Genetic Analysis of Parasitism in the Soybean Cyst Nematode *Heterodera glycines*

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ABSTRACT

A genetic analysis of parasitic ability in the soybean cyst nematode *Heterodera glycines* was performed. To identify and characterize genes involved in parasitism, we developed three highly inbred *H. glycines* lines, OP20, OP25 and OP50, for use as parents for controlled crosses. Through these crosses, we have identified genes in the inbred parents that control reproduction of the nematode on hosts that carry resistance genes. These genes, designated as *ror-** for reproduction on a resistant host, segregate in a normal Mendelian fashion as independent loci. Host range tests of F_1 generation progeny indicated that at least one parasitism gene in both the OP20 and OP50 lines for host PI 88788 was dominant. Parasitism genes in OP50 for hosts "Peking" and PI 90763 are recessive. Two types of single female descent populations, a single backcrossed BC_1F_2-derived and a double backcrossed BC_2F_1-derived, were established on the susceptible soybean cultivar "Lee 68." Host range tests for parasitism in these lines demonstrated the presence of two independent genes in OP50, one for host PI 88788 designated *ror-1* and one for host PI 90763 designated *ror-2*. OP20 carries two independent genes for parasitism on PI 88788, designated as alleles *kr3* and *kr4*.

SOYBEAN cyst nematode (SCN) (*Heterodera glycines*) is one of the most devastating pests of soybean on a worldwide basis, accounting for most of the approximately $2.5 billion in annual crop loss due to nematodes (SASSER and FRECKMAN 1987; NOEL 1992). *Heterodera glycines* is a obligate cross fertile species and a sedentary endoparasitic plant nematode. The life cycle consists of six stages: the egg, four juvenile stages and the sexually dimorphic adults. The second stage juvenile (J2) is the infective form. After the J2 penetrates the root, it migrates to an area near the vascular cylinder, where it establishes a complex feeding site (JONES 1981; ENDO 1992). SCN males migrate out of the root for mating within 15–20 days after infection. The adult female nematode produces 200–400 eggs, which remain primarily in her swollen, hardened body, forming a cyst (TRIANTAPHYLLOU and HIRSCHMANN 1962). Each life cycle takes 25–30 days and there may be several generations per growing season. The nematode egg is able to survive in the cyst for a number of years under very harsh environmental conditions (ALSTON and SCHMITT 1988; YOUNG 1992).

The ability of a nematode species to parasitize a host is measured by reproduction. In general, resistant hosts do not permit the female nematodes to develop to reproductive maturity, and host resistance is manifested as a hypersensitive response (KIRALY 1980). Parasitic ability, therefore, is a qualitative trait that the nematode either possesses or not. In addition, nematode populations are often quantitatively described by different levels of aggressiveness. Aggressiveness is generally defined as the relative level of reproduction that occurs on a given host genotype.

The genetic basis of the nematode-host interaction is poorly characterized. In the case of the potato cyst nematode, (*Globodera rostochiensis*)-potato interaction, a gene-for-gene relationship appears to be in operation (JANSSEN *et al.* 1991). In this system, nematode genes for parasitism are recessive. Potatoes carrying the dominant *H1* gene are resistant to certain pathotypes of *G. rostochiensis*, but those nematodes carrying recessive parasitism genes can reproduce. Pure parasitic and nonparasitic lines of *G. rostochiensis* have been selected, and crosses using these lines have revealed that parasitism is inherited at a single locus in a recessive manner (JANSSEN *et al.* 1990, 1991). Results from reciprocal crosses suggested that there is no evidence for sex-linked inheritance of parasitism. The expected segregation patterns of 3:1 nonparasitic to parasitic combined with the dominant nature of the *H1* resistance gene suggest that this interaction functions in a classical gene-for-gene type of mechanism (JANSSEN *et al.* 1991).

Genetic variability in *H. glycines* was detected almost as soon as host resistance was identified (ROSS and BRIM 1957; MILLER 1970). Initially, four races of *H. glycines* were designated based on reproduction on four resistant host differentials: "Peking," "Pickett" (resistance in Pickett is derived solely from Peking), PI 88788 and PI 90763 (GOLDEN *et al.* 1970). A fifth race was soon added to this system (INAGAKI 1979), and presently 16 races of *H. glycines* are proposed (RIGGS and SCHMITT

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1988). Nematode reproduction on one of the host differentials is considered positive if it exceeds 10% of that observed on the fully susceptible cultivar "Lee." In essence, races of *H. glycines* are field populations that possess a number of genotypes (TRIANTAPHYLLOU 1975; LEUDDERS and DROPKIN 1983). Selection pressure by cropping resistant cultivars is likely to alter the frequency of alleles for parasitism and, therefore, the race designation. The race concept as it is applied to *H. glycines* is not based on genotype, but rather on the predominant phenotype encountered at a particular time (NIBLACK 1992).

Early genetic studies on *H. glycines* variability were mainly by directional selection experiments on the various host differentials. In these studies, selection on a resistant host resulted in a gradual increase in the ability of the nematode population to reproduce on that host (MCCANN *et al.* 1982; YOUNG 1982). However, selection on one resistant differential had no effect on the nematode population's ability to parasitize a different host. From these studies, it was supposed that multiple genes in the nematode were involved in parasitism of resistant cultivars and that these genes could be separated into three relatively independent groups (TRIANTAPHYLLOU 1975). These groups corresponded to the ability to parasitize PI 88788, PI 90763 and Peking/Pickett, respectively. Later tests combining a primary directional selection on a resistant cultivar with a secondary selection on a cultivar with different resistance genes have further complicated the issue. In these experiments, secondary selection resulted in increased parasitic ability on the secondary host, but a loss of parasitism on the primary host (LUEDDERS and DROPKIN 1983; LEUDDERS 1985). Although it may be argued that these data support the idea that different alleles at the same locus are responsible for parasitic ability, the lack of fixation of parasitism genes during primary selection casts doubts on that conclusion (TRIANTAPHYLLOU 1987).

Results from controlled crosses between various races of *H. glycines* have suggested that parasitic ability is inherited in a dominant fashion, but no specific ratios were observed (TRIANTAPHYLLOU 1975; PRICE *et al.* 1978). This was most likely because the populations used for these experiments represented a mixture of genotypes rather than pure strains.

Our laboratory maintains a substantial collection of SCN populations from multiple locations in the United States. In addition to our field populations, we have over 70 populations that have been selected repeatedly on certain resistant soybean hosts. Although many of these lines still maintain a degree of heterogeneity due to the limited number of selection cycles that have been imposed, we have developed several highly inbred lines. These lines have been repeatedly selected and inbred by single female nematode inoculation for many generations. In particular, we are working with three highly homozygous lines, OP20, OP25 and OP50, which have been inbred for a minimum of 29 generations. Isozyme analysis has demonstrated that these three lines are homozygous at the esterase and glucose phosphate isomerase loci (ESBENSHADE and TRIANTAPHYLLOU 1988). Genetic analysis of esterase pattern indicates that the three observable phenotypes correspond to three codominant alleles at a single locus. No maternal inheritance patterns were detected in these studies (ESBENSHADE and TRIANTAPHYLLOU 1988). Host range testing using the standard soybean differential genotypes reveals that these lines are highly specific in their parasitic abilities. Unlike the standard race concept, parasitism of a differential by one of the inbred lines is either positive or negative. No cysts develop on resistant soybean lines. In this article, we report on a genetic analysis of parasitism on resistant soybeans by these inbred SCN lines.

MATERIALS AND METHODS

Parental lines: Three full sib inbred SCN lines, OP25, OP20 and OP50, all originating from North Carolina field isolates, were used in these experiments. The OP25 line was inbred on Lee 68 for 48 generations, OP20 was inbred on PI 88788 and OP50 was inbred on PI 90763 for 30 and 29 generations, respectively, in the greenhouse. Each line was also fixed for a different specific homozygous esterase marker. The host ranges of these lines were tested in the greenhouse with six replications and 500 eggs per plant on hosts Lee 68, Peking, PI 88788 and PI 90763. This experiment was repeated twice. Esterase phenotypic markers were also tested from randomly picked females from the susceptible host Lee 68 and individually run on a Phastgel using the Phast Electrophoresis System (Phamacia Biotech Inc.).

Crosses and F_1 generation testing: The inbred lines, OP25, OP20 and OP50 were maintained on Lee 68, PI 88788 and PI 90763, respectively, under greenhouse conditions with average day/night temperatures at 25°/20°. Controlled matings between a bulk sample of females from one line and a bulk sample of males from another line were conducted on a 2% agarose plate surface. All three combinations (OP25 × OP20), (OP25 × OP50) and (OP20 × OP50), including reciprocal crosses, were made. Eggs from freshly harvested females of each inbred line were inoculated onto ~20-day-old transplanted Lee 68 seedlings in 10-cm-diameter pots. Ten days later, the roots were carefully washed free of soil, and the plants were suspended in a 4-liter beaker with half-strength Hoagland's solution continuously aerated by bubbling air from aquarium pumps at 25°. Solutions were changed at 2-day intervals. Males usually began to emerge from the root 14 days after inoculation and sunk to the bottom of the beaker. They were recovered daily by passing the collected solutions through a 400-mesh sieve to concentrate the nematodes, and then they were stored at 4° for later use. Females were carefully removed from the root surface individually 25–30 days after inoculation, sterilized with 40 mg/ml streptomycin sulfate for 10 min, rinsed in tap water and transferred to a 2% agarose surface in 10-cm-diameter petri dishes. The water on the agarose surface around females was carefully removed and the dishes were kept at 25° for 3 days. Only white-colored, healthy-looking females were selected for mating; those that were beginning to turn brown were considered to be sib-

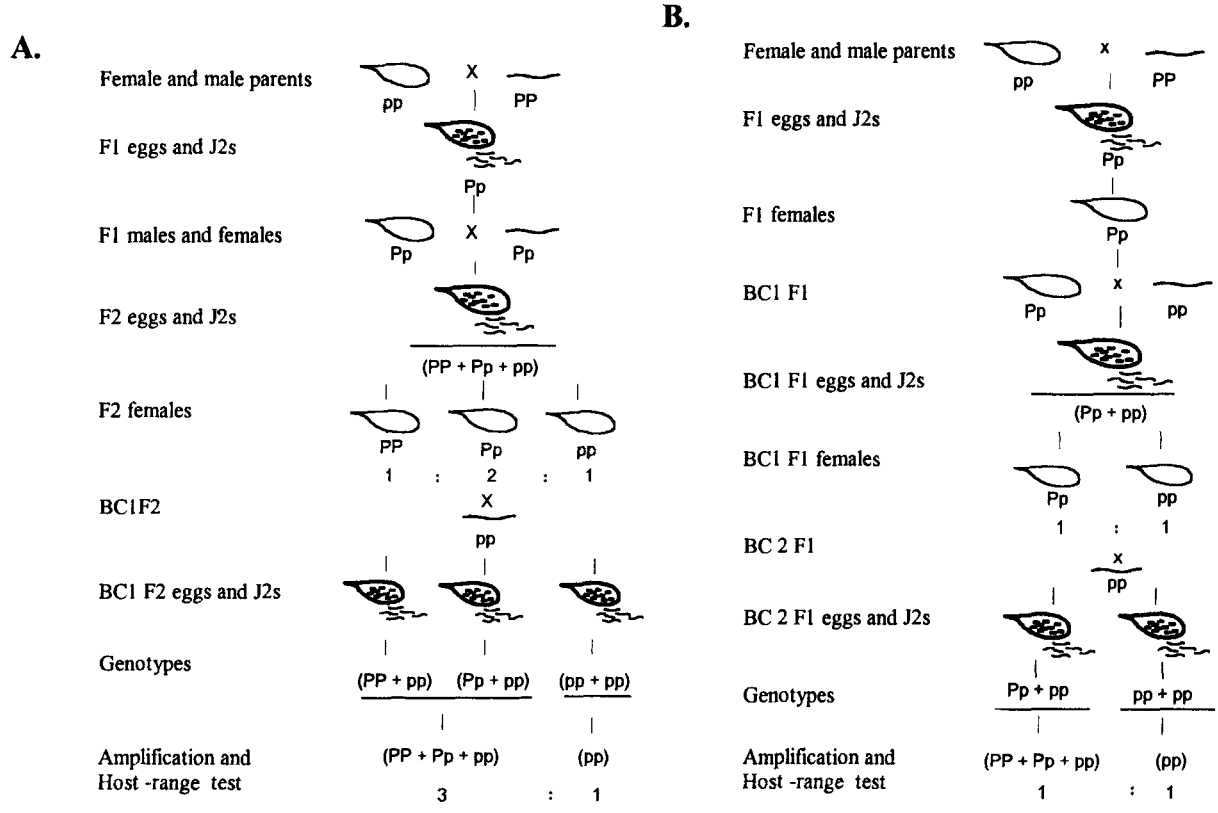

FIGURE 1.—Mating strategies to construct backcross 1 F_2 (BC_1F_2-derived) and backcross 2 F_1 (BC_2F_1-derived) generations. In both crosses the female and recurrent parent OP25 was nonparasitic on resistant hosts (pp), and the male parent, either OP20 or OP50, was parasitic (PP). The pear-shaped objects represent female nematodes and the long thin lines represent males. (A) In the BC_1F_2 derived, the individual eggs, juveniles and virgin females segregated for parasitism:nonparasitism at the F_2 generation. The backcross was made at this stage, and individual fertilized females were selected, gently disrupted to release the eggs and individual batches of eggs were inoculated to the susceptible soybean Lee 68. After several generations of amplification, individual plants were harvested and egg-containing cysts were bulked into individual progeny lines. These individual bulk lines were used for host range testing. Since the lines were individually bulked after amplification, observed ratios corresponded to phenotypes present in the lines. Therefore, the BC_1F_2-derived single female descent lines should have segregated into a 3:1 ratio for parasitism:nonparasitism for a single gene trait or 15:1 for two separate genes. (B) In the BC_2F_1 derived, the first backcross was made to F_1 virgin females arising from the original mating. The individual egg, juvenile and virgin female progeny from this backcross segregated for parasitism:nonparasitism at the BC_1F_1 generation. At this point, the second backcross to the nonparasitic recurrent parent (OP25) was made. Individual fertilized females were selected from matings, disrupted to release eggs and inoculated to Lee 68 as described previously. As with the BC_1F_2-derived crosses, amplification and individual bulking were completed. The individual bulk lines were used for host range tests. Using this strategy, the BC_2F_1-derived single female descent lines should have segregated into 1:1 ratio for parasitism:nonparasitism if one gene controls the trait or 3:1 if two independent genes are involved.

fertilized. The virgin females were transferred onto 2% agarose in 24-well, flat-bottom tissue culture plates with ~30 females per well. A drop of water that contained ~50 live males of the appropriate parental line were transferred into each well. The mating plates were incubated at 25°. All females that turned brown in the plate within the first 5 days after addition of males were removed to further control sib-mating. Twenty days after mating the remaining females were harvested, bulked based on each cross and crushed to release the F_1 eggs.

Eggs of the F_1 generation from (OP25 × OP20), (OP25 × OP50), (OP20 × OP50) and their reciprocal crosses were used for host range tests on Lee 68, Peking, PI 88788 and PI 90763 in the greenhouse, with five replications and 1,000 eggs per plant. There were two separate runs of this experiment. Average greenhouse temperatures were 25° day and 20° night. The F_1 generation females from each host were counted. Females were also randomly picked from the Lee 68 to determine the esterase phenotype. Because the alleles for esterase are co-dominant, sib-mated females could be detected by looking for homozygous F_1 progeny.

Mating and backcrossing: Analysis of segregation of parasitism genes is complicated by several factors, including the need to screen individuals for occurrence of parasitism, the obligate nature of nematode parasitism and uncertainty over how many genes could potentially be involved. To compensate for these problems, we designed a crossing strategy to maximize our chances of detecting rare phenotypes. The strategy we chose was to perform one or two backcrosses to the nonparasitic parent (Figure 1). Single cyst-descent lines were then established by culturing individual egg-containing females for several generations to increase numbers of nematodes for screening. This step was necessary because host range tests on individual F_2 worms are not reliable. In this way, we were able to determine accurately the number of genes controlling parasitic ability on a given soybean genotype. If a single gene

TABLE 1

Parental inbred line host range test results on standard soybean differentials

Line[a]	Lee 68[b]	Peking	PI 88788	PI 90763
OP20	44[c]	0	44	0
OP25	80	0	0	0
OP50	33	28	26	27

[a] OP20, OP25 and OP50 are *H. glycines* inbred lines developed by single female descent inoculation for a minimum of 29 generations on PI 88788, Lee and PI 90763, respectively.

[b] Lee 68, Peking, PI 88788 and PI 90763 are standard differentials for designating races of *H. glycines*. Lee 68 is completely susceptible to all *H. glycines* genotypes.

[c] Number of reproductively mature (*i.e.*, egg-containing) female *H. glycines* produced by a given inbred line on corresponding soybean differentials.

is involved in parasitic ability, the segregation ratio of the egg producing females in a BC_1F_2-derived generation should be 3:1, whereas in the double backcrossed BC_2F_1-derived generation it is 1:1. If two genes are involved, then the BC_1F_2-derived ratio is 15:1 and the BC_2F_1-derived ratio is 3:1.

OP25 was chosen as both the female parent and recurrent parent to initiate mating and backcrossing. The donor parent was either OP20 or OP50. For example, the BC_1F_2-derived generation for parasitic parent OP20 and nonparasitic parent OP25 was constructed as [(OP25 × OP20) × OP25], and the BC_2F_1-derived generation can be described as {[(OP25 × OP50) × OP25] × OP25}. All matings were carefully made as previously described. Generations were clearly separated without overlapping.

Single female inoculation and progeny lines: After all matings had been conducted, mature females with eggs were used to start progeny lines by single female inoculation on Lee 68. Females at the end of the reproductive process were recovered from the agarose surface and rinsed with tap water to remove both eggs and second stage juveniles outside the female bodies. Eggs from an individual intact mature female were released in a small glass dish and rinsed into a 10-cm-diameter pot with a 20-day-old transplanted Lee 68 seedling. Each susceptible Lee 68 plant was used to develop and amplify a single female descent progeny line. Approximately 100 days after the single female inoculation, the plants were washed individually and females from each plant were harvested as bulks for later tests.

Esterase segregation ratio test: After ~3 mo amplification from single female descent on Lee 68, progeny female nematodes from each plant were harvested as bulks. Segregation of esterase phenotypes among 116 progeny lines from {[(OP25 × OP20) × OP25] × OP25}, the BC_2F_1-derived population, were tested. Six white-colored young females were randomly selected from each bulk, individually tested for esterase markers, and phenotypes were determined.

Parasitism test of progeny lines: Progeny lines from single females were harvested. Each progeny line usually contained >500 mature females. Eggs were bulk collected from each line, concentrated on a sucrose gradient and pelleted in 1.5-ml microcentrifuge tubes. Each 5-μl egg pellet contained ~20,000 eggs. These eggs were then inoculated into 20-day-old transplanted differential host plants (either PI 88788 or PI 90763) in a 10-cm-diameter pot. There were two replications per line, and the experiment was conducted twice. Parasitism of each progeny line was evaluated 90–100 days after

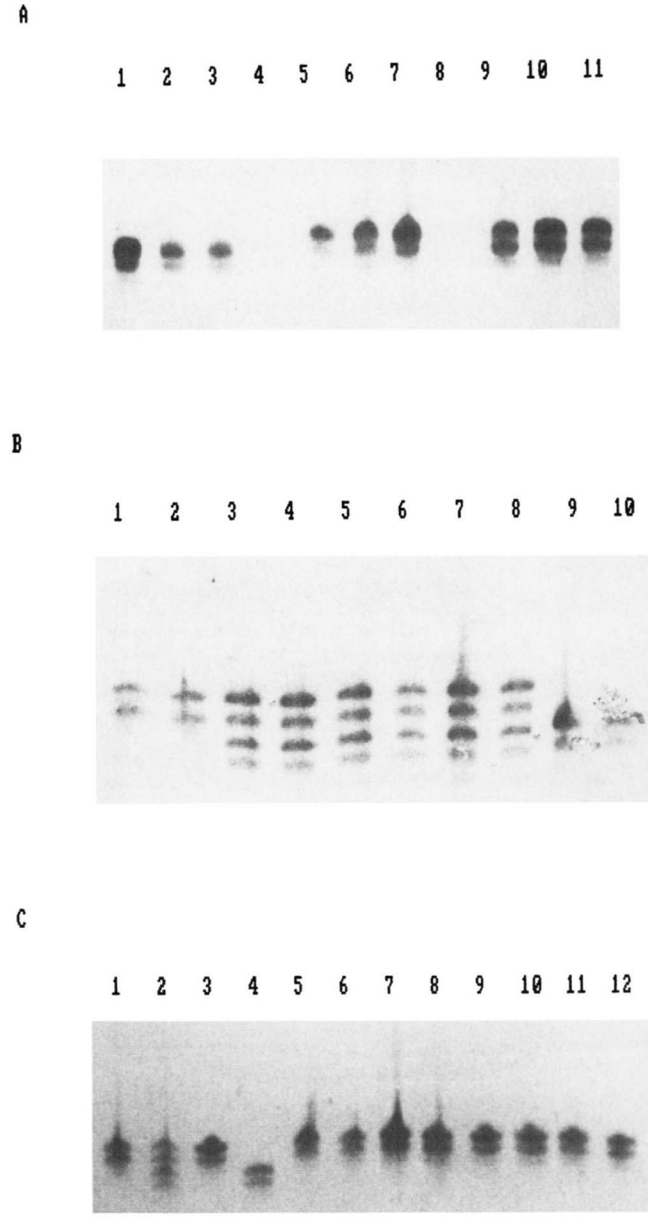

FIGURE 2.—(A) Single female esterase phenotypes of inbred lines OP20 (lanes 1–3), OP25 (lanes 9–11) and OP50 (lanes 5–7). (B) The female parent OP25 (lanes 1 and 2), male parent OP20 (lanes 9 and 10) and the F_1 generation individual female esterase phenotype (lanes 3–8). (C) The {[(OP25 × OP20) × OP25] × OP25} BC_2F_1-derived generation esterase phenotype, homozygous (lanes 7–12) *vs.* segregated (lanes 1–6).

inoculation. Because the recurrent parent OP25 was nonparasitic and cannot reproduce on either of the resistant hosts, parasitism among the progeny lines was evaluated based on the reproductive ability. If a certain line did not reproduce on these resistant hosts, the line was designated as nonparasitic. Otherwise, the line was classified as parasitic.

RESULTS

Parental lines: Parasitism test results of the three inbred lines, OP20, OP25 and OP50, are shown in Table

TABLE 2

F_1 generation host range test results from controlled crosses

Crosses[a]	Lee 68[b]	Peking	PI 88788	PI 90763
OP25 × OP20	157[c]	0	107	0
OP20 × OP25	198	0	119	0
OP25 × OP50	162	0	51	0
OP50 × OP25	189	0	74	0
OP20 × OP50	165	0	123	0
OP50 × OP20	153	0	118	0

[a] Crosses and reciprocal crosses between *H. glycines* inbred lines. Female parent is listed first.

[b] Lee 68, Peking, PI 88788 and PI 90763 are standard differentials for designating races of *H. glycines*.

[c] Number of reproductively mature (*i.e.*, egg-containing) female *H. glycines* produced by a given cross on corresponding soybean differentials.

1. The OP25 line only produced females on Lee 68, but had no females on the resistant hosts Peking, PI 88788 and PI 90763. The OP20 line produced equal numbers of females on both Lee 68 and PI 88788 and no females on Peking and PI 90763. However, the OP50 line produced almost equal numbers of females on all three of the resistant hosts.

The esterase phenotypes from the three parent lines also indicated that the individuals are homozygous and each population was homogeneous for each of these lines. Each line exhibited a characteristic esterase marker: OP25 as the AA line, OP50 as the BB line and OP20 as the CC line (Figure 2A).

Crosses and F_1 generation host range tests: Table 2 depicts the F_1 generation host range test results from crosses and reciprocal crosses of (OP25 × OP20), (OP25 × OP50) and (OP20 × OP50). The F_1 generation from (OP25 × OP20), (OP25 × OP50) and their reciprocal crosses produced females on PI 88788, indicating that at least one parasitism gene in both OP20 and OP50 to PI 88788 is dominant or partially dominant. No females were observed in the F_1 generation of either (OP25 × OP50) or (OP20 × OP50) or reciprocal crosses, on either Peking and PI 90763. Therefore, the parasitism gene(s) in the OP50 line to both Peking and PI 90763 are completely recessive. Additionally, results of reciprocal crosses indicated that there is no preferential effect of the donor parent on inheritance of parasitism genes.

Comparison of esterase phenotypes of both the parents and the F_1 progenies revealed that the F_1 generation was heterozygous, *e.g.*, the F_1 females from cross OP25 × O20 possessed co-dominant esterase markers (Figure 2B). No sib-mated individuals among the F_1 generation females were observed.

Progeny lines, esterase segregation ratio and parasitism gene number: Four kinds of progeny lines were established, including 260 lines from [(OP25 × OP20) × OP25], 116 lines from {[(OP25 × OP20) × OP25] × OP25}, 223 lines from [(OP25 × OP50) × OP25] and 130 lines from {[(OP25 × OP50) × OP25] × OP25}.

Esterase segregation ratios were evaluated from the {[(OP25 × OP20) × OP25] × OP25} populations. The female and recurrent parent OP25 is esterase AA, and the male parent OP20 is CC. Results from six single female esterase phenotype determinations for each of the 116 lines revealed 56 homozygous AA lines and 60 segregated lines (AA, AC and CC) (Figure 2C). The observed ratio was not significantly different from the 1:1 ratio ($P = 0.5–0.75$), which demonstrated that these progeny lines were suitable for further analysis of parasitism.

Results from the parasitism tests of OP25 × OP50 are depicted in Tables 3 and 4. Since the F_1 females from the original cross were all heterozygous for esterase, no further analysis of segregation was performed. The BC_1F_2-derived progeny lines yielded 48 nonparasitic and 175 parasitic phenotypes on the resistant host PI 88788. The observed ratio was not significantly different from the 1:3 ratio ($P = 0.10–0.25$). The BC_2F_1-derived lines segregated 63 nonparasitic to 67 parasitic, which is very close to the 1:1 ratio ($P = 0.5–0.75$) (Table 3).

TABLE 3

Host range test of OP25 × OP50 segregating progeny lines on PI 88788

Cross[a]	Lines[b]	Np:P[c]	Exp-rt[d]	χ^2	P
[(OP25 × OP50) × OP25]	223	48:175	1:3	1.437	0.10–0.25
{[(OP25 × OP50) × OP25] × OP25}	130	63:67	1:1	0.123	0.50–0.75

[a] OP50 was the parasitic and OP25 was the nonparasitic *H. glycines* inbred line on PI 88788. The mating processes [(OP25 × OP50) × OP25] (BC_1F_2 derived) and {[(OP25 × OP50) × OP25] × OP25} (BC_2F_1 derived) were developed by using OP25 as the female and recurrent parent and conducting one or two backcrosses to OP50.

[b] Numbers of single female-derived lines from the corresponding mating process.

[c] Numbers of the nonparasitic (Np) and the parasitic (P) lines on PI 88788. Parasitic lines were scored based on the presence of reproductively mature (*i.e.*, egg-containing) female *H. glycines;* nonparasitic lines were scored by the absence of any female nematodes.

[d] Expected segregation ratio.

TABLE 4

Host range test of OP25 × OP50 segregating progeny lines on PI 90763

Cross[a]	Lines[b]	Np:P[c]	Exp-rt[d]	χ^2	P
[(OP25 × OP50) × OP25]	223	57:166	1:3	0.037	0.75–0.90
{[(OP25 × OP50) × OP25] × OP25}	130	68:62	1:1	0.277	0.50–0.75

[a] OP50 was the parasitic and OP25 was the nonparasitic *H. glycines* inbred line on PI 90763. The mating processes [(OP25 × OP50) × OP25] (BC_1F_2 derived) and {[(OP25 × OP50) × OP25] × OP25} (BC_2F_1 derived) were developed by using OP25 as the female and recurrent parent and conducting one or two backcrosses to OP50.

[b] Numbers of single female-derived lines from the corresponding mating process.

[c] Numbers of the nonparasitic (Np) and the parasitic (P) lines on PI 90763. Parasitic lines were scored based on the presence of reproductively mature (*i.e.*, egg-containing) female *H. glycines;* nonparasitic lines were scored by the absence of any female nematodes.

[d] Expected segregation ratio.

These results show that there is a single gene in OP50 that confers the ability to parasitize PI 88788. We have designated this gene as *ror-1*(*kr1*) for reproduction on a resistant host (BIRD and RIDDLE 1994). Evaluation of the same progeny lines for the ability to parasitize PI 90763 yielded similar results. The BC_1F_2 derived was not significantly different from the 1:3 ratio ($P = 0.75$–0.9), and the BC_2F_1 derived segregated 1:1 ($P = 0.5$–0.75) (Table 4). These data reveal that OP50 carries a single gene for parasitism of PI 90763, which we have designated as *ror-2*(*kr2*).

Linkage analysis between *ror-1* and *ror-2* was performed. Evaluation of the 130 lines from the OP25 × OP50 BC_1F_2 derived revealed that 37 of the lines were nonparasitic on both resistant hosts, 36 lines were parasitic on both resistant hosts, 26 lines were parasitic only on PI 90763 and 31 lines were only parasitic on PI 88788. This is not significantly different from the 1:1:1:1 ratio for unlinked genes ($P = 0.5$–0.75). Therefore, it appears that *ror-1* and *ror-2* are unlinked genes that independently confer parasitic abilities on different soybean genotypes.

Results from the OP25 × OP20 progeny lines revealed a somewhat unexpected phenomenon. Evaluation of parasitism of PI 88788 by 260 BC_1F_2-derived lines showed a segregation pattern of 17 nonparasitic to 243 parasitic. This is very close to the 1:15 ratio for two separate genes controlling parasitism (Table 5, $P = 0.75$–0.9). The results from the BC_2F_1-derived progeny lines confirm this observation. These lines segregated very close to the 1:3 ratio ($P = 0.75$–0.9). These results indicate that OP20 carries two separate parasitism genes, both of which may confer parasitism of PI 88788. We have given these genes allele designations (*kr3*) and (*kr4*) since we do not yet know if either are alleles of *ror-1* or exist as separate loci.

DISCUSSION

Although most previous reports conclude that parasitic ability in *H. glycines* might be conditioned by oligogenic loci, our results from the inbred line crosses clearly demonstrate parasitic ability is inherited in a Mendelian fashion. Previous reports also concluded parasitism genes in this nematode were dominant or partially dominant, but in our tests we uncovered both dominant and recessive genes (TRIANTAPHYLLOU 1975, 1987). Both OP20 and OP50 carry dominant *ror-1* alleles for parasitism of PI 88788. In contrast, OP50 carries a recessive *ror-2* allele for parasitism of PI 90763.

TABLE 5

Host range test of OP25 × OP20 segregating progeny lines on PI 88788

Cross[a]	Lines[b]	Np:P[c]	Exp-rt[d]	χ^2	P
[(OP25 × OP20) × OP25]	260	17:243	1:15	0.037	0.75–0.90
{[(OP25 × OP20) × OP25] × OP25}	116	28:88	1:3	0.046	0.75–0.90

[a] OP20 was the parasitic and OP25 was the nonparasitic *H. glycines* inbred line on PI 88788. The mating processes [(OP25 × OP20) × OP25] (BC_1F_2 derived) and {[(OP25 × OP20) × OP25] × OP25} (BC_2F_1 derived) were developed by using OP25 as the female and recurrent parent and conducting one or two backcrosses to OP20.

[b] Numbers of single female-derived lines from the corresponding mating process.

[c] Numbers of the nonparasitic (Np) and the parasitic (P) lines on PI 88788. Parasitic lines were scored based on the presence of reproductively mature (*i.e.*, egg-containing) female *H. glycines;* nonparasitic lines were scored by the absence of any female nematodes.

[d] Expected segregation ratio.

Finally, linkage analysis has confirmed that *ror-1* and *ror-2* are separate, unlinked loci. In fact, all of the previous genetic analyses have been performed using field isolates without phenotypic control (*e.g.*, esterase markers). The heterogeneous nature of *H. glycines* among and within field isolates has been well characterized previously (TRIANTAPHYLLOU 1987). We therefore believe that the previous results are not accurate indications of the genetic basis of soybean parasitism in *H. glycines*.

After 20 generations of full-sib inbreeding, the inbreeding coefficient is ~0.989. Our *H. glycines* lines were inbred for a minimum of 29 generations and theoretically each inbred line is nearly homogeneous. The host range results demonstrated that the parasitism gene frequencies in OP25 to hosts Peking, PI 88788 and PI 90763 are reduced to 0%. The parasitism genes in OP20 are fixed for host PI 88788 and completely lost for hosts Peking and PI 90763. OP50 produced nearly equal numbers of females on all resistant and susceptible hosts, implying that parasitism genes from the original field isolate to the tested soybean genotypes are fixed in OP50.

In soybean, it is believed that both major and minor genes, dominant, partially dominant and recessive, are all involved to some degree in conferring resistance to *H. glycines* (TRIANTAPHYLLOU 1987). However, it is not clear which genes are essential and which are specific to certain nematode genotypes, if any. Interpretation is further complicated by the previous use of *H. glycines* field populations to evaluate resistant soybeans. We have developed pure lines of *H. glycines* that carry single genes for parasitic ability on soybeans and have demonstrated that *H. glycines* contains unlinked dominant and recessive genes for parasitism of various host genotypes. A further observation is that the two genes in OP20 controlling parasitism of PI 88788 may be acting additively. Examination of F_1 data from controlled crosses reveals that the presence of two genes (from OP20) results in twice as many females being formed on PI 88788 as when only one gene is present (from OP50) (Table 2). Although not verified, this is an intriguing possibility and may explain varying levels of aggressiveness between different nematode populations on the same host genotype. The lines we have developed will be useful for both further nematode analysis and also soybean genetics.

The mating technique we developed was a practical necessity because individually inoculated infective nematodes do not always penetrate soybean roots. This, in turn, makes direct analysis of segregating F_2 populations highly variable and inaccurate. Crosses and backcrosses were conducted using bulked individuals from each inbred line as parents, and single female descent populations were developed for testing the major and independent gene numbers. Using this strategy, dominance or recessiveness did not affect the analysis of gene numbers. For example, in the cross shown in Figure 1A, nonparasitism can only arise from individually derived lines that were homozygous to the nonparasitism allele in the F_2 generation. Parasitism would always be detected from lines that descended from heterozygous F_2 females due to segregation and random mating during the amplification process. Possible linkage between the parasitism genes can also be detected among the progeny lines, although the amplification process on susceptible host Lee 68 may cause linkage equilibrium within each line. Nevertheless, these lines are extremely useful for both classical and molecular genetic analysis.

During our analysis of parasitism genetics, we constructed ~1600 progeny lines from backcrosses such as {[(OP25 × OP50) × OP25] × OP25}. We have subjected these lines to a RAPD-PCR bulk segregant analysis. Numerous polymorphic bands have been detected among the inbred parent lines, and a genetic map of the *H. glycines* genome is being constructed by RAPD marker segregation and linkage analysis. Although genetic drift may affect frequency of a certain allele within each line, we have multiple lines that will allow us to correct for this potential problem. Recombination events that could cause linkage equilibrium within individual lines should not affect our genetic mapping purpose because we are using data from groups of lines. The marker density or linkage distance, therefore, can be calculated. Host range test results among these lines combining with RAPD bulk-segregant analysis will provide information for us to detect markers physically linked to parasitism genes as a prerequisite for map-based cloning of the parasitism gene(s).

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