

## Numerical Relationships between a Nematode Parasite, *Romanomermis culicivorax*, and Its Host Population, *Culex pipiens molestus* Larvae

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It is well established that the proper number of preparasitic or infective stage nematodes of *Romanomermis culicivorax* Ross and Smith, 1976 (*Reesimermis nielsenii* auct. partim.), a biocontrol agent of mosquitoes, need to be released into the mosquito larval habitat, for successful control. Thus Petersen and Willis (1970) demonstrated in the laboratory that exposure of a larval population to more than three times its number of preparasites makes for high incidence of parasitism. Percentage parasitism was shown to be higher at a host density of 0.7 than at the density 2.0 and 2.9 (Petersen, 1973a).

The present paper deals essentially with the results of experimental infection. Host and parasite were exposed to one another in varying numbers and densities. The data were analyzed to evaluate the effect of the host densities and the ratio of parasite to host on the percentage parasitism. The host-finding behaviour of the nematode population is also discussed.

### Materials and Methods

Cultures of *R. culicivorax* used in this study were kindly supplied by Dr. J. J. Petersen, Gulf Coast Mosquito Research Laboratory, Agricultural Research Service, USDA, Louisiana, USA. *Culex pipiens molestus* Forskal from a laboratory colony served as the host mosquito. In all tests, known numbers of

early second instar larvae were placed together with the preparasitic nematodes hatched five hours previously, in the plastic containers measuring 12×20 cm which were filled with 720 ml of distilled water. The containers were kept in the room at 25 C and given a continuous light aeration. Number of preparasitic nematodes were determined by means of a stereoscopic dissecting microscope, or by the method of Petersen and Willis (1970). The finely ground powder of commercial mouse-food were given as the food of mosquito larvae at quantity of 4 mg per larva on the first day.

Six groups of 24 (density 0.1/cm<sup>2</sup>) to 768 (density 3.2) *C. p. molestus* larvae were exposed to 8 groups of 24 to 3072 preparasitic nematodes. The actual combinations of host and parasite groups are indicated in Table 1. All the experimental infections were repeated from two to four times, depending on larval density. Ninety-six hours after exposure, the larvae were separated and dissected under a dissecting microscope. The number of parasitic nematodes recovered from each larva was recorded.

An additional series of exposure (paralleled by an uninfected control) was undertaken at a larval density of 0.4. These were kept until the pupation of the uninfected hosts, 150 mg of food being provided each day. The number of postparasitic nematodes hatched, the number of larvae paratized, the number

Table 1 Effect of host density and number of parasites exposed, on the proportion of larvae parasitized (P), mean number of nematodes per host (m) and the survival rate of preparasitic nematodes (s)

| No. of larvae (density) |   | No. nematodes exposed |      |      |      |      |      |       |       |
|-------------------------|---|-----------------------|------|------|------|------|------|-------|-------|
|                         |   | 24                    | 48   | 96   | 192  | 384  | 768  | 1,536 | 3,072 |
| 24(0.1)                 | P | 0.1                   | 0.18 | 0.59 | 0.29 | 1.0  |      |       |       |
|                         | m | 0.1                   | 0.2  | 0.8  | 1.2  | 2.6  |      |       |       |
|                         | s | 0.1                   | 0.1  | 0.2  | 0.15 | 0.16 |      |       |       |
| 48(0.2)                 | P | 0.12                  | 0.17 | 0.33 | 0.81 | 0.98 | 1.0  |       |       |
|                         | m | 0.11                  | 0.17 | 0.37 | 1.14 | 1.76 | 6.08 |       |       |
|                         | s | 0.22                  | 0.17 | 0.19 | 0.28 | 0.22 | 0.38 |       |       |
| 96(0.4)                 | P | 0                     | 0.11 | 0.15 | 0.51 | 0.9  | 1.0  | 1.0   | 1.0   |
|                         | m | 0                     | 0.11 | 0.15 | 0.63 | 1.6  | 4.2  | 6.04  | 15.14 |
|                         | s | 0                     | 0.22 | 0.15 | 0.31 | 0.4  | 0.52 | 0.37  | 0.47  |
| 192(0.8)                | P |                       | 0.02 | 0.14 | 0.33 | 0.7  | 1.0  | 1.0   | 1.0   |
|                         | m |                       | 0.02 | 0.14 | 0.41 | 1.03 | 2.2  | 4.4   | 12.45 |
|                         | s |                       | 0.08 | 0.28 | 0.41 | 0.52 | 0.55 | 0.55  | 0.77  |
| 384(1.6)                | P |                       |      | 0.02 | 0.16 | 0.31 | 0.4  | 0.56  | 1.0   |
|                         | m |                       |      | 0.02 | 0.16 | 0.33 | 0.5  | 0.72  | 2.75  |
|                         | s |                       |      | 0.08 | 0.32 | 0.33 | 0.25 | 0.18  | 0.34  |
| 768(3.2)                | P |                       |      |      | 0.08 | 0.12 | 0.24 | 0.28  | 0.93  |
|                         | m |                       |      |      | 0.08 | 0.12 | 0.24 | 0.34  | 2.34  |
|                         | s |                       |      |      | 0.32 | 0.24 | 0.24 | 0.17  | 0.58  |

of larvae pupating and the number of larvae died during the observation periods were recorded.

**Results**

The proportion of larvae parasitized (P) and the mean number of parasites per exposed host (m) are given in Table 1 by the combination of the host densities and the number of preparasites applied. As the number of preparasitic nematodes increased, so did the percentage parasitism following a pattern of sigmoid curves through the six different densities of host. When the percentage parasitisms were transformed to probit values (Y) and were plotted against the ratio of preparasite to host on the X-axis in the logarithmic scale, there were liner relationships observed (Fig. 1), except at the host densities of 1.6 and 3.2 in which the

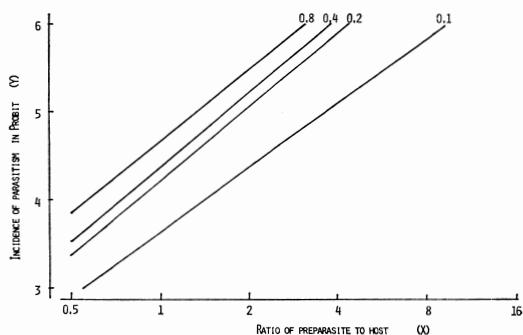


Fig. 1 Relationships between percentage parasitism in probit and the ratio of preparasite to host in log, for each of the host densities, 0.1~0.8, examined.

regression lines did not show a good fitness. The regression equations and the ratio of parasites to hosts which is required to give 95% parasitism (PR<sub>95</sub> values) were calculated as follows; when the density is

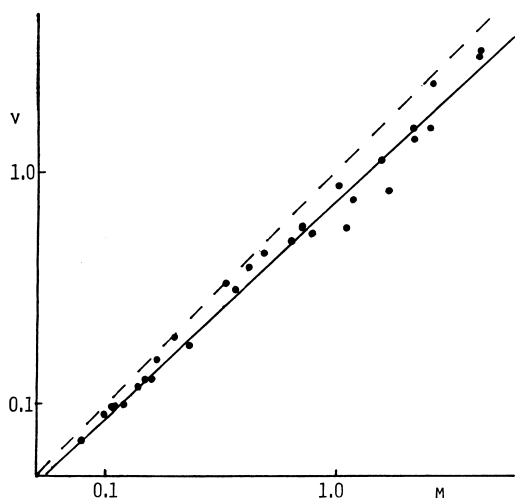


Fig. 2 Relationships between the mean number of parasites ( $m$ ) and the variances ( $v$ ) in log. Dotted line illustrates the randomness.

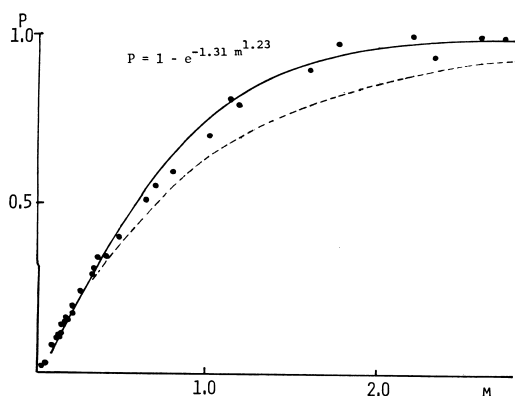


Fig. 3 Relationship between the proportions of larvae parasitized ( $P$ ) and the mean number of parasites per host ( $m$ ). Dotted line shows the expectation from Poisson distribution.

|      |                          |                  |
|------|--------------------------|------------------|
| 0.1, | $Y = 2.48 \log X + 3.59$ | $PR_{95} = 16.9$ |
| 0.2, | $Y = 2.78 \log X + 4.23$ | 7.3              |
| 0.4, | $Y = 2.83 \log X + 4.35$ | 6.5              |
| 0.8, | $Y = 2.66 \log X + 4.67$ | 5.5              |

$PR_{95}$  value was 16.9 at the host density of 0.1 and it decreased as host density increased up to 0.8.

The mean number of parasites ( $m$ ) was greater than its variance ( $v$ ); and the relation between  $m$  and  $v$  is shown in Fig. 2, together with a broken line representing

randomness in the spatial distribution of the nematodes. The points plotted lay towards the X-axis. This implies that the frequency distribution of the parasites in mosquito larvae is regular or represents under-dispersion.

The value  $m$  increased as the proportion of larvae parasitized ( $P$ ) increased for each of the host densities as shown in Table 1. The correlation between  $m$  value and  $P$  was highly significant. Fig. 3 shows  $P$  value equal to  $m$  at less than  $P=0.15$ , and their increased exponentially. The abscissa in Fig. 3 shows the mean numbers and ordinate the proportions of larvae parasitized. So that  $m$  may be estimated from  $P$  or *vice versa*. The incidence by Poisson distribution is also plotted with a broken line in Fig. 3. Where the frequency distribution of infection per host was fully random, the  $P$  value is to be expected; for this broken line incidence will be  $1 - e^{-m}$ . However, the formula  $P = 1 - e^{-am^b}$  ( $a$  and  $b$  being constant) was derived from the present results. By estimating the parameters  $a$  and  $b$ , the following formula was obtained.

$$P = 1 - e^{-1.31m^{1.23}}$$

The two parameters being greater than 1.0, this suggests also that the distribution pattern of the parasite population in its habitat, the host population, signifies under-dispersion. There were, however, some exceptions, e.g. the 0.2 larval density exposed to 768 parasites (i.e. a parasite-host ratio of 16:1), and 0.4 and 0.8 larval density for those exposed to 3072 (i.e. ratio 32 and 16 respectively). These three were excluded from the above calculations and graphs, for a few larvae survived even at day four due to heavy infections. As far as the survivors were examined,  $m$  values were quite high; and the variances were greater than  $m$ .

The survival rate of nematodes or recovery rate ( $s$ ), which is the proportion of nematodes recovered from mosquito larvae to the nematodes introduced to the habitat, following the formula;  $s = m \times \text{No. larvae exposed} / \text{No. preparasitic nematodes introduced}$ .

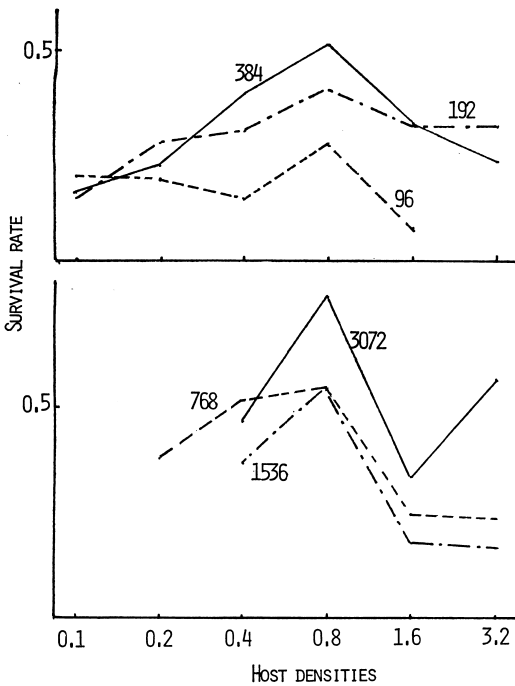


Fig. 4 Relationships between the survival rate of nematodes and host densities, for six different parasite population.

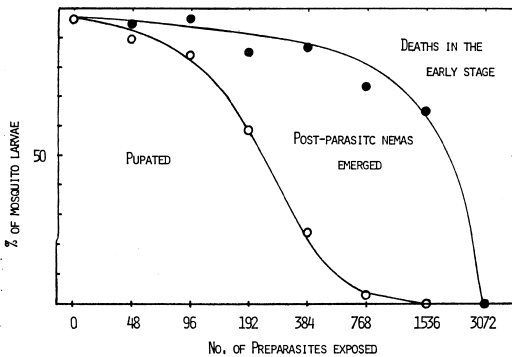


Fig. 5 The effect of the number of preparasitic nematodes on the pupation, and mortality in the early stage, for 96 mosquito larvae.

Values for  $s$  are in Table 1. The survival rates increased with host density rose in general. However the highest survival rate was recorded at host density 0.8 throughout 6 different preparasitic populations, with the exceptions of lowest two nematode populations. This was irrespective of the ratio of

preparasite to host, as shown in Fig. 4.

The larval mortality and the number of larvae emerging post-parasitic nematodes were observed for the series of larval density 0.4. Ninety-six percent of the larvae were survived and pupated, the remaining 4% dying, during the early larval instars of an unexposed control population (Fig. 5). Although some larval populations exposed to the lower densities of nematodes give similar results, the mortality increased as the number of parasites increased. This was particularly so for those larvae exposed to preparasitic nematodes with very high ratio. All the larvae were dead within two to five days in the case of exposure to 3072 nematodes (i. e. ratio 32) and no post-parasitic nematodes were found but more than 15 nematodes were dissected from a single dead larva.

### Discussion

It has been pointed out that both larval density and the ratio of preparasites to hosts at the time of exposure are important factors in determining the percentage parasitism (Petersen 1973 a, b, and Chen 1976). These authors used first instar larvae of *C. quinquefasciatus* as the host, and estimated the percentage of the uninfected controls commenced. Therefore, their data cannot be directly compared with those presented here. However, it appears generally the rule that higher levels of parasitism occur at higher densities if the ratio is same (where the range is below 0.8).

Effects of the two factors on the rate of parasitism were expressed using log-probit analysis. The regressions show the incidence of parasitism increasing as the ratio of preparasite to host (at the time of exposure) increases. The dosage of preparasitic nematodes needed to preclude mosquito breeding is easily approximated from Fig. 1; and can be calculated, using the above formulae, for the various larval densities. The higher dosage of nematodes induced the higher mortality of mosquitoes.

However, where the preparasitic nematode

densities become very high relative to those of the host, recycling of the nematodes in the habitat may be interfered. Host larvae and parasitic-stage nematodes were also eliminated when the preparasitic nematodes outnumbered the host population by 32 times. Chen (1976) also observed that with a ratio of up to 15 times, the number of nematodes harvested was lower compared with 10 times and 7.5 times; he assumed that some of the hosts were killed by overdosing with preparasitics. It seems overdose easily induces the parasitism more than the load of host and that the host simply cannot withstand an overdose of parasitic *R. culicivora*x, which invariably causes the early death of all mosquito larvae. This is, of course, disadvantageous with respect to the establishment of nematodes in a given area.

The survival rate of nematodes was also effected by host densities. The parasites must be more active than their host and the probability of contact of preparasitics to host increase when the host is abundant; yet, the survival rate declined beyond the host density 0.8 without respect to the ratio of preparasite to host, except where extremely small numbers (e. g. 24-48 nematodes) were introduced into container. There would be an optimum host density for preparasitic populations in the general environment. By way of contrast, the high density of the host larvae (above 0.8), may effect protection from the attack of the parasites.

In a previous paper (Kurihara, 1976) it was shown that the rate of parasitism was lower when the caged larvae were held at an edge or two specific locations than when the caged larvae were dispersed over more than three locations, though the same number of larvae and nematodes were exposed. Then, a host population heavily parasitized 14 hours previously were hardly infected at all when it was exposed to the infective parasites. This type of behaviour of the preparasitic nematodes was also evident from the present study. Two parameters of the formula  $P=1-e^{-am^b}$  indicated more than 1.0, and the mean values were greater than the

variances. This confirmed that the distribution pattern showed neither over-dispersion nor random but under-dispersion. These results give the general impression that the parasite distributes widely in the water with lack of aggregation, and each of the nematodes moving randomly through a rather limited area offering the possibility of host discovery.

The relationship of  $m$  and  $P$  values was slightly altered when the calculation was applied to the previous paper.  $P=1-e^{-1.62m^{1.42}}$  was given for larvae exposed more than 4 days. This almost resembles the equation of  $P=1-e^{-m}$ , where larvae were exposed for six hours only. Though the  $m$  values are a good prediction of percentage parasitism, it will be necessary to secure confirmations from field populations.

### Summary

The numerical relationships between an experimental population of *Romanomermis culicivora*x, and its host population, *Culex pipiens molestus* have been studied. Introducing the infective stage nematodes to second instar larvae in various combination permitted the incidence of parasitism to be examined. Regression analysis on the relation between percentage parasitism and the ratio of preparasite to host indicated that the ratio resulting in the high incidence of parasitism varies with host density. The ratios attained 95% parasitism or  $PR_{95}$  values were decreased as host density rose, up to the density 0.8/cm<sup>2</sup>. Extremely high ratios of preparasites to larvae proved unfavourable to the recycling of the nematodes in the habitat, due to early death of the host with consequent inhibition of parasite development. The proportion of parasites recovered from host to the preparasites exposed to the host increased with increase of the number of host larvae, and the trend was revised when the host density reached 0.8 for most of the parasite densities. Parasite distribution in mosquito larvae appeared to represent an under-dispersion.

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蚊幼虫寄生線虫 *Romanormis culicivorax* がその宿主チカイエカ への  
寄生を高める要因—とくに両者の個体数の関係について

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*Romanormis culicivorax* は、蚊幼虫に寄生し、宿主を殺す線虫で、人工的に放流して蚊の天敵利用防除をする試みが注目されている。一定数の感染幼虫期線虫に対しては宿主密度が低い程、寄生率の高まることを前報(栗原1976)にしめしたが、その後宿主と感染幼虫両者の密度をさまざまに変えた組み合わせで、寄生率と、宿主選択の生態を検討した。

0.1~0.8/cm<sup>2</sup> の4段階の幼虫密度と8段階の感染幼虫投与量(ここでは感染幼虫数:蚊幼虫数の比で示されているが)の組み合わせの感染実験の結果、寄生率と投与量の間に関係式が作られ、これによつて、各密度ごとに95%の寄生率をえるための投与量 PR<sub>95</sub> を求めることができた。PR<sub>95</sub> の値は密度が高まるにつれて低下した。すなわち少ない投与で寄生率が高まる。高密度1.6と3.2では投与が各8倍と4倍の際に急激に寄生率が高まり、前述のような関係式はえられない。

感染幼虫数が宿主数に対して著しく多数だと(比が高いと)、すべての宿主が過重の寄生を受けて、若齢のうちに死滅してしまい、寄生をした線虫も成長できない。このことは蚊の防除の目的には望ましいことだが、天敵である本種線虫を発生源内に定着させて生活史をくり返

させる意図には向いていない。

宿主幼虫1匹あたりの平均寄生数  $m$  と、その幼虫群の寄生率  $P$  との間に、 $P=1-e^{-am^b}$  の関係式を求めえた。これを図示することにより、 $m$  の値を求めて、 $P$  値またその逆を推定することができる。この定数  $a$  と  $b$  は1より大であり、また  $m$  の分散の値は  $m$  より小さい。このことから、この寄生虫の宿主群内での分布は、一様性のある非集中型の分布型をとると推察される。感染幼虫が、既に寄生を受けた宿主を避けて未感染の宿主を求める傾向のあらわれと考えられる。多くの動物寄生虫で、宿主群の特定個体に偏在する集中分布型をしめす例が知られているが、それとは異なり蚊防除に利用するには好都合な宿主攻撃性をもつといえよう。

宿主の密度は、一定数の感染幼虫に対し、宿主体内に侵入定着に成功する比率  $s$  をも支配している。宿主密度が高まる程、 $s$  値も高まる。しかし密度0.8で最大の  $s$  値がえられ、それ以上では逆に  $s$  が低下した。特に少数の感染幼虫群を除いて、この傾向は一定している。宿主密度0.8は、本線虫の宿主発見・侵入に最適な密度であり、逆にこれより高い密度の宿主は、本線虫の行動を阻害するものと解される。