THE INFLUENCE OF MULTIPLE MATING ON THE REPRODUCTION AND GENETICS OF HETERODERA ROSTOCHIENSI S AND H. SCHACHTII

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Females of Heterodera rostochiensis Woll. embedded in agar or peat were inseminated more readily than in sand. Females removed from their host plants with small pieces of root attached lived longer and produced more eggs than those taken from roots. The behaviour of males in the presence of females was observed, or was deduced from tracks left on an agar surface or from their behaviour towards attractant substances secreted by the females and absorbed in agar discs.

Males of H. rostochiensis attracted each other slightly but were much more strongly attracted to females. When females were in groups, males at a distance responded to the group as a whole, rather than to individuals, but when near to the group they tended to be 'captured' by a single female near the edge of the group.

To estimate the proportions of ineffective males and sterile females in matings and the influence of multiple matings on fecundity, females were mated with different numbers of males. Males of H. rostochiensis and H. schachtii Schm. seemed able to inseminate up to ten females. Fecundity depended more on the age of the females when taken from the roots than the number of males that inseminated them.

When several males collected around a single female they obstructed each other in attempts to copulate, but several must have succeeded in inseminating the female, counteracting the loss of fecundity caused by sterile or impotent males of which there were many.

The genetic effects of multiple matings on inheritance were explored theoretically, using as an example the ability of some larvae of H. rostochiensis to become females in the roots of eelworm resistant potatoes bred from Solanum tuberosum ssp. andigena. Kort's data from single cyst cultures seemed to fit the theory that this ability depended on a recessive gene occurring infrequently. It seems that neither the first nor the last mating was the only effective one and multiple mating would cause a greater diversity of genes in the offspring of one female. Promiscuity and the ability of males to mate several females successfully, are advantageous in species such as H. rostochiensis and H. schachtii, in which the sex ratio ranges greatly and males may be outnumbered nine to one in sparse populations. Because eggs are concentrated in cysts, the progeny from cysts tend to invade roots in groups and clusters of females result. Grouping enhances their attractiveness to males. The males leave the females they have recently mated, move away and so have the opportunity of fertilising others.

Although many workers have studied population fluctuations of cyst-forming nematodes when different host and non-host crops are grown, few have paid attention to reproduction, yet a study of this subject might suggest new methods of control, aimed to prevent multiplication rather than to kill eggs and larvae. Diminishing the population of eggs and larvae can improve crop yields greatly, but usually it diminishes them to a point where multiplication is fastest, so numbers
after cropping may be greater than they would otherwise be, whereas controlling reproduction would decrease numbers after harvest (Jones, 1969).

In an obligate bisexual species the first step in the reproductive cycle is the union of the sexes. Green (1966) showed that *Heterodera* females produced substances that alert and attract males, and Peters (1952) and others observed males, sometimes in great numbers, clustering around single females. From the frequency of new cysts on the roots of susceptible and resistant potato varieties in single-cyst cultures, Jones (1967) deduced that females of *H. rostochiensis* may be inseminated by as many as six or seven males. There is, therefore, reason to suppose that males mate more than once and that females are mated successfully by several males. Sex attractants, promiscuity and the grouping of eggs in cysts or egg masses have many advantages in maintaining old and establishing new infestations of species such as *H. rostochiensis*, in which the sex ratio differs greatly according to root conditions and larvae and males can move only short distances.

This paper describes experiments on mutual attraction between males, the attraction of males to groups of females and the effects of multiple matings on fecundity. The influence of multiple mating on genetic characters controlled by simple dominant or recessive alleles is deduced and its implications discussed.

**MATERIALS AND METHODS**

In our work *H. rostochiensis* means the so-called pathotype A which is distinct from pathotypes B and E and does not interbreed with them (Parrott in litt.). These pathotypes are evidently different species or sub-species and are as yet undescribed.

Virgin males of *H. rostochiensis* and *H. schachtii* were collected as described by Green (1966), except that the plants were supported by slotted aluminium sheets which darkened the bowls or funnels and left the roots freely suspended in the water. Except where otherwise stated, undamaged females taken from the roots were mated on 0.8% Oxoid Ionagar No. 2, in 5 or 10 cm diameter polystyrene Petri dishes. The dishes were incubated at 15°C±1°C in the dark for at least 21 days before examining the females to see whether they contained eggs. Behaviour of males was observed or deduced from tracks left on the agar surface. Such tracks were either photographed or permanent casts were made with nail varnish (Green 1966). The attractiveness of secretions to males was assessed by impregnating 5 mm diameter agar discs with the secretion and using these as targets on 5 cm diameter agar plates. The degree of attraction was scored in a logarithmic scale ranging from 0.70 to 2.54 in twenty steps, corresponding to the twenty-one possible positions five males can occupy (5, 0, 0; 4, 1, 0; .......... 0, 0, 3) within the target, inner and outer areas into which the Petri dishes were divided (Greet, Green & Poulton, 1967). A score of about 1.00 indicates random movement unaccompanied by attraction, whereas a score of 2.54 indicates that all males reached the target.