.6–6.1)	4.1 ± 0.12 bc (3.3–5.2)	4.2 ± 0.12 bc (3.0–5.6)	4.8 ± 0.06 a (3.1–6.6)
Excretory pore to head end	43.8 ± 2.67 bc (21.1–74.0)	30.5 ± 1.90 d (11.1–51.8)	46.1 ± 2.29 b (18.5–79.9)	40.1 ± 2.09 c (22.2–62.9)	30.1 ± 2.80 d (11.8–60.7)	52.2 ± 2.64 a (22.2–74.0)	43.8 ± 2.78 bc (24.9–74.0)	42.2 ± 0.94 bc (17.8–80.1)
Stylet knob width/height	2.0 ± 0.04 d (1.7–2.4)	2.2 ± 0.05 ab (1.7–2.7)	2.0 ± 0.04 cd (1.7–2.5)	2.0 ± 0.05 cd (1.7–2.5)	2.3 ± 0.04 a (1.9–2.8)	2.1 ± 0.04 cd (1.7–2.5)	2.1 ± 0.05 bc (1.8–2.9)	1.7 ± 0.02 e (1.3–2.4)

All linear measurements in µm.

Values are means ± SE (range). Means followed by the same letter within a row are not different according to the Waller-Duncan k-ratio *t*-test (k-ratio = 100).

TABLE 3. Morphometric comparison of second-stage juveniles of hypotriploid and triploid populations of *Meloidogyne arenaria*.

Character	Hypotriploid populations (N = 30)							Triploid populations (pooled; N = 150)
	1	2	3	4	5	6	7	8
Body length	452.4 ± 4.07 b (400.0–489.6)	411.4 ± 3.74 c (360.0–448.0)	439.3 ± 5.04 b (400.0–489.6)	374.0 ± 2.26 d (352.0–393.6)	361.0 ± 3.5 d (304.0–393.6)	401.7 ± 3.95 c (345.6–438.4)	373.8 ± 8.13 d (320.0–467.2)	503.6 ± 4.26 a (391.6–605.2)
Greatest body width	14.6 ± 0.09 bc (13.8–15.9)	14.8 ± 0.14 bc (13.3–16.4)	15.0 ± 0.09 ab (14.2–16.3)	14.6 ± 0.05 cd (13.8–14.8)	14.2 ± 0.12 d (12.6–15.7)	14.6 ± 0.08 cd (13.8–15.5)	14.6 ± 0.07 cd (13.3–15.2)	15.3 ± 0.09 a (12.8–17.8)
Body width at anus	10.6 ± 0.07 cd (9.6–11.3)	11.1 ± 0.09 a (10.0–12.0)	10.8 ± 0.10 bc (9.6–11.8)	10.5 ± 0.08 de (9.6–11.5)	10.3 ± 0.07 ef (9.6–11.1)	10.2 ± 0.07 f (9.6–11.0)	10.3 ± 0.07 f (9.7–11.1)	10.9 ± 0.05 ab (9.7–12.8)
Stylet length	11.4 ± 0.05 a (10.8–11.8)	10.7 ± 0.08 e (9.7–12.0)	11.2 ± 0.08 bc (10.4–11.9)	10.8 ± 0.04 d (10.4–11.2)	10.6 ± 0.07 e (9.8–11.7)	11.3 ± 0.06 ab (10.7–12.1)	10.7 ± 0.09 e (9.8–11.5)	11.1 ± 0.03 c (10.1–11.9)
Stylet base to head end	15.0 ± 0.06 ab (14.2–15.7)	14.0 ± 0.08 d (13.0–14.8)	15.1 ± 0.07 a (14.2–15.9)	14.3 ± 0.04 c (14.1–14.8)	13.7 ± 0.06 e (13.3–14.3)	15.0 ± 0.05 ab (14.8–15.6)	14.0 ± 0.11 d (13.2–15.5)	14.8 ± 0.05 b (13.4–16.2)
DGO distance	3.4 ± 0.06 c (2.8–4.2)	3.3 ± 0.07 c (2.6–4.1)	3.5 ± 0.06 c (3.0–4.1)	3.1 ± 0.03 d (2.8–3.6)	3.9 ± 0.07 a (3.3–5.3)	3.5 ± 0.05 c (2.8–4.1)	3.7 ± 0.07 b (2.8–4.4)	3.7 ± 0.04 b (2.8–4.7)
Head end to metacorpus valve	60.3 ± 0.35 a (56.5–64.6)	55.9 ± 0.37 c (51.8–59.2)	58.0 ± 0.55 b (51.8–63.9)	52.3 ± 0.27 d (49.2–55.8)	52.0 ± 0.48 d (45.9–60.0)	58.5 ± 0.41 b (54.0–62.0)	52.8 ± 0.74 d (46.6–63.7)	60.9 ± 0.43 a (49.4–71.2)
Excretory pore to head end	86.7 ± 0.57 b (79.2–91.0)	81.4 ± 0.53 c (74.7–90.3)	86.9 ± 0.76 b (76.2–94.2)	80.5 ± 0.51 cd (75.1–86.6)	77.7 ± 0.64 e (70.4–85.5)	82.6 ± 0.58 c (75.0–90.7)	78.5 ± 1.11 de (71.0–96.2)	89.8 ± 0.56 a (75.0–105.2)
Tail length	54.1 ± 0.48 ab (48.1–60.3)	47.2 ± 0.54 c (42.6–54.4)	52.0 ± 0.60 b (46.6–59.2)	46.9 ± 0.44 c (42.9–51.8)	40.5 ± 0.59 d (31.8–46.9)	48.7 ± 0.41 c (44.4–55.4)	42.6 ± 1.11 d (33.3–55.9)	56.0 ± 0.53 a (43.6–69.4)
a	30.9 ± 0.31 b (27.6–34.1)	27.8 ± 0.28 d (24.1–30.6)	29.4 ± 0.40 c (24.6–33.1)	25.6 ± 0.15 e (24.0–27.0)	25.4 ± 0.25 e (22.9–28.5)	27.6 ± 0.27 d (22.5–29.6)	25.6 ± 0.55 e (21.6–32.9)	33.1 ± 0.29 a (22.4–40.5)
Body length/ head end to metacorpus valve	7.5 ± 0.07 bc (6.9–8.2)	7.4 ± 0.06 c (6.9–8.1)	7.6 ± 0.06 b (6.8–8.3)	7.1 ± 0.04 d (6.5–7.5)	7.0 ± 0.07 ef (6.3–8.0)	6.9 ± 0.06 f (6.1–7.4)	7.1 ± 0.09 de (6.2–8.0)	8.3 ± 0.04 a (7.3–9.6)
c	8.4 ± 0.08 c (7.6–9.4)	8.7 ± 0.09 b (7.9–9.7)	8.5 ± 0.07 c (7.9–9.3)	8.0 ± 0.06 d (7.4–8.6)	9.0 ± 0.11 ab (7.7–10.6)	8.3 ± 0.09 c (6.9–9.0)	8.8 ± 0.15 ab (7.2–11.1)	9.0 ± 0.05 a (7.5–10.9)
d	5.1 ± 0.04 a (4.6–5.4)	4.3 ± 0.05 cd (3.6–4.7)	4.8 ± 0.06 b (4.2–5.5)	4.5 ± 0.05 c (4.0–5.1)	3.9 ± 0.06 e (3.2–4.6)	4.8 ± 0.05 b (4.1–5.3)	4.1 ± 0.10 d (3.4–5.5)	5.1 ± 0.05 a (3.8–6.6)
Excretory pore %	19.2 ± 0.10 e (18.4–20.7)	19.8 ± 0.15 d (17.8–21.5)	19.8 ± 0.17 d (18.1–21.7)	21.5 ± 0.13 a (20.3–23.7)	21.6 ± 0.16 a (19.3–23.2)	20.6 ± 0.16 c (18.0–22.3)	21.1 ± 0.25 b (17.8–23.4)	17.9 ± 0.07 f (15.8–21.8)

All linear measurements in μm.
Values are means ± SE (range). Means followed by the same letter within a row are not different according to the Waller-Duncan k-ratio t-test (k-ratio = 100).

largest values for nine characters but have the shortest excretory pore position among all populations.

Males

Twelve of the 18 characters evaluated were helpful in distinguishing some of the populations (Table 4). Population 5 produced only a few males, all of which were small and were not measured.

Body length: In general, body length in males of *Meloidogyne* species shows extensive intraspecific variation due to the varying environmental conditions existing during development. On the average, the hypotriploid populations of *M. arenaria* have shorter males as compared to the pooled triploid populations. Population 2 has the shortest males among all populations. Population 7 has the stoutest males (smallest a ratio), whereas population 1 and the triploids have the most slender males.

Stylet: Stylet length and knob width overlap between hypotriploid and triploid populations and are not useful differentiating features. Stylet-knob height, however, is significantly different between the two ploidy groups. As in the females, all hypotriploids have lower stylet knobs and consequently larger knob-width/height ratios than the triploids. Among all populations, population 4 has the lowest knobs and population 6, on the average, the widest knobs.

Dorsal esophageal gland orifice (DGO): The DGO distance is the best differentiating character in males. It is significantly different among all populations, except for hypotriploid population 1 and the triploid populations, which have very similar DGO distances. Population 4 has the shortest distance. Its DGO range does not overlap with that of population 1 and the triploids; thus population 4 can be separated from these populations by DGO distance.

Body length/head end to metacorpus valve: The hypotriploid and triploid populations overlap in esophagus-length ratio, although this is a good character for distinguishing some hypotriploid populations. Populations 4 and 6, for example, can be separated by their highest (shortest esophagus) and lowest (longest esophagus) ratio, respectively. Population 1 is similar to the triploids in this ratio.

Spicule and gubernaculum length: Spicule length overlaps between the hypotriploid and triploid populations. Among the hypotriploids, however, spicule length is the second best differentiating character after DGO distance. Population 6 has the longest spicules and can be separated from all other hypotriploids by this character. The same is true for population 2, which has the shortest spicules. Similar relationships are present in gubernaculum length.

Among the hypotriploids, in general, population 2 has the shortest males and is characterized by the shortest stylet, tail, spicule, and gubernaculum lengths. Population 4 has the smallest stylet knobs (smallest height and width) and shortest DGO distance among all populations. It also has the shortest esophagus as compared to body length (largest ratio). Population 6 is distinguished among all populations by its longest stylet with widest knobs, longest spicules and gubernaculum, and proportionally longest esophagus. The triploids have the largest means for body length, knob height, esophagus length, excretory pore distance, and a ratio.

Stepwise discriminant analysis

Females

The stepwise discriminant analysis (SDA) entered five of the six morphometric characters of females into the discriminant function in the following sequence, in decreasing significance: stylet-knob height (canonical correlation, CC = 0.3189), stylet length (CC = 0.2362), DGO distance (CC = 0.1802), stylet-knob width (CC = 0.1504), and excretory-pore distance from head end (CC = 0.1268). These were the canonical variables that best separated the populations. Stylet-knob height adds more to the separation of the populations than any other of the five characters considered, whereas excretory-pore distance adds the least. These findings confirm the

TABLE 4. Morphometric comparison of males of hypotriploid and triploid populations of *Meloidogyne arenaria*.

Character	Hypotriploid populations (N = 30)						Triploid populations (pooled; N = 150)
	1†	2	3	4	6	7	8
Body length	1,598.1 ± 28.11 b (1,215.0–1,903.5)	1,295.6 ± 24.54 d (1,012.5–1,539.0)	1,466.6 ± 24.39 c (1,215.0–1,903.5)	1,479.9 ± 26.88 c (1,255.5–1,782.0)	1,504.4 ± 17.33 bc (1,239.3–1,701.0)	1,446.4 ± 25.56 c (1,134.0–1,644.3)	1,720.2 ± 23.45 a (979.0–2,278.5)
Greatest body width	34.1 ± 0.67 d (25.4–45.7)	32.5 ± 0.63 d (25.9–39.7)	39.4 ± 0.69 ab (31.1–47.1)	38.2 ± 0.94 b (29.6–51.8)	38.9 ± 0.58 ab (33.3–44.4)	40.5 ± 0.91 a (33.3–52.5)	36.4 ± 0.39 c (27.4–47.5)
Body width at stylet knobs	18.5 ± 0.14 d (17.0–20.0)	18.9 ± 0.15 d (17.0–21.0)	20.6 ± 0.18 a (18.3–22.2)	20.3 ± 0.18 a (18.0–22.2)	19.1 ± 0.13 bc (17.8–20.7)	19.6 ± 0.21 b (17.1–21.8)	19.1 ± 0.13 bc (15.4–22.3)
Body width at excretory pore	27.9 ± 0.29 c (23.2–29.6)	26.8 ± 0.36 c (22.2–31.5)	30.7 ± 0.38 a (26.7–36.0)	30.4 ± 0.43 a (25.6–37.0)	30.5 ± 0.38 a (26.2–35.2)	30.1 ± 0.41 ab (26.2–36.9)	29.1 ± 0.26 b (21.6–39.7)
Stylet length	22.2 ± 0.11 c (20.6–23.5)	21.5 ± 0.13 d (19.6–22.2)	22.6 ± 0.13 bc (21.6–24.9)	21.5 ± 0.12 d (20.1–22.8)	23.7 ± 0.13 a (22.2–24.8)	22.7 ± 0.15 b (21.2–24.4)	22.7 ± 0.12 b (19.8–28.4)
Stylet-knob height	2.7 ± 0.04 d (2.2–3.1)	2.9 ± 0.03 c (2.4–3.3)	3.0 ± 0.04 b (2.6–3.4)	2.3 ± 0.04 e (1.9–2.7)	3.0 ± 0.05 b (2.6–3.4)	3.0 ± 0.05 b (2.4–3.7)	3.5 ± 0.04 a (2.8–4.6)
Stylet-knob width	5.1 ± 0.05 bc (4.4–5.7)	5.0 ± 0.05 bc (4.4–5.7)	4.9 ± 0.07 c (4.3–5.9)	4.4 ± 0.06 d (3.8–5.0)	5.3 ± 0.06 a (4.8–5.9)	5.2 ± 0.06 ab (4.4–5.7)	4.9 ± 0.04 c (3.8–5.9)
DGO distance	5.5 ± 0.17 a (3.6–7.4)	3.5 ± 0.08 e (2.7–4.1)	4.9 ± 0.13 b (3.4–5.8)	2.8 ± 0.06 f (2.2–3.5)	4.0 ± 0.12 d (2.8–5.2)	4.5 ± 0.15 c (2.8–6.1)	5.8 ± 0.08 a (3.7–7.9)
Head end to metacorpus valve	97.6 ± 1.23 c (74.0–114.7)	83.3 ± 1.14 e (70.3–99.2)	93.7 ± 1.00 d (83.0–107.0)	77.5 ± 0.92 f (66.6–88.1)	101.3 ± 1.13 b (87.3–114.7)	95.6 ± 0.89 cd (85.1–105.5)	105.3 ± 0.91 a (82.8–121.3)
Excretory pore to head end	161.0 ± 1.75 c (144.3 ± 185.0)	148.0 ± 2.86 d (101.8 ± 181.3)	162.4 ± 2.58 bc (127.4–202.4)	145.0 ± 1.83 d (123.6–173.2)	167.9 ± 2.07 ab (148.0–196.1)	163.8 ± 2.09 bc (139.1–192.4)	172.8 ± 1.60 a (119.3–213.2)
Tail length	13.0 ± 0.33 bc (10.2–18.9)	12.3 ± 0.27 c (8.9–14.8)	13.7 ± 0.35 ab (10.5–18.5)	14.0 ± 0.21 a (10.0–15.5)	14.0 ± 0.38 a (10.4–19.1)	14.0 ± 0.33 a (7.4–17.0)	13.5 ± 0.13 ab (10.7–16.7)
Spicule length	33.6 ± 0.28 b (29.6–37.0)	29.3 ± 0.33 e (25.8–32.6)	31.6 ± 0.37 c (26.7–34.6)	30.3 ± 0.30 d (27.8–34.0)	37.2 ± 0.45 a (31.5–42.2)	31.2 ± 0.25 c (29.6–34.4)	31.7 ± 0.24 c (26.7–39.4)
Gabernaculum	8.7 ± 0.16 bc (7.4–10.5)	7.5 ± 0.13 d (6.3–9.0)	9.0 ± 0.12 ab (7.9–10.6)	7.7 ± 0.14 d (6.9–10.1)	9.4 ± 0.18 a (7.4–11.8)	8.7 ± 0.11 c (7.4–10.4)	9.0 ± 0.17 bc (7.3–10.4)
a	47.2 ± 0.88 a (37.3–56.5)	39.9 ± 0.55 b (34.2–46.0)	37.6 ± 1.02 bc (27.4–54.6)	39.0 ± 0.58 b (32.0–44.3)	38.9 ± 0.63 b (30.4–44.0)	36.1 ± 0.80 c (28.1–44.0)	48.1 ± 0.78 a (30.0–63.7)
c	124.9 ± 3.55 a (94.4–167.8)	107.2 ± 3.32 b (72.0–151.1)	109.5 ± 3.51 b (81.9–162.8)	106.5 ± 2.41 b (84.8–140.2)	109.0 ± 2.63 b (84.9–152.5)	105.3 ± 3.41 b (82.1–175.1)	126.6 ± 2.36 a (87.9–190.4)
Body length/ head end to metacorpus valve	16.4 ± 0.34 b (13.3–21.9)	15.6 ± 0.27 cd (11.9–19.5)	15.7 ± 0.21 c (13.1–18.7)	19.1 ± 0.30 a (15.8–23.2)	14.9 ± 0.23 d (12.6–17.6)	15.1 ± 0.21 cd (12.8–17.0)	16.5 ± 0.19 b (11.6–20.4)
Stylet knob width/ height	1.9 ± 0.03 a (1.6–2.2)	1.8 ± 0.02 b (1.5–2.1)	1.6 ± 0.03 c (1.3–2.0)	1.9 ± 0.03 a (1.6–2.3)	1.8 ± 0.04 b (1.5–2.2)	1.8 ± 0.03 b (1.5–2.0)	1.5 ± 0.01 d (1.1–1.8)
Excretory pore %	10.1 ± 0.16 b (8.7–12.5)	11.5 ± 0.28 a (6.9–15.0)	11.1 ± 0.22 a (8.9–15.1)	9.9 ± 0.15 b (7.7–11.6)	11.2 ± 0.16 a (9.8–12.8)	11.4 ± 0.20 a (9.6–14.8)	10.2 ± 0.09 b (7.8–13.0)

All linear measurements in µm.
Values are means ± SE (range). Means followed by the same letter within a row are not different according to the Waller-Duncan k-ratio t-test (k-ratio = 100).
† Population 5 produced only a few dwarf males, measurements not included.

results of the standard descriptive statistics (Table 2). The parameters chosen by the SDA include those most frequently used by taxonomists as good separating characters in the differentiation of *Meloidogyne* females.

The classification matrix (Table 5) resulting from the SDA of 325 females based on these five characters shows that 92.7% of the individuals of the triploid populations (population 8) were identified correctly, whereas a much smaller percentage of females could be identified correctly in the hypotriploid populations (populations 1–7). Population 3 had only 8.0% correctly identified females, which means that most of the females of this population were incorrectly allocated to other populations.

Furthermore, the SDA determined the first two canonical variables that were used as the two axes on which the population means (centroids) and all the individuals were plotted (Fig. 1). Population means show that the pooled triploid populations are quite distinct from the hypotriploids. Graphic representation using maximum convex polygonals encompassing the widest outlying members of each population and connecting them by straight lines shows a great amount of overlap among the individuals of the hypotriploid populations. Only populations 4 and 7 can be separated by this analysis. The triploids are clearly set off to the left of the canonical plot and are not overlapped by populations 2 and 4. A small degree of overlap, however, occurs with hypotriploids 1, 6, and 7 and more overlap with 3 and 5. Pop-

TABLE 5. Classification matrices resulting from SDA of 325 females, 360 J2, and 330 males of *Meloidogyne arenaria* based on 5, 7, and 12 morphometric characters, respectively.

Population	Percentage of correct identifications	Number of nematodes allocated into populations								
		1	2	3	4	5†	6	7	8	Total
					Females					
1	52.0	13	2	3	0	2	0	4	1	25
2	72.0	0	18	0	3	3	0	1	0	25
3	8.0	7	1	2	2	1	6	4	2	25
4	56.0	3	1	0	14	5	0	1	1	25
5	52.0	1	3	0	5	13	0	2	1	25
6	56.0	3	1	4	0	3	14	0	0	25
7	32.0	1	0	2	1	4	8	8	1	25
8	92.7	2	0	8	0	0	1	0	139	150
Total	68.0	30	26	19	25	31	29	20	145	325
					J2					
1	73.3	22	0	3	0	0	4	0	1	30
2	76.7	1	23	1	1	3	0	1	0	30
3	53.3	5	1	16	2	0	2	1	3	30
4	83.3	0	0	2	25	0	0	3	0	30
5	66.7	0	2	0	0	20	0	8	0	30
6	86.7	2	0	2	0	0	26	0	0	30
7	16.7	2	0	2	4	14	2	5	1	30
8	84.7	2	10	9	2	0	0	0	127	150
Total	73.3	34	36	35	34	37	34	18	132	360
					Males					
1	93.3	28	0	0	0		0	2	0	30
2	100.0	0	30	0	0		0	0	0	30
3	73.3	3	0	22	0		0	4	1	30
4	100.0	0	0	0	30		0	0	0	30
6	90.0	0	0	0	0		27	3	0	30
7	70.0	1	2	5	0		1	21	0	30
8	83.3	0	0	2	0		0	2	20	150
Total	87.3	32	32	29	30		28	32	21	330

† Population 5 produced only a few dwarf males; measurements are not included.

ulations 3, 5, and the triploids exhibit a high degree of variability as indicated by large-size polygonals, whereas populations 1 and 4 cover smaller polygonals and thus show lower morphometric variability.

Second-stage juveniles

The SDA chose 7 out of 14 morphometric characters to separate the populations on the basis of canonical correlation. These included, in decreasing significance, distance of excretory pore to head end expressed as a percentage of body length (CC = 0.2746), stylet base to head end (CC = 0.1726), DGO distance (CC = 0.1264), body length/head end to metacorpus valve (CC = 0.0915), tail length (CC = 0.0786), stylet length (CC = 0.0683), and body width at anus (CC = 0.0617). The parameters stylet base to head end, DGO distance, and tail length have been found in general by taxonomists to have good taxonomic value in J2. However, the position of the excretory pore, selected by the SDA as the best variable, has not been recognized extensively as an important distinguishing character of J2.

The classification matrix (Table 5) resulting from the SDA of 360 J2 based on the seven characters chosen shows that 70% or more of the J2 were correctly identified in populations 1, 2, 4, 6, and 8. In populations 3, 5, and 7, only 53.3, 66.7, and 16.7%, respectively, could be placed correctly.

Canonical plots using maximum convex polygonals for all J2 of each population show a great amount of overlap of most hypotriploid populations, although several pairs of populations do not overlap (Fig. 2). As in the females, the triploids are clearly set off to the left of the hypotriploids. No overlap exists between the triploids and populations 4 and 6, and a very small overlap occurs with 5. A distinct overlap occurs with populations 1, 2, 3, and 7. Populations 2, 5, 7, and the triploids have the largest polygonals and thus exhibit the highest degree of variability, whereas population 4, with the smallest polygonal, shows the lowest morphometric variability of all populations.

Males

The characters of the SDA selected as the best variables for separating the males of the seven populations included the following 12 out of 18 in decreasing canonical correlation: DGO distance (CC = 0.2945), spicule length (CC = 0.1053), stylet-knob height (CC = 0.0546), body length/head end to metacorpus valve (CC = 0.0300), body width at stylet knobs (CC = 0.0190), stylet-knob width (CC = 0.0132), stylet length (CC = 0.0100), stylet-knob width/height (CC = 0.0080), greatest body width (CC = 0.0065), ratio a (CC = 0.0056), body length (CC = 0.0047), and gubernaculum length (CC = 0.0041). Morphometric features associated with stylet, DGO, spicule, and gubernaculum of males have been shown, generally, to be helpful in *Meloidogyne* taxonomy, whereas length and width measurements in males have been less useful.

The classification matrix, based on the SDA of these 12 variables for a total of 330 males, shows a high degree of correct identifications ranging from 70–100% (Table 5). All males of populations 2 and 4, for example, were identified correctly, and only two males of 30 specimens of population 1 and three males of population 6 were assigned to different populations.

Two-dimensional canonical plots with maximum convex polygonals of each population (Fig. 3) appear completely different from those of the females and J2. Canonical centroids of populations 1, 3, and 7 are closely grouped and their polygonals overlap. They overlap also to some extent with populations 2, 6, and 8. Centroids of populations 2, 4, 6, and 8 are widely separated, although maximum convex polygonals of populations 2 and 4 overlap marginally. Populations 3, 6, and 8 exhibit considerable variability as indicated by large-size polygonals, whereas the remaining populations have smaller polygonals and thus lower variability.

Cluster analysis

The BMDP 2M cluster analysis of cases was employed to group the 325 females, 360 J2, and 330 males (males of population 5 were not included) of all populations into clusters according to their relative degree of similarity based on the mean values of the same morphometric characters used for each life stage in the standard descriptive statistics. Dendrograms of the resulting clusters indicating the sequence of clustering and the amalgamated distances are presented in Figs. 4–6.

Females (Fig. 4)

Populations 7 and 3 are clustered in the first step as having the smallest distance between them (1.225) and consequently being very similar to each other. Populations 1 and 6 are added in the next two steps to this cluster. In the following step, populations 2 and 5 are grouped in another cluster (distance of 2.102), to which population 4 is added in the next step. The two clusters are then united into one cluster to which population 8 (the triploids) is added in the last step.

Second-stage juveniles (Fig. 5)

The hypotriploid populations 5 and 7 are most similar to each other (distance: 1.787) and are clustered in the first step. Populations 3 and 1, which are grouped in another cluster, follow in the next step. Populations 6, 4, and 2 are added to the latter cluster in the following three steps. The two clusters are subsequently united into one cluster, to which the triploid population 8 is added in the last step.

Males (Fig. 6)

Populations 7 and 3 have the smallest distance between them (2.341) and are clustered in the first step, to which population 6 is added in the next step. In the third step, the triploid population 8 and the hypotriploid population 1 (distance of 5.170) are grouped in another cluster. The two clusters are then united into one (distance of 5.263), to which populations 2 and 4 are added in the last two steps (distance of 5.457 and 5.820, respectively).

Discussion

The various morphometric characters examined were not equally useful across all life stages for precise characterization of the populations of *M. arenaria*. Body length of males varied greatly within populations; however, on the average, the hypotriploids had shorter males than the triploids. A similar trend was present in J2,

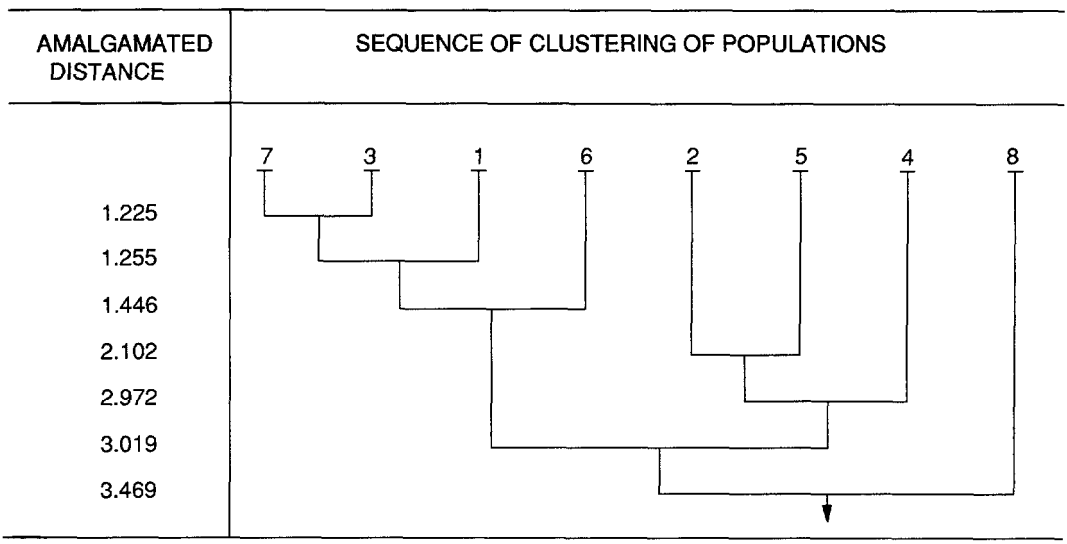

Fig. 4. Dendrogram resulting from cluster analysis of females of eight populations of *Meloidogyne arenaria* based on mean values of six morphometric characters.

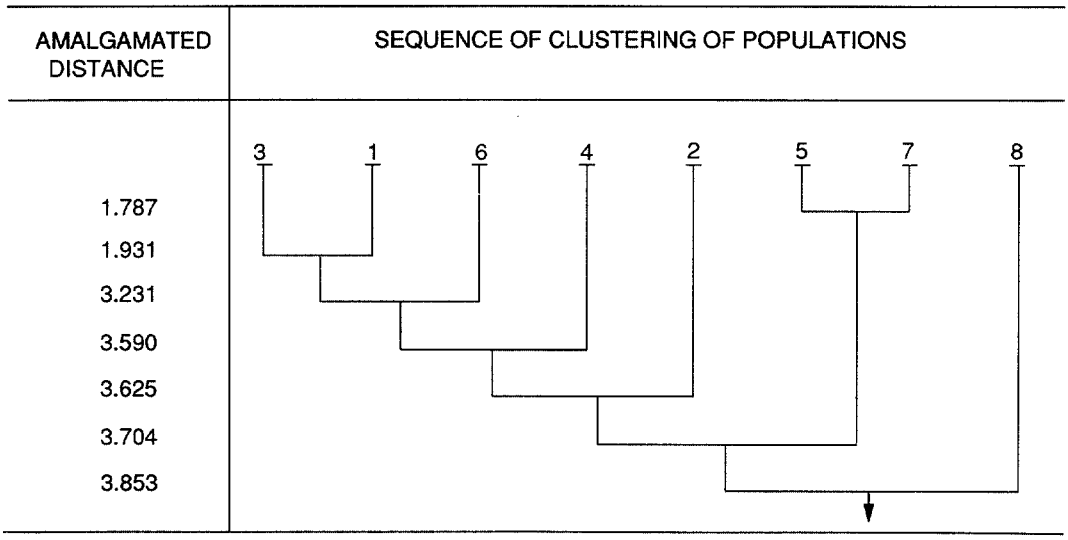

FIG. 5. Dendrogram resulting from cluster analysis of J2 of eight populations of *Meloidogyne arenaria* based on mean values of 14 morphometric characters.

where body length overlapped among all populations, but the hypotriploids had generally smaller means. With respect to stylet length, most females of the hypotriploid populations had greater means, and stylet length measured to the head end in J2 was also helpful to separate certain populations. Stylet-knob dimensions, especially stylet-knob height, are characteristic features that can be used to distinguish the females and males of the hypotriploid from those of the triploid populations. Morphometric variation of the various stylet parts is generally low in all life stages of *Meloidogyne* species (9). Stylet length was found to be the least variable character in each life stage of four populations of *M. arenaria* from Florida (14). Dorsal esophageal gland orifice distance, which is also a useful character in *Meloidogyne* species (9),

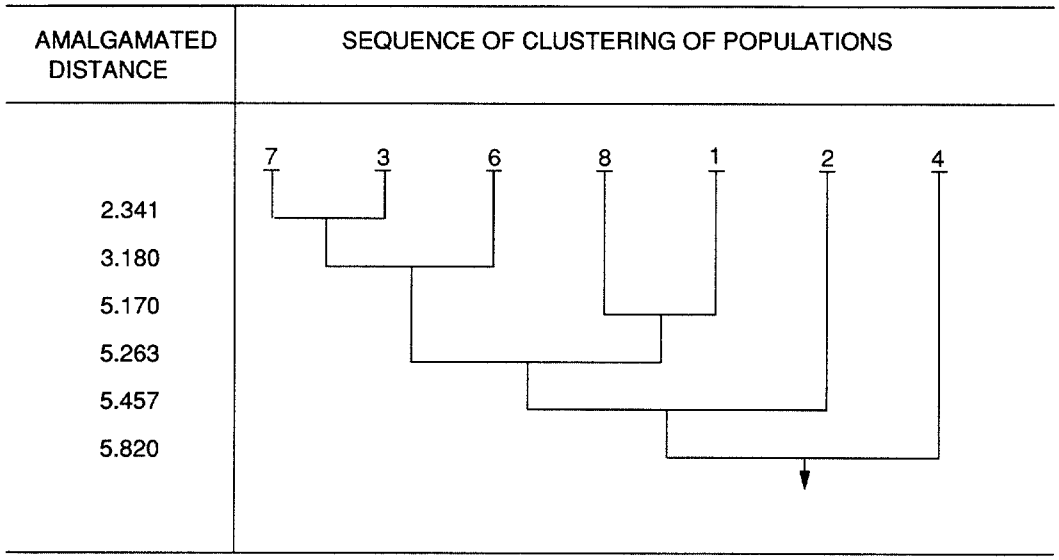

FIG. 6. Dendrogram resulting from cluster analysis of males of seven populations of *Meloidogyne arenaria* based on mean values of 18 morphometric characters.

was helpful in separating J2 of certain populations. In females, most hypotriploids had a shorter mean DGO distance. In males, this character was significantly different among most populations and was the best differentiating character. Body length/esophagus-length ratio was a good distinguishing feature among males of hypotriploids. The latter ratio and excretory pore position were useful distinguishing characters in J2. These characters had been proposed also in J2 of other *M. arenaria* populations for a more precise definition of this species (14). Spicule length was reliable for distinguishing the hypotriploid populations.

When the results of the SDA were illustrated, using maximum convex polygonals, and compared among females, males, and J2, different relationships between populations were expressed for each life stage. These differences may be partially due to the types of characters available in each life stage and the different numbers of variables selected by the SDA. In the case of males, the triploid vs. the hypotriploid populations were grouped quite differently from those of the females and J2, which appeared basically similar. In general, the triploids were set off from the hypotriploid populations in each life stage, although overlap of certain hypotriploid with triploid populations occurred to some degree. In females, the hypotriploid populations 2 and 4 could be distinguished from the pooled triploids. In J2, populations 4 and 6 and most individuals of population 5 were different, and in males, populations 2, 4, and 6 showed no overlap. Population 4 could be clearly separated from the pooled triploids in all three life stages. Among the hypotriploids, many populations overlapped and could not be separated from each other using this analysis. In females, only populations 4 and 7 did not overlap. In J2, several populations could be distinguished from each other. In males, the results appeared different as compared to females and J2. Populations 1, 3, and 7 of the hypotriploids were closely grouped, whereas populations 2, 4, and 6 were widely spaced in addition to being separated distinctly from population 8. Morphometrics of males obviously contribute more to the differentiation of the populations than those of females or J2. The degree of variability of each population, expressed by the size of its polygonals, varied between the three life stages. The hypotriploid population 4 showed the lowest variability throughout females, J2, and males. In a morphometric study of three populations of *Anguina amsinckiae* parasitizing three different host plants, some differences were found between females and males when canonical variables were compared (15). Although much overlap occurred in dimensions of certain characters in both sexes from each host species, the males were not as separable as the females.

With respect to the degree of similarity among all populations based on cluster analysis, it was also obvious that each life stage exhibited a different trend. In females and males, populations 7 and 3 were most similar, whereas in J2 populations 7 and 5 showed the closest affinity. The triploid population 8 was least similar to all hypotriploids in females and J2, as it was added in each case in the last step. In males, however, it was clustered in the third step together with population 1.

It is difficult to integrate morphology with morphometrics because expressions of morphological characters are weighted differently and allow more subjective judgement than measurements. However, certain morphological features can be expressed in quantitative terms. The ratio stylet-knob width/height, for example, describes more objectively the shape of the knobs (index of flatness). When the results of this morphometric study were compared with previous morphological data of the same populations (17), no consistent relationships were evident between the hypotriploid and the triploid populations. The relationships of the various populations differed between the three life stages based on qualitative as well as quantitative data, and no definite conclusions could be

drawn. In general, however, it can be concluded that all hypotriploid populations are more similar to each other than they are to the pooled triploids with respect to qualitative as well as quantitative features. A combination of morphological and morphometric data provides a more complete characterization of a population than either type of information considered alone. Morphological and morphometric differences observed among populations, in the present study, were not sufficiently striking to merit describing any of the populations as new species. The populations compared should, therefore, be considered intraspecific variants of *M. arenaria.*

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