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Selection Against *Heterodera glycines* Males by Soybean Lines with Genes for Resistance[1]

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Abstract: Soybeans with genes for resistance select against *Heterodera glycines* with the corresponding genes for avirulence. There may be a differential effect of sex with some specific gene interactions, which would influence the magnitude of gene frequency changes. No effect on *H. glycines* males was detected with one selected nematode population and the resistant soybean line PI88788. The selective effect of PI89772 against male nematodes was greater with two inbred nematode populations than with one selected (on PI88788) population, presumably due to differences in *H. glycines* gene frequencies. 'Peking' also had few males with the one inbred nematode population, whereas Forrest and 'Pickett 71' had intermediate numbers. Apparently Forrest and Pickett 71 did not get all the Peking genes for resistance that affect male as well as female nematode development. Other *H. glycines*-soybean genes stop only females, since there were few or no cysts, except on the susceptible soybean Williams. The number of males' phenotype will help identify specific genes in both organisms.

Key words: *Glycine max*, soybean cyst nematode, gene frequency, sex ratio.

"Resistance" of soybean (S), *Glycine max* (L.) Merr., to soybean cyst nematodes (CN), *Heterodera glycines* Ichinohe, is classified by relative numbers of cysts per plant that are phenotypes of the CN-S symbiosis (13). Nematode males and the specific genetic complement of populations have received relatively little attention. Triantaphyllou (18) selected populations for increased ability to form cysts on the resistant soybean lines 'Peking', 'Pickett', and PI88788. He suggested that gene frequencies would describe population structure of *H. glycines* better than races did. The central feature of population genetics is the Hardy-Weinberg equilibrium with genotypic frequencies of $p^2 + 2pq + q^2$. This equilibrium is reached without selection, but soybean genes for resistance exert selection pressure on CN. The natural selection, with intensity s, might be against nematodes with "aa" genotypes with q^2 frequency. The effect has been defined as $(1 - s)q^2$ (6). This becomes $0q^2$ with $s = 1$ and "aa" nematodes do not reproduce.

The intensity of selection against males (s_m) and females (s_f) may be different. Then the effect would be $[1 - \frac{1}{2}(s_m + s_f)]q^2$ (3). Thus, the "aa" contribution to reproduction would be $\frac{1}{2}q^2$ rather than $0q^2$, with the obvious effect of less change in gene frequency with selection. Knowledge about natural selection on males would be helpful in interpreting results of selection experiments. Endo (5) found no males or females in Peking, whereas Ross (16) indicated that male nematodes were common. They used different populations which presumably differed in their frequencies of one gene that interacted with a Peking gene for resistance to stop the development of both male and female nematodes. The interactions of most CN-S genes (for avirulence–resistance) that produce the cysts' phenotype may affect females only (18). My objective was to show that some genes do affect the development of males.

Materials and Methods

The nematode populations used were P88, P89, I1, and I2. The "P" designation was used by Acedo et al. (1) for populations selected for ability to reproduce on PI88788 (P88) or on PI89772 (P89) (15). Inbreds I1 and I2 were developed, from a field population near Center, Missouri, through nine generations of inbreeding on 'Williams' soybean. Inbreeding was performed by single cyst transfers without any control of male number. Soybean lines used were Forrest, Peking, Pickett 71, Williams, PI88788, and PI89772. Seedlings of PI88788 and PI89772 were transplanted

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TABLE 1. Numbers of males and females of *Heterodera glycines* populations selected on PI88788 (P88) or on PI89772 (P89) that developed on PI88788 and on PI89772 soybeans.

H. glycines population	Test	Soybean line			
		PI88788		PI89772	
		Males	Females	Males	Females
P88	1	310	594	181*	11
P88	2	165	185	34*	3
P89	1	319	96	335	415
P89	2	14	4	11	17

* Significantly fewer males than PI88788, $P = 0.05$, *t*-test.

into 0.91-m-d microplots infested with either P88 or P89. Five days later six uniform seedlings of each CN-S combination were transferred into aerated hydroponic culture maintained at 27 C. A −N nutrient solution (19) was changed weekly, with 5.0 mM $Ca(NO_3)_2 \cdot 4H_2O$ instead of $CaCl_2$ and Sequestrene as the iron source. Males were collected in 30-μm-pore sieves every 2–3 days and counted. Females and cysts were counted after males stopped emerging. The test was repeated once, in a completely random design with six replications.

Peking, Williams, and PI89772 seedlings were planted into crocks containing either I1 or I2. Three seedlings of each combination were transferred to determine numbers of males and females as above. Another test was conducted using Forrest, Pickett 71, Peking, and Williams with the I2 population.

RESULTS AND DISCUSSION

PI88788 was effective against only females of P89, since the numbers of males were about the same as on PI89772 (Table 1). With P88, PI89772 had significantly fewer males than PI88788. The differences in numbers of cysts was as previously reported (15). Similar results were obtained with PI89772 and PI209332 by Halbrendt (9), with inbreds developed on PI209332 and PI89772 (14). PI88788 and PI209332 appear to be genetically similar (13,20). Each PI has been evaluated for males with only one population, an insufficient sample to determine that none of their genes for resistance affect male development. The selective effect of PI89772 (and Peking) on males of I1 and I2 (Table 2) appeared to be greater than with P88. Not all of Peking's genes for resistance got transferred to Forrest and Pickett 71, since they sometimes have more cysts than Peking (14,20). The 0, 1, and 2 females (Table 2) may not be different; however, Forrest and Pickett 71 have many more males than Peking but fewer than Williams. These results can be explained by only two genes affecting the development of males, but the frequencies of nematode genes for avirulence and (or) the intensities of selection (s_m) must be somewhat less than 1.0.

The number of CN-S genes affecting males probably is greater than two. Fox (7) reported that few males developed on Peking, but many developed on PI90763. Peking, PI89772, and PI90763 were grouped as being genetically similar (13,20), but they may differ in at least one gene affecting males. The number of males is a phenotype that should help show the number of genes involved and identify discrete single gene effects. This identification will require developing more inbreds with appropriate gene frequencies.

Theoretical aspects of sex ratios were discussed by Karlin and Lessard (10), and 1:1 is the norm in diploid populations. There are many autosomal genes which produce minor variations in sex ratios (3); effective selection on such genetic variation was cited in 1919 (4). In *H. glycines* sex appears to be genetically determined; there may be some sex-determining chromatin even though there are no sex chromosomes per se (8). Males in the male : female ratio are favored because of crowding (11) and high temperature (17). The opposite effect may occur with isolated root segments in vitro since 2.3:36 and 1:28 were observed (12). The in vitro method was reported to give reliable results on resistance; there was significant variation in numbers of females (2 to 36) but not of males (0.3 to 5.0). Sex ratios per se do not

TABLE 2. Numbers of males and females of *Heterodera glycines* inbreds (I1 and I2) that developed on soybean lines.

H. glycines	Soybean line									
	Williams		PI89772		Peking		Forrest		Pickett 71	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
I1	340	68	6	1	5	0				
I2	1,934	205	36	0	46	0				
I2	582	265			72	0	408†	2	336*	1

† Significantly fewer males than Williams, $P = 0.05$ and < 0.10†, analysis of variance.

appear to be particularly useful with CN-S; it is the relative numbers of both that are important for gene identification. Reduced numbers of males and females were reported also with *H. avenae* on oats (2). Relative differences in numbers of males with different CN-S may reflect the action of different soybean genes and also different nematode gene frequencies. Thus one important use for the males' phenotypes would be to identify the populations essential for transferring soybean genes into cultivars that block the production of males.

The CN-S genes affecting male development probably function soon after penetration. Syncytia in Peking were degenerating 4–5 days after infection; few third-stage juveniles and no males were observed (5). Syncytia associated with males normally may be degenerating by 9 days after infection, whereas females may feed through 21 days (1). There is a longer time for female development to be affected and probably more injury to the plants. Cultivars with few males and females might be damaged less than cultivars with no females and many males in highly infested fields.

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