Genetic variation in progeny allocation in *Trichogramma maidis*

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Abstract

A considerable amount of work has been recently devoted to the study of progeny allocation strategies by insect parasitoids under different environmental constraints (see Waage, 1986, for a review). All these studies present each of the oviposition strategies (e.g. number of eggs laid per host, superparasitism) as a phenomenon which is progressively settled by natural selection during the course of generations. However, this optimal progeny allocation theory is conceivable only if the variation in wasp's biological traits involved are under a genetic control on which natural selection could act. We have decided therefore to look for a genetic variability in these traits in a *Trichogramma maidis* Pintureau & Voegelé (Hym.; *Trichogrammatidae*) population.

Based on histological staining which allows to count the number of *Trichogramma* eggs deposited in each host (*Ephestia kuehniella* Zeller), we compared different wasp isofemale lines (i.e. families) each of which was submitted to different conditions (i.e. parasite/host ratios).

The results show that the control of superparasitism seems to be genetically determined. This appears true both for the average number of eggs deposited in each host, and for the frequency distribution of wasp eggs among hosts.

The evolutionary consequences of such results and their possible applications in order to improve the wasp's mass-rearing conditions are discussed.

Introduction

As pointed out by Van Dijken & Waage (1987), the term 'superparasitism' is often used to describe two different mechanisms involved in progeny allocation by insect parasitoids. Some authors use this word to explain the fact that females lay more eggs in or on a host than can develop to the imaginal state (Salt, 1934; Van Lenteren, 1981). Others use it to describe the decision by the ovipositing female to allocate one or more eggs to previously parasitized hosts (see

Waage, 1986 for a review). Furthermore, in this last case, two different mechanisms seem to be involved: one in which a female lays eggs in or on hosts which she has previously attacked (self-superparasitism), and an other in which a female lays eggs in or on hosts previously attacked by other conspecifics (conspecific superparasitism) (Bakker *et al.*, 1985; Waage, 1986; Van Dijken & Waage, 1987; Hubbard *et al.*, 1987; Waage, 1988).

Because supernumerary eggs allocated to hosts have a lower chance to become adult than others,

all these so-called superparasitism mechanisms have been widely considered as disadvantageous and so as a maladaptive mistake (Fiske, 1910; Van Lenteren, 1981). Recently, however, different theoretical and experimental studies have shown that superparasitism can often be advantageous if an egg allocated to a host still has a finite probability of survival in competition with previously deposited ones (Van Alphen & Nell, 1982; Parker & Courtney, 1984; Charnov & Skinner, 1984, 1985; Bakker et al., 1985; Van Alphen et al., 1987; Hubbard et al., 1987; Waage, 1988). Thus, all these studies support the hypothesis that observed progeny allocation strategies (e.g. number of eggs laid per host, superparasitism) are adaptive, and they are therefore implicitly based on the assumption that such strategies are progressively settled by natural selection during the course of generations.

Such an evolutionary approach gives, at least in part, answers to different questions such as: (a) why some parasitoid species are solitary and others gregarious? (Waage, 1986; Le Masurier, 1987), and (b) how can we produce natural enemies that are more efficient to control pests in biological control programs? (Waage, 1988).

However, this appealing optimal progeny allocation theory is conceivable only if the wasp's biological traits involved are under a genetic variation on which natural selection could act. Showing the existence of such variation could contribute to a better understanding of host-parasite interactions (Boulétreau, 1986). It could also give us means, by applying genetic selection programs (Mackauer, 1976; Roush, 1979), to avoid as much as possible mechanisms such as superparasitism (Wajnberg & Pizzol, 1989). Indeed, in large-scale production of natural enemies for inundative biological control, this phenomenon often leads both to a decrease in the fitness of the surviving parasitoids (i.e. fecundity, longevity, searching rate, etc.) (Chacko, 1969; Waage, 1986) and to the emergence of the population with a male-biased sex-ratio (Waage, 1986, 1988).

We decided therefore to look for a genetic (i.e. polygenic) variability of some of the biological traits involved in a *Trichogramma maidis*

Pintureau & Voegelé (Hym., *Trichogrammatidae*) (= *T. brassicae*) population. This wasp, which is a parasitoid of the eggs of several moth species, was chosen because it is one of the most intensively produced insect over the world for inundative biological control programs (DeBach, 1974; Stinner, 1977; Pak, 1988). Furthermore, whatever the hosts attacked, *Trichogramma* females show a high variation in the number of eggs laid per host (e.g.: Klomp & Teerink, 1962, 1967 for *T. embryophagum*), and seems therefore to be a good model for such experimental study.

We used an histological staining procedure which allows us to count the number of *Trichogramma* eggs deposited inside each host (the eggs of the mediterranean flour moth *Ephestia kuehniella* Zeller (Lep.; *Pyralidae*)). We compared, with the isofemale lines method (Parsons, 1980), different wasp families, each of which was submitted to different conditions (i.e. parasite/host ratios). The results suggest that the distribution of wasp eggs over the hosts is genetically determined, both for the average number of eggs deposited in each host (i.e. superparasitism intensity) and for the frequency distribution of eggs among available hosts.

The evolutionary consequences of such results and their possible applications in order to improve the wasp's mass-rearing conditions are discussed.

Materials and methods

The *T. maidis* strain used in the experiments was reared from about thirty parasitized *Ostrinia nubilalis* Hübner egg masses collected during summer 1986 in Alsace (France). This strain probably originated from the Moldavian population (Pintureau & Voegelé, 1980) released in this area for biological control against the European Corn Borer. Its electrophoretic pattern for esterases was the same as the one described by Pintureau & Babault (1981). From the time of capture onwards, the strain was maintained under laboratory conditions for 60 generations on *E. kuehniella* eggs, at 25 °C, L12:D12.

Experiments. Ten mated *T. maidis* females, taken arbitrarily from the laboratory mass-rearing were used to found ten isofemale lines (i.e. ten families). After two generations of multiplication, *Ephestia kuehniella* eggs were offered to mated females (less tan 24 hours old) from each line, in glass tubes (1 × 7 cm), according to different female/host ratios. In order to accurately describe superparasitism, these ratios took four different values: $1/10$, $1/4$, $1/2$ or $1/1$. This was done in two different ways: (1) the number of females was kept constant and equal to 10, and the number of hosts offered was: 100, 40, 20 or 10 eggs, or (2) a fixed number of hosts (40) and different numbers of females (4, 10, 20 or 40). The whole experimental procedure is summarized in Fig. 1.

After 24 h of parasitization (at 25 °C), females were removed and host eggs were histologically stained in order to count the number of *Trichogramma* eggs deposited inside each of them. The staining procedure is described below:

90 min of fixation in Carnoy (acetic acid, chloroform and 100% ethyl alcohol in a proportion of 1:3:6), followed by a coloration during 120 min with Toluidine Blue (in saturation in 70% ethyl alcohol). Then, a selective decoloration was done through successive incubations in ethyl alcohol with increasing concentration: 15 min in 70%, 15 min in 80%, 15 min in 80% with added acetic acid, 15 min in 96% and 15 min in absolute ethyl alcohol. Finally, hosts were dehydrated in toluene for 15 min, mounted on slides with Canada Balsam and observed under a standard microscope.

In order to increase the relevance of the statistical analyses, two replicates were done for each isofemale line and for each female/host ratio. Thus, a total of 6600 hosts were stained and the number of wasp eggs deposited inside each of them was recorded.

For the different traits observed, the isofemale lines were compared with an analysis of variance, after pooling the two replicates done in each case.

Results

Frequency distributions of wasp eggs per host for all the female/host ratios and for the ten isofemale lines are given on Fig. 2. Data corresponding to line B are missing for one experimental condition (10 females/40 hosts), the corresponding hosts were lost during the staining procedure. These data have been treated as standard missing values in all analyses of variance.

There are substantial variations in such frequency distributions between the different experimental conditions and the ten families studied. Are such variations statistically significant?

Variations in superparasitism intensity. If we quantify superparasitism intensity by the average number of parasite eggs deposited inside each host (Fiske, 1910), it becomes possible to compare statistically this intensity between the different isofemale lines and the different experimental conditions.

Results given in Table 1 show that there is a strong female/host ratio effect on this parameter: the more this ratio increases, the more the average number of eggs deposited in each host rises correspondingly (Fig. 3).
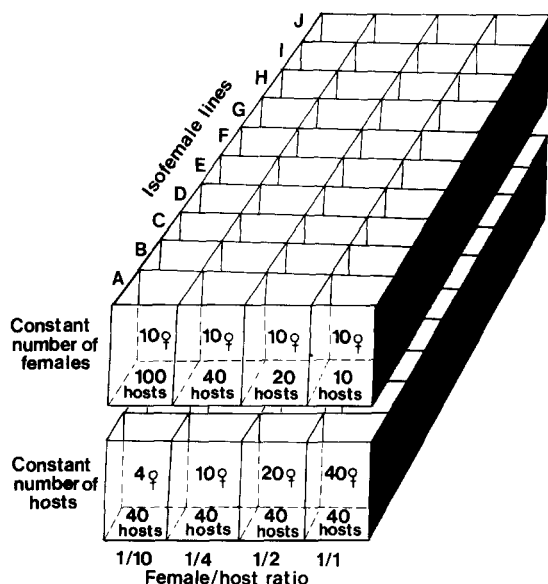

Fig. 1. Diagrammatic representation of the experimental design. For each isofemale line, four female/host ratios were studied. This was done either with a fixed number of wasp females and a varying number of hosts, or with a fixed number of hosts and an increasing number of females.
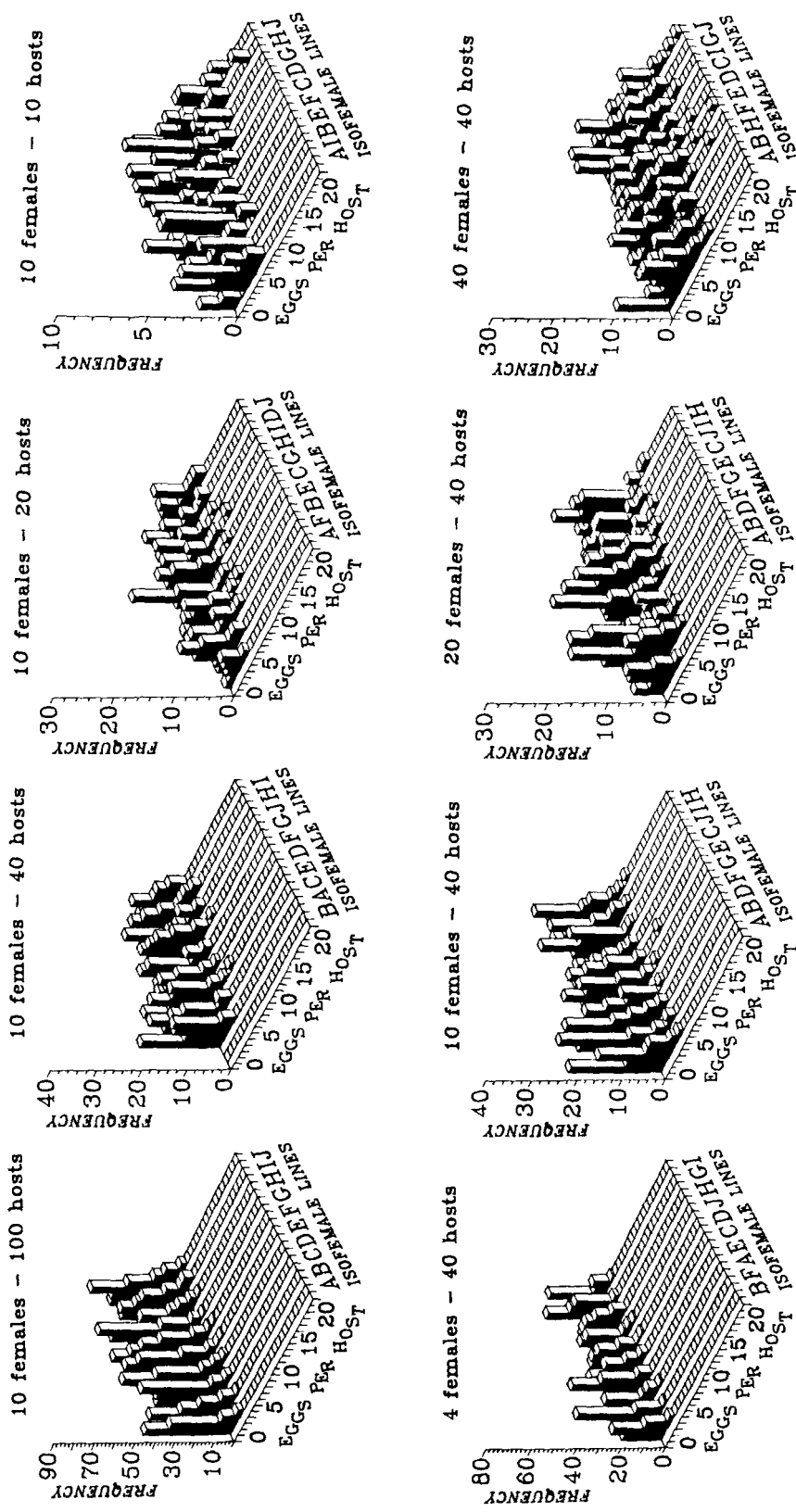
Fig. 2. Distributions of wasp eggs per host for all of the female/host ratios and for the ten isofemale lines studied. In each graph, lines are ordered according to increasing mean value of wasp eggs per host.
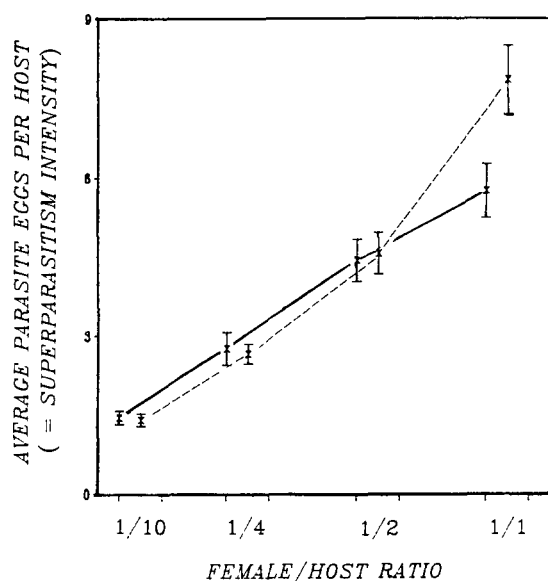
Fig. 3. Changes in the superparasitism intensity with different female/host ratios (means ± s.e.). Variation in female/host ratio was done either with a fixed number of wasps and a varying number of hosts (solid line) or with a fixed number of hosts and an increasing number of females (broken line).

Additionally, we can see that there is also a strong isofemale line effect (P < 0.01). This result shows that superparasitism intensity is a family characteristic and seems to be therefore genetically determined in the *Trichogramma* populations studied. This seems to be confirmed by the fact that the sorting out of lines in Fig. 2, on the average number of parasite eggs per host, is statistically the same for all the eight experimental conditions (Friedman test: Chi^2 = 50.4; 9 d.f.; P < 0.01).

Variations in the way female wasps distribute their eggs among available hosts. Figure 4 shows the frequency distribution of first level risks of the 79

Table 1 Analysis of variance of the average number of parasite eggs deposited in each host (a : P < 0.01)

Source of variation	d.f.	Variances	F
Female/host ratio	7	50.12	66.8 a
Isofemale line	9	6.25	8.3 a
Error	62	0.75	
Total	78	5.82	

tests (chi^2) done to compare the observed egg distributions (Fig. 2) with random distributions of similar means (Poisson distributions). If a Poisson process is a good description of the set of observed frequency distributions, these first level risks would be uniformly distributed over the whole range between 0 and 1. However, it can be shown from Fig. 4 that more than 26% of the chi^2 tests are significant at the 5% level (more than 15% are significant at the 1% level). The hypothesis that, in this experiment, wasp females distributed their eggs randomly among hosts, must therefore be rejected. We will thus now try to describe the way they distribute their progeny among hosts, and to determine if there is a genetic variability of this new feature.

One of the parameters frequently used to describe dispersions is the variance/mean ratio which, in this case would indicates, at constant average values, if wasp females distributed randomly their eggs among hosts or not. Values of one indicate that the variance is approximately equal to average, which is characteristic of a random (Poisson) distribution (Bartko *et al.*, 1968). Values smaller than one show that we are dealing with more regular distributions, and, on the other hand, a variance/mean ratio larger than one indi-
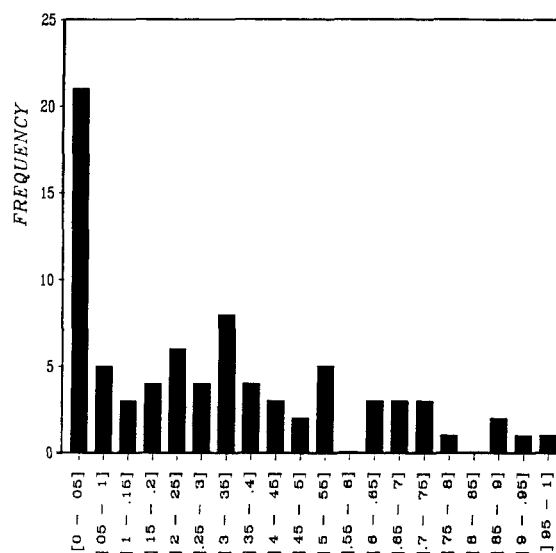

Fig. 4. Frequency distribution of first level risks of tests done to compare all observed egg distributions (Fig. 2) with Poisson distributions of similar means (n = 79).

Table 2 Analysis of variance of the variance/mean ratio of the distributions shown in Fig. 2 (a : P < 0.01)

Source of variation	d.f.	Variances	F
Female/host ratio	7	1.095	7.9 a
Isofemale line	9	0.386	2.8 a
Error	62	0.139	
Total	78	0.253	

cates that ovipositing wasp females lay their eggs in an aggregative manner.

In spite of the fact that this parameter is not normally distributed, analysis of variance was done here again to compare values of this ratio between the different isofemale lines and the different experimental conditions. Indeed, such statistical procedures are known to be not very sensible to non-gaussian situations (Atiqullah, 1962). Table 2 shows that there is a strong female/host ratio effect on that new parameter. This result, shown in Fig. 5, suggests that, when this ratio increases, wasp females progressively aggregate their eggs among hosts more intensively.
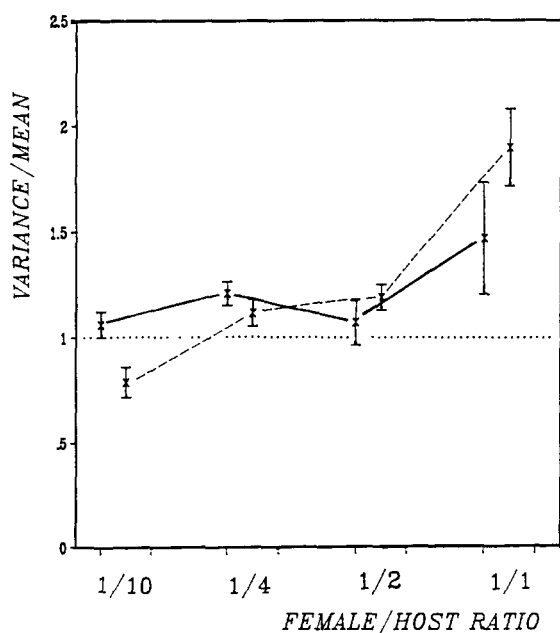
Fig. 5. Changes in the variance/mean ratio with different female/host ratios (means ± s.e.). Variations in female/host ratio was done either with a fixed number of wasps and a varying number of hosts (solid line) or with a fixed number of hosts and an increasing number of females (broken line).
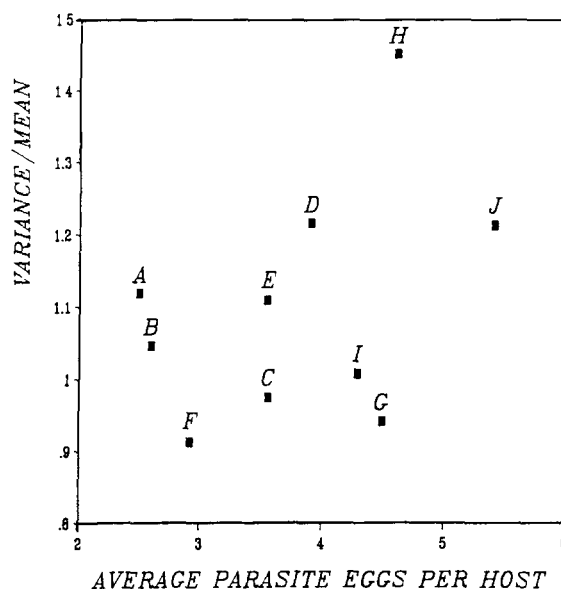
Fig. 6. Relationship between the two traits calculated on each family: average number of wasp eggs per host (superparasitism intensity) and variance/mean ratio. Points represent *Trichogramma* isofemale lines which are labeled as in Fig. 2. Data shown are mean values of the eight female/host ratio conditions. Spearman rank correlation = 0.297 (NS).

More interesting, there is also a significant isofemale lines effect (P < 0.01), which suggests that distribution of wasp eggs among hosts is a family feature: some lines aggregate their eggs more intensively than others. Thus, this trait seems also to be genetically determined.

Finally, Fig. 6 shows that there is no significant correlation between the two traits studied (average number of eggs per available host, and variance/mean ratio) among families. This suggests that these two characters do not seem to be controlled by the same genetic mechanism.

Discussion

Results show that there is substantial variation in progeny allocation in the mass-reared *Trichogramma maidis* population studied. This variation was shown both in the intensity at which female wasps superparasitize the hosts (as measured by the average number of parasite eggs deposited per host) and in the way wasps distribute their eggs among hosts (as measured by the variance/mean

ratio of the frequency distributions of parasite eggs per host). For both traits, two types of variation were found: variation in response to changes in the wasp female/host ratio, and variation between single-female founded families. This second source of variation suggests that these two features are genetically determined in the wasp population studied.

The experimental design used here is not based on the exhaustive recording of the behaviour of each wasp. Therefore, these data are only the overall outcome of different biological processes which could have occurred during the course of the experiment.

Thus, concerning the mean number of eggs deposited per host (i.e. superparasitism intensity), the fact that the variations seem to be genetically determined could be the consequence of the existence of a genetic variation only in one or in several of the different biological mechanisms involved. These biological events are, among others: (1) the allocation by a female, during a single ovipositor insertion, of a clutch of one or more eggs in unparasitized hosts (Klomp & Teerink, 1962, 1967), (2) the laying by a female of one or more additional eggs in hosts which she has previously attacked (self-superparasitism) or in hosts attacked by another conspecific (conspecific superparasitism) (Van Dijken & Waage, 1987; Hubbard et al., 1987; Waage, 1986, 1988), (3) behavioural and physiological mechanisms that lead to the avoidance of superparasitism by wasp females (Salt, 1961; Rogers, 1975) (see below), (4) chemical (e.g. pheromonal) and/or mechanical interferences between parasiting adult females (Rogers & Hassell, 1974; Rogers, 1975), and (5) all of the possible interactions between these different processes, most of which remain to be analysed.

The genetic variation observed in the rate of superparasitism could just be the consequence of a genetic variability in the clutch size deposited by females in unparasitized hosts. Such an explanation is, however, not so likely because, with E. kuehniella as host, only one to two Trichogramma eggs are usually laid per oviposition (Klomp & Teerink, 1962, 1967), and this range of

variation, even if genetically determined, is not high enough to explain the magnitude of the genetic variability observed. It seems more likely that this variability is the consequence of a genetic variation in other biological mechanisms such as the intensity of self- and/or conspecific superparasitism. In particular, this variability could be the result of a genetic variation in fecundity, variability which is already known to exist in the present population (Chassain, 1988). Indeed, genetical differences in fecundity between wasps can lead to a corresponding variation in the number of eggs in the female's oviduct. In return, such variability in the 'egg pressure' could lead to a variation in the modalities of the progeny allocation in the wasp population (Waage, 1986).

Moreover, as predicted by optimal progeny allocation theory, females should lay in each host a number of eggs that depends on the form of the function relating clutch size to individual fitness (Lack, 1947; Charnov & Skinner, 1984, 1985; Waage & Godfray, 1985; Waage, 1986, 1988). It is possible that the observed genetic variability is a consequence of a genetic variation in such fitness function in the population, or in all the possible mechanisms that are involved here (Charnov & Skinner, 1985). Further investigations must therefore be performed in order to find out what are the real biological features implicated here.

Figure 3 shows that the rate of superparasitism increases progressively with the female/host ratio. This well-known phenomenon (Rogers, 1975; Waage, 1986, 1988; Van Alphen, 1988) work here differently when changes in female/host ratio are obtained by fixing the number of wasps or with a constant number of hosts: the increase in the rate of superparasitism is more important when variations in female/host ratio are obtained with a fixed number of hosts and an increasing number of females $(F = 6.40; \ P < .02)$. The difference becomes statistically significant only with a female/host ratio of $^1/_1$ ('t' test; $P < .02$). With a constant number of hosts, the increase in the female/host ratio corresponds to an increase in the absolute number of wasps. This would lead to an increase in the frequency of conspecific relative to self-superparasitism. In such conditions, we

may predict from theory that the increase in clutch size through superparasitism would be greater than when the number of female remains constant, because females will lay eggs in parasitized hosts more frequently during conspecific than during self-superparasitism (see Waage, 1986). In contradiction with our results, such a difference between self- and conspecific superparasitism was not found by Van Dijken & Waage (1987) for *T. evanescens* Westwood. Their experiments were done by offering an individual female ten *Mamestra brassicae* L. eggs (unparasitized or parasitized). In the present one, however, conditions are much more stringent: the female/host ratio has been intentionally kept higher (up to $1/1$), and eggs of *E. kuehniella* are much smaller than those of *M. brassicae* (i.e. smaller amount of resource for which parasitoid larvae are competing, and thus smaller global fitness realized by mothers through offspring produced per host) (Klomp & Teerink, 1967; Pak, 1988).

Table 2 shows that there is also a significant variation in the way wasp females distribute their eggs among hosts at a given rate of superparasitism. This result has been obtained by studying variations in the variance/mean ratio of frequency distributions of parasite eggs per host. What is exactly the biological meaning of this parameter? Only a few studies have been devoted so far to the analysis of the biological mechanisms implicated in the frequency distribution of parasitoid's eggs. In these studies, authors have defined and estimated (with mathematical models) a parameter which describes the rate at which parasites avoid superparasitism ('1 − δ' in Bakker *et al.*, 1972; 'v' in Rogers, 1975). In these models: parasites are assumed (1) to encounter their hosts at random, and (2) to avoid superparasitism when they encounter any already parasitized hosts, during a proportion (v) of the encounters (using Rogers' notation).

Figure 7 shows the relationship between avoidance (v) and variance/mean ratios, (calculated from Rogers' model). There is a clear, non-linear, relation between these two parameters: the more parasitoids avoid superparasitism, the lower the variance/mean ratio is (i.e. the more wasps uni-
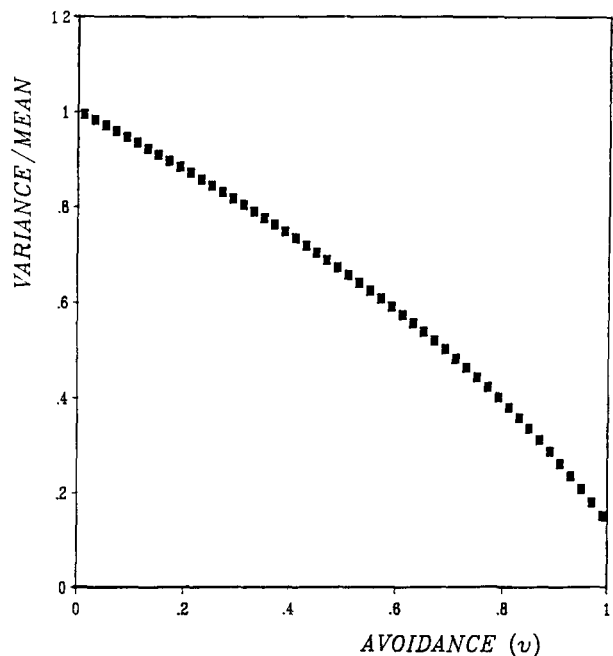

Fig. 7. Relationship between the proportion of avoidance (v) of superparasitism (Rogers, 1975) and the variance/mean ratio. This relationship has been calculated using the example given on Table 2 in Rogers (1975): parasites make a total of 200 random encounters with 100 hosts, and distributions of eggs per host are calculated with different values of v, from 0 to 1, with a step of .02 (see text).

formly distribute their eggs). The proportion of avoidance (v) has not been calculated here because the first two categories of frequency distributions on parasite eggs (zero and one) were not always large enough to get accurate statistical estimations (Rogers, 1975). In spite of this, the variation observed in variance/mean ratio seems, at least in part, to be in relation with variations in the level of avoidance of superparasitism.

Thus, Fig. 5 suggests that the rate of avoidance decreases progressively when the female/host ratio increases. A similar result was obtained by Rogers (1975).

Moreover, the significant variation in the variance/mean ratio between the ten isofemale lines (Table 2) also suggests that there is a genetic variability in the rate of avoidance of superparasitism in the present population.

Mathematical models which are designed to describe frequency distributions of wasp eggs over the hosts, were initially proposed to explain

the fact that such distributions are often much more uniform than predicted by random (i.e. Poisson) processes (Bakker *et al.*, 1967, 1972; Rogers, 1975). This is however not always the case here, where all types of frequency distributions of eggs, from uniform to aggregative, are obtained (variance/mean ratios are uniformly distributed between .9 to 1.5, cf. Fig. 6). Therefore, biological mechanisms other than superparasitism avoidance must be involved in the variations observed. In particular, the genetic variation in the way females distribute their eggs among hosts could be the result of the genetic variability in the way individual *T. maidis* females spatially distribute their eggs among hosts within a cluster (Chassain & Boulétreau, 1987).

Investigations must now be carried out in order to better understand what are the exact biological mechanisms involved in the observed genetic variability. Such work would lead to a better understanding of the evolution of parasitoid populations in response to variations in environmental conditions. They will provide the basic information needed to perform genetic selection of mass-reared populations in order to reduce superparasitism as much as possible, and, therefore, to produce insects that will be more efficient for biological control programs by inundative wasp releases (Stinner, 1977; Wajnberg & Pizzol, 1989).

Finally, the experiment presented here was done by offering hosts to mated wasps females. In this case, and for other 'semi-gregarious' species, it could be misleading to study solely progeny allocation without considering sex allocation (Waage & Ng, 1984; Waage, 1986). So, it would be of prime interest to perform experiments in order to determined if sex allocation of *T. maidis* females is also under a genetic control.

Résumé

Variation génétique de l'allocation à la descendance chez Trichogramma maidis

Récemment, de nombreux travaux ont été publiés dans le but de mieux comprendre les différentes stratégies de reproduction (i.e. répartition de la progéniture parmi les hôtes) présentées par les insectes parasitoides sous différentes contraintes environnementales (cf. Waage, 1986, pour une synthèse). L'ensemble de ces travaux considère chacune de ces stratégies (e.g. nombre d'œufs déposés par hôte, décision de superparasiter) comme un phénomène progressivement mis en place par la sélection naturelle au cours des générations. Ceci n'est cependant concevable que si les caractères biologiques en cause chez les parasites sont sous la dépendance d'une variation génétique sur laquelle la sélection naturelle peut agir.

Les expériences présentées ici ont donc eu pour but de voir si ces caractères sont effectivement déterminés génétiquement dans une population de *Trichogramma maidis* Pintureau & Voegelé (Hym.; *Trichogrammatidae*).

Grâce à l'utilisation d'une coloration histologique qui permet de compter le nombre d'œufs de trichogrammes déposés dans chaque hôte, différentes lignées isofemelles (i.e. familles) ont été comparées, et ce selon différentes conditions expérimentales correspondant à différentes valeurs du rapport nombre de femelles parasites sur nombre d'hôtes offerts.

Les résultats obtenus montrent que le phénomène du superparasitisme semble être déterminé génétiquement. Ceci est constaté aussi bien au niveau du nombre moyen d'œufs de trichogramme déposés par hôte, que dans les caractéristiques des distributions des œufs de parasites parmi les hôtes.

Les conséquences évolutives de tels résultats, ainsi que leurs applications possibles dans le but d'améliorer les conditions d'élevage de masse de ces insectes, sont discutées.

Acknowledgements

version of the manuscript. This work has been supported by the 'A.I.P./I.N.R.A.' 'Génétique des populations de trichogrammes' (n° 9/4702).

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