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Intraspecific Variability of *Rotylenchulus reniformis* from Cotton-growing Regions in the United States[1]

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Abstract: Reniform nematode (*Rotylenchulus reniformis*) is a major pest of cotton in the southeastern United States. The objective of this study was to examine the variation of reniform nematode populations from cotton-growing locations in the United States where it is prevalent. Multivariate analysis of variance and discriminant analysis were used to determine the variability of morphology in males and immature females. Reproduction indices of populations were measured on selected soybean and cotton genotypes in the greenhouse. High variability in morphometrics and reproduction was observed within all the populations, and several differences were found among populations. DNA sequences of the nuclear ribosomal first internal transcribed spacer region (ITS1) were compared among populations from the United States and to sequences of populations from Brazil, Colombia, Honduras, and Japan. No polymorphic nucleotide sites were observed among the amphimictic populations. Only a parthenogenic population from Japan was distinct. The phenotypic polymorphism of the species in the United States could impact the effectiveness of management strategies based on host plant resistance.

Key words: cotton, genetic variation, morphometrics, reniform nematode, reproductive index, ribosomal DNA, *Rotylenchulus reniformis*.

The reniform nematode (*Rotylenchulus reniformis*) is considered an important emerging problem in cotton (*Gossypium hirsutum*) production in the southeastern United States (Koenning et al., 2004). Management practices to control this nematode in cotton include the use of nematicides and crop rotation (Davis et al., 2003; Koenning et al., 2004). However, environmental and economical reasons make host plant resistance the preferred method for nematode management. In recent years, research efforts to develop commercial upland cotton cultivars with resistance to reniform nematode have increased (Koenning et al., 2004). Information on the variability among and within populations of this nematode present in the cotton-growing regions of the United States, necessary for the development of resistant cotton cultivars, does not exist. Genetic variability of reniform nematode could impact the effectiveness and longevity of management strategies based on host plant resistance, as has been the case with several cyst and root-knot nematode species in various crops (Anwar et al., 2000; Kaplan et al., 1999; Niblack et al., 2002; Noe, 1992; Riggs et al., 1981; Van der Beek et al., 1999; Zhou et al., 2000). Also, the potential existence of infraspecific variants with differential reproduction abilities should be investigated to verify the reliability of a single isolate or population for resistance-screening purposes.

Several authors have reported morphometric variability among populations of reniform nematode and among individuals of a single population (Dasgupta et al., 1968; Germani, 1978; Lehman and Inserra, 1989; Linford and Oliveira, 1940; Nakasono, 1983; Robbins, 1994; Sivakumar and Seshadri, 1971; Soares et al., 2003; Van der Berg, 1978). Dasgupta et al. (1968) examined specimens from diverse geographic origins and characterized the species as "polymorphic," reporting the occurrence of populations without males, and of individuals ranging in size from small to large. In Japan, Nakasono (1983) identified three morphologically distinguishable groups based on size (small, medium, and large) and three biological types based on frequency of male occurrence (male-numerous, male-rare, and male-absent). This naturally occurring, intraspecific variability has not been correlated to host species, geographic origin, population density, or any environmental factor.

Literature on phenotypic variation in reniform nematode related to host preferences is scarce, and no standardized tests exist to identify variants within this species. Dasgupta and Seshadri (1971) proposed the existence of two "races" in India, based on a study of reproduction on cowpea, castor, and cotton, where 9 of 10 morphologically similar populations of reniform nematode were able to reproduce on the three hosts, and one would reproduce only on cowpea. Vadhera et al. (1999) subsequently confirmed this observation. Nakasono (1983) used a system analogous to the race determination scheme for soybean cyst nematode (Golden et al., 1970) to characterize differences among populations of reniform nematode in Japan, by comparing reproduction on nine plant species. His results suggested variations in host preferences within populations and implied that the genetic structure of the populations could be altered over successive generations. McGawley and Overstreet (1995), in a study including reniform nematode populations from Arkansas, Hawaii, Louisiana, Mississippi, and Texas, reported

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variations among populations in reproduction and damage to cotton and soybean. No genetic markers correlated to differential responses on plant hosts, or to any other type of infraspecific variation in *R. reniformis,* have been reported.

The development of effective management strategies is directly related to the ecological significance of the phenotypic variation in *R. reniformis* and the correlation of such variation with genetic diversity in the nematode. This is particularly true when considering that the pattern of distribution of reniform nematode in the United States reflects the pattern of cotton production in the southeastern states (Heald and Robinson, 1990). The objective of this study was to examine the phenotypic and genetic variation of reniform nematode populations from locations representing the main cotton-growing regions in the United States. To establish a possible range limit for molecular variation, populations from Brazil, Colombia, Hawaii, Honduras, and Japan also were examined.

Materials and Methods

Nematode sources: Morphological variation and reproduction were examined for 13 populations that represented the principal cotton-growing regions in the United States where reniform nematodes are present, and Hawaii (Table 1). Table 1 includes abbreviations for populations used in the text. Each population was maintained separately in the greenhouse on soybean (*Glycine max*) cv. Braxton. Additional populations preserved in saline solution were obtained from Brazil, Colombia, Honduras, and Japan and were included in comparative DNA analyses (Table 1). All populations, except for one parthenogenetic population from Japan (JP), had abundant males and were considered to be amphimictic.

Morphological variation: Specimens to be measured were extracted from soil by the centrifugal flotation technique (Jenkins, 1964), mounted in water, and heat-narcotized. A total of 520 individuals (20 immature females and 20 males for each of the 13 populations) were measured. Twelve morphometric variables (body length, stylet length, position of vulva, spicule length, tail length, length of hyaline portion of tail, position of dorsal oesophageal gland orifice, position of excretory pore, maximum width, oesophageal length, and anal width) were selected and subjected to canonical analysis. The de Man's formula ratios a, b, c, and c′ were also calculated. Multivariate analysis of variance (MANOVA) was used to determine if significant differences existed among populations. The DISCRIM procedure in SAS version 8.2 (SAS Institute, Cary, NC) was used to perform discriminant analysis. Canonical variable scores were generated, and the values for males and females were plotted separately to indicate how populations differed. Morphometrics of the populations were compared with published data for the species.

Reproduction tests: The hosts selected for the reproduction tests were soybean cv. Forrest, considered resistant to reniform nematode (Robbins et al., 2001); soybean cv. Braxton, considered highly susceptible (Robbins et al., 2001); cotton cv. Deltapine 50, considered highly susceptible (Robinson et al., 1999); and *Gossypium longicalyx,* considered highly resistant (Yik and Birchfield, 1984). Seeds of the selected hosts were germinated in vermiculite. At the primary leaf stage, the seedlings were transplanted to sterilized fine sand in 500-cm^3 pots and inoculated with 3,000 nematodes/

Table 1. Geographic origin and host of each *Rotylenchulus reniformis* population examined.

Origin	Abbreviation	Host	Source
Huxford, Alabama	ALH	Cotton	K. McLean, Auburn University
Limestone, Alabama	ALL	Cotton	K. McLean, Auburn University
Mississippi Co., Arkansas	ARM	Cotton	T. Kirkpatrick, University of Arkansas
Pinebluff, Arkansas	ARP	Cotton	R. Robbins, University of Arkansas
Florida	FL	*Sanseviera* sp.	B. Adams, University of Florida
Belckley, Georgia	GA	Cotton	R. Davis, USDA
Oahu, Hawaii	HWC	Cowpea	B. Sipes, University of Hawaii
Oahu, Hawaii	HWP	Pineapple	B. Sipes, University of Hawaii
Baton Rouge, Louisiana	LA	Cotton	C. Overstreet, Louisisana State University
Glendora, Mississippi	MS	Cotton	G. W. Lawrence, Mississippi State University
North Carolina	NC	?	S. Koenning, North Carolina State University
St. Matthews, South Carolina	SC	Cotton	J. Mueller, Clemson University
College Station, Texas	TX	Cotton	A. F. Robinson, USDA
Brazil	BRC	Cotton	G. L. Asmus, EMBRAPA Agropecuária Oeste
Brazil	BRS	Soybean	G. L. Asmus, EMBRAPA Agropecuária Oeste
Antioquia, Colombia	CB	Banana	C. Volcy, Universidad Nacional de Colombia
Santander, Colombia	CT	Tobacco	C. Volcy, Universidad Nacional de Colombia
Honduras	HND	Eggplant	L. F. Durán, FHIA
Japan	JA	Sweet potato	H. Iwahori, National Agricultural Research Center
Japan	JP[a]	Sweet potato	H. Iwahori, National Agricultural Research Center

[a] Parthenogenetic population. All other populations in this study were considered amphimictic.

pot. Fallow pots were also included as controls. The plants were kept in the greenhouse, where the ambient temperature was maintained between 28 ° and 34 °C. Sixty days after inoculation, the vermiform stages present in the soil were extracted by centrifugal-flotation (Jenkins, 1964) and the reproductive index (RI = final population/initial population) was calculated. The 13 populations from the United States were evaluated simultaneously, with 10 replications in time. Inoculations were made from 28 May to 25 June and the respective extractions from 28 July to 25 August 2001. The correlation of the geographic origin of a nematode population with its RI was determined by ANOVA. Pair-wise comparisons between population means were performed with Student's t-test at $P = 0.05$. All analyses were done using JMP statistical software (SAS Institute, Cary, NC).

Genetic comparisons: DNA was extracted from individual immature females, using the Sigma REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich Co., St. Louis, MO). Amplification of the nuclear rRNA first internal transcribed spacer region (ITS1) by polymerase chain reaction was accomplished with primers Ren1F (5′-GGT AGC TGT AGG TGA ACC TGC TG-3′) and Ren1R (5′-TCT TAT CGG TGG ATC ACT CGG CT-3′), designed from a *R. reniformis* genomic DNA sequence (GenBank Accession No.AY335192) submitted by H. Iwahori and Z.I. Sano in July 2003 (unpubl.). These primers amplify a 3′ portion of the 18S gene, the entire ITS1 region, and a portion of the 5.8S gene. The PCR protocol was 40 cycles of 94 °C for 45 seconds, 54 °C for 45 seconds, and 72 °C for 60 seconds. The amplified fragment was cloned in pDrive vector (Qiagen, Valencia, CA) and transformed into *Escherichia coli* Qiagen EZ Competent cells. Plasmid preparations were made using the QIAprep Spin Miniprep Kit from bacterial colonies containing the inserts. Sequencing of the plasmid preparation was done using an ABI Prism 377 DNA sequencer (PE Applied Biosystems, Foster City, CA) at the University of Arkansas for Medical Sciences DNA Sequencing Facility (Little Rock, AR). ITS1 fragments were amplified and cloned from two to five individuals of each population, and forward and reverse sequences were obtained from two to five clones from each individual. Sequences were aligned and compared using the computer program BioEdit Sequence Alignment Editor (Hall, 1999).

Results

Morphological variation: Body length ranged from 345 µm to 560 µm for immature females and from 360 µm to 525 µm for males. The ranges and means for body length of immature females of all populations are shown in Figure 1. All population ranges overlapped extensively. The greatest variation was found in the population from pineapple in Hawaii. Populations from the continental locations had similar levels of variation in their body length (Fig. 1). The ranges and means of all measurements in males and immature females are presented in Tables 2 and 3, respectively. The *R. reniformis* populations in this study mostly fall within the range of variation reported in other countries, with a tendency toward the larger sizes. The lowest and highest values for some of the measurements are presented in Table 4. Previous reports of body length of immature females range from 291 µm in the United States (Robbins, 1994) to 514 µm in India (Sivakumar and Seshadri, 1971). A comparison with the species type (Linford and Oliveira, 1940), neotype, and topotypes (Dasgupta et al., 1968), which are all specimens collected from Hawaii, reveals that the morphometrics of the populations considered in this study are consistent with these references (Table 5).

The MANOVA indicated there were differences among populations ($P = 0.05$). The first two canonical variables generated were plotted for both the immature female and the male data. For females, canonical variable 1 was most highly correlated with body length, position of vulva, and position of excretory pore (Table

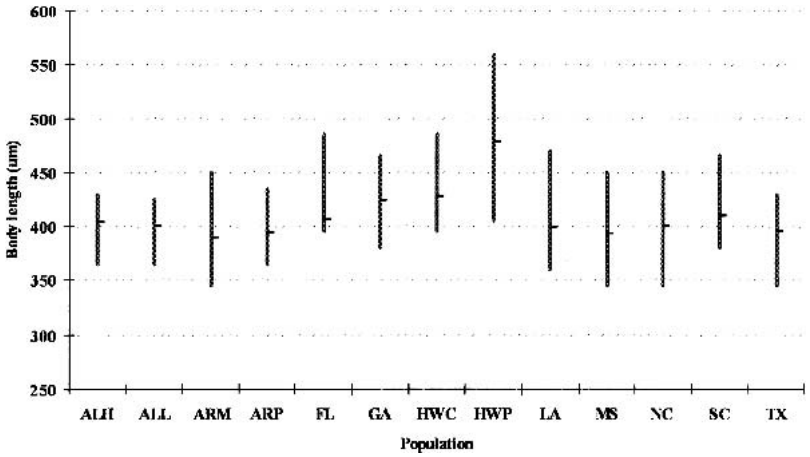

Fig. 1. Ranges and means of immature female body length of the different *Rotylenchulus reniformis* populations. Vertical bars indicate ranges ($n = 20$); horizontal bars indicate mean values.

TABLE 2. Ranges and means of morphological measurements of male *Rotylenchulus reniformis* in populations.

Population (n = 20)	Stylet length (µm)	Body length (µm)	Spicule length (µm)	Tail length (µm)	h[a] (µm)	Excretory pore (µm)	Max. width (µm)	a	Oesophageal length (µm)	b	c	Anal width (µm)	c′
ALH	13–16	375–480	18–23	23–26	4–8	75–100	13–18	23.4–31.9	100–125	3.3–4.0	12.2–18.3	8–11	2.3–4.5
	14.85	410	20.9	29.05	6	82.5	15.0	27.56	112.8	3.6	14.2	10.2	2.9
ALL	13–18	375–445	18–24	24–32	4–8	70–95	13–17	24.1–31.9	95–125	3.3–4.1	13.0–16.9	9–12	2.2–3.3
	15.1	404.3	20.4	28	5.8	81.2	14.4	28.2	110.0	3.7	14.5	10.65	2.6
ARM	14–16	385–485	19–26	23–37	5–8	70–90	14–17	26.6–32.3	100–125	3.3–4.3	13.1–18.3	8–12	2.3–4.5
	14.7	424.8	21.1	29.5	5.4	81	14.9	28.6	111.0	3.8	14.6	9.9	3.0
ARP	14–17	385–485	19–26	23–37	5–8	70–90	14–17	26.6–32.3	100–125	3.5–4.3	13.1–17.8	8–12	2.2–4.1
	14.8	430.5	21.0	29.3	5.5	82.5	15.1	28.6	112.0	3.9	14.9	10	3.0
FL	13–18	405–505	19–25	26–34	5–8	80–100	13–19	23.7–33.7	100–140	3.3–4.1	12.7–16.6	9–12	2.4–3.4
	14.9	429.8	20.8	28.9	5.9	84	15.4	28.1	114.8	3.8	15.1	10.3	2.9
GA	13–16	365–480	19–24	26.32	4–9	60–100	14–20	22.3–31.0	90–125	3.4–4.2	12.8–16.4	9–13	2.2–3.6
	14.8	427.8	21.7	30.6	5.7	84	15.7	27.4	114.5	3.7	14.1	10.5	2.9
HWC	13–18	405–505	19–25	26–34	5–8	80–100	13–19	23.7–33.7	100–140	3.3–4.1	12.7–16.6	9–12	2.4–3.4
	15.1	443.3	22.1	30.3	6.1	87	15.3	29.2	117.5	3.8	14.7	10.7	2.9
HWP	13–18	395–525	18–34	23–32	4–9	70–110	14–20	26.0–33.9	105–140	3.1–4.4	13.2–17.5	10–13	2.3–2.9
	15.7	456.5	22.1	29.0	5.9	91.5	15.5	29.6	118.8	3.9	15.9	11.6	2.5
LA	14–16	390–475	19–24	27–36	4–8	65–90	14–17	23.2–31.7	95–130	3.2–4.3	12.0–16.3	9–12	2.5–3.6
	15.1	432.8	21.3	30.6	6.2	80.8	15.4	28.2	117.3	3.7	14.2	10.55	2.9
MS	13–16	360–480	18–24	24–36	5–9	70–90	13–18	22.9–32.7	95–120	3.6–4.1	12.2–17.3	8–12	2.0–4.5
	14.3	416.5	20.7	29.9	6.5	80.3	15.0	28.0	108.5	3.8	14.0	10.1	3.0
NC	14–17	385–485	19–26	23–37	5–8	70–90	14–17	26.6–32.3	100–125	3.5–4.2	11.3–18.3	8–12	2.3–4.1
	15.3	424.8	21.8	30.3	6.4	80.3	15.0	28.45	112.8	3.8	14.1	9.8	3.1
SC	13–16	375–480	18–23	23–36	4–8	75–100	13–18	23.4–31.9	100–125	3.3–4.0	12.2–18.3	8–11	2.3–4.5
	14.9	410	20.9	29.1	6	82.5	15.0	27.6	112.8	3.6	14.2	10.2	2.9
TX	13–16	360–450	18–25	24–33	5–9	70–95	14–17	22.5–30.4	95–125	3.5–4.1	11.3–15.8	8–12	2.3–4.0
	14.2	406.3	20.9	29.4	6.7	79	15.0	27.3	109.0	3.7	13.9	10.1	2.9

[a] h: length of hyaline portion of the tail.

6), whereas canonical variable 2 was mostly defined by stylet length, position of the dorsal oesophageal gland orifice, and oesophageal length (Table 7). For males, canonical variable 1 was most highly correlated to body length, position of excretory pore, and anal width. Canonical variable 2 in males was defined by a combination of body length, length of spicule, tail length, oesophageal length, and maximum body width.

The plot of the mean values of the first two canonical variables for females (Fig. 2) illustrates that the populations from Hawaii (HWC and HWP) differ from the others in the first canonical axis, primarily because of the higher frequency of larger body sizes. The population from Pinebluff, Arkansas, (ARP) differs from the others in the second canonical axis, indicating a tendency toward smaller stylet and dorsal oesophageal gland orifice closer to the stylet base. The plot of the mean canonical variable scores for the males shows the Hawaiian population from pineapple (HWP) differs from the others in the first canonical value (Fig. 3). The population from Limestone, Alabama, (ALL) differs in the second canonical axis, indicating more uniformity toward the smaller male sizes in this population. The overlapping of the morphometrics and the statistical differences of the means suggest a more diverse composition of the reniform populations in Hawaii, covering a wider range of body sizes than that found in the continental United States.

Reproduction tests: Differences occurred in the reproduction of the populations on the selected hosts (Table 8). In Braxton soybean, the mean RI ranged from 2.8 (ALH) to 62.1 (ALL), and the widest range of variation on this host was for ARP (Fig. 4). The population from Limestone, Alabama, (ALL) was distinct from all other populations, including ALH, in its high and more uniform reproduction on this host. Reproduction of ALH, HWC, and LA were notably low in all replications. On Forrest soybean, the mean RI ranged from 0.5 (HWP) to 8.4 (GA). All populations showed expected low values on this resistant host, except for GA, which showed individual values as high as 16.51 (Fig. 5). On Deltapine 50 cotton (Fig. 6), the mean RI ranged from 0.3 (HWP) to 55.7 (TX). The ARM and TX populations were quite variable and had the highest RI. All other populations were more uniform with lower RI values on Deltapine 50. Reproduction of ALH, GA, HWC, and HWP on cotton was notably low. All populations behaved similarly in *G. longicalyx* (RI 0.05 to 2.3) and in fallow soil (RI 0.05 to 1.87), with limited to no reproduction.

Braxton was a better host for most populations. Forrest was resistant to all populations, except GA, to which it was only moderately resistant. ALH, HWC, and HWP reproduced poorly on all hosts.

Genetic comparisons: DNA sequencing of the ITS1 PCR-amplified product yielded a 348-bp amplicon. Among the 20 amphimictic populations studied, no polymorphic nucleotide sites were observed. A parthenogenic population from Japan was distinguished from other populations based on 11.78% (41/348 bp) ITS1 sequence divergence (Fig. 7).

TABLE 3. Ranges and means of morphological measurements of female *Rotylenchulus reniformis* in populations.

Population (n = 20)	Stylet length (µm)	Body length (µm)	Vulva (µm)	Vulva (%)	Tail length (µm)	h[a] (µm)	DEG[b] (µm)	O[c] (%)	Excretory pore (µm)	Max. width (µm)	a	Oesophageal length (µm)	b	c	Anal width (µm)	c′
ALH	17–21	365–430	255–310	66–74	25–38	3–8	12–22	63–110	75–100	15–18	21.5–28.7	115–165	2.8–3.5	10–16.3	8–12	2.3–3.7
	19.4	403.8	284.3	70.4	28.9	5.3	15.6	80.6	85.3	16.2	25.1	128.5	3.2	14.1	10	2.9
ALL	17–20	365–425	255–330	66–78	18–40	3–9	13–22	68–116	75–100	15–19	21.1–26.6	110–150	2.6–3.5	10–22.8	9–13	2.0–3.5
	19.2	399.8	284.5	71.2	28.9	5.3	16.3	84.8	88.3	16.9	23.7	128	3.1	14.3	10.7	2.7
ARM	16–20	345–450	240–325	68–76	16–31	2–9	10–18	56–100	65–110	14–19	21.4–27.5	90–150	2.8–3.5	13.3–20.7	7–12	2.4–4.1
	18.6	389.8	278.5	71.5	26.7	5.3	15.3	82.1	84.3	15.8	24.8	131.8	3.0	15.2	9.8	2.7
ARP	17–20	365–435	260–325	70–75	20–30	3–8	12–27	67–135	72–100	14–17	25–27.1	110–160	2.4–3.5	13.2–19.5	7–11	2.2–3.5
	18.4	393.8	284	72.1	26.7	5.8	17.1	92.6	83.0	15.1	26.1	135.5	2.9	14.9	9.1	3.0
FL	18–21	395–485	255–330	68–76	21–36	3–9	14–22	70–122	90–110	15–20	20.3–30.3	135–160	2.5–3.1	11.8–20.0	9–12	2.1–3.6
	18.7	405.5	290.5	71.7	29.0	5.3	15.9	85.2	91.3	16.2	25.3	140.8	2.9	14.2	10.0	2.9
GA	18–21	380–465	275–335	69–74	22–34	3–12	14–21	67–105	80–100	14–17	23.8–31.0	115–155	2.8–3.5	13.3–20.7	8–11	2.4–4.1
	19.8	424.3	304	71.65	29.1	6.3	17.1	86.6	91.8	15.5	27.4	134	3.2	14.7	9.8	3.0
HWC	18–21	395–485	290–330	68–76	21–36	3–9	14–22	70–122	90–110	15–20	20.3–30.3	135–160	2.5–3.1	11.8–20.0	9–12	2.1–3.6
	18.8	426.8	306	71.78	28.7	5.4	16.8	89.9	97.5	16.5	26.1	150.3	2.8	15.2	10.3	2.8
HWP	18–22	405–560	295–460	69–75	21–44	4–11	11–28	58–133	85–120	15–22	22.5–31.9	135–200	2.4–4.1	11.5–20.2	7–17	2.0–3.7
	20.1	478.5	343.5	71.7	31.7	6.8	17.5	86.9	102.3	17.8	27.1	161.5	3.0	15.4	11.2	2.9
LA	17–21	360–470	225–345	68–77	21–33	3–8	12–17	67–94	75–105	14–19	21.2–29.4	105–160	2.4–3.5	12.4–19.0	9–13	2.0–3.7
	19.5	398.5	287	72.0	27.0	5.4	15.2	77.9	88.3	16.3	24.6	134.3	3.0	14.9	9.8	2.8
MS	16–22	345–450	240–325	68–76	16–31	2–9	10–18	56–100	65–110	14–19	21.4–27.5	90–150	2.8–3.5	13.3–20.7	7–12	2.4–4.1
	18.8	392.5	282.5	72.0	26.9	6.0	14.9	79.3	88.5	16.4	24.1	132.0	3.0	15.1	10.1	2.7
NC	17–21	345–450	275–335	67–74	22–34	3–9	12–17	63–88	80–100	14–18	22.2–28.1	100–165	2.3–4.0	11.7–15.7	8–11	2.4–3.7
	19.4	400.3	284.3	71.0	29.2	5.6	14.6	75.6	88	16	25.1	129.8	3.1	13.8	9.7	3.0
SC	18–21	380–465	275–335	69–74	22–34	3–12	14–21	67–105	80–100	14–17	23.8–31.0	115–155	2.8–3.5	13.3–20.7	8–11	2.4–4.1
	19.5	410	292	71.2	29.4	6	16.5	84.8	92.1	15.5	26.5	130.3	3.2	14.1	9.7	3.0
TX	18–21	345–430	240–305	69–74	18–33	3–7	12–20	60–111	80–100	14–17	21.5–27.0	120–165	2.5–3.4	11.7–19.2	7–11	2.5–3.7
	19.2	395	282.5	71.5	27.4	5.3	15.8	82.6	90	15.9	25.0	131.8	3.0	14.6	9.8	2.8

[a] h: length of hyaline portion of the tail.
[b] DEG: position of dorsal oesophageal gland orifice.
[c] O: (dorsal oesophageal gland orifice to stylet base distance/stylet length) × 100.

TABLE 4. Comparison of values found in this study for reniform nematode populations from the United States with the lowest and highest values found in published data for reniform nematode immature females.

	Published measurements		Measurements in this study	
Character	Minimum value Location (reference)	Maximum value Location (reference)	Minimum value Location[a]	Maximum value Location[a]
Body length (μm)	291.7 Arkansas, U.S. (Robbins, 1994)	514 India (Sivakumar & Seshadri, 1971)	345 ARM, MS, TX, NC	560 HWP
Stylet (μm)	11.6 Brazil (Soares et al., 2003)	25.4 Brazil (Soares et al., 2003)	16 ARM, MS	22 HWP, MS
Vulva (%)	55 Cape Verde Islands (Germani, 1978)	84 Brazil (Soares et al., 2003)	66 ALH, ALL	78 ALL
Tail length (μm)	19 Hawaii, U.S. (Dasgupta et al., 1968)	38 Cape Verde Islands (Germani, 1978)	16 ARM, MS	44 HWP
h (μm)	4 Madagascar (Germani, 1978) Hawaii, U.S. (Dasgupta et al., 1968)	12 Sudan & Cape Verde Islands (Germani, 1978)	2 ARM	12 SC
EP (μm)	62 Cape Verde Islands (Germani, 1978)	102 Cape Verde Islands (Germani, 1978)	65 ARM, MS	120 HWP
a	19.3 Hawaii, U.S. (Linford & Oliveira, 1940)	37 Ethiopia (Germani, 1978)	20.3 FL, HWC	31.9 HWP
b	2.1 Cape Verde Islands (Germani, 1978)	4.3 Hawaii, U.S. (Dasgupta et al., 1968)	2.3 NC	4.1 HWP
c	11 Senegal & Cape Verde Islands (Germani, 1978)	37 South Africa (Van der Berg, 1978)	10 ALH, ALL	22.8 ALH
c′	2.1 Martinique (Germani, 1978)	5.0 Cape Verde Islands (Germani, 1978)	2.0 ALL, HWP, LA	4.1 ARM, GA, MS, SC

[a] ALH: Huxford, Alabama; ALL: Limestone, Alabama; ARM: Mississippi Co., Arkansas; ARP: Pinebluff, Arkansas; FL: Florida; GA: Belckley, Georgia; HWC: Oahu, Hawaii (cowpea); HW: Oahu, Hawaii (pineapple); LA: Baton Rouge, Louisiana; MS: Glendora, Mississippi; NC: North Carolina; SC: St. Matthews, South Carolina; TX: College Station, Texas.

All amphimictic populations were identical for their ITS1 sequence, but several are distinguishable by their morphometrics and(or) host preferences. Both populations from Hawaii tend to be larger and more variable in size (Figs. 1–3) and did not reproduce well in our greenhouse tests (Figs. 4–6). The population from Limestone, Alabama, (ALL) tends to be more uniformly small (Figs. 1–3) and was different from all others—even the population from Huxford, Alabama, (ALH)—by its high level of reproduction on Braxton soybean (Fig. 4). ARP tends to have smaller immature females (Fig. 2) and high and variable reproduction on Braxton and Deltapine 50 (Figs. 4, 6). The populations from Georgia and Texas are not distinguishable morphometrically (Figs. 2, 3), but their host preferences are distinct, as evidenced by the ability of GA to better reproduce on Forrest soybean (Fig. 5) and the marked preference for cotton of TX (Fig. 6).

DISCUSSION

The notably larger size (body length > 450 μm, see reference values in Table 5) of several individuals in the Hawaiian populations and, less frequently, in some of the continental populations has been reported from the Cape Verde Islands (Germani, 1978), India (Siva-

TABLE 5. Comparison of morphometrics of type, neotype, topotype populations of *Rotylenchulus reniformis* with the populations from cotton-growing locations in the United States used in this study.

	Type (Linford & Oliveira, 1940) Hawaii, U.S. (*n* = ?)	Neotype (Dasgupta et al., 1968) Hawaii, U.S.	Topotypes (Dasgupta et al., 1968) Hawaii, U.S. (*n* = 26)	Populations U.S. (*n* = 260)
Body length (μm)	376 (321–432)	400	340–420	345–560
Stylet (μm)	18.8 (16–20)	16	16–18	16–22
Vulva (%)	72	72	68–73	66–78
Tail length (μm)	–	24	19–26	16–44
h (μm)	–	6	4–8	2–12
EP (μm)	–	77	73–90	65–120
O%	–	82	81–106	56–135
A	19.3	24	22–27	20.3–31.9
B	3.1	3.8	3.6–4.3	2.3–4.1
C	15.0	16	14–17	10–22.8
c′	–	2.9	2.6–3.4	2.0–4.1

TABLE 6. Correlations between the first two canonical variables and the morphometric parameters of *Rotylenchulus reniformis* immature females. The larger the standardized coefficient, the greater the contribution of the respective variable to the discrimination between groups.

Variable	Canonical variable 1	Canonical variable 2
Stylet length	0.293	–0.427
Body length	0.808	0.178
Position of vulva	0.748	0.247
Tail length	0.319	–0.110
Hyaline portion of tail	0.156	0.143
Dorsal oesophageal gland	0.153	0.370
Excretory pore	0.702	–0.102
Maximum width	0.480	–0.298
Oesophagus	0.622	0.474
Anal width	0.377	–0.234

kumar and Seshadri, 1971), and the United States in Florida (Lehman and Inserra, 1989) and Arkansas (Robbins, 1994). Nakasono (1983) compared a population from Hawaii with Japanese isolates and placed it along with the larger-sized Japanese reniform nematodes, consistent with our observation of higher frequency of larger sizes in the populations from Hawaii.

In the host preference tests, the low reproductive indices of the Hawaiian populations on cotton and soybean can alternatively be an indication of suboptimal environmental conditions for these nematodes in our greenhouse. Reproduction tests can be highly variable (Riggs et al., 1988; Zhang et al., 1998), and many factors can alter the expression of a genotypic trait. Differences in the frequencies of phenotypes, accounting for the statistical differences when comparing means, are probably a result of selection. Reproduction tests in a greenhouse can be misleading when used as a means to characterize populations because of the selection process that transfer and adaptation from a host in the field to a different species/cultivar in the greenhouse entails (Viney, 2001). Nevertheless, whether the differences are related to differential response to the host or to the environment, the differences reflect probable

TABLE 7. Correlations between the first two canonical variables and the morphometric parameters of *Rotylenchulus reniformis* adult males. The larger the standardized coefficient, the greater the contribution of the respective variable to the discrimination between groups.

Variable	Canonical variable 1	Canonical variable 2
Stylet length	0.388	0.013
Body length	0.586	0.682
Spicule length	0.243	0.398
Tail length	–0.067	0.467
Hyaline portion of tail	–0.139	0.074
Excretory pore	0.647	0.046
Maximum width	0.167	0.459
Oesophagus	0.377	0.405
Anal width	0.591	–0.092

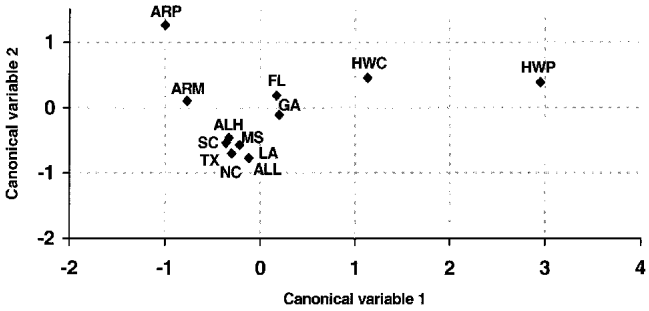

FIG. 2. Plot of the means of the first two canonical variables for morphological data of the immature females of *Rotylenchulus reniformis* populations. Correlations with the original variables are presented in Table 6.

genetic differences in the Hawaiian populations. The development of DNA-based markers (e.g., microsatellites, AFLP markers) for direct assay of genotypes would provide a more reliable way to evaluate populations.

Even though we detected clear differences among populations, these populations do not constitute fixed discrete infraspecific categories. The frequency of occurrence of the different phenotypes (body sizes or host preference) conforming the populations during consecutive cycles needs to be observed to assess the stability of this distribution with respect to a normal distribution of phenotypes in the population. Selection, defined as the differential survival of phenotypes when exposed to specific environmental conditions (Caswell and Roberts, 1987), determines the frequency of phenotypes in a population by acting on genotypes. Our results show an initial indication of the variability present within the species in the United States. Still, diversity within populations needs to be further characterized.

The extensive overlapping of morphological and reproductive data as well as the lack of genetic differentiation in the ITS region suggest there is no geographic component to the variability of populations in the cotton-growing areas. Heald and Robinson (1990) found no consistent relationship between the presence of reniform nematode in different regions in the United States and soil texture, soil pH, rainfall, or irrigation regime. Considering there is no major geographical

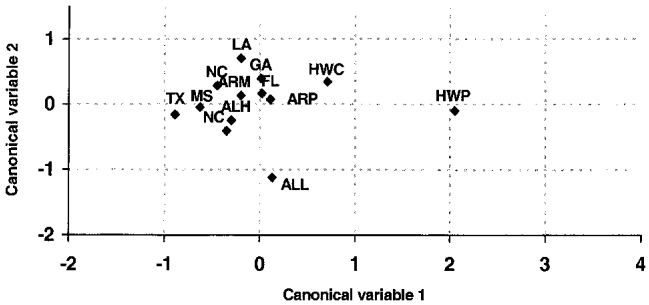

FIG. 3. Plot of the means of the first two canonical variables for morphological data of the males of *Rotylenchulus reniformis* populations. Correlations with the original variables are presented in Table 7.

TABLE 8. Mean reproductive indices (RI = final population/initial population) of the *Rotylenchulus reniformis* populations on soybean cv. Braxton, soybean cv. Forrest, and cotton dv. Deltapine 50, in descending order.

Soybean cv. Braxton		Soybean cv. Forrest		Cotton cv. Deltapine 50	
Population	RI	Population	RI	Population	RI
ALL	62.1a	GA	8.4a	TX	55.7a
ARP	38.5b	LA	4.1b	ARP	33.5b
TX	30.3b	ARP	2.9b	LA	18.5bc
MS	29.3b	ALL	2.5b	MS	14.9bc
GA	26.7b	MS	2.1b	FL	13.5bc
ARM	22.0bc	FL	1.7b	ARM	9.4bc
FL	13.1bcd	ARM	1.7b	ALL	8.7bcd
SC	12.8bcd	SC	1.3bc	SC	5.6bcd
HWP	11.4bcd	HWC	0.8bcd	NC	4.1bcd
NC	8.7bcd	TX	0.8bcd	GA	1.2bcd
LA	6.1bcd	NC	0.6bcd	HWC	0.8bcd
HWC	3.5bcd	ALH	0.5bcd	ALH	0.4bcd
ALH	2.8bcd	HWP	0.5bcd	HWP	0.3bcd

Data are means of 10 replications. Means followed by the same letter in each column are not significantly different (LSD, $P \leq 0.05$).

barrier to restrict passive transport by human activities among the cotton-growing locations in the southeastern United States, we hypothesize that *R. reniformis* is either a reproductively congruent population or, more likely, represents recent, very rapid radiation from a common origin. Implicit in the latter scenario is the imposition of a genetic bottleneck resulting from a limited number of introductions. Lack of genetic differentiation among populations also has been observed for soybean cyst nematode, another widespread plant-parasitic nematode introduced to the United States. *Heterodera glycines* exhibits high variability within populations in the United States, and shifts in genetic structure of field populations can be monitored in response to host selection pressures (Niblack et al., 2002). However, attempts to characterize differences between races or geographic populations with neutral genetic markers may yield no differences (Sui et al., 1999). Similarly, in France, 94.6% of the genetic variability observed in *Heterodera schachtii* is within fields, and there is a low

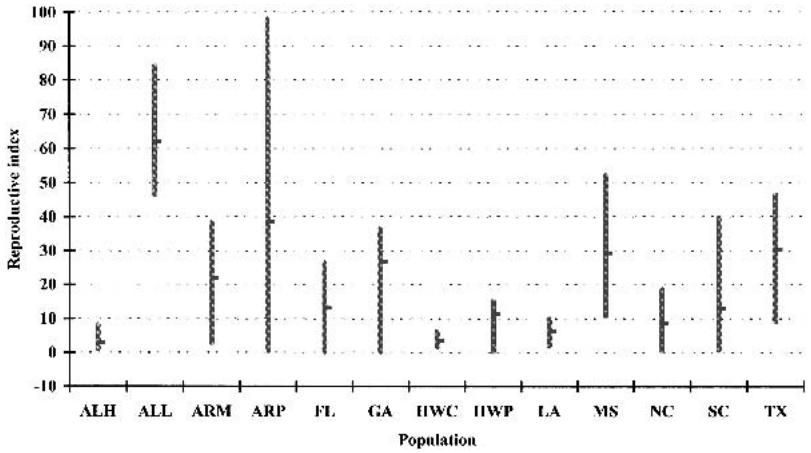

FIG. 4. Ranges and means of reproductive indices of *Rotylenchulus reniformis* populations on soybean cv. Braxton. Vertical bars indicate ranges ($n = 10$); horizontal bars indicate mean values.

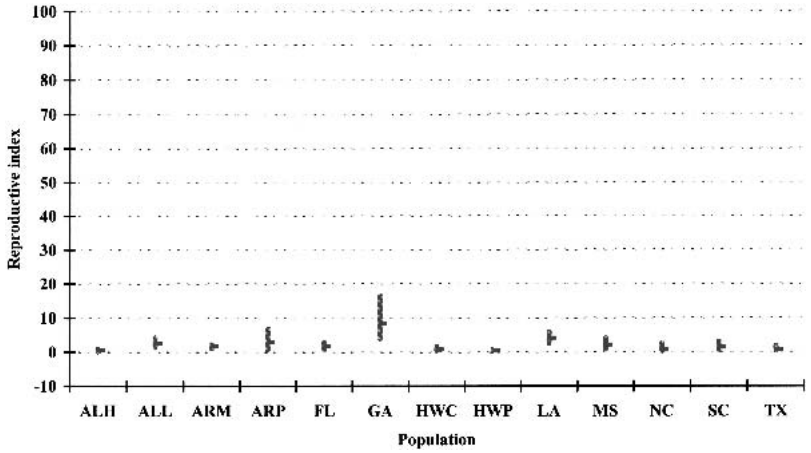

FIG. 5. Ranges and means of reproductive indices of *Rotylenchulus reniformis* populations on soybean cv. Forrest. Vertical bars indicate ranges ($n = 10$); horizontal bars indicate mean values.

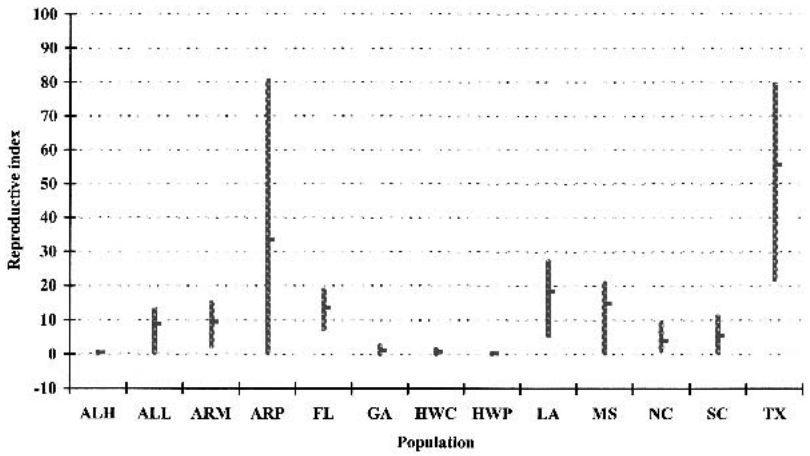

FIG. 6. Ranges and means of reproductive indices of *Rotylenchulus reniformis* populations on cotton cv. Deltapine 50. Vertical bars indicate ranges (n = 10); horizontal bars indicate mean values.

differentiation of populations among fields and regions (Plantard and Porte, 2004), suggesting significant gene-flow at these spatial scales.

The variability of the ITS1 region has been regarded as useful for the study of intraspecific variation in several plant-parasitic nematodes (Blok et al., 1998; Ibrahim et al., 1994; Zheng et al., 2000). In some species this marker has been considered too variable to be used for determining the relationships among nematode populations, presenting polymorphisms within individual nematodes (De Ley et al., 1999; Hugall et al., 1999). The fact that no polymorphisms are present in the ITS region of reniform nematode from 20 locations in the United States, as well as four other countries, could suggest that the genetic variants present within the populations probably diverged very recently from a relatively small base of origin. However, this needs to be confirmed by further phylogenetic analyses including other markers informative at the population level (e.g., micorsatellites, mitochondrial markers, other nuclear markers).

The Japanese parthenogenetic reniform nematode, distinct in its ITS1 sequence, was originally described as a different species, *Rotylenchulus nicotiana* (Nakasono and Ichinohe, 1967), and later synonymized (Dasgupta et al., 1968) with *R. reniformis*. Dasgupta et al. (1968) did not give taxonomic significance to the absence of males and suggested it was affected by environmental factors. Nakasono (1983) later established that frequency in male occurence was not affected by environmental changes but rather was determined genetically, and proved parthenogenetic populations were reproductively isolated from the amphimictic.

Studies including populations of *R. reniformis* from other continents and other species in the genus are needed to elucidate the origin, evolution, and history

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A    1    GGTAG CTGTA GGTGA ACCTG CTGCT GGATC ATTAC ACGTG ATTCT CACAT
P    1    GGTAG CTGTA GGTGA ACCTG C---T GGATC ATTAC ACGTG ATTCT CACAT

A   51    TCACC ACCTA TCTGT GTTGT ATAAA TGTTC CCCGA ACCTT GAAAC ATCCA
P   51    TCACC ATCTA TCTGT GT-GT AGATA TTGTC CCCCG AACTT GAAAC ATCCA

A  101    GTTTA AAGTG GCTGT TCGCC ACACT AACAA ACCGT TTAAA CCAGG GGGAC
P  101    GTTTA AAGTG GCTGT TCGCC ACACT AACAA ATCGT TTAGA TCAGG GGGAC

A  151    ACTGA TCAAA AACGG CTGCG CTGGC GTCTC TGCGT TGTTG AGCAG TTGTT
P  151    ACAAA A-ACC AACGG CTGCG CTGGC GTCTC TGCGT TGTTG AGCAG TTGTT

A  201    GCAT- -TGCT A-ATG TGCTA CGTCC GTGGC TGTGA TGAGA CAACG CGTTA
P  201    GCATG CCATC ATATA TGCTA CGTCC GTGGC TGTGA TGAGA CAACG CGGTA

A  251    GGACT GCG-- TACCT TGTTA GTCTG GTATG CGGTT TAAGA CTCAA TGAGC
P  251    GGACT GCACA TACCT TGCCA CTCTG GTTTG CGGTT TAAGA CTCAA TGAGC

A  301    GCCCT GGTTT GGCGC CGCCA GCATT TTTTT TCAAA CAAAA ACTTT TTA--
P  301    GCCCT GGTTT GGCGC CGCCA GCATT ATTTT TTCAT TAAAA ACTTT TTAAC

A  351    CAAAA AAAAC AAAAT TCTAG TCTTA TCGGT GGATC ACTCG GCT
P  351    CAAAA AAAAC AACAT TCTAG TCTTA TCGGT GGATC ACTCG GCT
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FIG. 7. Nucleotide sequence of the amplified ITS1 region of *Rotylenchulus reniformis* populations including portions of the 18S gene and 5.8S gene. Ribosomal gene sequences are in italics, and primer sequences are underlined. Gaps in the sequence alignment are noted by dashes. Bold letters indicate differences between amphimictic (A) and parthenogenetic (P) populations. Sequence A represents all amphimictic populations in Table 1, and sequence P represents the parthenogenetic population examined (JP).

of dissemination of this nematode. Additionally, methods must be devised to identify genetic variants within populations to enable the monitoring of shifts in the structure of populations and the design of durable management strategies.

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