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Temperature Effects on Race Determination in *Heterodera glycines*[1]

A. J. Palmateer,[2] M. E. Schmidt,[3] S. R. Stetina,[4] and J. S. Russin[3]

Abstract: Currently there are 16 possible races for *Heterodera glycines,* and these are differentiated based on ability of a nematode population to develop on a set of four differential soybean genotypes. Because results are based on numbers of nematode females that develop to a specific stage rather than on the reproductive capability of these females, race determinations based on female indices may not represent results obtained after several reproductive cycles of *H. glycines.* Counting numbers of eggs and juveniles, and then developing corresponding indices, would allow reproduction to be considered in making race determinations. Our objectives were to compare the present race identification scheme for *H. glycines* based on female indices with those using egg and juvenile indices and to examine the effect of temperature on race designations using female, egg, and juvenile indices. Race designations for *H. glycines* populations from two locations in Illinois were determined at 20, 27, and 30 °C in a water bath. The numbers of females, eggs, and juveniles (at 19 days) were recorded, and an index based on each life stage was calculated. Race determinations based on female, egg, or juvenile indices were inconsistent when conducted at 20 °C, which demonstrates that this temperature is not suitable for identifying races of *H. glycines.* However race designations at 27 and 30 °C were consistent for all three indices. This indicates that counting females, eggs, or juveniles should be equally reliable when race determinations are conducted at these two temperatures, and choice of method would depend on investigator preference or research objective.

Key words: Glycine max, Heterodera glycines, host differential, host pathogen interaction, nematode, race determination, reproductive index, soybean, soybean cyst nematode.

Soybean cyst nematode, *Heterodera glycines* Ichinohe, is the most serious soybean (*Glycine max* (L.) Merrill) pest in the United States and is present in nearly every soybean-producing state (Niblack, 1999). Soon after *H. glycines* was found in North Carolina in 1954, more than 4,000 soybean germplasm lines were screened against a field population of this nematode and several were found to have resistance (Caviness, 1992). Shortly thereafter, populations of this nematode species were reported from other areas that were able to develop on lines derived from these initial sources of resistance. The initial study of *H. glycines* populations led to the designation of four distinct races of this pathogen (Golden et al., 1970), but the system was expanded in 1988 to include 16 races (Riggs and Schmitt, 1988).

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[2] Graduate Assistant, [3]Associate Professors, and [4]Postdoctoral Researcher, Department of Plant, Soil, and General Agriculture, Southern Illinois University, Carbondale, IL 62901. Present address of first author: Department of Entomology and Plant Pathology, Auburn University, AL 36849. Present address of third author: University of Minnesota Southwest Research and Outreach Center, Lamberton, MN 56152.

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E-mail: jrussin@siu.edu

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The current scheme designating 16 races is based on the ability of the *H. glycines* population to develop on a set of four differential soybean genotypes (Riggs and Schmitt, 1988). These genotypes are 'Peking', 'Pickett', PI 88788, and PI 90763, and they are tested in conjunction with a standard susceptible cultivar such as Lee or Essex (Riggs and Schmitt, 1991). Races are determined by dividing the average number of females and cysts on each differential genotype approximately 30 days after inoculation by the average number of females and cysts on the susceptible cultivar, then multiplying the result by 100 to obtain a female index. A female index that is less than 10% of that on the susceptible cultivar indicates resistance, whereas a female index greater than or equal to 10% indicates moderate resistance to susceptibility (Schmitt and Shannon, 1992). Races are designated according to the combination of indices on all four differentials.

Race determination in *H. glycines* is based on developmental rather than reproductive parameters. That is, results are based on numbers of females that develop to a specific stage rather than on the reproductive capability of these females. Race determina-

tions based on female indices may not represent results obtained after several reproductive cycles of the nematode (Triantaphyllou, 1975). Counting numbers of eggs and juveniles, and then developing corresponding indices, would allow reproduction to be considered in making race determinations. However, no published work has compared the relationship between race designations based on female development with those based on egg and juvenile production. The objectives of the current research were to: (i) compare the present race identification scheme for *H. glycines* based on female indices with that using egg and juvenile indices and (ii) examine the effect of temperature on race designations using female, egg, and juvenile indices.

Materials and Methods

Two isolates of *H. glycines* were used in all studies. These were collected in 1993 from soybean fields in Elkville and Sand Ridge, Illinois, and were determined to be races 3 and 4, respectively, according to methods described by Riggs and Schmitt (1988). The nematode populations were maintained on the susceptible soybean cultivar Hutcheson. All experiments were conducted in a greenhouse between June 1999 and December 1999 where ambient temperatures ranged from 24 to 35 °C under natural light. Both populations of *H. glycines* were increased on 'Hutcheson' that was grown in 15-cm-diam. plastic pots that contained approximately 1.6 kg of soil.

For inoculum preparation, 'Hutcheson' soybean plants were removed from pots 30 days after inoculation. Roots were washed thoroughly using a strong stream of water to dislodge females and cysts from roots onto nested 710-µm over 250-µm-pore sieves. Material on the 250-µm-pore sieve was centrifuged for 6 minutes at 1,475*g*. Approximately 40 ml water was added to each tube, and females and cysts were ruptured by grinding with a motorized pestle for 60 seconds (Niblack et al., 1993) to release eggs. Eggs captured on a 25-µm-pore sieve were retained for inoculum.

Influence of temperature on race determinations for the *H. glycines* populations was examined in greenhouse trials at the Horticulture Research Center of Southern Illinois University, Carbondale. Three temperatures (20, 27, and 30 °C) were maintained in water baths. For each temperature and race, four soybean differential genotypes ('Peking', 'Pickett', PI 88788, and PI 90763) along with the susceptible 'Hutcheson' were planted in a completely randomized design with four replications. Six additional 'Hutcheson' soybean plants were included to periodically assess development of females and determine an appropriate harvest date if development required less than 30 days at higher temperatures.

Soybean seeds were germinated in a 1:1 vermiculite-perlite medium in the greenhouse, and a single 3-day-old seedling (radicle length = 20 mm) was transplanted into each of 26 tubes made from polyvinyl chloride (20.2 cm long and 2.5-cm diam.) placed in a plastic bucket (20-cm diam.). The tubes and buckets were filled with steam-sterilized soil (75% sand, 15% silt, 10% clay; pH 6.2) and maintained at 20, 27, or 30 °C in water baths. The 20 °C temperature was chosen because it is typical of soil temperatures in southern Illinois at soybean planting time (J. S. Russin, unpubl.). The 27 °C temperature is optimum for *H. glycines* race determination (Riggs and Schmitt, 1988), and the 30 °C temperature is optimum for soybean growth and development (Scott and Aldrich, 1983). Four or five days after transplanting, a depression (1-cm diam. and 4 cm deep) was made approximately 1 cm away from the soybean seedling in the culture tube. A suspension of 2,000 *H. glycines* eggs in 5 ml tap water was pipeted into each depression, which then was filled with soil.

At 20 days after inoculation and every 2 days thereafter, one 'Hutcheson' plant was examined for white or yellow females on roots. Because nematodes developed at different rates based on temperature, the trial durations also varied based on temperature. Trials conducted at 30 °C were harvested 26 days after inoculation, whereas those at 20

and 27 °C were harvested 30 days after inoculation.

Plant shoots were removed by cutting the stem 1 cm above the soil line. Tubes containing roots in soil were placed individually in 1-liter beakers containing 500 ml of tap water to separate the roots from the soil. Roots were placed on nested 710-µm over 250-µm-pore sieves and washed using a strong stream of water to dislodge the cysts and females. These were counted using a stereomicroscope at ×30 magnification. A female index was derived for each soybean differential genotype by dividing the number of females and cysts observed on the differential by the number of females and cysts observed on 'Hutcheson' and then multiplying the result by 100 to obtain a percentage. Race designations were determined according to the procedure described by Riggs and Schmitt (1988).

After the females and cysts were counted, they were ruptured using a motorized pestle. Released eggs were washed through nested 75-µm over 25-µm-pore sieves. Eggs retained on the 25-µm-pore sieve were transferred to petri dishes (90 mm × 90 mm) containing 40 ml tap water (pH 6.8) and incubated on a laboratory bench top at room temperature (approximately 24 °C) for 19 days. Eggs and juveniles were stained using acid fuchsin (Southey, 1986) and counted using a stereomicroscope at ×60 magnification. Race designations again were determined according to the procedure described by Riggs and Schmitt (1988), but egg and juvenile indices rather than female indices were calculated. The egg index was derived by dividing the mean number of eggs and juveniles observed on each soybean differential by the mean number of eggs and juveniles observed on 'Hutcheson' and then multiplying by 100. The juvenile index was derived by dividing the mean number of juveniles observed on each soybean differential by the mean number of juveniles observed on 'Hutcheson' at 19 days and then multiplying by 100. The number of eggs per female was derived by adding the number of eggs and juveniles and then dividing the sum by the number of females and cysts.

Race designations at different temperatures were compared qualitatively. Data for eggs per female were analyzed according to ANOVA using PC SAS version 6.12 (SAS Institute, Inc., Cary, NC), and means were compared using least significant difference at $P \leq 0.05$. All experiments were conducted twice. For each race and temperature, data from both trials were combined for analysis unless otherwise noted.

Results and Discussion

Production of females and cysts, eggs, and juveniles was greater at 27 °C than at 20 or 30 °C for both populations of *H. glycines* used in these studies (Tables 1–6), which suggests that 20 and 30 °C were sub-optimal for nematode development. Numbers of females and cysts or eggs on 'Hutcheson' at these temperatures were less than 30% of those at 27 °C. These results agree with those of Anand et al. (1995), who found that populations of females and cysts at 26 °C were 2- to 6-fold greater than those at 20 and 30 °C.

At 20 °C, the number of females and cysts per root system for the Elkville population on all four soybean differentials confirmed this population as race 3 (Table 1). However, race designations differed when egg or juvenile indices were calculated. This population was designated as race 5 based on egg indices but as race 6 based on juvenile indices. The number of eggs per female did not vary among soybean genotypes.

A treatment by trial interaction was detected for the Sand Ridge population at 20 °C; therefore, data for individual trials at this temperature were analyzed separately (Table 2). Results from trial 1 showed that numbers of females and cysts, eggs, and juveniles confirmed this population as race 4. However, in trial 2, lower numbers for females and cysts, eggs, and juveniles per root system on PI 90763 changed the race designation to race 2 based on all three indices. The number of eggs per female was greatest on 'Peking' in trial 1 and on 'Peking' and PI 90763 in trial 2.

TABLE 1. Numbers of females and cysts, eggs, and juveniles of the Elkville population of *Heterodera glycines* that developed on five soybean genotypes at 20 °C.

Soybean genotype	Females and cysts/ root system	Female index[a,b]	Eggs/root system	Egg index[b,c]	Juveniles/ root system	Juvenile index[b,d]	Eggs/ female
'Hutcheson'	13.8	100	543.5	100	321.3	100	23.8 a
'Pickett'	1.3	9.4 (–)	215.8	39.7 (+)	93.1	30.0 (+)	30.5 a
'Peking'	0.5	3.6 (–)	17.4	3.2 (–)	3.5	1.1 (–)	17.6 a
PI 88788	0.8	5.8 (–)	95.4	17.6 (+)	21.0	6.5 (–)	6.0 a
PI 90763	0.3	2.2 (–)	23.9	4.4 (–)	6.3	2.0 (–)	10.0 a
Race designation[e]		race 3		race 5		race 6	

For each soybean genotype, values are means of eight replicates in two trials. For eggs/female, means followed by the same letter did not differ ($P > 0.05$).

[a] Female index = (number of females and cysts on differential genotype/number on 'Hutcheson') × 100.

[b] (–) = number of females, eggs, or juveniles on a differential genotype <10% of that on 'Hutcheson'; (+) = number of females, eggs, or juveniles on a differential genotype ≥10% of that on 'Hutcheson'.

[c] Egg index = (number on differential genotype/number on 'Hutcheson') × 100.

[d] Juvenile index = (number on differential genotype/number on 'Hutcheson') × 100. Counts were made after 19 days.

[e] Race designation according to Riggs and Schmitt (1988).

At 27 °C, the Elkville population was confirmed as race 3 (Table 3) and the Sand Ridge population was confirmed as race 4 (Table 4) by all three indices. The number of eggs per female for both Elkville and Sand Ridge populations did not vary among all genotypes tested (Tables 3 and 4). This temperature proved to be the most suitable of all three temperatures for reproduction of the nematode. This finding agrees with several published reports (Riggs and Schmitt, 1988, 1991), which found the optimum temperature for development of female *H. glycines* to be approximately 28 °C.

Although 30 °C was sub-optimal for nematode development, all three indices confirmed the Elkville population as race 3 (Table 5) and the Sand Ridge population as race 4 (Table 6). The number of eggs per female on 'Pickett' was greater than that on PI 90763 and 'Hutcheson'.

Race designations based on female, egg,

TABLE 2. Numbers of females and cysts, eggs, and juveniles of the Sand Ridge population of *Heterodera glycines* that developed on five soybean genotypes at 20 °C.

Soybean genotype	Females and cysts/ root system	Female index[a,b]	Eggs/ root system	Egg index[b,c]	Juveniles/ root system	Juvenile index[b,d]	Eggs/ female
			Trial 1				
'Hutcheson'	35.3	100	1,008.0	100	362.3	100	29.0 b
'Pickett'	20.8	58.9 (+)	459.0	45.5 (+)	125.3	34.6 (+)	26.3 b
'Peking'	3.8	10.6 (+)	244.5	24.3 (+)	54.8	15.1 (+)	82.3 a
PI 88788	10.0	28.4 (+)	275.8	27.4 (+)	68.0	18.8 (+)	31.3 b
PI 90763	7.3	20.6 (+)	230.5	22.9 (+)	46.5	12.8 (+)	33.5 b
Race designation[e]		race 4		race 4		race 4	
			Trial 2				
'Hutcheson'	50.0	100	1,333.5	100	692.5	100	26.8 b
'Pickett'	60.3	120.5 (+)	1,429.3	107.2 (+)	312.8	45.2 (+)	25.8 b
'Peking'	5.8	11.5 (+)	367.3	27.5 (+)	167.8	24.2 (+)	60.8 a
PI 88788	16.8	33.5 (+)	430.3	32.3 (+)	195.8	28.3 (+)	24.3 b
PI 90763	4.0	8.0 (–)	129.8	9.7 (–)	43.0	6.2 (–)	43.5 ab
Race designation		race 2		race 2		race 2	

For each soybean genotype, values within each trial are means of four replicates. For eggs/female, means followed by the same letter did not differ ($P > 0.05$).

[a] Female index = (number of females and cysts on differential genotype/number on 'Hutcheson') × 100.

[b] (–) = number of females, eggs, or juveniles on a differential genotype <10% of that on 'Hutcheson'; (+) = number of females, eggs, or juveniles on a differential genotype ≥10% of that on 'Hutcheson'.

[c] Egg index = (number on differential genotype/number on 'Hutcheson') × 100.

[d] Juvenile index = (number on differential genotype/number on 'Hutcheson') × 100. Counts were made after 19 days.

[e] Race designation according to Riggs and Schmitt (1988).

TABLE 3. Numbers of females and cysts, eggs, and juveniles of the Elkville population of *Heterodera glycines* that developed on five soybean genotypes at 27 °C.

Soybean genotype	Females and cysts/ root system	Female index[a,b]	Eggs/ root system	Egg index[b,c]	Juveniles/ root system	Juvenile index[b,d]	Eggs/ female
'Hutcheson'	366.5	100	6,405.0	100	2,167.4	100	23.8 a
'Pickett'	0.4	0.1 (–)	91.5	1.4 (–)	33.0	1.5 (–)	30.5 a
'Peking'	0.3	0.1 (–)	55.9	0.9 (–)	40.6	1.9 (–)	17.6 a
PI 88788	1.0	0.3 (–)	44.0	0.7 (–)	18.0	0.8 (–)	6.0 a
PI 90763	0.9	0.2 (–)	65.4	1.0 (–)	31.3	1.4 (–)	10.0 a
Race designation[e]		race 3		race 3		race 3	

For each soybean genotype, values are means of eight replicates in two trials. For eggs/female, means followed by the same letter did not differ ($P > 0.05$).

[a] Female index = (number of females and cysts on differential genotype/number on 'Hutcheson') × 100.

[b] (–) = number of females, eggs, or juveniles on a differential genotype <10% of that on 'Hutcheson'; (+) = number of females, eggs, or juveniles on a differential genotype ≥10% of that on 'Hutcheson.'

[c] Egg index = (number on differential genotype/number on 'Hutcheson') × 100.

[d] Juvenile index = (number on differential genotype/number on 'Hutcheson') × 100 after 19 days.

[e] Race designation according to Riggs and Schmitt (1988).

and juvenile indices were inconsistent at 20 °C, which indicated that this temperature was not reliable for designating race in *H. glycines*. There may be several explanations for this. Although nematode establishment and development in roots likely were inhibited at this temperature (Ross, 1964), changes in race designation may have resulted from certain individuals within the nematode populations that were more able than others to infect and reproduce at 20 °C. The fact that soybean plants grow more slowly at 20 °C (Scott and Aldrich, 1983) may modify their susceptibility to *H. glycines*. Also, competition from other organisms such as bacteria and fungi that grow well at 20 °C may make the environment less suitable for hatch and root infection by the nematode than at more optimal temperatures.

Egg production per *H. glycines* female was relatively consistent at each temperature and averaged 24.4 eggs per female across all temperatures and trials (Tables 1–6). These numbers were lower than those reported by Tefft et al. (1982), who observed an average of 323 eggs per female, and by Young (1992), who reported that females may produce 200 to 600 eggs. With so few females, a very small change in number could affect whether a differential genotype received a ''+'' or ''–'' designation relative to reproduction on 'Hutcheson'. It is further possible that all eggs and juveniles produced were

TABLE 4. Numbers of females and cysts, eggs, and juveniles of the Sand Ridge population of *Heterodera glycines* that develop on five soybean genotypes at 27 °C.

Soybean genotype	Females and cysts/ root system	Female index[a,b]	Eggs/ root system	Egg index[b,c]	Juveniles/ root system	Juvenile index[b,d]	Eggs/ female
'Hutcheson'	131.8	100	2,919.8	100	1,394.8	100	23.6 a
'Pickett'	178.6	135.6 (+)	3,952.6	135.4 (+)	1,322.8	94.8 (+)	22.8 a
'Peking'	50.1	38.1 (+)	1,272.3	43.6 (+)	625.1	44.8 (+)	26.4 a
PI 88788	76.8	58.3 (+)	1,828.4	62.6 (+)	681.0	48.8 (+)	28.1 a
PI 90763	44.0	33.4 (+)	1,233.5	42.3 (+)	615.9	44.2 (+)	81.3 a
Race designation[e]		race 4		race 4		race 4	

For each soybean genotype, values are means of eight replicates in two trials. For eggs/female, means followed by the same letter did not differ ($P > 0.05$).

[a] Female index = (number of females and cysts on differential genotype/number on 'Hutcheson') × 100.

[b] (–) = number of females, eggs, or juveniles on a differential genotype <10% of that on 'Hutcheson'; (+) = number of females, eggs, or juveniles on a differential genotype ≥10% of that on 'Hutcheson'.

[c] Egg index = (number on differential genotype/number on 'Hutcheson') × 100.

[d] Juvenile index = (number on differential genotype/number on 'Hutcheson') × 100. Counts were made after 19 days.

[e] Race designation according to Riggs and Schmitt (1988).

TABLE 5. Numbers of females and cysts, eggs, and juveniles of the Elkville population of *Heterodera glycines* that developed on five soybean genotypes at 30 °C.

Soybean genotype	Females and cysts/ root system	Female index[a,b]	Eggs/ root system	Egg index[b,c]	Juveniles/ root system	Juvenile index[b,d]	Eggs/ female
'Hutcheson'	52.9	100	1,149.9	100	668.4	100	21.8 a
'Pickett'	0.1	0.3 (–)	2.1	0.2 (–)	1.86	0.3 (–)	0.6 b
'Peking'	0	0 (–)	0	0 (–)	0	0 (–)	0 b
PI 88788	0	0 (–)	0	0 (–)	0	0 (–)	0 b
PI 90763	0	0 (–)	0	0 (–)	0	0 (–)	0 b
Race designation[e]		race 3		race 3		race 3	

For each soybean genotype, values are means of eight replicates in two trials. For eggs/female, means followed by the same letter did not differ ($P > 0.05$).

[a] Female index = (number of females and cysts on differential genotype/number on 'Hutcheson') × 100.

[b] (–) = number of females, eggs, or juveniles on a differential genotype <10% of that on 'Hutcheson'; (+) = number of females, eggs, or juveniles on a differential genotype ≥10% of that on 'Hutcheson'.

[c] Egg index = (number on differential genotype/number on 'Hutcheson') × 100.

[d] Juvenile index = (number on differential genotype/number on 'Hutcheson') × 100. Counts were made after 19 days.

[e] Race designation according to Riggs and Schmitt (1988).

not recovered in our experiments. This may have resulted from accidental rupture of females and release of eggs when spraying roots, loss of eggs exuded by the females prior to or during harvest, or hatching of eggs prior to completion of the trial.

The race designation procedure described by Riggs and Schmitt (1988), which involves female indices, is likely quicker than using egg or juvenile indices for those experienced in the process. Determining egg indices adds the steps of rupturing females and staining eggs, which require additional time and effort. However, stained eggs are much easier to count than cysts. This could result in more accurate race determinations, especially if inexperienced personnel are used to process large numbers of samples. Determining juvenile indices was the most time-consuming step because of the period required for egg hatch. Because egg and juvenile indices were identical at 27 and 30 °C, counting juveniles would not be advantageous if race determination were the sole objective. Another problem with juvenile indices concerns the conditions under which eggs are incubated for hatch. Laboratory conditions differ greatly from those in soil, and many soil minerals, nutrients, and root exudates known to be important for nematode egg hatch (Clarke and Perry, 1977) are absent. Some investigators have used zinc sulfate or zinc chloride to stimulate egg hatch (Behm

TABLE 6. Numbers of females and cysts, eggs, and juveniles of the Sand Ridge population of *Heterodera glycines* that developed on five soybean genotypes at 30 °C.

Soybean genotype	Females and cysts/ root system	Female index[a,b]	Eggs/ root system	Egg index[b,c]	Juveniles/ root system	Juvenile index[b,d]	Eggs/ female
'Hutcheson'	31.6	100	329.6	100	108.4	100	10.6 c
'Pickett'	12.3	38.7 (+)	276.0	83.7 (+)	95.9	88.5 (+)	23.1 a
'Peking'	10.3	32.4 (+)	188.6	57.2 (+)	68.4	63.1 (+)	19.8 ab
PI 88788	9.6	30.5 (+)	171.9	52.1 (+)	58.1	53.6 (+)	18.4 ab
PI 90763	6.6	21.0 (+)	101.9	30.9 (+)	16.6	15.3 (+)	16.3 bc
Race designation[e]		race 4		race 4		race 4	

For each soybean genotype, values are means of eight replicates in two trials. For eggs/female, means followed by the same letter did not differ ($P > 0.05$).

[a] Female index = (number of females and cysts on differential genotype/number on 'Hutcheson') × 100.

[b] (–) = number of females, eggs, or juveniles on a differential genotype <10% of that on 'Hutcheson'; (+) = number of females, eggs, or juveniles on a differential genotype ≥10% of that on 'Hutcheson'.

[c] Egg index = (number on differential genotype/number on 'Hutcheson') × 100.

[d] Juvenile index = (number on differential genotype/number on 'Hutcheson') × 100. Counts were made after 19 days.

[e] Race designation according to Riggs and Schmitt (1988).

et al., 1995; Tefft et al., 1982; Thompson and Tylka, 1997), but this was not the case in our study. It is recognized that the juvenile index may primarily be a measure of hatching behavior of the nematode eggs. However, our results clearly showed that juvenile indices were identical to female and egg indices for designating race at both 27 and 30 °C, which suggests potential for the juvenile index as a measure of reproductive capacity.

Two conclusions can be drawn from the current study. Race determinations based on female, egg, or juvenile indices were inconsistent when conducted at 20 °C, which demonstrates that this temperature is not suitable for identifying races of *H. glycines*. Race designations at 27 and 30 °C were consistent for all three indices and matched the original race designations in every case. However, nematode development was less at 30 °C than at 27 °C, which indicates that counting females and cysts, eggs, or juveniles should be equally reliable when race designations are conducted at 27 °C. The choice of method would depend on investigator preference or research objective; however, it is more efficient to base race designation on number of females and cysts.

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