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Relationship of cyst nematode gene frequencies to soybean resistance *

V. D. Luedders

Department of Agronomy, University of Missouri, Columbia, MO 65211, USA

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Summary. Soybean (S, *Glycine max* (L.) Merr.) lines with relatively few cysts of soybean cyst nematode (CN, *Heterodera glycines* Ichinohe) populations are usually called CN-resistant. The phenotype of number of cysts per plant is of the CN-S (Cyst Nematode-Soybean) association and determined by the interactions of genes for avirulence-resistance. The acronym "alins" was proposed for these alleles for incompatibility, with "xalin" representing the interaction X of one microsymbiont m-alin with its host h-alin. These alins are dominant in the gene-for-gene model but may be mostly recessive with CN-S. Definitive genetic studies have been hindered by the heterogeneity of sexually reproducing CN populations and lack of the appropriate genetic models. Loegering's abstract interorganismal genetic model was modified so that one model represented all four possible interactions of dominant-recessive alins for an incompatible phenotype. This involved redefining the Boolean algebra symbol 1 to represent both the alins AND their frequencies. The model was used to derive the relationship:

$$E(\text{cysts}) \propto \Pi(1-p_d)^2\,(1-q_r{}^2)$$

where the expectation E of cysts (of any CN-S combination, as proportion of number of cysts on a check cultivar) is proportional to the product Π of CN genotypic frequencies expressed as functions of m-alin frequencies. Each m-alin is at a different locus, i.e., $p_d+q_r \neq 1$. The number of terms multiplied for each CN-S is equal to the number of alins in the S line (or F_2 plant). There are too many unknowns in the equation to solve for any of them. The relationship does explain the continuous distributions of phenotypes that were nearly always observed. Basic genetic principles were used to concurrently derive the models and to obtain discontinuous distributions of numbers of cyst phenotypes in segregating generations due to one recessive alin in a CN-"susceptible" soybean line.

Key words: *Glycine max* – *Heterodera glycines* – Genes for avirulence-resistance

Introduction

Soybean cyst nematode (CN, *Heterodera glycines* Ichinohe) populations can severely reduce soybean (S, *Glycine max* (L.) Merr.). yields. Sources of CN resistance were identified, with some of the genes for resistance put into cultivars to reduce damage (Hartwig 1981). Studies on S inheritance of CN resistance (and of CN parasitic ability) have been less than definitive, probably due to heterogeneity of the CN populations used (Luedders 1983; Triantaphyllou 1987). Soybean resistance to CN has been characterized by relatively few cysts per plant, which is one phenotype of the CN-S (Cyst Nematode-Soybean) association and is determined by the genes of both symbionts. The host category "resistant", and its alternative, "susceptible", do not adequately reflect the continuous distributions of numbers of cysts per plant. Such continuous variation of phenotypes can be due to genetic variation of both CN populations and S lines (Luedders 1985; Young 1984). There has been no satisfactory explanation of the continuous distributions of CN-S phenotypes or of why several S genes were necessary for "resistance" to CN populations. A related inadequacy is race designations of CN populations based on their ability to form cysts on S differentials with several genes for resistance.

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The explanation for the continuous distributions of cyst numbers may involve the gene frequencies of the sexually reproducing nematodes. Triantaphyllou (1975) suggested that frequencies of genes for parasitism would describe cyst nematode population genetic structure better than race designations do, but no model was presented. The existence of races suggests that there is a gene-for-gene relationship. This concept was developed by Flor (1971), who stated that breeders were using the principles, either consciously or unconsciously. The principles developed by Flor are not appreciated by many. Ellingboe (1983) indicated surprise at how few pathologists and breeders had taken the time to understand the genetics of host-parasite interactions when the rules were so simple. As a rule, genes for resistance and avirulence are dominant. With CN-S, however, many of these genes appear to be recessive, and the appropriate models of CN-S genetic interactions, including gene frequencies, were not obvious (Luedders 1983). Until recently, no specific genes had been identified in either organism (Luedders 1987). Thus, the dilemma appeared to be that models were necessary to help identify individual genes, which would aid in formulating the additional, alternative models.

In this report, I discuss some possible symbiont (host-parasite) gene interactions and models, and develop a model relating CN gene frequencies to the CN-S phenotypes of numbers of cysts per S plant.

Gene-for-gene specificity

The term "gene-for-gene" appears to be interpreted in several ways, although Loegering (1984) indicated that it designated a concept and should never be translated literally. A concept is useful only if it helps explain, or can be directly applied to, real biological systems. The term has become more or less synonymous with the model of Ellingboe (1976), where the + 's represent more compatible

	P_	pp
R_	−	+
rr	+	+

phenotypes of the association than the − or incompatible phenotype. Wheeler (1975) considered such a model to follow Flor's rule: a resistant reaction occurs if, and only if, a gene for resistance interacts with a gene for avirulence. Ellingboe (1982) has made a similar statement, but a term for such specific interactions is lacking, which may be part of the reason for the lack of appreciation and use of the concept. The following two acronyms are proposed to facilitate the discussion and development of the models describing CN-S relationships:

ALIN – an allele for incompatibility (−), plural alins
XALIN – the interaction (X) of two alins, one h-alin and one m-alin, for a − phenotype.

The [dominant] alins, R and P, in Ellingboe's model above are frequently called genes for resistance and avirulence; the general prefixes h- for host and m- for microsymbiont indicate which or whose allele for incompatibility is being referred to. The − phenotype [Wheeler's (1975) host resistance] results from only one xalin, the interaction of two highly specific alins. Such xalins were the basis for the development of the gene-for-gene concept by Flor (1971). There is extensive genetic variation for this specificity, which Ellingboe (1976) considered to be superimposed on a basic compatibility.

Basic compatibility

Basic compatibility enables two species to be symbionts so that specificity can occur. The relationship in Victoria blight of oats is probably basic compatibility (Ellingboe 1976), and can be represented as:

		host cell lysis: yes	host cell lysis: no
fungal toxin	yes	+	−
	no	−	−

The relationship is completely different from the "specificity" in Ellingboe's model. In Victoria blight, toxin production is dominant in the fungus as is protoplast lysis in the host, i.e., "avirulence" and resistance are recessive. Also, four alleles represent the genetic variation of Victoria blight. There appears to be extensive genetic variation in CN-S, but neither model seems to apply to the recessive alins involved in incompatible phenotypes.

Basic compatibility may be a series of signals – responses; one example may be the toxin-lysis of Victoria blight. Mutations have been for loss of toxin and lysis, with the phenotype of the association going from + to −. Such changes might be expected since the specific interaction was for a + phenotype compared to − with the genes of specificity in Ellingboe's model. Thus, mutations of alins should, and do, change phenotypes from − to +. Most mutations of genes of basic compatibility would be expected to result in loss of symbiotic ability, and have been actively generated by transposons in *Rhizobium meliloti* Dangeard (Long et al. 1982). Isolates of parasites

do lose pathenogenicity spontaneously. Such mutations may also be a genetic load in CN; only 22%–33% of the juveniles in one and two juvenile inoculations developed into adult nematodes (Evans and Fox 1977; Koliapanos and Triantaphyllou 1972). Several recessive host genes give resistance to certain symbiotic species, e.g. *ml-o* in barley to *Erysiphe graminis* f. sp. *hordei* (Wiberg 1973), soybean *rjl* to *Bradyrhizobium japonicum* (Williams and Lynch 1953) and *rxp* to *Xanthomonas phaseoli* var. *sojense* (Hartwig and Lehman 1951), and several alfalfa alleles to *Rhizobium meliloti* (Peterson and Barnes 1981). Uninoculated barley plants homozygous for the *ml-o* allele develop necrotic flecks, which may indicate that it is a mutation of a gene necessary for normal growth. These recessive resistances may be the mutations of normal host genes that had been activated by their symbionts' signals. Resistance may be the proper term here, although it is avoided where incompatible phenotypes of associations appear to be due to xalins.

Models

Loegering (1978, 1984) constructed a general, abstract model to represent all possible gene-for-gene relationships, including Victoria blight of oats. It was to eliminate all confusion of parasite and host-oriented terms including dominance and resistance. He used the Boolean algebra symbols 1 and 0, the 1 s represented the definitive genotypes and a definitive aegricorpus or phenotype of the association. Browder (1985) pointed out that Loegering erred in adding, rather than multiplying the symbols. Thus the rules of ordinary multiplication apply and only $1 \times 1 = 1$. The L model is Loegering's basic model, with the lettering changed for CN-S.

		S	
		1	0
CN	1	1	0
		CN-S	
	0	0	0

L model

The model was at least partly based on Wheeler's (1975) statements using the term "definitive"; with Flor's (1971) rule, the resistant reaction is definitive in that it specifies the (haploid) genotypes of both plant and pathogen. He stated that with dominance, diploid plants and dicaryotic pahtogens could be considered as haploids. The relevent research used homogeneous parasite clones. Loegering (1984) presented variations with the internal 1 in the three other positions, always with external $1 \times 1 =$ internal 1. This seems to be keeping the intraorganismal dominant and recessive genotypes in constant positions and varying their interactions at the interorganismal level.

The only definitive 1 in the L model is the 1 CN-S (zero cyst) phenotype, which can result from one xalin. I will use the external 1 s as the alins AND their frequencies. Thus, the L model includes the four possible interactions of dominant and recessive CN-S alins, and also eliminates any confusion of dominance and the need to define the external 0 s beyond the frequencies of alins in the one xalin model. Use of the symbolism beyond one xalin can be confusing, because h-alins are frequently at the same locus. The model is very useful conceptually but its application to real biological systems may not be obvious. Caution must be used in representing phenotypes by something else, be it 1 0, – +, or resistant susceptible. The relevance of the model may be demonstrated by adapting the information of Layton et al. (1984) on soybean phytophthora root rot as follows:

		soybean line		
Phytophthora		Mukden	Sanga	PI 157409
"race"	Alins	1 a	1 b	1 b, 4 a
1	1 a 1 b 4 a	–	–	–
2	1 a – 4 a	–	+	–
3	– 1 b 4 a	+	–	–

The phenotypes are usually very distinct, with the inoculated plants either dead (+) or healthy (–). The "dominant" *Rps* 1 a and 1 b (alins), previously shown to be at locus 1, were also called recessive genes for susceptibility to some races. Both alleles can function at the same time since heterozygous lab plants have a – phenotype when inoculated with a mixture of races 2 and 3. Dominance refers to ratios; co-dominance seems to be an inapt term. The definitive – phenotypes with PI 157409 only indicated that it had at least one alin. The 15–: 1 + segregation indicated two xalins. The m-alin designations of 1 a, 1 b, and 4 a may give more information about isolates than do race designations. Sanga may not have the 1 b allele that was named using D 60-9647. Apparently, F_2 progeny of intercrosses of Sanga, PI 84637, and D 60-9647 did not segregate for 1 b, but the only sure conclusion is that they have alins at the same locus. Keeling (1982) called Sanga resistant to race 17 but PI 171442 and Tracy were susceptible. Tracy has two *Rps* alleles, 1 b from D 60-9647 and 3(?) from PI 171442. Several similar, impossible combinations were obtained by M. A. Hobe (unpublished data), (courtesy A. F. Schmitthenner, OARDC, Woster/OH). Rennie and Be-

versdorf (1987) indicated that Sanga was susceptible to their isolate of "race 17". Keeling called Sanga resistant since the 27 resistant (4 with lesions) and 9 susceptible plants were a 3:1 ratio.

The definitive 1 CN-S phenotype in the L model can be zero cysts due to only one xalin. In studies of inheritance of resistance, crosses of S lines with few and with many cysts (resistant × susceptible) resulted in continuous distributions of phenotypes or numbers of cysts per F_2 plant. Such results indicated the segregation of several S alins, with the phenotypes determined by the frequencies of specific xalins. These can be expressed most clearly as functions of m-alin frequencies. The usual relationships for gene $(p+q=1)$ and genotypic $(p^2+2pq+q^2=1)$ frequencies will be used even though the Hardy-Weinberg equilibrium may never obtain, especially when several loci (linkage disequilibrium) are involved (Falconer 1981). Alins at several CN loci affect the phenotypes with S lines that have several alins, but alins at all CN loci will be represented by p and q, with frequencies $p_d\,(+q=1)$ for each dominant and $q_r\,(+p=1)$ for each recessive alin. Note that $p_d+q_r\neq 1$, because two m-alins generally are not at the same locus. Considering the frequencies of the m-alin involved in each xalin eliminates some potential complications. The intensity of natural selection, s, affects the genotypic frequencies as $1-s$ (Falconer 1981). Thus a m-alin is a lethal with $s=1.0$, e.g. $(1-s)q_r{}^2=0$. The $s=1$ will be assumed to keep the model simple. The soybean alin is considered to be the selecting agent, decreasing the frequencies of nematode alins, the basis for increasing numbers of cysts on CN "resistant" S lines in selection experiments.

The proportion of a CN population genetically capable of becoming cysts on an S line involving only one xalin with a recessive m-alin is p^2+2pq, or in terms of the alin $(1-q_r{}^2)$. With the m-alin dominant, only q^2 of the population is genetically capable of becoming cysts in a soybean with only that one alin. This might be more clearly expressed in terms of the dominant m-alin or $(1-p_d)^2$. These expectations are shown in Table 1, but it may not be clear what they mean in terms of phenotypes. The clearest interpretation of many CN-S phenotypes, i.e., cyst numbers of different combinations of populations-lines, is shown to be the numbers of cysts of each population on the S lines as proportions relative to a standard or check cultivar. Such proportions or phenotypes might be roughly proportional to the expectations in Table 1. Numbers of cysts of one population can vary continuously from none to many on different S lines and F_2 segregates from crosses. The reasons for this have not been totally clear, but the CN-S phenotypes, expressed as proportions, appear to depend on the number and identity of h-alins and the frequencis of "their" m-alins. Thus, the number of expectations (proportions as functions of alin frequencies in Table 1) that must be considered for any CN-S phenotype is equal to the number of alins in an S line or segregate. These individual expectations are multiplied (Π, the product operator) to obtain an expectation E of cysts for each CN-S in the frequency model: $E(\text{cysts}) \propto \Pi\,(1-p_d)^2\,(1-q_r{}^2)$. The $\propto$ might be best interpreted as roughly proportional too, since an exact relationship with CN alin frequencies may not be possible. The 1.00 of the standard may not be the maximum phenotype observed in a test. Lehman and Dunn (1987) reported that the differential S cultivar Pickett was 333% and 260% (3.33 and 2.60) of the susceptible check Lee with two field CN populations. The frequency model shows that a low E (expectation of cysts) can be obtained by multiplying several intermediate numbers (e.g.) $0.5^6=0.016$) or many 1 s and only one 0. Distributions of CN-S phenotypes on S F_2 plants depend on CN alin frequencies as well as on the number and dominance of the segregating S alins.

Table 1. Effect of frequencies of one dominant (p_d) or one recessive (q_r) cyst nematode allele for incompatibility on the expectation of cysts (proportion of a population genetically capable of becoming cysts) on a soybean line with the one interacting allele for incompatibility

Allele for incompatibility		
Frequency	Dominant	Recessive
	Expectation $(1-p_d)^2$	$1-q_r^2$
0	1.00	1.00
0.1	0.81	0.99
0.2	0.64	0.96
0.3	0.49	0.91
0.4	0.36	0.84
0.5	0.25	0.75
0.6	0.16	0.64
0.7	0.09	0.51
0.8	0.04	0.36
0.9	0.01	0.19
1.0	0	0

Applications

Some of the concepts developed in this paper were applied even before satisfactory models of CN-S gene interactions and their frequencies were realized. The effect of products of probabilities for cyst success was discussed previously (Luedders 1983), but a satisfactory relationship and a sound biological basis for the expectation of cysts were elusive. The distribution of genes for CN resistance in the S germplasm was, and is, unknown. However, it seemed unreasonable that a few "resistant" S lines

should have all of the many alins. The majority of lines were considered to be susceptible to CN, since they had more than some arbitrary numbers of cysts. This could have been due to low alin frequencies in those CN populations. Thus, nematodes were inbred and then evaluated with CN-susceptible soybeans. Specific CN inbreds were inbred for more generations, with artificial selection (among CN inbreds) for fewer cysts on specific S lines. The discontinuous distributions of phenotypes of F_2 plants and their F_3 progeny indicated that the zero cyst phenotype of the CN-"susceptible" PI88287 (with one specific inbred) was due to one recessive h-alin (Luedders 1987). This shows that CN "resistance" can result from one xalin, and is evidence for the validity and usefulness of the concepts discussed.

Nematode alin frequencies can be adjusted by natural selection and/or inbreeding with artificial selection for no cysts on individual S lines for definitive genetic studies. Such studies may not be possible if the effects of CN alins are ignored or if continuous CN-S phenotypes are separated into resistant and susceptible categories. Hancock et al. (1987) postulated that one recessive gene (with heritability estimates of 61%–64%) in the source of resistance PI90763 gave resistance to CN "race X" even though the distributions of phenotypes were continuous, indicating the segregation of more than one alin. The three "susceptible" parents had only about half as many cysts as Lee, which may have influenced the numbers of cysts on progeny plants. Plants with <10% of the number of cysts on Lee were classified as resistant, even though the maximum on PI90763 was only 0.5% of Lee. Thus, an unknown number of segregates with a phenotype of cysts numbers greater than that of PI90763 was considered to have its genotype. Rao and Anand (1986) reported that F_1 plants from crosses of CN-resistant and susceptible lines had fewer cysts than the susceptible parents and concluded incomplete dominance for susceptibility. An alternate interpretation would be that at least one dominant h-alin was present but the frequencies of the m-alins and xalins were less than 1.0, so that there were moderate numbers of cysts on the F_1 plants.

Nematode alin frequencies can help explain the ranges in numbers of cysts and the necessity for several genes for resistance – alins and xalins – to get few cyst phenotypes with most CN populations. The frequency model is useful for planning research and interpreting results, but cannot be used directly to determine either the number of xalins or any exact frequencies. A zero or low probability of cysts is possible with a high frequency of only one m-alin, or lower frequencies of several. The numbers in Table 1 are precise, as they can be in models and computer simulations; however, determining the potential CN-S phenotypes is subject to sampling deviations. The 0.7 frequency of one dominant alin (Table 1) might result in a relatively few or zero cyst phenotype due to one xalin, even though the theoretical expectation is 0.09. Conversely, the 0.84 expected with the 0.4 frequency of one recessive alin could appear as 1.0 (or perhaps greater), relative to the check cultivar or standard of comparison. The intensity of selection s might be less than one, so there could be some cysts even with a m-alin frequency of unity. Thus it may not be possible to precisely estimate the frequencies of nematode alins. In spite of this limitation, CN alin frequencies are a necessary consideration for any genetic implications based CN-S phenotypes.

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References

Browder LE (1985) Parasite: host: environment specificity in the cereal rusts. Annu Rev Phytopathol 23:201–222

Ellingboe AH (1976) Genetics of host-parasite interactions. In: Heitefuss R, Williams PH (eds) Physiological plant pathology. (Encyclopedia of plant physiology, N.S.4). Springer, Berlin Heidelberg New York, pp 761–778

Ellingboe AH (1982) Host resistance and host-parasite interactions: a perspective. In: Mount MS, Lacy GH (eds) Phytopathogenic prokaryotes, vol 2. Academic Press, New York, pp 103–117

Ellingboe AH (1983) Genetical aspects of interaction between plant hosts and their soilborne pathogens. Phytopathology 73:941–944

Evans DM, Fox JA (1977) The sex ratio of *Heterodera glycines* at low population densities. J Nematol 9:207–210

Falconer DS (1981) Introduction to quantitative genetics, 2nd edn. Ronald Press, New York

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296

Hancock JA, Hancock FG, Caviness CE, Riggs RD (1987) Genetics of resistance in soybean to "race X" of soybean cyst nematode. Crop Sci 27:704–707

Hartwig EE (1981) Breeding productive soybean cultivars resistant to the soybean cyst nematode for the Southern United States. Plant Dis 65:303–305

Hartwig EE, Lehman SG (1951) Inheritance of resistance to the bacterial pustule disease in soybeans. Agron J 43:226–229

Keeling BL (1982) Four new physiologic races of *Phytophthora megasperma* f. sp. *glycinea*. Plant Dis 66:334–335

Koliapanos CN, Triantaphyllou AC (1972) Effect of infection density on sex ratio of *Heterodera glycines*. Nematologica 18:131–137

Layton AC, Athow KL, Laviolette FA (1984) Inheritance of resistance to *Phytophthora megasperma* f. sp. *glycinea* in a soybean plant introduction. Plant Dis 68:1080–1083

Lehman PS, Dunn FA (1987) Distribution of Florida populations of the soybean cyst nematode with previously described genetic variation. Plant Dis 71:68–70

Loegering WQ (1978) Current concepts in interorganismal genetics. Annu Rev Phytopathol 16:309–320

Loegering WQ (1984) Genetics of the pathogen – host association. In: Bushnell WR, Roelfs AP (eds) The cereal rusts. Academic Press, New York London, pp 165–192

Long SR, Meade HM, Brown SE, Ausubel FM (1982) Transposon-induced mutants of *Rhizobium meliloti*. In: Panopoulos NJ (ed) Genetic engineering in the plant sciences. Praeger, New York

Luedders VD (1983) Genetics of the cyst nematode-soybean symbiosis. Phytopathology 73:944–948

Luedders VD (1985) Selection and inbreeding of *Heterodera glycines* on *Glycine max*. J Nematol 17:400–404

Luedders VD (1987) A recessive gene for a zero cyst phenotype in soybean. Crop Sci 27:604–606

Peterson MA, Barnes DK (1981) Inheritance of ineffective nodulation and non-nodulation traits in alfalfa. Crop Sci 21:611–616

Rao AP, Anand SC (1986) Screening for cytoplasmic/maternal effects in resistance to soybean cysts nematodes. Soybean Genet Newslett 13:132–133

Rennie BD, Beversdorf WD (1987) On the response of the *Rpsl-b* allele to race 17 of *Phytophthora megasperma* f. sp. *glycinea*. Soybean Gene Newslett 14:82–84

Triantaphyllou AC (1975) Genetic structure of races of *Heterodera glycines* and inheritance of ability to reproduce on resistant soybeans. J Nematol 3:356–364

Triantaphyllou AC (1987) Genetics of nematode parasitism on plants. In: Veech JA, Dickson DW (eds) Vistas on Nematology. Painter, DeLeon Springs/Fl, pp 354–363

Wheeler H (1975) Plant pathogenesis. (Advanced series agricultural Sc. 2) Springer, Berlin Heidelberg New York

Wiberg A (1973) Mutants of barley with induced disease resistance to powdery mildew. Hereditas 75:83–100

Williams LJ, Lynch DL (1953) Inheritance of a non-nodulating character in soybean. Agron J 64:28–29

Young LD (1984) Changes in the reproduction of *Heterodera glycines* on different lines of *Glycine max*. J Nematol 16: 304–309