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Esterase Allozymes of Soybean Cyst Nematode, *Heterodera glycines*, from China, Japan, and the United States

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Abstract: Individual females from 19 populations of *Heterodera glycines* from China, Japan, and the United States were analyzed for esterase allozyme polymorphism. Eight esterase electrophoretic phenotypes were resolved. Four putative loci, *est-1, est-2, est-3,* and *est-4,* were identified, having one, one, two, and one allele, respectively. The four loci expressed six genotypes in the four Chinese populations. Loci *est-2, est-3,* and *est-4* were identified in five Japanese populations and expressed five genotypes, whereas only loci *est-2* and *est-3* were identified in 10 populations from the United States and expressed four genotypes. Putative alleles at each locus were defined as characters for data analysis. Phylogenetic analysis using parsimony (PAUP) was utilized to determine relationships among the 19 populations. More loci and alleles in populations from China and phylogenetic similarities among populations from Japan and the United States are consistent with a founder effect resulting from dissemination of progenitor *H. glycines* from China to Japan and subsequent introductions of founder populations from Japan to the United States.

Key words: allozyme, biogeography, colonization, esterase polymorphism, founder effect, *Heterodera glycines,* parsimony, phylogenetic analysis, soybean cyst nematode.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, was first observed in China ca. 1880 and at approximately the same time in Japan (Noel, 1992). In 1955 the first occurence of the nematode in the United States was reported in North Carolina (Winstead et al., 1955). Few electrophoretic studies have been done with *H. glycines,* and most have investigated interspecific and intraspecific relationships (Dickson et al., 1970; Dickson et al., 1971; Esbenshade and Triantaphyllou, 1988; Ishibashi, 1970; Pozdol and Noel, 1984; Radice et al., 1988). Radice et al. (1988) examined enzymes in several populations of *H. glycines* including representatives from Asia and the United States and resolved activity in 20 of 42 enzymes investigated. Of those 20 enzymes, 10 were polymorphic, but only esterase and acid phosphatase were resolvable for individual females. Esbenshade and Triantaphyllou (1988) found a population of *H. glycines* from North Carolina polymorphic for esterase and investigated the inheritance of esterase in highly inbred lines developed from that population. Our objective was to investigate esterase allozyme polymorphisms and relationships among populations of *H. glycines* from China, Japan, and the United States to determine whether a founder event (Carson and Templeton, 1984; Mayr, 1970; Templeton, 1980) occurred and thus provide information on the geographic origin of the nematode.

Materials and Methods

Populations of *H. glycines* from China, Illinois, and Tennessee were collected by the first author. Populations from Japan were obtained courtesy of Dr. Haruo Inagaki, and those from North Carolina were kindly provided by Dr. D. P. Schmitt. The origin of the populations and their acronym are provided in Table 1. Nematodes were maintained on cv. Lee 68 soybean (*Glycine max* (L.) Merr.) in greenhouse cultures. Individual females were selected from roots, placed in 0.05 M phosphate buffer, pH 7.2 on ice. Females were crushed individually on a 2-mm × 2-mm piece of filter paper moistened with phosphate buffer and placed on a polyacrylamide isoelectric focusing gel (pH 4.0–6.5).

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TABLE 1. Esterase phenotypes expressed in 19 populations of *Heterodera glycines* from China, Japan, and the United States.

Country and population	Phenotype							
	1	2	3	4	5	6	7	8
China								
Heilongjiang (HLJ)				+	+	+		
AnDa, Heilongjiang (HLJ1)			+	+	+			
BaoQing, Heilongjiang (HLJ2)				+	+	+	+	+
Harbin, Heilongjiang (HLJ3)	+	+		+		+		
Japan								
Hokkaido (HKO)					+	+		
Hokkaido 2 (HKO2)					+	+		
Hokkaido 3 (HKO3)						+		
Nagano (NGO)	+			+				
Tokkaido (TKO)						+		
United States								
Illinois 1 (IL1)				+		+		
Illinois 2 (IL2)				+	+	+		
Illinois 3 (IL3)				+				
Illinois 4 (IL4)				+				
Illinois 5 (IL5)						+		
North Carolina 1 (NC1)						+		
North Carolina 2 (NC2)				+		+		
North Carolina 14 (NC14)						+		
Tennessee 3 (TN3)				+	+			
Tennessee 4 (TN4)				+	+			

Proteins were separated using a Phastsystem (Pharmacia, Inc., Piscataway, NJ). Esterase (EC 3.1.1.-) allozymes were visualized using alpha-naphthyl acetate as the substrate, which resolves carboxylesterase, the major soluble esterase found in *H. glycines* (Esbenshade and Triantaphyllou, 1986; Murphy et al., 1990; Noel and Mayasich, 1991). Phenotypes were assigned to the electromorphs (May, 1992), and the reference electromorphs from IL3 were assigned the value "80" (*est-3**80) and "100" (*est-2**100). Loci were numbered beginning with "1" for the fastest migrating (anodal) locus. On all gels, an IL3 female was placed on each of the two outside lanes.

Each putative genotype was considered as an independent character for data analysis. Genotypes either shared by at least two populations or that did not occur in all populations were considered informative for phylogenetic inference. Thus, of the six putative genotypes, only four were analyzed (Table 2). A data matrix was compiled using these independent characters for the 19 populations comprised of 169 individuals from the three countries (Sneath and Sokal, 1973). Statistical analysis was performed using phylogenetic analysis using parsimony (PAUP) version 3.1 to determine relationships among the four informative genotypes and the 19 populations (Swofford, 1993). An exhaustive search was performed, and a 50% majority consensus tree was obtained (Swofford and Olsen, 1990). Pairwise distances among the 19 populations were calculated. Since Chinese population HLJ2 represented the most diversified genotypes, it was used as the outgroup for analysis (Swofford, 1993).

RESULTS

Electrophoresis resolved distinct electromorphs of esterase phenotypes (EEP) for populations of *H. glycines* from China compared to those from Japan and the United States (Fig. 1). Eight EEPs were identified for 169 individual females of *H. glycines* representing 19 populations from the three countries (Figs. 1,2A; Table 1). All eight EEPs were resolved in the populations from

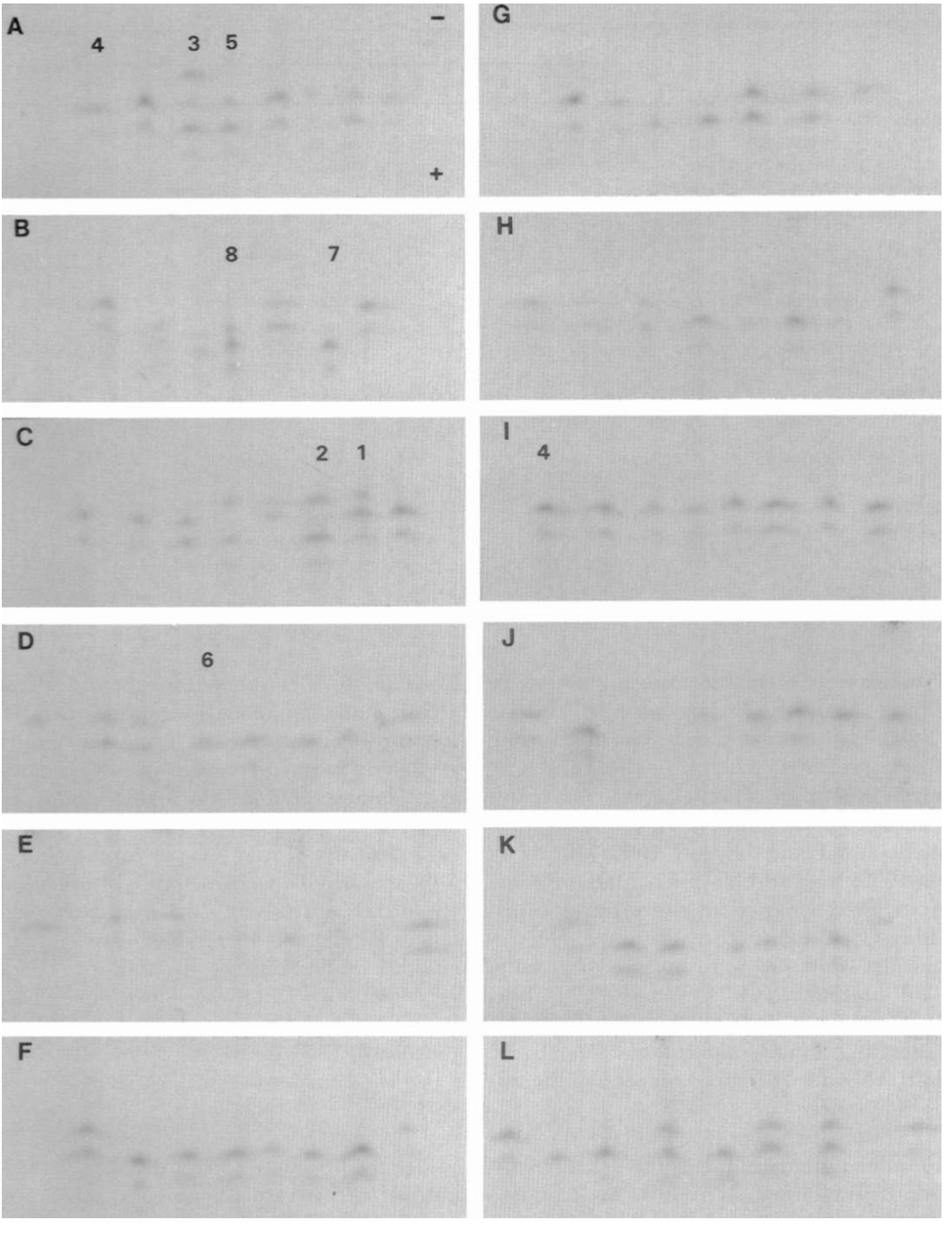

FIG. 1, A–L. Representative electrophoretic gels illustrating esterase phenotypic diversity of *Heterodera glycines* from China (A–C), Japan (D–F), and the United States (G–L). Numbers above a lane indicate a female representing that particular phenotype. See Table 1 for phenotypes expressed in different populations. Each outside lane on a gel is the electromorph reference (*est-3**80 and *est-2**100) expressed by an Illinois 3 female (phenotype 4). A = AnDa, B = BaoQing, C = Harbin, D = Hokkaido 2, E = Nagano, F = Tokkaido, G = Illinois 1, H = Illinois 2, I = Illinois 3, J = North Carolina 2, K = North Carolina 14, and L = Tennessee 3.

TABLE 2. Putative esterase genotypes represented in 19 populations of *Heterodera glycines* from China, Japan, and the United States.

Population[a]	Genotype					
	1	2	3	4	5	6
HKO, HKO2, TN3, TN4	–	–	+	+	+	–
HKO3, IL5, NC1, NC14, TKO	–	–	–	+	+	–
HLJ, IL2	–	+	+	+	+	–
HLJ1	+	+	+	–	+	–
HLJ2	–	+	+	+	+	+
HLJ3	+	+	–	+	+	–
IL1, NC2	–	+	–	+	+	–
IL3, IL4	–	+	–	–	+	–
NGO	+	+	–	–	+	–

[a] Acronym and origin of populations are provided in Table 1.

China. Each EEP was represented by more than one individual except for EEP 3 and EEP 7, which were found in the AnDa (HLJ1) and BaoQing (HLJ2) populations, respectively. Each of the four populations from China expressed three to five EEPs. Four EEPs were resolved in the populations from Japan. Two of the Japanese populations, HKO3 and TKO, expressed only EEP 6, whereas HKO and HKO2 expressed EEP 5 and EEP 6. Population NGO expressed EEPs 1 and 4 and was the only population other than HLJ3 from China to express EEP1. Only EEP 4, EEP 5, and EEP 6 were resolved in populations from the United States. In 5 of 10 populations from the United States, IL3, IL4, IL5, NC1, and NC14, only one EEP was resolved. Four populations, IL1, NC2, TN3, and TN4, expressed two EEPs. Population IL2 expressed EEP 4, EEP 5, and EEP 6.

Among the eight EEPs, six putative genotypes and five allelic products were identified (Fig. 2B). Genotype 1 was identified as a monomer with one allele (*est4**100). Genotypes 2, 3, and 4 were identified as a dimer with two alleles (*est3**80, *est3**100, and *est3**120). Genotype 5 (*est-2*) and genotype 6 (*est-1*) also were identified as monomers. Among 46 individuals representing the four populations from China, the six genotypes were expressed by the four putative loci. Five genotypes were observed among 32 individuals representing five populations from Japan and were expressed by loci *est-2, est-3,* and *est-4*. Among 91 individuals in 10 populations from the United States, four genotypes were expressed by loci *est-2* and *est-3*.

Among the six putative genotypes, the allelic products *est-4**100, *est-3**80, *est-3**100, and *est-3**120, which comprised genotypes 1, 2, 3, and 4, were considered as informative as they either were represented by more than one population or were not found in all populations (Table 2). Thus, genotype 5 found in all populations and genotype 6 found only in HLJ2 from China were not included. An exhaustive search was conducted using PAUP, and 135,135 trees were evaluated. The 30 shortest trees, each with a length of seven steps and a consistency index (CI) of 0.571, were resolved. A 50% majority consensus tree (length = 7; CI = 0.571) was determined (Fig. 3), and pairwise mean and absolute distances also were calculated (Table 3). The analysis demonstrated that HLJ2 was most closely related to HLJ and IL2. Populations HLJ3, HLJ1, NGO, IL3, IL4, IL1, and NC2 comprised a group more closely related to each other than to the two groups—HKO, HKO2, TN3, and TN4, and HKO3, TKO, IL5, NC1, and NC14—both of which consisted only of populations from Japan and the United States.

DISCUSSION

Allozymes are gene products that provide molecular markers for analyzing single locus variation in populations (Bandoni et al., 1995; Emberton, 1995; May, 1992; Sahuquillo and Lumaret, 1995; van der Bank,

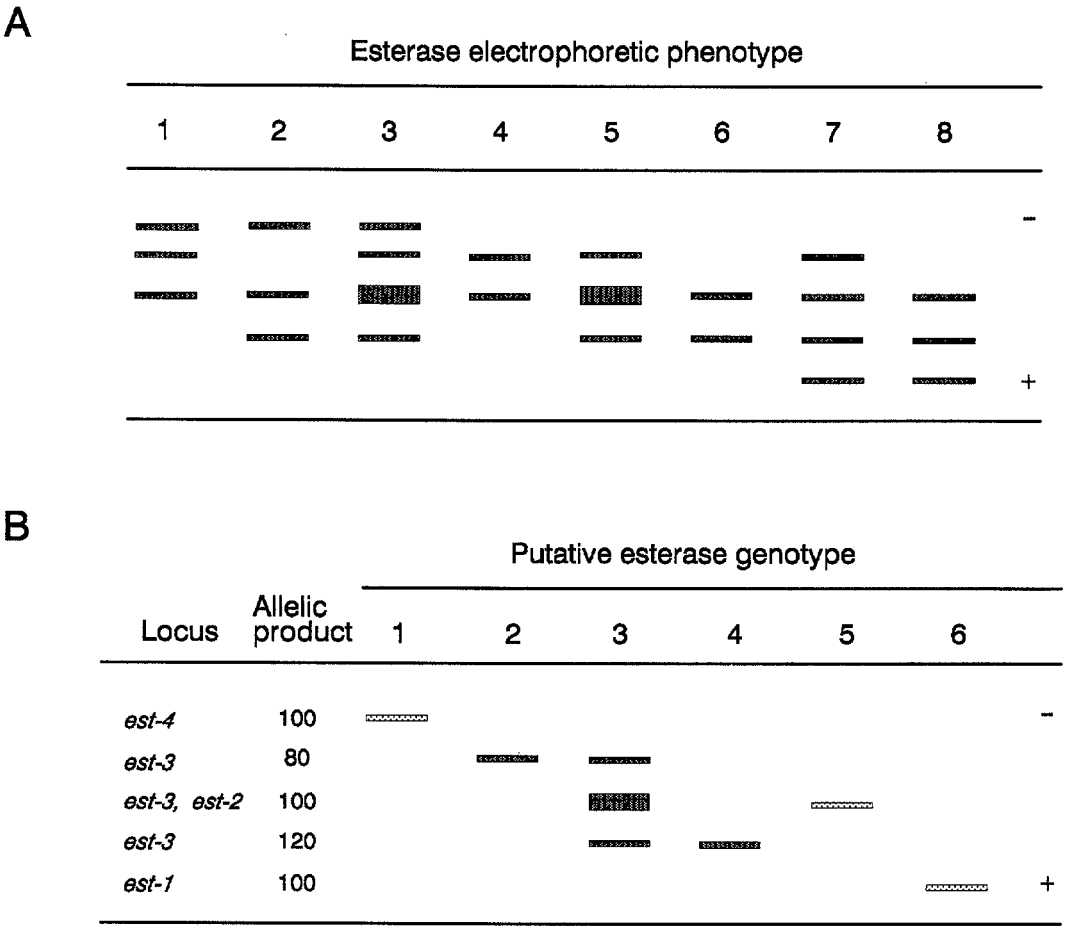

FIG. 2. Electrophoretic phenotypes (A) and putative genotypes (B) of esterase for 19 populations of *Heterodera glycines* from China, Japan, and the United States.

1994). Genetic analysis of several allozymes from individuals is desirable (Murphy et al., 1990), but the small size of an individual female of *H. glycines* prevents analysis of several enzyme systems of one individual. However, in some organisms several enzymes can be resolved, but the percentage of polymorphic loci is highly variable ranging from 15% for birds to 60% for marine invertebrates. Some organisms are similar to *H. glycines* in that they are highly polymorphic for esterase, and those polymorphisms have provided valuable allelic frequency data (Avise and Selander, 1972; Baker and Moeed, 1987; Gorman et al., 1975; Hunt and Selander, 1973; Klautau et al., 1994; St. Louis and Barlow, 1988). Our sample sizes were within accepted ranges (Archie et al., 1989; Gorman and Renzi, 1979; Karakousis and Kyriakopoulou-Sklavounou, 1995). Radice et al. (1988) investigated isozymes of several populations of *H. glycines* and were able to resolve activity for acid phosphatase and esterase in single individuals. Acid phosphatase also was resolved in preliminary work done during the research reported herein (data not shown). Radice et al. (1988) did not observe the level of polymorphism for esterase reported herein. This is not surprising, given the advances in instrumentation and availability of ultra-thin gels, which permit routine electrophoresis of individual females of cyst and root-knot species. Coefficients of similarity were higher than reported herein (Radice et al., 1988). Similarities among populations from Asia

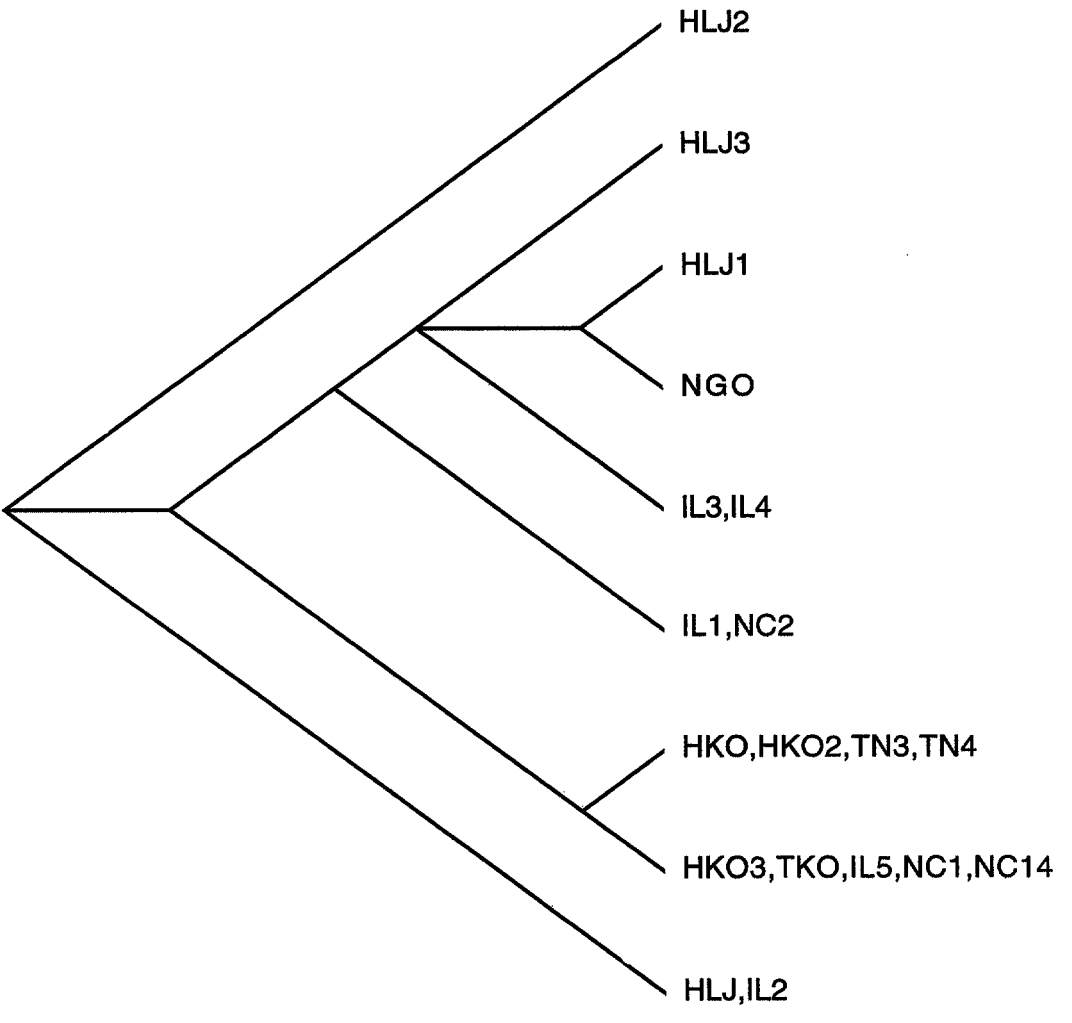

FIG. 3. A 50% majority consensus tree (length = 7; consistency index (CI) = 0.571) derived from 30 equal shortest trees (length = 7; CI = 0.571) using PAUP to determine phylogenetic relationships based on putative esterase genotypes among 19 populations of *Heterodera glycines* from China, Japan, and the United States. Acronyms and origin of populations are provided in Table 1.

and the United States reported for that research were due in part to the inability to resolve enzymatic polymorphism of individual females, thus necessitating electrophoresis of mass homogenates of several individuals. Electrophoresis of homogenates of several females would preclude genotypic data to calculate similarity indices. Esbenshade and Triantaphyllou (1988) investigated the genetic basis of esterase polymorphism in highly inbred lines of *H. glycines* and reported three alleles expressed by a single esterase locus. They reported that the esterases probably were monomeric in spite of a unique double banding pattern, and that the two bands probably resulted from two proteins produced by the same gene rather than two genes located close together. The latter is more common in allozyme analysis and reported herein. We report the existence of three monomers and a dimer in our populations (May, 1992).

Heterodera glycines was reported for the first time in the United States in North Carolina (Winstead et al., 1955). Since that time, nematologists have differed in their opinion of the origin of *H. glycines* in the United States. Some believe that the nematode is

TABLE 3. Pairwise mean distances and absolute distances based on informative esterase genotypes of allelic characters for 19 populations of *Heterodera glycines* from China, Japan, and the United States.

Population(s)[b]	Pairwise distance[a]								
	1	2	3	4	5	6	7	8	9
1 HLJ2	–	0.50	0.00	0.50	0.25	0.25	0.50	0.50	0.75
2 HLJ3	2	–	0.50	0.50	0.75	0.25	0.50	0.50	0.25
3 HLJ, IL2	0	2	–	0.50	0.25	0.25	0.50	0.50	0.75
4 HLJ1	2	2	2	–	0.75	0.75	1.00	0.50	0.25
5 HKO, HKO2, TN3, TN4	1	3	1	3	–	0.50	0.25	0.75	1.00
6 IL1, NC2	1	1	1	3	2	–	0.25	0.25	0.50
7 HKO3, IL5, NC1, NC14, TKO	2	2	2	4	1	1	–	0.50	0.75
8 IL3, IL4	2	2	2	2	3	1	2	–	0.25
9 NGO	3	1	3	1	4	2	3	1	–

[a] Above diagonal are mean distances with 1.00 = most distant; below diagonal are absolute distances with 4 = most distant.
[b] Acronym and origin of populations are provided in Table 1.

indigenous to North America and is derived from an ancient ancestor once widespread in Asia and North America (Ferris et al., 1985). An alternate hypothesis is that *H. glycines* has a more recent history, having been imported in soil from Japan in the late 1800s and early 1900s to provide inoculum of *Bradyrhizobium japonicum* (Kirchner) Jordan for nodulation of soybean (Noel, 1992). The data reported herein support a founder effect (Bonnell and Selander, 1974; Carson and Templeton, 1984; Easteal, 1985; Grant and Grant, 1995; Hunt and Selander, 1973; Mayr, 1970; Prakash et al., 1969; Sytsma and Schaal, 1985; Templeton, 1980), with colonizing *H. glycines* in Japan having arisen from a progenitor ancestral line in China. Founder events result in reduced heterozygosity and reduced genetic variability, including loss of rare alleles (Avise and Selander, 1972; Baker and Moeed, 1987; Gorman et al., 1975; Selander, 1976; St. Louis and Barlow, 1988). All genotypes and rare alleles were found among only four populations from China. No new alleles or genotypes were found among larger numbers of populations and individuals from Japan and the United States. Requirements of PAUP precluded analysis of the rare allele found in genotype 6 in Chinese population HLJ2 since that allele was not shared with any other population. Thus, the inability to utilize the rare allele minimized the diversity of Chinese populations indicated in the consensus tree and the pairwise mean and absolute distances. In particular, the analysis indicated that HLJ2 was identical to HLJ and IL2. Population IL2 was unique in that it was the only population from Japan and the United States to express both alleles for *est-3*. With sufficient evolutionary time, those populations that become isolated may, through mutation, regain heterozygosity and rare alleles found in the progenitor population. The similarities among populations from Japan and the United States indicate that infestations in the United States probably originated from several colonizations from Japanese sources. The hypothesis of Chinese origin and movement of *H. glycines* to Japan and subsequent introduction into the United States is consistent with the origin of the soybean and its domestication (Hymowitz, 1970). The soybean is believed to have originated in central and northern China. Domestication first occurred in northeast China about 1100 B.C. in what is now known as Heilongjiang Province. Cultivated soybean was introduced into Japan between 200 B.C. and 300 A.D. Soybean seed was imported into the United States frequently in the late 1800s and early 1900s, but plants resulting from those seeds were not nodulated because *B. japonicum* is not native to North America (Noel, 1992). In order for soybean in the United States to fix nitrogen, inoculum in the form of soil and potted plants growing in field soil was imported from Japan on several documented occasions (Noel, 1992). This soil was dis-

bursed to various experiment stations, and farmers subsequently spread soil from field to field as inoculum to nodulate soybean. The rapid "spread" of *H. glycines* throughout the United States following its discovery is explained by agricultural activities for several decades following the nematode's introduction prior to its discovery in North Carolina.

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