Preservation of Avirulence Genes of Potato Cyst Nematodes Through Environmental Sex Determination: A Model Involving Complete, Monogenic Resistance

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ABSTRACT

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Host-parasite compatibility strongly influences the sex determination of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). The probability of a juvenile developing into a male may be higher in the case of poor compatibility than in the case of full compatibility. As a result, avirulence toward the resistant host may lead to the production of more males. In contrast, virulence favors the production of females. These conflicting sexual trends imply opposing selection pressures by a resistant host. This may create an equilibrium frequency between the avirulent and the virulent nematodes, thereby preserving host resistance. In this way, parasite populations with environmental sex determination could maintain their genetic diversity at the expense of their reproduction rate. The equilibrium frequency of avirulence and the matching level of durable resistance are derived mathematically for complete, monogenic resistance.

The sex differentiation of potato cyst nematodes (*Globodera rostochiensis* (Woll.) Behrens and *G. pallida* (Stone) Behrens) is determined by their environment. If feeding conditions are good, then the probability of a juvenile becoming female is high. If feeding conditions are poor, then development into males occurs more frequently (1,2,8,14). Females require good feeding conditions for egg formation and so remain connected to the roots (5), whereas males stop feeding after the third molt, leave the roots, and move toward the females to copulate. The feeding conditions are mostly determined by competition between nematodes for feeding sites (9,14), root diameter (8), and host-parasite compatibility.

If a host-nematode combination is not fully compatible, the transfer of nutrients from plant to nematode is restricted (12). This gives rise to a high male:female ratio (7,15). Janssen et al (4) inoculated in petri dishes 400 avirulent *G. rostochiensis* juveniles (AA) on roots of a cultivar that carried the *H1* resistance gene; they found no females and 49 males. The inoculation of 400 juveniles on a susceptible cultivar yielded 220 females and fewer males, e.g., eight. A test with Aa juveniles provided similar results. With virulent juveniles (aa) on the resistant host, again the number of females was high and the number of males low.

Apparently, avirulence favored the production of males, whereas virulence favored the production of females. These conflicting trends may lead to an equilibrium in the frequency of avirulent nematodes and virulent nematodes rather than to a complete loss of avirulent nematodes. This implies that a breakdown of host resistance is prevented.

In this paper, the equilibrium proportion of avirulent nematodes and the matching level of durable resistance are derived in a mathematical way for complete, monogenic resistance.

THE MODEL

Frequency course of avirulent nematodes. Consider a host with complete, monogenic resistance that forces avirulent juveniles to become males, thus preventing the formation of avirulent females. Then, all females produced are virulent and therefore homozygous recessive for the virulence gene a (3). As a consequence, the progeny of these females cannot be homozygous for avirulence (AA). The probability P of AA offspring equals zero: $P(AA) = 0$ and

$$P(aa) + P(Aa) = 1. \quad (1)$$

For the males from this progeny on the resistant host it holds that:

$$m = m_{aa}\, P(aa) + m_{Aa}\, P(Aa), \quad (2)$$

where m = proportion of eggs that develop into males ($0 \leq m \leq 1$), $m_{aa} = m$ for homozygous recessive eggs (aa, virulent), and $m_{Aa} = m$ for heterozygous eggs (Aa, avirulent).

The frequency of the A allele equals 0 for the aa males and ½ for the Aa males, giving for the sperm:

$$P_m(A) = \frac{\frac{1}{2}\, m_{Aa}\, P(Aa)}{m}.$$

Substitution of m using equation 2 gives

$$P_m(A) = \frac{\frac{1}{2}\, m_{Aa}\, P(Aa)}{m_{aa}\, P(aa) + m_{Aa}\, P(Aa)}. \quad (3)$$

As, according to equation 1, $P(aa)$ equals $1 - P(Aa)$, the term $P(aa)$ can be eliminated:

$$P_m(A) = \frac{m_{Aa}\, P(Aa)}{2\, m_{aa}\,(1 - P(Aa)) + 2\, m_{Aa}\, P(Aa)}.$$

Substituting α for m_{aa}/m_{Aa} and Q for $P(Aa)$, this equation can be rewritten as

$$P_m(A) = \frac{Q}{2\alpha(1 - Q) + 2Q}.$$

As all females are homozygous recessive ($P_f(a) = 1$), the proportion of *Aa* nematodes in generation $t+1$ equals $P_m(A)$ of generation t:

$$Q_{t+1} = \frac{Q_t}{2\alpha(1 - Q_t) + 2Q_t}. \quad (4)$$

Figure 1 shows the relationship between Q_t and Q_{t+1} for some values of α. From this the development of Q over time can be derived, as shown in Figure 2. If the initial proportion of avirulent nematodes is low, then Q_0 lies on the left-hand side of the intersection of the curve and the 45° line in Figure 2 and Q increases. In contrast, if Q starts at a high value, it decreases. In Figure 2, the frequency of avirulent nematodes Q attains a final value that is greater than 0. Avirulent nematodes are not completely removed from the population, but the resistant host brings about an equilibrium $\hat{Q}$ in the avirulence frequency. When the virulence allele predominates, the resistant host supplies selection pressure for avirulence rather than virulence, producing an increase in Q.

Durable, incomplete resistance to a continuously heterogeneous population. With complete, monogenic resistance, the level of resistance to a population equals the proportion of avirulent nematodes Q in that population, as avirulent nematodes produce no cysts and virulent nematodes reproduce normally. If the proportion of avirulent nematodes has reached its equilibrium level $\hat{Q}$, then the level of resistance also equals $\hat{Q}$. Since $\hat{Q}$ is an equilibrium level, this incomplete resistance is durable.

Thus, although in this model resistance to avirulent individuals is complete and fully virulent nematodes are present, a durable, incomplete resistance may emerge at the population level. This phenomenon is caused by the equilibrium frequency of avirulent nematodes, which in turn stems from conflicting selection pressures by the resistant host via the two sexes.

Derivation of the equilibrium frequency of avirulent nematodes. $\hat{Q}$, and thus the level of durable resistance, can be derived analytically. In the case of equilibrium, Q is constant over time ($Q_t = Q_{t+1} = \hat{Q}$). This corresponds to the intersections of the $Q_t = Q_{t+1}$ line with the curves in Figures 1 and 2. Equation 4 then becomes

$$\hat{Q} = \frac{\hat{Q}}{2\alpha(1 - \hat{Q}) + 2\hat{Q}},$$

which can be rewritten as

$$2\alpha\hat{Q} - 2\alpha\hat{Q}^2 + 2\hat{Q}^2 - \hat{Q} = 0$$

and simplified to

$$\hat{Q}\{(2 - 2\alpha)\hat{Q} + (2\alpha - 1)\} = 0.$$

The roots are $\hat{Q} = 0$ and

$$\hat{Q} = \frac{1 - 2\alpha}{2(1 - \alpha)}. \quad (5)$$

The first solution refers to the case of the initial absence of A in the population ($Q_0 = 0$), no mutation of a to A, and no immigration of A during selection. As $P(aa) = 1 - Q$, the second solution can be transformed into

$$\hat{P}(aa) = \frac{1}{2(1 - \alpha)}. \quad (6)$$

Because $\hat{P}(aa) \leq 1$, this solution is biologically meaningful only if $2(1 - \alpha) \geq 1$. Therefore, $\alpha \leq \frac{1}{2}$, i.e., $m_{aa}/m_{Aa} \leq \frac{1}{2}$. Thus, if a completely monogenically resistant host is grown continuously, an equilibrium frequency of avirulent nematodes exists only if the proportion of *Aa* eggs that develop into males (m_{Aa}) is more than twice as great as the proportion of *aa* eggs that

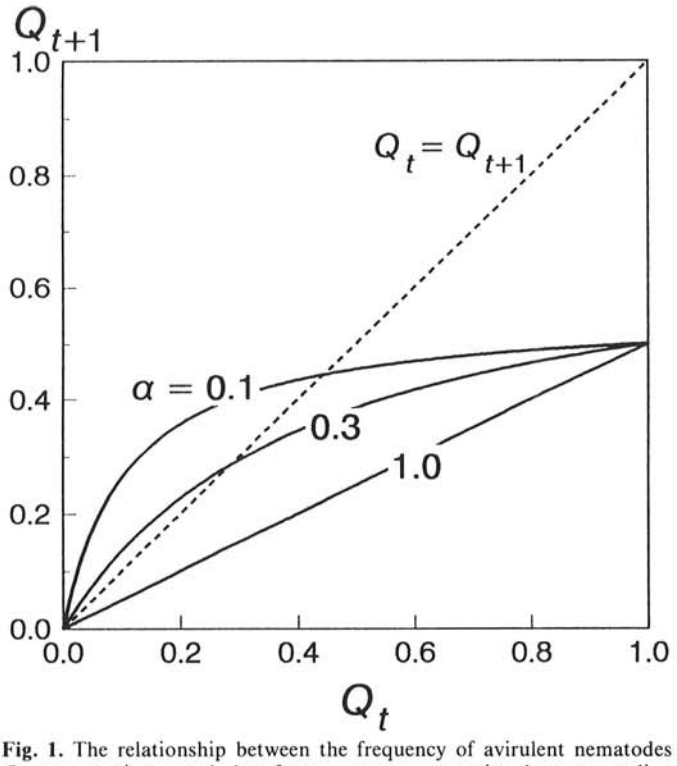

Fig. 1. The relationship between the frequency of avirulent nematodes Q at generation t and that frequency one generation later, according to equation 4. The monogenic resistance of the host forces avirulent nematodes to the male development path, thus preventing the formation of avirulent females. α = The ratio between the proportion of eggs that develop into males in the case of virulence and that proportion in the case of avirulence.

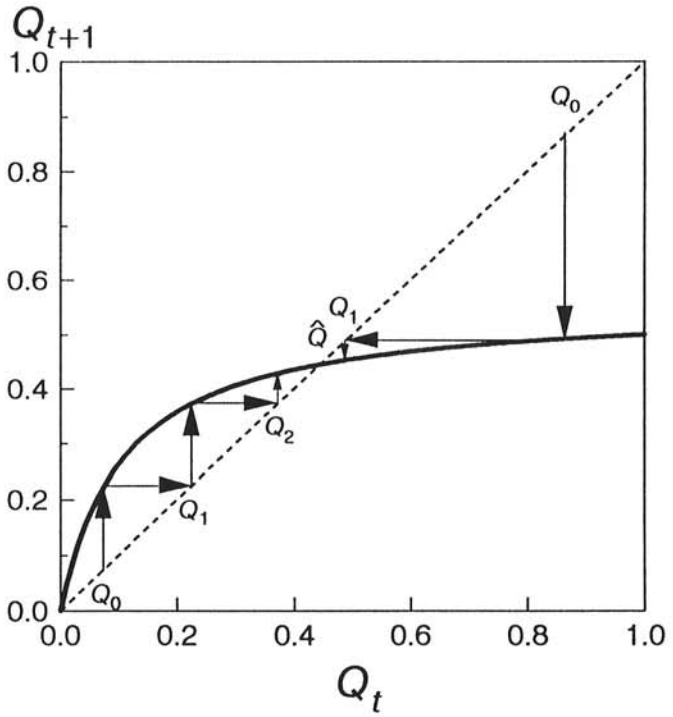

Fig. 2. As Figure 1 for $\alpha = 0.1$. The 45° line represents the $Q_t = Q_{t+1}$ situation. Starting at avirulence frequency Q_0, the frequency of avirulent nematodes in the next generation Q_1 can be read from the solid curve. Using this frequency as the new starting point of Q_t (arrow to 45° line), the curve gives the value of Q_2. This process is continued until the population arrives at the equilibrium point $\hat{Q}$. The same procedure can be followed for genotype frequencies greater than the equilibrium value, showing again that the genotype frequency converges to the stable equilibrium point at the intersection of the curve and the 45° line.

develop into males (m_{aa}). Otherwise, the avirulence allele will finally be lost from the population. The equilibrium $\hat{Q}$ is stable for $\alpha < ½$ (see Appendix).

DISCUSSION

Overlapping generations. For the above derivation of the equilibrium frequency of avirulence, it was assumed that the nematode generations do not overlap. Under field conditions, however, not all eggs in the cysts hatch when a potato crop is grown; a proportion will hatch in later years. This leads to overlapping generations and therewith to a reduction of the selection rate. The final virulence level and the level of durable resistance are not affected by an overlap of generations (13).

Implications. As shown, environmental sex determination may lead to an equilibrium proportion of avirulent nematodes on a resistant host and thus to a level of durable resistance. As a consequence, the reproduction rate of the nematode population is lower than in the case of complete selection for virulence. Through environmental sex determination, parasite populations could maintain their genetic diversity at the expense of their reproduction rate.

This result may be relevant to practice. Genetic diversity with respect to virulence implies mixtures of pathotypes. The existence of such mixtures may hamper the pathotype determination of populations in soil samples.

As appears from Figure 1, the equilibrium proportion of avirulent nematodes is always less than 0.5. Consequently, the level of resistance at equilibrium is under 50%. Unfortunately, this is too susceptible to arouse practical interest among potato growers.

Other host-parasite combinations. This theory on incomplete selection for virulence can be applied to other parasite species that show environmental sex determination. Müller (10) showed that the sex determination of beet cyst nematodes (*Heterodera schachtii* Schmidt) is affected by host resistance. Moreover, he reared a population of *H. schachtii* repeatedly on a resistant host, and the relative susceptibility increased from 2.5 to 52% after six generations (11). This level, however, could not be raised further through three additional generations on the host (J. Müller, *unpublished*; 6). Müller's findings may provide support for the present theory or, alternatively, may point to an extra resistance gene that conferred partial resistance toward which the population was not virulent.

APPENDIX

It can be proved that the equilibrium frequency $\hat{Q}$ is stable for $\alpha < ½$ for complete, monogenic resistance. Equation 4 is identical to

$$Q_{t+1} = Q_t \frac{1}{2\alpha(1 - Q_t) + 2Q_t}.$$

Q increases if Q_{t+1} exceeds Q_t, which is the case if

$$\frac{1}{2\alpha(1 - Q_t) + 2Q_t} > 1.$$

That is, $2\alpha(1 - Q_t) + 2Q_t < 1$

$$Q_t < \frac{1 - 2\alpha}{2(1 - \alpha)}.$$

The right-hand term equals $\hat{Q}$ according to equation 5. In other words, Q increases if it is lower than its equilibrium frequency. In analogy, it can be shown that Q decreases if $Q > \hat{Q}$. This indicates the stability of equilibrium $\hat{Q}$.

In addition, stability without oscillation requires:

$$\left| \left(\frac{dQ_{t+1}}{dQ_t}\right)_{Q_t = \hat{Q}} \right| < 1.$$

Differentiation of Q_{t+1} with respect to Q_t and substitution of Q_t by $\hat{Q}$ using equation 5 yield 2α for the left-hand term. If $\hat{Q} > 0$, then $\alpha < ½$ and consequently $|2\alpha| < 1$. This is a formal proof for the stability of $\hat{Q}$ and thus for its complement $\hat{P}(aa)$.

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