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Inter- and Intraspecific Variation in Wild-type and Single Female-derived Populations of *Xiphinema americanum*-group Nematodes[1]

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Abstract: Ten populations of *Xiphinema americanum*-group nematodes were reared from individual females to evaluate inter- and intraspecific variation under identical host and environmental conditions. Data indicated that morphometric variability of *X. americanum* was the result of genetic variation rather than phenotypic plasticity and that genetic heterogeneity was greater than previously thought. Morphometrics of single female derived (SFD) populations identified different genotypes present in the field populations. Stylet length was the least variable morphometric character of SFD populations, but collectively stylet measurements of all individuals formed an uninterrupted continuum ranging from 107–148 µm. Range and frequency of stylet measurements of field populations could be accounted for by the relative proportion of different genotypes in the population. Nine SFD populations were identified as *X. americanum* sensu stricto, and one SFD population was similar to *X. californicum*.

Key words: development, genetic, morphometric, morphotype, nematode, phenotype, species, variation, virus-vector, *Xiphinema americanum, X. californicum.*

Morphometric variability of *X. americanum* has frequently been mentioned in the literature. In a redescription of *X. americanum* sensu stricto (s. s.), Lamberti and Golden (16) reported differences between three populations they collected and Cobb's original material. The most obvious difference was in stylet length (= odontostyle + odontophore). Although the mean stylet length in each population differed, the range of measurements overlapped, and some specimens essentially fit the description of those in all populations. These authors concluded that all four populations were *X. americanum* s. s. and that the morphometric differences were "probably normal biological variations which might be caused by pedoclimatic and nutritional factors."

In a study of seasonal fluctuation of *Xiphinema* spp. populations in Pennsylvania, Jaffee et al. (13) worked with mixed populations of *X. americanum* and *X. rivesi*. They found little difference in the morphometrics of *X. rivesi* collected from apple and peach, whereas *X. americanum* from apple had shorter stylets than specimens collected from peach. In the same peach orchard samples, these authors also found specimens of *X. californicum* coexisting with *X. americanum* and *X. rivesi*. Georgi (10) reported morphometric variation in *X. americanum* populations from New York orchards. Morphometric differences in her populations were attributed to host and region, with regional differences usually greater than host differences. Georgi noted that variation in stylet length was the most obvious difference between populations. Her collections also provided the first report of *X. californicum* in New York. In her results, stepwise discriminant analysis (SDA) of morphometric data from 13 populations failed to clearly separate *X. americanum, X. californicum,* and *X. rivesi*.

Griesbach and Maggenti (11) used techniques similar to those used by Georgi in a study of 12 *Xiphinema* populations that included *X. americanum* and *X. rivesi* from the East Coast and *X. californicum* and nine other field populations of *X. americanum*-group nematodes from California. Their analysis failed to distinguish *X. americanum* and *X. californicum* in field populations, but *X. rivesi* was distinct from others.

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Lack of uniquely distinguishing characters for *X. americanum*-group species has generated controversy over the usefulness of the present nomenclature (12,17). Controversy over speciation in the *X. americanum*-group will continue until phenotypic plasticity within the group and the influence of host and environment on the morphometric criteria used for species separation is understood. In this regard, information from field collections is of limited value, as it is difficult to determine the host and because of the complexity of environmental interactions. Furthermore, two or more species frequently occur together in nature (7,10,13). Therefore, the probability exists that data collected from morphologically similar species may unwittingly be mixed, invalidating statistical tests. This paper reports the results of research to determine intraspecific variation in *X. americanum*-group nematodes by studying the progeny of single females under controlled conditions.

Materials and Methods

Rearing: Xiphinema were obtained from two sites used in the study by Jaffee et al. (13): an apple orchard (Red Delicious on MM.106) at the Pennsylvania State University Fruit Research Laboratory in Biglerville, Pennsylvania, and a peach orchard (Loring on Halford) in Arendtsville, Pennsylvania. The two orchards were 3 miles apart, and both were on gravelly loam soil. Nematodes were extracted from soil samples (100 cm^3) by a modified decanting and sieving method with a final separation on Baermann funnels and held 6–20 hours at room temperature (21–24 C). Individual adult females were hand-picked and transferred to styrofoam cups (240 ml) containing 200 cm^3 sterile soil into which was planted sudan grass (cv. Piper). Subsamples of nematodes from the field (= wild type) populations were preserved in TAF (4) for reference. *Xiphinema* from apple were designated "wild-type apple" (WTA) and those from peach as "wild-type peach" (WTP). Approximately 210 individual females from each population were transferred to separate cups.

Cups were maintained on a greenhouse bench with a cheesecloth shade to block direct sunlight. Watering was by hand held hose with a mist nozzle; the amount of water was determined by visual examination of the soil. Care was taken to prevent overwatering. The grass was maintained between 4–15 cm high by clipping, and mean greenhouse temperatures ranged from 27 (summer) to 21 C (winter). Nematodes were assayed over a 3–13 month period. Soil was removed from cups and nematodes extracted according to the procedure described above. All recovered nematodes were returned to clean soil and replanted with sudan grass as above. Nematodes were extracted a second time 6–13 months after their return to soil. Single female derived (SFD) populations consisting of 100 or more nematodes were split, one portion going back to soil and the remainder being heat killed and preserved in TAF.

Morphology and morphometric studies: Standard descriptive morphometric and allometric characters were determined for 15–30 adult specimens from each SFD population and the two wild-type populations. Measurements were obtained from digitized video images using video imaging software on a Macintosh II computer. Body length and anterior to vulva distance were measured at ×80 on a stereoscope, while all other measurements were taken at ×400 on a light microscope. All nematodes from the 10 SFD populations possessed offset lip regions and conoid tails; therefore, only morphometrics of similar appearing wild-type nematodes were included in the data set. Wild-type *Xiphinema* with lip regions continuous with the body and/or rounded tails were omitted. Data were analyzed by standard descriptive statistics, analysis of variance (Tukey-Kramer, $P = 0.01$), and stepwise discriminant analysis (BMDP7M).

Results

Rearing: Relatively few nematodes were recovered within the initial 13 months of the project. Two or more nematodes were

recovered from only 48 of the 420 cups (11.4%) to which individual females had been added. Fewer than 42 nematodes were recovered from each of 45 cups, but from each of the remaining three cups at least 100 nematodes were recovered, with one cup yielding more than 400 specimens (Table 1). Not all of the populations recovered from cups successfully reproduced or survived the second year. By the end of the second year, only 30 populations survived (7.1%), and numbers varied greatly. Some populations that initially reproduced well declined in the second year, while others increased. The largest population after 2 years was 1,659 nematodes.

The earliest that nematodes were extracted for the purpose of determining reproduction was 100 days after inoculation, at which time first and second-stage juveniles (J2) were found and occasionally an adult, which was assumed to be the original female. The third-stage juvenile was first found at 4 months, and all of the population had only three juvenile stages. The presence of multiple adults confirmed that nematodes had developed from egg to maturity, and this was first observed 225 days after inoculation.

Morphology and morphometric studies: Two wild-type (WTA, WTP) and nine SFD populations (ABB, AH, AI, AY, PU, PV, PHH, PLL, PE) were each identified as *X. americanum* s. s. Population PG was similar to *X. californicum*. Morphometric and allometric characters of all populations are listed in Table 2 in order of increasing variability as determined by the average coefficient of variability of all SFD populations. Except for population PE, stylet measurements of *X. americanum* specimens were typical for the species. Morphometrics of population PE were similar to specimens collected from rose by Lamberti (14) at Beltsville, Maryland, and the stylet lengths were in the upper range of published measurements for the species.

With the exception of body length, every morphometric character showed statistically significant differences between some populations. However, the only characters that distinguished population PG (the *X. californicum*-like population) from all others were measurements of the stylet, odontostyle, and anterior to guide ring. Population PE was distinguished from all others only by measurements of the stylet and odontostyle. No other character separated any single population from all other populations (Table 3).

Stylet lengths were normally distributed in all populations except the wild-type from peach, which appeared slightly skewed because a few nematodes with relatively long stylets appeared in the sample (Fig. 1). Ten of the 12 populations had mean stylet lengths between 113.7 and 120.4 μm, but two of the SFD populations (PE and PG) had distinctly longer stylets with mean lengths of 128.5 and 140.9 μm, respectively. However, the range of stylet measurements overlapped between populations and formed a continuum ranging from 107 to 148 μm (Fig. 1, Table 2).

Stepwise discriminant analysis showed SFD populations PE and PG as being dif-

TABLE 1. Summary data showing the number of progeny recovered from single female derived (SFD) populations of *Xiphinema* over 2 years.

	Year 1		Year 2	
No. SFD populations†	No. nematodes	Time (months)	No. nematodes	Time (months)
43	<20	3–13	‡	6–13
1	40	9	—	6–13
1	41	8	—	6–13
1	116	9	1,659	11
1	136	13	178	11
1	448	13	107	11

† Number of populations from the initial 420 single nematodes that were maintained on sudan grass in the green house.
‡ Of 45 populations examined after 6–13 months, 18 died, 18 increased or decreased by ±60 nematodes, and 9 increased to 111–1,152 nematodes.

TABLE 2. Descriptive morphometrics and ratios of 10 single female derived and two wild-type *Xiphinema americanum*-group populations.

Apple orchard populations					Peach orchard populations						
WTA 15	ABB 15	AH 15	AI 15	AY 15	WTP 15	PU 15	PV 30	PHH 30	PLL 15	PE 16	PG 15
Total stylet (μm)											
117.0 ± 2.9† (114–124)‡	119.0 ± 2.4 (112–121)	119.3 ± 3.3 (114–125)	118.5 ± 0.9 (117–119)	113.7 ± 2.3 108–117)	120.4 ± 5.2 (112–133)	115.8 ± 5.8 (107–130)	114.7 ± 2.4 (110–119)	118.6 ± 2.0 (114–122)	115.4 ± 2.9 (107–118)	128.5 ± 2.8 (123–134)	140.9 ± 3.5 (134–148)
Odontostyle (μm)											
69.5 ± 2.6 (65.0–77.0)	70.7 ± 1.4 (68.0–73.0)	72.0 ± 2.1 (67.6–75.3)	70.7 ± 0.5 (70.0–71.0)	68.5 ± 2.2 (65.7–73.6)	73.2 ± 4.8 (66.0–82.7)	71.1 ± 4.0 (65.1–81.0)	67.5 ± 1.7 (65.0–71.0)	69.7 ± 1.6 (66.0–73.0)	69.9 ± 1.7 (67.3–72.8)	78.7 ± 2.5 (73.7–82.3)	88.9 ± 2.8 (84.9–95.7)
Anterior to guide ring (μm)											
62.0 ± 2.9 (58.4–68.0)	63.2 ± 1.4 (60.0–65.0)	63.9 ± 3.3 (57.0–69.9)	62.0 ± 1.6 (58.0–65.0)	60.8 ± 1.8 (55.3–62.6)	65.2 ± 4.9 (60.0–77.6)	61.3 ± 3.1 (56.5–68.1)	59.6 ± 1.6 (56.0–63.0)	60.2 ± 1.7 (58.0–63.0)	64.2 ± 1.8 (60.0–66.0)	67.7 ± 1.9 (64.3–71.9)	76.3 ± 2.3 (71.9–80.4)
Diameter at guide ring (μm)											
25.8 ± 1.1 (22.4–27.1)	26.0 ± 0.0 (26.0–26.0)	25.3 ± 0.8 (23.7–26.5)	25.5 ± 0.9 (24.0–26.0)	24.9 ± 2.3 (22.7–31.1)	26.0 ± 0.7 (24.0–27.0)	25.3 ± 0.9 (23.7–27.0)	25.6 ± 0.8 (24.0–26.0)	24.8 ± 1.0 (24.0–26.0)	24.0 ± 0.8 (22.7–26.0)	23.5 ± 0.7 (21.8–24.6)	26.9 ± 1.8 (24.0–30.1)
Odontophore (μm)											
47.5 ± 2.0 (44.0–51.0)	48.3 ± 1.4 (44.0–50.0)	47.3 ± 2.0 (43.9–50.4)	47.7 ± 1.2 (46.0–49.0)	45.2 ± 1.8 (41.6–49.2)	47.2 ± 1.4 (44.6–50.0)	44.7 ± 4.6 (34.8–50.4)	47.1 ± 1.4 (44.0–49.0)	48.9 ± 1.6 (46.0–52.0)	45.6 ± 2.6 (37.0–47.9)	49.8 ± 1.4 (46.9–51.5)	52.0 ± 1.8 (49.0–54.8)
V											
50.3 ± 1.0 (48.5–52.1)	50.0 ± 1.0 (48.4 ± 51.8)	50.1 ± 1.6 (48.2–54.0)	49.8 ± 1.5 (48.3–54.2)	48.8 ± 4.9 (32.0–52.7)	50.7 ± 1.8 (48.0–54.4)	51.4 ± 1.8 (47.6–54.8)	48.3 ± 3.6 (40.4–51.9)	48.9 ± 2.2 (42.3–51.8)	50.3 ± 1.3 (47.9–52.6)	51.6 ± 1.7 (48.2–54.7)	51.2 ± 2.0 (47.5–55.3)
Length (mm)											
1.73 ± .19 (1.48–2.11)	1.83 ± .08 (1.70–1.98)	1.84 ± .10 (1.64–1.97)	1.82 ± .07 (1.72–1.98)	1.73 ± .09 (1.60–1.90)	1.71 ± .09 (1.56–1.84)	1.77 ± .10 (1.57–1.91)	1.77 ± .05 (1.66–1.90)	1.74 ± .11 (1.42–1.90)	1.81 ± .11 (1.61–2.00)	1.79 ± .10 (1.62–1.96)	1.85 ± .09 (1.72–2.07)
Diameter at lip (μm)											
10.0 ± 0.8 (9.1–12.3)	11.4 ± 1.2 (8.9–13.7)	10.1 ± 0.3 (9.6–10.8)	10.0 ± 0.5 (9.0–11.0)	10.9 ± 0.6 (10.0–11.8)	10.6 ± 0.7 (9.4–11.8)	10.4 ± 0.5 (9.4–11.0)	10.1 ± 0.7 (8.1–11.0)	10.2 ± 0.8 (8.8–11.8)	10.5 ± 0.4 (10.0–11.4)	10.4 ± 0.7 (8.7–11.4)	10.6 ± 0.3 (10.1–11.0)

					Diameter at anus (μm)					
20.9 ± 1.8	21.6 ± 1.1	20.5 ± 1.0	20.5 ± 1.3	21.5 ± 1.8	20.6 ± 1.0	21.3 ± 1.0	21.1 ± 1.4	21.3 ± 1.4	19.9 ± 1.3	18.8 ± 1.0	20.7 ± 0.9
(17.0–23.7)	(20.0–23.7)	(18.5–22.1)	(18.4–22.7)	(19.3–25.8)	(18.5–21.8)	(19.0–23.6)	(18.1–23.5)	(17.3–24.0)	(17.7–21.6)	(17.3–20.8)	(18.3–22.3)
					Tail length (μm)					
34.4 ± 3.2	37.1 ± 2.0	37.7 ± 2.3	34.8 ± 2.5	33.4 ± 1.5	32.5 ± 2.2	34.6 ± 2.8	33.0 ± 2.8	33.1 ± 2.8	35.6 ± 1.3	33.1 ± 1.9	33.8 ± 1.8
(29.8–40.3)	(33.7–39.7)	(33.6–41.6)	(31.4–40.7)	(31.0–36.2)	(28.9–36.3)	(29.1–40.0)	(28.6–39.0)	(29.0–38.3)	(32.3–36.8)	(29.5–36.1)	(30.8–36.4)
					c'					
1.7 ± 0.2	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.6 ± 0.1
(1.4–2.0)	(1.5–1.9)	(1.7–2.0)	(1.5–1.9)	(1.3–1.7)	(1.4–1.8)	(1.5–1.9)	(1.3–2.0)	(1.4–1.8)	(1.5–2.0)	(1.5–2.0)	(1.5–1.8)
					c					
50.6 ± 5.6	49.2 ± 2.5	48.8 ± 2.5	52.6 ± 3.2	51.8 ± 2.5	53.0 ± 4.2	51.4 ± 4.4	54.0 ± 4.2	52.8 ± 5.2	50.8 ± 2.7	54.3 ± 4.9	54.8 ± 3.8
(42.1–58.5)	(45.7–54.2)	(43.4–53.8)	(46.5–57.8)	(46.6–55.5)	(46.3–58.6)	(42.8–59.2)	(46.8–60.8)	(41.1–60.9)	(44.2–55.0)	(45.1–65.3)	(49.4–60.8)
					a					
46.0 ± 3.7	47.9 ± 3.8	47.9 ± 4.4	48.8 ± 3.1	45.1 ± 5.4	44.4 ± 2.5	42.0 ± 4.4	46.4 ± 1.8	46.4 ± 2.9	48.5 ± 3.5	49.9 ± 2.6	41.9 ± 5.1
(39.2–52.8)	(41.4–53.5)	(40.8–54.0)	(43.2–55.2)	(34.1–50.6)	(40.9–48.9)	(33.6–49.0)	(43.3–50.2)	(38.5–51.8)	(40.5–52.7)	(45.2–54.0)	(35.8–50.7)
					Diameter at vulva (μm)					
37.7 ± 2.9	38.3 ± 3.4	38.7 ± 4.7	37.5 ± 2.8	38.9 ± 5.5	38.7 ± 2.9	42.6 ± 5.6	38.2 ± 1.4	37.6 ± 2.7	37.4 ± 3.3	35.8 ± 1.4	44.6 ± 5.2
(33.1–43.3)	(33.3–44.8)	(30.4–46.3)	(32.0–42.5)	(33.2–52.9)	(34.5–42.9)	(35.1–54.9)	(34.0–40.1)	(30.7–42.8)	(33.8–45.8)	(34.2–38.4)	(35.1–50.4)
					J. diameter (μm)					
8.4 ± 1.2	7.3 ± 0.6	7.8 ± 0.5	8.6 ± 0.8	8.4 ± 0.9	7.9 ± 1.1	7.8 ± 1.1	8.1 ± 0.8	8.9 ± 1.3	7.1 ± 1.0	7.7 ± 0.8	9.2 ± 0.9
(5.9–10.5)	(6.0–8.3)	(7.3–8.9)	(7.1–10.0)	(6.8–9.7)	(5.6–9.3)	(6.3–10.0)	(6.7–10.3)	(7.1–11.9)	(5.0–8.7)	(6.0–8.8)	(8.4–10.2)
					J'					
1.1 ± 0.2	1.1 ± 0.1	1.2 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.3 ± 0.1	1.1 ± 0.1
(0.8–1.6)	(1.0–1.5)	(0.9–1.5)	(0.6–1.4)	(0.8–1.1)	(0.9–1.3)	(0.8–1.2)	(0.8–1.2)	(0.7–1.3)	(0.8–1.2)	(1.0–1.5)	(0.9–1.2)
					J. length (μm)					
9.2 ± 2.1	8.3 ± 1.1	9.6 ± 1.3	7.5 ± 1.3	7.5 ± 0.9	8.6 ± 1.4	7.4 ± 1.0	8.0 ± 1.2	8.3 ± 1.5	7.3 ± 1.5	9.9 ± 1.3	10.1 ± 0.8
(5.2–14.0)	(7.0–10.9)	(6.8–11.3)	(4.5–10.0)	(5.6–9.2)	(7.2–11.8)	(6.0–8.9)	(5.8–10.9)	(5.6–11.6)	(4.1–9.3)	(7.5–12.8)	(8.0–11.3)

† Mean ± standard deviation.
‡ Range.

TABLE 3. Analysis of variance of selected morphometric and allometric characters between *X. americanum* s. s. and a *X. californicum*-like population.

Pop.	*n*	Stylet (μm)	L (mm)	G. ring (μm)	Tail (μm)	V	c'
				X. americanum s. s.			
AY	15	113.7a†	1.73a	24.9abc	33.4a	48.8ab	1.57ab
PV	30	114.7ab	1.77a	25.6cd	33.0a	48.3a	1.57ab
PLL	15	115.4abc	1.81a	24.0ab	35.6ab	50.3ab	1.79cd
PU	15	115.8abc	1.77a	25.3bc	34.6ab	51.4b	1.63abc
WTA	15	117.0abcd	1.73a	25.8cd	34.4ab	50.3ab	1.66abc
AI	15	118.5bcd	1.82a	25.5bcd	34.8ab	49.8ab	1.70abcd
PHH	30	118.6cd	1.74a	24.8abc	33.1a	48.9ab	1.56a
ABB	15	119.0cd	1.83a	26.0cd	37.1b	50.0ab	1.72bcd
AH	15	119.3cd	1.84a	25.3bc	37.7b	50.1ab	1.84d
WTP	15	120.4d	1.71a	26.0cd	32.5a	50.7ab	1.58ab
PE	16	128.5e	1.79a	23.5a	33.1a	51.6b	1.76cd
				X. californicum-like			
PG	15	140.9f	1.85a	26.9d	33.8a	51.2b	1.64abc

† Mean values in a column followed by the same letter are not significantly different (Tukey-Kramer, $P = .01$).

ferent from each other and from all other populations, but the remaining SFD populations and wild-type population were virtually indistinguishable from each other. Stepwise discriminant analysis correctly classified all members of populations PE and PG, but members of other populations were identifiable only about half of the time. A two-dimensional canonical plot of all females ($n = 211$) from all populations revealed three distinct clusters (Fig. 2). Because of the extreme overlap of the *X. americanum* populations (except PE), the populations AI, AH, AY, and ABB are grouped, as are populations PHH, PV, PU, and PLL, to minimize confusion.

The original data set was modified twice to determine the effect of removing selected variables on the separation of nematodes by SDA. First, stylet and odontostyle measurements were omitted from the data set and then stylet, odontostyle, and anterior to guide ring were omitted as these characters were the most useful for separation of the species. Removal of the stylet and odontostyle measurements lessened the ability of the computer program to correctly identify individuals of population PE but had little meaningful effect on other nematodes. Removal of the stylet, odontostyle, and anterior to guide ring measurements prevented SDA from completely separating any population from all of the others. A classification summary of all three SDA is in Table 4.

DISCUSSION

Rearing: The appearance of various developmental stages in the cups resulted from eggs deposited sometime after transfer of the original females into cups. Allowing for a margin of error, e.g., not knowing the lag time before egg laying and not finding stages at their earliest appearance, it can be estimated that in this study, under the prevailing greenhouse conditions and temperatures, J2 developed between 70 and 90 days after transfer of the females, third stage between 80 and 110 days, and adults between 170 and 210 days. Based on these developmental rates, second-generation nematodes may have appeared after 180 days, and presumably the five largest populations after 8 months were mixtures of first- and second-generation nematodes (Table 1).

Morphology and morphometric studies: Lip region morphology and shape of the tail are used as principal characters to distinguish putative members of the *X. americanum* group (14,15). In our study, all specimens from SFD populations possessed offset lip regions and conical tails without any discernible inter- or intrapopulational variation. Thus our data support the value

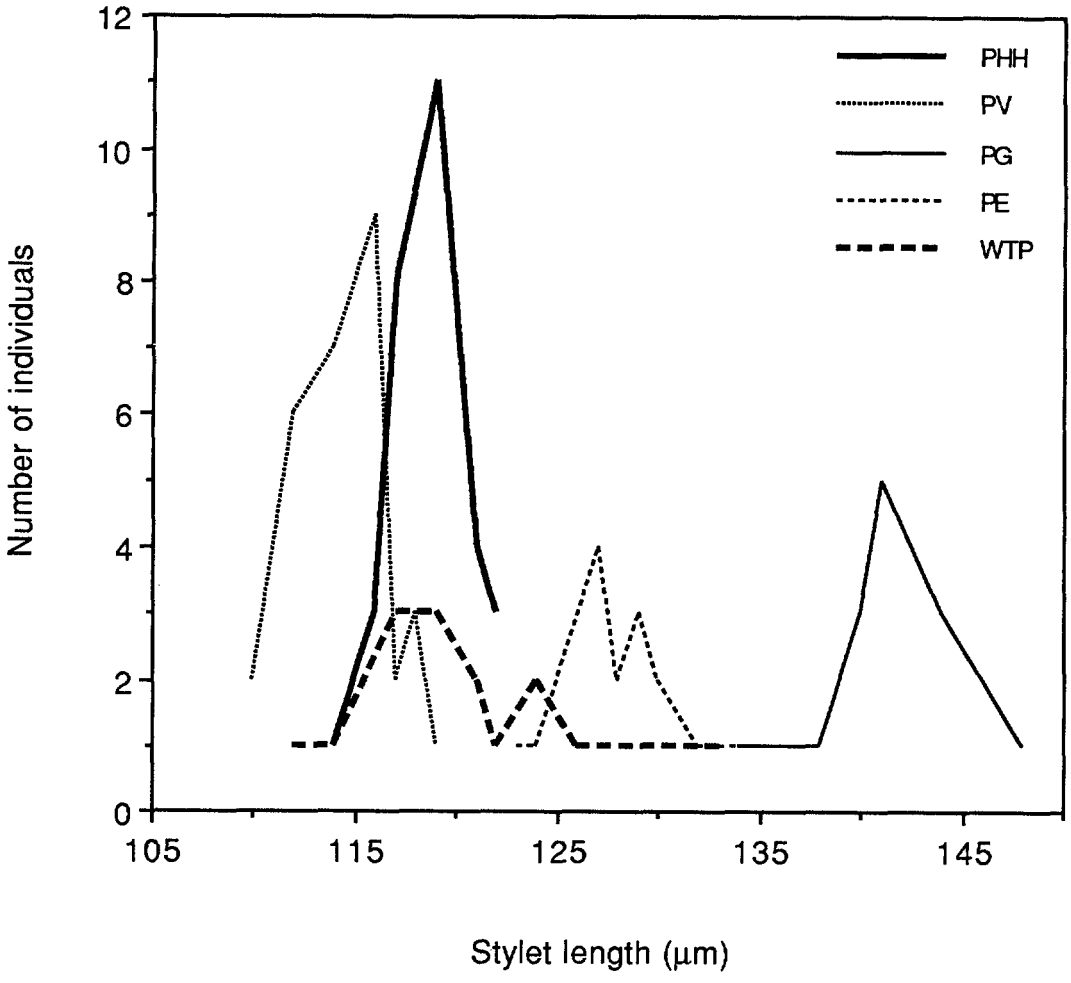

FIG. 1. Frequency distribution of stylet lengths of one wild-type and four single female derived *X. americanum*-group populations from peach.

of lip and tail morphology as diagnostic species characters. These results differ from those of Griesbach and Maggenti (11), who reported variation of these characters in field populations that they considered to be monospecific.

Differences in stylet length and other morphometric characters reported among various populations of a species have frequently been regarded as normal biological variation resulting from nutritional and environmental factors (2,5,6,21). However, the SFD populations used in this study developed under identical environmental conditions. Therefore, the morphometric differences between PE, PG, and the other SFD populations are best explained as genetic variation (Tables 2,3). These data show that intraspecific variation of some *X. americanum*-group characters (e.g., stylet length) may be attributed more to genetic heterogeneity than previously supposed. This could explain why a relatively broad range of stylet measurements has been reported for *X. americanum* s. s. but not *X. rivesi*, despite the fact that both nematodes are frequently found together with the same host and environmental conditions (7,10,13). Presumably, host and environmental factors also affect the phenotype, but the extent of these influences has not been determined.

In wild-type populations of parthenogenic species, such as *X. americanum*-group

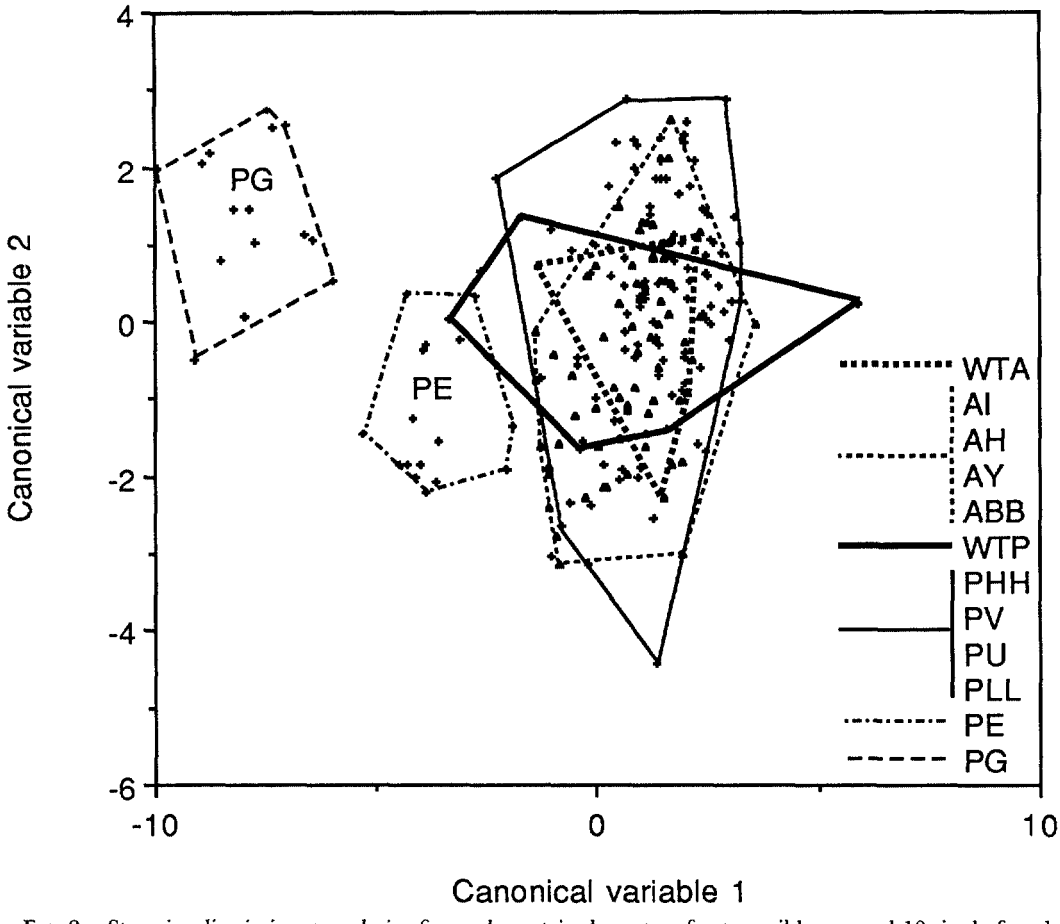

FIG. 2. Stepwise discriminant analysis of morphometric characters for two wild-type and 10 single female derived *X. americanum*-group populations: scatterplot of the first two canonical variables. Nematodes from apple indicated by a triangle and nematodes from peach indicated by a plus sign.

nematodes, genetic characters may not necessarily display a normal distribution. Since interbreeding does not take place, the population does not represent a common gene pool, and specific genotypes may be present in varying proportions (12,23). Nematodes from the wild-type peach population showed a relatively broad range of stylet measurements, and apparently two or more genotypes were present in the sample. Most nematodes had short stylets similar to SFD populations PU, PV, PHH, and PLL, but a few individuals had distinctly longer stylets like specimens of population PE. In effect, the single female derived populations were isolated genotypes and stylet measurements showed a normal distribution over a relatively narrow range (Fig. 1). These data suggest that the high degree of morphometric variability reported for some field populations of *X. americanum*-group nematodes results from the relative proportion of different genotypes in the population.

Morphometrics of SFD population PG were most similar to descriptions of *X. californicum,* although with some small differences (14,15). These differences were typical of the subtle variation reported for other species of the *X. americanum*-group where the authors have assumed that they were dealing with monotypic, wild-type populations. Jaffee et al. (13) earlier identified *X. californicum* from the same orchard where the parent of population PG

TABLE 4. Stepwise discriminant analysis classification summary: Results of the original and two modified data sets.

Xiphinema population	Correct identifications (%)		
	Original data	Data set 2†	Data set 3‡
	X. americanum s. s.		
WTA	13.3	33.3	20.0
PU	40.0	60.0	53.3
WTP	46.7	60.0	46.7
AY	46.7	53.3	46.7
PV	46.7	36.7	36.7
AI	53.3	53.3	73.3
AH	60.0	73.3	80.0
PLL	60.0	60.0	66.7
PHH	60.0	70.0	70.0
ABB	86.7	86.7	86.7
PE	100.0	93.8	87.5
	X. californicum-like		
PG	100.0	100.0	80.0

† Omitting measurements of stylet and odontostyle.
‡ Omitting measurements of stylet, odontostyle, and anterior to guide ring.

was obtained. This identification was independently confirmed by A. M. Golden, USDA, Beltsville. These authors provided only the stylet measurements of their specimens, which ranged from 132–136 μm. These measurements overlapped the range of stylet measurements obtained from SFD populations PE (*X. americanum*) and PG (*X. californicum*-like) in the present study (Table 2). Altogether, the data provided by Jaffe et al. and the present study show that specimens of *X. americanum* and *X. californicum* (or *X. californicum*-like) from a single orchard site displayed an uninterrupted continuum of stylet measurements, which ranged from 107–148 μm (Table 2).

Nematodes from SFD population PG differed significantly from those of the other 11 populations in our study only in the mean lengths of the odontostyle, stylet, and anterior to guide ring. The measurements of the other characters overlapped between all the populations, including population PG. Since the stylet measurement consists of the odontophore and odontostyle, the significance of this measurement may be attributed to the difference recorded in the odontostyle measurement alone. Therefore the morphometrical differences available in our study to distinguish the *X. americanum* populations from *X. californicum*-like populations are only the measurements of the odontostyle and the anterior to guide ring.

In their description of *X. californicum*, Lamberti and Bleve-Zacheo (14) reported the morphometrics of populations from California and Mexico. Assuming these to be monotypic populations, the odontostyle measurements were 79–85 μm and 78–94 μm, respectively, which overlap with populations PG, PE, PU, and WTP in our study. Odontostyle measurements of populations PE and PU derived from single females, and of WTP, a wild-type population from peach, overlap with all the other populations in our study. Similar overlap exists in the anterior to guide ring measurements between our populations and those reported by Lamberti and Bleve-Zacheo.

Results obtained in this study demonstrate unambiguously that several morphotypes (genetically and/or phenotypically induced) may exist within wild-type populations of *X. americanum*-group nematodes. This possibility also applies to other parthenogenic nematode species. Some morphometrical differences may be present that can be used to distinguish morphotypes, but examination of several morphotypes has revealed that these differences form a continuum. Where the measurements for a character fit a normal distribution curve, the standard deviation (S.D.) rule may be applied to distinguish morphotypes (i.e., 68% of the morphotype will be distributed within plus or minus one S.D. of the mean, 95% within two S.D., and 99% within three S.D. of the mean). However, significant morphometrical differences in only two characters, as reported here with *X. americanum* s. s. and the *X. californicum*-like population, do not appear acceptable for practical identification of these species.

This study has shown that genetic variation may have a greater role in the reported morphometric variability of *X. americanum*-group nematodes than previ-

ously supposed, whereas head and tail shapes were found to be stable characters. These data emphatically reinforce the importance of determining the significance of relatively subtle morphometric differences if they are to be useful for the separation of putative species.

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