The Sex Ratio of *Heterodera glycines* at Low Population Densities[1]

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Abstract: The sex ratio of the Arkansas 1 isolate of *Heterodera glycines* was determined in experiments in which 'Lee' soybean was inoculated with either one or two larvae. A 3:1 male to female sex ratio was established for this isolate under the test conditions used. No influence of one nematode on the penetration and development to adult of another nematode in the same root was detected in double larval inoculations. *Key Words:* cyst nematodes, nematode-nematode interaction, soybean.

Sex ratios in the Heteroderidae vary with many environmental conditions (1, 2, 5, 6, 13, 15, 16). It is desirable, in studies of factors influencing the sex ratio of *Heterodera glycines* Ichinohe, to establish as a point of reference a sex ratio that is characteristic of a given isolate of *H. glycines* under standardized conditions which allow the expression of the inherent potential of sex. Sex ratios observed under different conditions of nematode development may then be compared to that which is innate for a particular isolate. The objective of this study was to determine the sex ratio of the Arkansas isolate 1 of *H. glycines* under standardized conditions.

MATERIALS AND METHODS

A population of *H. glycines,* Arkansas 1 isolate, was maintained in the greenhouse on 'Lee' soybean. Cysts were collected by rinsing the soybean roots and potting medium forcibly with running water and sieving the water through a 710-μm and a 250-μm screen series. Cysts were collected on the 250-μm screen and crushed with a modified Seinhorst cyst crusher (12). The resulting suspension of broken cysts, larvae, eggs, and organic matter was sieved through a 75-μm and a 26-μm screen series. Eggs and larvae were collected on the 26-μm screen and placed on Baermann funnels to provide freshly hatched larvae.

Seeds of a susceptible soybean, *Glycine max* 'Lee', were germinated in a 1:1 v/v mixture of Weblite® and vermiculite. Single and double larval inoculations were prepared in modified plastic Petri dishes by inoculating the primary root tips of uniform seedlings, 5 to 7 days of age, which had primary roots 6 to 12 cm long. The single larval inoculation procedure was as follows: A small drop of water was placed in the Petri dish bottom and a single, second-stage larva was transferred to the drop. The seedling root tip was placed in the water drop with the nematode and a small cone of fine, dry Weblite was used to cover the seedling tip and hold it close to the nematode. Coarse white sand was then used to fill the remaining space in the Petri dish around the cone of Weblite. The sand prevented movement of the Weblite about the root tip and served as a water reservoir. The seedling shoot protruded through a 1.5-cm "V" notch cut in the periphery of an opaque plastic Petri-dish lid and was watered through a 1-cm^2 hole cut in the periphery of the lid 90° around the circumference from the notch. The double larval inoculations were prepared in the same manner except that two second-stage larvae were transferred to the root tip. Inoculated plants were placed on support benches with continuous illumination by Westinghouse Cool White® fluorescent lamps (500 lux).

In separate experiments, 100 single larval inoculations, as described in the preceding paragraph, were incubated at either 23 C (Experiment 1) or 28 C (Experiment 2) for 5 days, and 47 double larval inoculations were incubated at either 23 C (Experiment 3) or 28 C (Experiment 4) for 5 days. The plants were washed free of Weblite and sand immediately after the 5-day penetration period, returned to the Petri dishes with tap water, and watered with 1X Hoagland's solution (7) as needed. The solutions were sieved through a 38-μm screen 17 and 29 days after inoculation to recover nematodes in the solutions. The

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roots were harvested 29 days after inoculation, fixed and stained in a 1:1 v/v solution of glacial-acetic acid and ethanol containing acid fuchsin, and then cleared in a saturated solution of chloral hydrate.

Binomial distribution models (11) were used to analyze nematode-nematode interactions in Experiments 3 and 4 (Table 2, 3). In the first model, in which it was assumed that penetration and subsequent development to adult by one larva would occur independently of a second larva in the same root, p = percentage of larvae which did not develop to adults and q = percentage of larvae that matured to adults. In the second model, in which it was assumed that sexual differentiation of a nematode larva would occur independently of a second nematode in the same root, x = percentage of females and y = percentage of males. The observed values obtained in these experiments were compared to the expected values predicted by the models (3) and tested with chi-square.

RESULTS AND DISCUSSION

Males predominated in single and double larval inoculation experiments at 23 and 28 C (Table 1) and there was no significant difference between the percentage of males among adults developing in single or double larval inoculations at 23 and 28 C. No nematodes were observed in the stained and cleared root systems.

A sex ratio of 75% males to 25% females (a 3:1 male to female sex ratio) was obtained for the Arkansas 1 isolate of *H. glycines* under these experimental conditions. In the individual experiments, the percentage of males among adults varied only slightly from a low of 71% males to a high of 74% males. Although the sex ratios in the four experiments were not significantly different, the ratio of 50% males to 50% females was rejected only in Experiment 1 with 73% males and in Experiment 4 with 74% males. There were too few observations to permit rejection of this ratio in Experiments 2 and 3, although 71% and 72% males were reported for these experiments, respectively. In contrast, the ratio of 75% males to 25% females was accepted in all four experiments. A homogeneity chi-square of 0.106 with three degrees of freedom was calculated to determine whether the results from the four experiments were sufficiently uniform to be analyzed together. The chi-square value was accepted, an indication that the results could be pooled and the "total" data chi-square values (Table 1) represented true tests of the sex-ratio hypotheses. The rejection of $P = 0.001$ of the 1:1 sex ratio for the "total" data indicated that the observed deviations from this sex ratio could happen by chance alone only one time in a thousand, whereas the alternate 3:1 sex ratio was a good fit to the observed data.

The assumption that the presence of one nematode in a root would not influence the penetration and development of a second

TABLE 1. Distribution of males and females of *Heterodera glycines* developing at low nematode population densities.

Experiment	Total adults	Percentage males	Chi-square tests[a] 1 male: 1 female	3 males: 1 female
Single larval inoculations				
Experiment 1 (23 C)	23	73.9	4.39*[b]	0.02
Experiment 2 (28 C)	24	70.8	3.38	0.06
Double larval inoculations				
Experiment 3 (23 C)	21	71.4	3.05	0.02
Experiment 4 (28 C)	31	74.2	6.35*	0.01
Combined Total	99	72.7	19.56***[b]	0.17

[a]Chi-square tests computed with Yates' correction for continuity.
[b]Asterisks (*, ***) indicate different than those expected at $P = 0.05$ and 0.001, respectively.

nematode in the same root was tested by using the data of Experiments 3 and 4 (Table 2). The calculation of a homogeneity chi-square of 0.864 with one degree of freedom indicated that the results of the double larval inoculations could be pooled. The observed values of zero, one or two adults per plant were in substantial agreement with the expected values (3) predicted by the binomial distribution model (χ^2 = 1.17). Thus, the presence of one nematode did not increase or decrease the chance of another nematode developing in the same root at the low numbers of the present study.

Although the presence of 78% males in roots with one larvae appears to be different from the presence of 50% males in roots with two larvae (Table 3), there was an insufficient number of observations in the two nematodes/root class to make a valid comparison with the expected values predicted from the binomial distribution model (3). Thus, no conclusions can be drawn about the effect of one larva on the sexual differentiation of a second nematode in the same root.

These predominantly male sex ratios were in contrast to the predominately female sex ratios reported by Ross (10) for an isolate of *H. glycines* from Castle Haynes, N. C. Koliopanos and Triantaphyllou (8), in an experiment of single larvae inoculations of an isolate from Wilmington, N. C. on 'Lee' soybean, obtained 64% males, a result which was statistically different (P = 0.05) from a 1:1 ratio. However, in their experiment with inoculum levels of 20, 200, 1,000, and 5,000 larvae/plant, they obtained 49-50% males at the three lowest levels and 63% males at the 5,000 level. They attributed the increased male to female ratio at high infection densities to differential death-rate of male and female larvae under conditions of food stress and crowding. In another experiment reported by Evans and Fox (4), the Arkansas 1 isolate had 77% males under conditions which limited possible differential death of the sexes as a cause of the unbalanced sex ratio. Although death of larvae of *H. glycines* on soybean and, very likely, differential death of the sexes occur under some conditions, the Arkansas 1 isolate in most of our experiments had approximately 75% males, a result which may have been caused by some phenomenon other than differential death.

TABLE 2. Distribution of mature adults of *Heterodera glycines* in soybean roots developing from a double larval inoculation system.

Experiment	Survival[a] %	No. adults/plant 0	1	2
No. 3 (23 C)	22.3	27	19	1
No. 4 (28 C)	33.0	20	23	4
Total of observed values		47	42	5
Expected values[b]		49	38	7

[a]Percentage of larvae that became adults.
[b]Based on a binomial distribution model with a survival rate of 28%.

TABLE 3. Distribution of sex of adult *Heterodera glycines* developing from a double larval inoculation system.

Experiment	Survival[a] %	Distribution of sexes: One nematode/plant Male	Female	Two nematodes/plant 2 males	Male-female	2 females
No. 3 (23 C)	22.3	14	5	0	1	0
No. 4 (28 C)	33.0	19	4	2	0	2
Total of observed values		33	9	2	1	2
Expected values[b]		28	10	4	3	0

[a]Percentage of larvae that became adults.
[b]Based on a binomial distribution model with a survival rate of 28% and a sex-ratio of 75% males.

The sex ratios obtained in the single larval inoculations for amphimictic species of cyst nematodes range from 9% males in *Globodera rostochiensis* (14) to 75% males in the Arkansas 1 isolate of *H. glycines*. Trudgill (14) demonstrated a continuous range (approximately 9 to 89% males) of sex ratios in *G. rostochiensis* in tomato and potato as a result of nematode density. He also demonstrated (15) the sex ratio of *G. rostochiensis* can be altered from 33 to 94% males by DL tyrosine without altering the number of adults. Johnson and Viglierchio reported a range of sex ratios in *H. schachtii* from 22 to 50% males with 27% males at the optimum temperature for development. They concluded that the sexes of this species have a differential ability to develop to maturity.

Since a 1:1 sex ratio is not consistently expressed in amphimictic species of cyst nematodes, alternatives to the hypothesis that a strong genetic mechanism of sex determination and differential death of the sexes are the sole cause of unbalanced sex ratios should be considered. The wide range of sex ratios and the demonstrated influence of environmental factors on sex ratios in these species are phenomena similar to those reported for *Meloidogyne graminis* (17) for which there is some evidence for polygenic control of sex. This relationship may be analagous to the concept of genetic control of sex in mammals (9) in which the phenotypic sex is the result of the interaction of the genotypic sex and the sex hormones. The variability of nematode sex ratios, in response to environmental factors, may indicate that these factors exert an influence on the nematode's hormonal system during embryogenesis and post-embryogenesis. Although results of limited experiments suggest that both environmental and genetic factors influence sex expression of some populations of *H. glycines*, more extensive experiments are needed to characterize the phenomena regulating sex expression of the amphimictic species of cyst nematodes.

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