

SUPERPARASITISM AND HOST DISCRIMINATION  
BY ASOBARA TABIDA NEES (BRACONIDAE:  
ALYSIINAE), A LARVAL PARASITOID OF DROSOPHILIDAE

by

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SUMMARY

This paper presents the results of a study of superparasitism and host discrimination by *Asobara tabida* Nees. We found that:

- (1) *A. tabida* females are able to distinguish unparasitized hosts from those previously parasitized by themselves or by a conspecific;
- (2) There is no evidence that *A. tabida* females are able to distinguish hosts in which they laid an egg themselves from hosts parasitized by conspecifics;
- (3) *A. tabida* females, unlike those of *Leptopilina heterotoma* cannot discriminate between hosts with different numbers of eggs;
- (4) Superparasitism may occur because:
  - (a) inexperienced females of *A. tabida* may initially lay two eggs during one oviposition.
  - (b) a female *A. tabida* may re-attack a host after oviposition within the period needed for building up the factor which causes avoidance of superparasitism.
  - (c) the restraint to oviposit in parasitized hosts breaks down when a female *A. tabida* only meets parasitized hosts and does not lay eggs during a period of at least 8 hours.
  - (d) females that have never oviposited in unparasitized hosts do not refrain from oviposition in parasitized hosts.

We discuss whether superparasitism by insect parasitoids can be adaptive under particular circumstances.

INTRODUCTION

Many insect species are known to distribute their eggs in such a way, that the distribution is in proportion to the (limited) amount of food for their progeny's development. PROKOPY (1981) reviewed this subject for phytophagous insects, listing examples for Coleoptera, Lepidoptera and Diptera. The best known examples of insects that are able to distribute their eggs in proportion to the amount of food are found among the hymenopterous parasitoids; for a review see VAN LENTEREN (1981). The selective advantage of such an egg distribution for an individual insect seems obvious: it should not lay eggs in places where intraspecific com-

petition will probably reduce the survival chance of its offspring by subjecting it to scramble competition. However, the problem is worth some further analysis. When there is only one ovipositing female in a habitat, it is obvious that minimizing the competition for food amongst her offspring will contribute to the maximization of the number of offspring. When the female shares the habitat with ovipositing conspecifics, and she avoids to lay eggs in places where these have already laid theirs, she not only reduces the degree of intraspecific competition for her own offspring, but as a consequence of that, also for the offspring of the other females. This is obviously not an act of altruistic behaviour and therefore we would expect this behaviour to disappear when it is advantageous under certain conditions to lay additional eggs in places where conspecifics already laid theirs. In such situations conditional strategies can occur, which, when they are recognized, provide us with functional explanations of phenomena that earlier were classified as mistakes made by imperfect animals. Without claiming that animals are perfect and do not make errors, we will try to show that for hymenopterous parasitoids superparasitism is not always disadvantageous.

One situation in which superparasitism can be advantageous is that of a gregarious parasitoid that lays one or more male eggs in a previously parasitized host. The larvae hatching from the eggs laid by the second female share the available food with the larvae from the eggs laid by the first female. This occurs when hosts are limiting and results in wasps that are smaller than those that would have hatched when no superparasitism had occurred. However, superparasitism by gregarious parasitoids does not involve a serious mortality risk for the few eggs laid by the second female. The sex ratio of wasps hatching from the eggs of the first female is usually female biased. Therefore the males hatching from the eggs of the second female have a good chance of mating with females hatching from the first batch and thereby the second female can increase the chance of propagating her genes at a low cost (HAMILTON, 1967; WERREN, 1980). Gregarious parasitoids are able to discriminate between parasitized and unparasitized hosts (*e.g.* VAN ALPHEN, 1980), and it depends on the ratio of parasitoids to hosts in the habitat whether superparasitism is advantageous or not. Clearly the laying of additional eggs that are male in an already parasitized host can only be advantageous when this host had previously been parasitized by a conspecific female; the laying of additional male eggs in a host previously parasitized by the same female is not advantageous. Therefore the question arises whether a parasitoid is able to recognize hosts previously parasitized by herself from those parasitized by other females.

A similar argument as the one used for gregarious parasitoids above,

can be given for solitary parasitoids which utilize hosts that occur in aggregations. Such a situation is called quasi-gregarious by VAN DEN ASSEM *et al.* (1980), because the mating strategies which occur in this situation may be similar to those of a truly gregarious parasitoid. As an example one can think of egg batches exploited by egg parasitoids (WAAGE, 1982) or clusters of fly pupae attacked by solitary pupal parasitoids. The first female that exploits such a patch usually produces a female biased sex-ratio, so again it pays for the second female that arrives to have some male offspring in that patch. This can be achieved by laying some male eggs in hosts not utilized by the first female, or by superparasitizing hosts already utilized by the first female. Whether it is advantageous to superparasitize hosts depends in the latter case on the survival chance of the egg laid in the already parasitized host, because in hosts superparasitized by solitary parasitoids one of the parasitoid larvae eliminates the other and eventually only one wasp hatches.

While dissecting *Drosophila* larvae parasitized by *Asobara tabida* we repeatedly found a freshly hatched *A. tabida* larva with an injured *A. tabida* egg in its mandibles. EIJSACKERS and BAKKER (1971) found that male eggs of *L. heterotoma* hatch 3 hours before female eggs laid at the same time. If it is a general pattern that the first egg to hatch in a host has the best chance to survive and that male eggs have a shorter developmental time than female eggs, then the probability that the male egg laid by the second female survives may be large enough to allow the same strategy as described above for gregarious parasitoids.

NELL & VAN LENTEREN (1982) while studying host discrimination of *Pachycrepoideus vindemiae*, a pupal parasitoid of *Drosophila*, which lays its eggs in the space between pupa and puparium, found that in 64.6% of all observed superparasitizations the second wasp destroyed the egg laid by the first female, before laying an egg herself. This is an excellent way to increase the survival chance of the second egg.

Superparasitism needs not to be part of a sex ratio game to be advantageous. When no unparasitized hosts can be found, it is always better to lay eggs in hosts that have already been parasitized by other females if you are a solitary parasitoid, even if the survival chance for the second egg is small. As an example may serve *Tetrastichus* spec., the gregarious egg parasitoid of the twelve spotted asparagus beetle, *Crioceris duodecimpunctata*. In laboratory experiments this parasitoid lays 6 egg (s.d. = 2.4) per oviposition, and is well able to discriminate between parasitized and unparasitized hosts (VAN ALPHEN, 1980). In the field at low host densities 12–18 wasps hatched from one host, a number that must result from superparasitism; 92% of all collected hosts appeared to be parasitized. Under these circumstances the chance of a searching female to find an unparasitized host is very small, so pre-

sumably superparasitism under these circumstances does not mean wastage of eggs. Although the superparasitized hosts may yield smaller wasps that lay fewer eggs and live shorter than larger wasps, a larger number of smaller offspring may represent more potential search time than a few larger offspring and therefore find more hosts than the large wasps together, when host density is low. VAN LENTEREN (1981) reviews the literature on parasitoids that superparasitize when they have not encountered unparasitized hosts for a long time. This phenomenon seems to be widespread.

Thus it appears that superparasitism under certain conditions can be advantageous. With this idea in mind we will now discuss a special situation in which superparasitism occurs. It has been shown for a number of species that females that had not oviposited before do not refrain from oviposition in already parasitized hosts JACKSON, 1966; RABB & BRADLEY, 1970; VAN LENTEREN, 1976; KLOMP *et al.*, 1980). VAN LENTEREN (1976) en KLOMP *et al.* (1980) give a causal explanation for this phenomenon that discrimination between parasitized and unparasitized hosts has to be learnt by experience with unparasitized hosts. Both authors refer to THORPE's (1956) definition: "learning is that process which manifests itself by adaptive changes in individual behaviour as a result of experience". In their view the change from accepting parasitized hosts by an inexperienced wasp to rejecting them after experience with unparasitized hosts is learning according to Thorpe's definition, because host discrimination is adaptive. However, the question is not only whether the ability to discriminate between parasitized and unparasitized hosts is adaptive, but also whether it is adaptive that this ability is only manifest after experience with unparasitized hosts. NELL & VAN LENTEREN (1982) have shown that the ability to discriminate between parasitized and unparasitized hosts is innate in *Pachycrepoideus vindemiae*, hence there is an alternative that at first sight seems less wasteful of eggs than the acceptance of parasitized hosts by inexperienced wasps.

Three possible explanations have to be considered:

- 1) The ability to discriminate between parasitized and unparasitized hosts is manifested only after experience with unparasitized hosts, because inexperienced wasps are unable to discriminate; this behavioural constraint is *not* functional.
- 2) The ability to discriminate between parasitized and unparasitized hosts is manifested only after experience with unparasitized hosts, because inexperienced wasps are unable to discriminate; this behavioural constraint is functional.
- 3) The ability to discriminate between parasitized and unparasitized hosts is manifested only after experience with unparasitized hosts, but

inexperienced wasps are able to discriminate. However, they accept parasitized hosts because it is the best strategy under the circumstances.

The experiments performed by VAN LENTEREN (1976) and KLOMP *et al.* (1980) allow no choice between these 3 possibilities, therefore their conclusion that discrimination must be learnt is premature.

This paper presents the results of a study of host discrimination by *Aobara tabida* Nees, a larval parasitoid of *Drosophila* in western Europe. The work is part of a comparative study of the foraging behaviour of the parasitoids of fruit inhabiting *Drosophila*, and in this paper we compare the discrimination behaviour of *A. tabida* with that of *L. heterotoma*, another member of the parasitoid complex. VAN LENTEREN (1976) made a detailed causal analysis of superparasitism in this species. He listed the following possible causes of superparasitism:

- 1) a female lays more than one egg at an oviposition;
- 2) a female does not recognize hosts parasitized by other females;
- 3) a female lays a second egg after the first oviposition within the period needed for building up the factor which causes avoidance of superparasitism;
- 4) two or more females lay eggs simultaneously in one host;
- 5) a female's tendency to oviposit increases when she encounters only parasitized hosts for a long period; she will then lay eggs in these hosts;
- 6) a female has not learnt to discriminate.

The hypotheses 1, 3 and 4 are more of ethological than of ecological interest, and 4 is only a special case of 3. In *L. heterotoma* they were found to be unimportant and led to few if any cases of superparasitism. With *A. tabida* we did no special experiments to test these hypotheses, but we will comment on them when data available from other sources allow such.

Hypothesis 2 is inadequately formulated. As we have seen, a female belonging to a solitary parasitoid species that lays an egg in a host previously parasitized by herself always wastes one egg, but when she lays an egg in a host previously parasitized by a conspecific, her offspring may survive. Therefore, parasitoids may behave differently towards hosts containing eggs of conspecifics as compared to hosts containing their own eggs. However, when unparasitized hosts are available, one would expect that parasitoids reject as well hosts parasitized by other females as hosts previously parasitized by themselves. Hence, we first show that a female *A. tabida* discriminates between unparasitized hosts and hosts she has previously parasitized herself. We then show that *A. tabida* females discriminate between unparasitized hosts and hosts parasitized by other females. After that we test the hypothesis that *A. tabida* females discriminate between hosts parasitized by conspecifics and those parasitized by herself, in the absence of unparasitized hosts.

A substantial amount of superparasitism by *L. heterotoma* was found when the wasps were deprived of unparasitized hosts and encountered only parasitized hosts for a long period (hypothesis 5). We tested whether this was also true for *A. tabida*.

Because it is difficult to prove that discrimination has to be learnt, we reformulated the 6th possible cause of superparasitism mentioned by VAN LENTEREN (1976). Hence, we tested the hypothesis that *A. tabida* females lacking experience with unparasitized hosts do not refrain from oviposition in hosts previously parasitized by conspecifics.

#### MATERIAL AND TECHNIQUES

*The hosts:* Wildtype *Drosophila melanogaster* flies, strain "WW", were used both for rearing the parasitoids and as hosts in our experiments. Early second instar fly larvae were used in all the experiments. Larvae reared at 25° C reach this stage about 24 hours after hatching.

*The parasitoids:* The *A. tabida* wasps, strain "Leiden", used in this study were collected in the garden surrounding the laboratory. In september 1975 banana-baited jars were exposed to the garden populations of *Drosophila* and its larval parasitoids for two weeks. After this period all the jars contained several hundred *Drosophila* larvae (mainly *D. subobscura* and *D. melanogaster*) and in each jar a number of *A. tabida* females were present. The jars were closed with foam plastic plugs and placed in a 20°C climate room with a 16 hour photo period to prevent diapause. Three weeks later the first *A. tabida* offspring had hatched, and after four weeks several hundred wasps had been collected from the jars. A number of pairs from each jar were chosen at random and used to establish a laboratory culture.

*Rearing of the wasps:* 24-hours old larvae of *D. melanogaster* were transferred to small glass jars (100 cm<sup>3</sup>; 4 cm diameter) containing an agar base (20 cm<sup>3</sup>) with a thin strip of suspended baker's yeast on top (0.5 cm<sup>3</sup> yeast suspension slightly dried by a ventilator). Each jar contained about 150 larvae. Two pairs of *A. tabida* were placed in each jar. The jars were placed in a climate room at 20° C with a relative humidity of 70% ± 5%. To prevent diapause, daylength was maintained at 16 hours. The wasps were removed after 24 hours, and some extra yeast was added to provide the host larvae with enough food for their development. Flies that emerged from unparasitized hosts were removed after two weeks. The wasps emerged after 26–29 days and 1–4 days later the young wasps were removed from the rearing jars, placed in clean jars containing an agar bottom and a piece of blotting paper wetted with a sugar solution and stored at 10° C until used in the experiments.

*The experiments* were done in a climate room at a temperature of 20° C  $\pm$  1° C, and an RH of 60%  $\pm$  5%. Unless stated otherwise we used experienced females, *i.e.* females which had been allowed to parasitize hosts for 2 h., one day before the experiments were done. These "experienced" females have no difficulties with handling their hosts, as unexperienced wasps sometimes appeared to have when encountering their first hosts. (This phenomenon has been discussed for *L. heterotoma* by SAMSON-BOSHUIZEN *et al.*, 1974). Experienced females were obtained by placing 5 ♀ and 5 ♂ *A. tabida* in a jar with 150 hosts. With this procedure there is no absolute certainty that each individual female indeed parasitized one or more hosts. However, we chose this procedure because isolated females often wait several hours before they start to search for hosts, while in groups they usually start within a quarter of an hour. The males were added to promote that the females used in the experiments were mated. The males did not interfere with the searching females, because they remained on the sides of the glass jar and only pursued females when these moved to the sides of the jar.

To observe the parasitoids, we put them in a small petridish (diameter 4.5 cm; height 8 mm) containing an agar base (10 cm<sup>3</sup>) on which a thin spot of suspended yeast had been placed (diameter 2 cm). The searching wasp was observed using a low power stereo microscope (magnification 6.4–16 x). The petridish was illuminated with a cold light source (optical glass fibers). To determine whether an egg had been laid in a particular host, the larva was dissected in a droplet of water by pulling them apart with a pair of fine tweezers on a microscope slide using a dark field stereo microscope (Olympus J.M.). usually the transparent parasitoid eggs pop out and are clearly visible against the dark background. When no egg was found at first inspection, the remnants of the host were carefully examined. A magnification of 25  $\times$  is sufficient to discover the tiny freshly laid eggs of *A. tabida*. Shortly after an egg has been laid it starts to swell. We used this property of the parasitoid eggs to distinguish the first laid egg in superparasitized hosts from a second egg laid in the host some minutes before dissection.

*Observations:* in most experiments the behaviour of the wasps was observed continuously and the duration of each particular behavioural component was recorded on tape.

*Marking technique:* to distinguish between two categories of host larvae, one group was coloured by allowing them to feed in yeast coloured with carmine. After a few hours feeding the guts of the larvae are filled with red yeast, which is clearly visible through the skin of the larvae and remains a good mark for about 1 hour.

A preliminary series of 5 experiments (table I) was carried out, in which individual *A. tabida* females searched during half an hour on a yeast patch of 2 cm diameter with 16 uncoloured hosts and 16 carmine coloured hosts. This experiment showed that this method of colouring

TABLE I

The number of encounters with carmine coloured and uncoloured host larvae, and the number of ovipositions in both categories of larvae.

| exp.<br>nr | nr. of encounters * |                          | $\chi^2$         | nr. of ovipositions |                          | $\chi^2$         |
|------------|---------------------|--------------------------|------------------|---------------------|--------------------------|------------------|
|            | uncoloured          | coloured<br>with carmine |                  | uncoloured          | coloured<br>with carmine |                  |
| 1          | 32                  | 28                       | 0.13             | 14                  | 13                       | 0.02             |
| 2          | 27                  | 23                       | 0.16             | 13                  | 8                        | 0.60             |
| 3          | 18                  | 19                       | 0.01             | 11                  | 12                       | 0.02             |
| 4          | 22                  | 18                       | 0.20             | 8                   | 9                        | 0.03             |
| 5          | 16                  | 14                       | 0.07             | 11                  | 11                       | 0.00             |
| tot.       | 115                 | 102                      | 0.57<br>P > 0.95 | 57                  | 53                       | 0.67<br>P > 0.95 |

has no influence on the chance that they are found by the parasitoid. The number of parasitizations in both categories of larvae does not differ either. Hence, the colouring of larvae with carmine has no effect on their attractiveness to *A. tabida*.

*Collecting parasitized larvae:* one or 2 experienced *A. tabida* females were placed in a petridish with 30 hosts and observed continuously. When a wasp assumed its oviposition posture, the lid of the petridish was slowly removed and after the oviposition had been finished, the parasitized larva was removed with a fine paintbrush. This could be done without severely disturbing the parasitoids. The removed larva was replaced by an unparasitized one. We repeated this procedure until enough parasitized larvae had been collected.

Procedures specific to a particular experiment will be described in the section dealing with that experiment.

### 1. Number of Eggs Laid per Oviposition

No special experiments were designed to test whether superparasitism by *A. tabida* occurs because the wasp lays more than one egg per oviposition, but in the course of our study of *A. tabida* 8262 larvae have been dissected in which the parasitoid inserted her ovipositor once. Most of these larvae contained one egg and some contained no egg at all. How-



ever, when we dissected hosts immediately after they had been parasitized by females that oviposited for the first time in their life, we found 2 freshly laid eggs in the host in 5 out of 32 occasions. One of the parasitoids laid another 2 eggs during her second oviposition. All the remaining females laid but one egg per host during subsequent ovipositions. These observations indicate that the laying of more than one egg per oviposition is not important as cause of superparasitization in *A. tabida*, but that inexperienced females may initially lay more than one egg during an oviposition. BELING (1932) found that inexperienced females as well as experienced females of *Nemeritis canescens* occasionally laid 2 or 3 eggs during an oviposition, although they normally laid only one egg. VAN LENTEREN (1976) claimed that this was also true for *Aphelinus semiflavus* and cited WILBERT (1964), but this publication lacks the information from which such a conclusion can be drawn. WILBERT (1964) never claimed that *A. semiflavus* laid more than one egg during an oviposition.

## 2. Recognition of Hosts Parasitized by the Female Herself

To investigate whether a female of *A. tabida* discriminates between unparasitized hosts and hosts parasitized by herself we analysed the distribution of *A. tabida* eggs in the host larvae from the experiments at density 32 of VAN ALPHEN & GALIS (in prep.).

*Material and techniques.* In these experiments, experienced females of *A. tabida* were allowed to search individually on a patch with a diameter of 2 cm containing a yeast suspension made from 0.125 g of dried yeast. The patch contained 32 *D. melanogaster* larvae. We observed the wasps continuously and counted the total number of encounters and the number of acceptations and rejections. The experiment lasted till the wasp left the patch for more than one minute. After the experiments all 32 larvae were dissected.

*Results.* Table II shows the distribution of *A. tabida* eggs amongst the hosts in the 9 experiments. All the distributions are very regular and are significantly different from a Poisson distribution. One would expect a Poisson distribution when (1) the wasps searched randomly, (2) when they had an equal chance to encounter parasitized and unparasitized hosts and (3) when they did not discriminate between parasitized and unparasitized hosts. GALIS & VAN ALPHEN (1981) have shown that *A. tabida* leaves a mark on a patch on which it has searched. When this mark is not placed after the patch has been exploited, but is left during searching, it may allow the parasitoid to avoid the already searched

TABLE II

The distribution of *A. tabida* eggs over *D. melanogaster* larvae, when experienced females are allowed to search for 32 hosts till they give up and leave the patch. A comparison is made with a Poisson distribution.

| exp<br>nr | nr<br>diss | nr<br>enc | nr<br>rej | number of eggs per larva: |      |     |      |     |     |     |     | $\bar{m}$ | $\chi^2$ | P   |      |         |
|-----------|------------|-----------|-----------|---------------------------|------|-----|------|-----|-----|-----|-----|-----------|----------|-----|------|---------|
|           |            |           |           | 0                         |      | 1   |      | 2   |     | 3   |     |           |          |     | 4    |         |
|           |            |           |           | obs                       | exp  | obs | exp  | obs | exp | obs | exp |           |          |     | obs  | exp     |
| 1         | 32         | 86        | 52        | 0                         | 10.6 | 30  | 11.7 | 2   | 6.4 | 0   | 2.4 | 0         | 0.9      | 1.1 | 45.5 | < 0.001 |
| 2         | 32         | 70        | 38        | 0                         | 11.8 | 32  | 11.8 | 0   | 5.9 | 0   | 2.0 | 0         | 0.5      | 1.0 | 54.8 | < 0.001 |
| 3         | 32         | 49        | 22        | 5                         | 14.4 | 27  | 11.5 | 0   | 4.6 | 0   | 1.2 | 0         | 0.3      | 0.8 | 33.1 | < 0.001 |
| 4         | 32         | 60        | 28        | 0                         | 11.8 | 32  | 11.8 | 0   | 5.9 | 0   | 2.0 | 0         | 0.5      | 1.0 | 54.8 | < 0.001 |
| 5         | 32         | 77        | 45        | 0                         | 11.8 | 32  | 11.8 | 0   | 5.9 | 0   | 2.0 | 0         | 0.5      | 1.0 | 54.8 | < 0.001 |
| 6         | 32         | 52        | 21        | 1                         | 11.8 | 31  | 11.8 | 0   | 5.9 | 0   | 2.0 | 0         | 0.5      | 1.0 | 54.8 | < 0.001 |
| 7         | 32         | 51        | 22        | 3                         | 13.0 | 29  | 11.7 | 0   | 5.3 | 0   | 1.6 | 0         | 0.4      | 0.9 | 40.6 | < 0.001 |
| 8         | 32         | 66        | 34        | 1                         | 11.8 | 31  | 11.8 | 1   | 5.9 | 0   | 2.0 | 0         | 0.5      | 1.0 | 54.8 | < 0.001 |
| 9         | 32         | 48        | 19        | 3                         | 13.0 | 29  | 11.7 | 0   | 5.3 | 0   | 1.6 | 0         | 0.4      | 0.9 | 40.6 | < 0.001 |

part of the patch, and thereby to search more or less systematically. VAN ALPHEN & GALIS (in prep.) have shown that *A. tabida* encounters fewer parasitized hosts than expected when the wasp would search randomly and would have an equal encounter chance with unparasitized and parasitized hosts. This reduced probability of finding parasitized hosts may be a consequence of a non-random search pattern by the parasitoid, caused by the mark she left and/or the lower activity of parasitized larvae. This latter reduces their chance of being discovered by a searching parasitoid (see: VAN ALPHEN & DRIJVER, in prep.). Hence, two other mechanisms may also contribute to the regular egg distributions. Therefore, we cannot conclude from a regular distribution of eggs over the hosts that an *A. tabida* female is able to discriminate between unparasitized hosts and hosts previously parasitized by herself. However, because the wasps were observed continuously, data on the total number of encounters and the number of rejections are also available (table II). These data show that *A. tabida* re-encounters hosts which have previously been parasitized. This information together with the regular egg distribution demonstrates that an *A. tabida* female is able to discriminate between unparasitized hosts and hosts parasitized by herself.

These results show that we cannot conclude that a parasitoid is able to discriminate between parasitized and unparasitized hosts based on a comparison between an observed distribution and an expected (Poisson) distribution. When the observed distribution of eggs per host is more regular than expected, causes other than the ability to discriminate may be responsible for this distribution.

## 2a. Recognition of Hosts Parasitized by Other Females

To determine whether *A. tabida* females are also able to discriminate between unparasitized hosts and hosts parasitized by a conspecific we did the following experiment.

*Material and technique.* An experienced female *A. tabida* was placed in a petridish with a yeast spot containing 15 unparasitized larvae and 15 larvae parasitized by another female. The experiment lasted half an hour during which the behaviour of the wasp was recorded on tape. Each larva which was parasitized during the experiment was replaced by a larva of the same category. The experiment was done 8 times, of which 4 replicates with the unparasitized larvae coloured red with carmine, and the other experiments with the parasitized larvae coloured red.

*Results.* Table III gives the number of rejections and the number of ovipositions in both parasitized and unparasitized hosts. We scored as rejections, those encounters in which *A. tabida* pierced the host's skin with its ovipositor and kept the ovipositor inserted for a period of 2–5 s. We scored as ovipositions, those encounters in which *A. tabida* pierced the host with its ovipositor and kept the ovipositor inserted for a period longer than 10 s. Dissection of the hosts after the experiments showed that no eggs were laid during the insertions shorter than 5 s, while 92%

TABLE III

The numbers of ovipositions in and rejections of parasitized and unparasitized hosts, when they are offered in equal numbers to experienced *A. tabida* females.

| exp<br>nr. | parasit    |     | unparasit  |     | $\chi^2$ | P       |
|------------|------------|-----|------------|-----|----------|---------|
|            | acc        | rej | acc        | rej |          |         |
|            | red        |     | uncoloured |     |          |         |
| 1          | 0          | 9   | 13         | 1   | 19.2     | < 0.001 |
| 2          | 0          | 15  | 18         | 4   | 23.9     | < 0.001 |
| 3          | 4          | 35  | 43         | 0   | 67.3     | < 0.001 |
| 4          | 3          | 36  | 21         | 0   | 66.2     | < 0.001 |
|            | uncoloured |     | red        |     |          |         |
| 5          | 3          | 11  | 18         | 3   | 14.5     | < 0.001 |
| 6          | 3          | 29  | 21         | 1   | 39.1     | < 0.001 |
| 7          | 1          | 40  | 59         | 3   | 87.2     | < 0.001 |
| 8          | 2          | 103 | 61         | 2   | 151.4    | < 0.001 |
| tot.       | 16         | 278 | 254        | 14  | 448.2    | < 0.001 |

of the hosts in which the ovipositor had been inserted for more than 10 s contained an egg. All 8 wasps rejected significantly more parasitized hosts than unparasitized hosts.

The conclusion we draw from these experiments is that *A. tabida* females are able to discriminate between unparasitized hosts and hosts parasitized by other females.

2b. *Discrimination between Hosts Parasitized by a Conspecific and Hosts Parasitized by the Female Herself (No Unparasitized Hosts Present)*

VAN LENTEREN (1976) tested the hypothesis whether *L. heterotoma* was able to recognize hosts parasitized by other females in experiments in which individual *L. heterotoma* females searched on a patch containing 25 unparasitized hosts and 25 hosts parasitized by another female. Larvae parasitized during the experiment were replaced by larvae of the same category so that there was no depletion of unparasitized larvae. Under these circumstances one may expect that *L. heterotoma* will reject the hosts parasitized by a conspecific when it has the ability to recognize them. VAN LENTEREN did these experiments in order to determine whether an inability of *L. heterotoma* to recognize hosts parasitized by conspecifics, could explain the substantial amount of superparasitism found by BAKKER *et al.* (1967, 1972). However, in most of the experiments by BAKKER *et al.* (1967, 1972), more than one female searched the patch during a long time, resulting in a high proportion of superparasitized larvae. Superparasitism under these circumstances may then occur because females are able to distinguish hosts in which they had laid an egg from those parasitized by conspecifics. Additional eggs may be laid in hosts previously parasitized by a conspecific, when the parasitoid is unable to find unparasitized hosts during a long period. Such behaviour can be functional for the following reason. When a parasitoid superparasitizes a host in which she has previously oviposited herself, one egg is always wasted, since at most only one of the female's eggs will develop into a new parasitoid. When a parasitoid lays an egg in a host previously parasitized by a conspecific, her egg has some chance to survive. Therefore, we expected, that if *A. tabida* had the ability to discriminate between hosts parasitized by herself and those parasitized by other females, that she would manifest this only when she was unable to find unparasitized hosts for some time. However, an experimental test of this hypothesis is difficult since *A. tabida*'s foraging decisions on a patch are influenced by experience on the previous patch (VAN ALPHEN & VAN HARSEL, in prep.). It is impossible to obtain hosts parasitized by the female used in the experiment without giving her experience on a patch containing unparasitized hosts. This makes it

likely that she will reject a patch which contains only parasitized hosts. Yet, we decided to give it a try and did the following experiment.

*Material and technique.* Several hours before the experiment was done, we collected larvae which had been parasitized by a female that was to be used later in the experiment and larvae parasitized by a conspecific. The 2 types of larvae were kept separately and one group was placed in yeast coloured with carmine. This permitted us to recognize the two types of larvae in the experiments. In half of the experiments the larvae parasitized by the female used in the experiment were coloured red, in the other half the hosts parasitized by the other females were red. Eight larvae of each type were placed in a 2 cm diameter yeast patch and the wasp was allowed to search for them until she left the petridish for more than 5 minutes. The behaviour of the wasp was continuously observed and recorded on tape. The experiment was done 12 times.

*Results.* Table IV gives the number of contacts leading to rejection or acceptance, with both types of hosts. It also shows how long the wasp

TABLE IV

The numbers of ovipositions in and rejections of hosts parasitized by the female used in the experiment and hosts parasitized by other females. For each experiment patch time and number of patch visits are given.

| exp<br>nr   | parasitized by: |     |       |     | patch<br>time in<br>min | nr of<br>patch<br>visits |
|---|-----------------|-----|-------|-----|-------------------------|--------------------------|
|   | self            |     | other |     |                         |                          |
|   | acc             | rej | acc   | rej |                         |                          |
| larvae parasitized by the<br>tested female are red      |                 |     |       |     |                         |                          |
| 1   | 0               | 10  | 0     | 8   | 11                      | 5                        |
| 2   | 0               | 7   | 0     | 6   | 13                      | 3                        |
| 3   | 0               | 17  | 0     | 15  | 19                      | 9                        |
| 4   | 0               | 11  | 0     | 9   | 13                      | 2                        |
| 5   | 0               | 9   | 0     | 12  | 12                      | 3                        |
| 6   | 0               | —   | 0     | 1   | 1                       | 1                        |
| larvae parasitized by the<br>tested female not coloured |                 |     |       |     |                         |                          |
| 7   | 0               | 10  | 0     | 17  | 15                      | 3                        |
| 8   | 0               | 8   | 0     | 8   | 8                       | 3                        |
| 9   | 0               | 0   | 0     | 2   | 8                       | 1                        |
| 10  | 0               | 21  | 0     | 23  | 16                      | 1                        |
| 11  | 0               | 1   | 0     | 4   | 2                       | 3                        |
| 12  | 0               | 11  | 0     | 12  | 10                      | 2                        |

stayed on the patch and how many times it visited the patch. The results indicate that parasitized hosts of both types were rejected. The wasps were reluctant to stay on the patch, just as we expected. The experiments do not show that *A. tabida* is able to distinguish between larvae previously parasitized by itself from those parasitized by a conspecific. To force an *A. tabida* female to accept parasitized hosts she should be confined with parasitized hosts for a much longer time. Our marking technique however does not allow us to do experiments for longer than one hour. After one hour the gut of the larvae no longer contains red yeast. Therefore we did not pursue this question any further.

### 3. The Period Needed for Building up the Factor which Causes Avoidance of Superparasitism

No special experiments were done to test this hypothesis but when collecting parasitized larvae and during other experiments we sometimes observed that a wasp re-attacked the larva it had just parasitized.

*Results and discussion.* We observed 51 times that a parasitoid attacked the same host a second time. Fig. 1 shows the numbers of second attacks that result in oviposition and those that result in rejection. The time between first oviposition and second attack was used to classify the data in 10 intervals. If the second attack occurred more than 90 s after the first oviposition the host was always rejected. This suggests that it takes at most 90 s before the parasitoid can recognize a host as parasitized. *A. tabida* paralyzes a host before oviposition, but the effect of paralysis is highly variable. Sometimes a larva goes limp for about one minute, but more often the effect is hardly noticeable. Possibly, the rejections of parasitized hosts within a period of 90 s are not in response to the factor

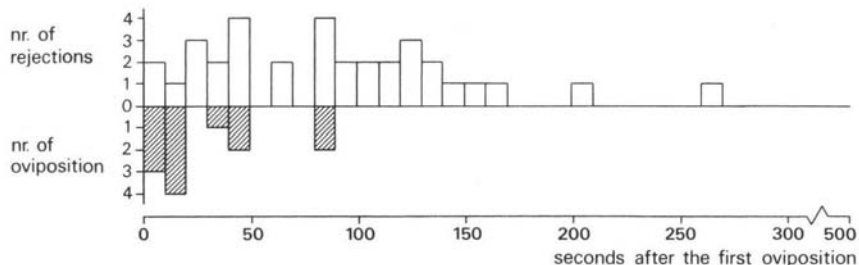


Fig. 1. The number of rejections (open columns) and the number of ovipositions (black columns) after the first oviposition. Re-encounters more than 90s after the first oviposition always result in rejection of the host. Five rejections between 300–500 s after the first oviposition are not shown in the figure.

which later causes the avoidance of superparasitism, but in response to the physical condition of the host caused by the paralysis. We have observed thousands of ovipositions, yet we have seen only 51 re-attacks, of which only 12 led to superparasitization. Hence, re-attacks shortly after the first oviposition are not an important cause of superparasitization.

#### 4. *Simultaneous Ovipositions by Two or More Females in One Host*

While collecting parasitized larvae, we often allowed more than one wasp to search a patch. Yet, we have never observed 2 or more wasps ovipositing simultaneously in the same larva. Moreover, *A. tabida* has a stiff ovipositor and always stands over its host during oviposition. This makes it highly improbable that simultaneous ovipositions in one larva will occur.

#### 5. *An Increased Tendency to Oviposit*

Females of many species of parasitoids are able to discriminate between parasitized and unparasitized hosts, but have been observed to oviposit in parasitized hosts when they have not encountered unparasitized hosts for a long period (for references see: VAN LENTEREN, 1976, 1981). VAN LENTEREN (1976) explains this breakdown of the restraint to oviposit in parasitized hosts by assuming that the tendency to oviposit increases when a parasitoid only meets parasitized hosts and does not lay eggs during some time. Her tendency to migrate from the exploited patch also increases, but when the parasitoid is forced to stay near that patch her tendency to oviposit will eventually become stronger than the restraint to oviposit in parasitized hosts and superparasitism will occur. However, after one or two eggs have been laid the tendency to oviposit decreases again and the wasp will reject parasitized hosts until the tendency to oviposit again becomes stronger. The behaviour of *L. heterotoma*, when confined with 25 hosts for a 7 hour period, fits well with this descriptive model.

We tested whether a breakdown in the restraint to oviposit, when *A. tabida* failed to encounter unparasitized larvae during a long period, could also cause superparasitism in *A. tabida*.

*Material and technique.* Experienced *A. tabida* females were allowed to search individually on a 2 cm diameter patch made of 0.125 g suspended yeast. The patch contained 32 *D. melanogaster* larvae. Preliminary experiments in which the wasps were kept for 8 hours in a petridish containing 32 larvae, showed that within this period no break-

down of the restraint to oviposit occurred. We therefore decided to confine them in the petridishes for about 20 hours under continuous light. After 20 h the wasps were removed and placed with unparasitized hosts to determine whether they were still capable of laying eggs. The larvae which had been exposed to the parasitoids for 20 h were dissected to obtain the number and the distribution of *A. tabida* eggs over the hosts. Twenty female parasitoids were tested. The behaviour of the wasps was not observed.

*Results and discussion.* Table V shows the number and distribution of eggs laid in the 20 experiments. The results show that after 20 h of exposure superparasitism occurs, though to a varying degree. Because preliminary experiments, which lasted 8 h had shown that *A. tabida* strongly refrained from oviposition in parasitized hosts apparently the restraint to oviposit breaks down when *A. tabida* fails to encounter unparasitized hosts for a period longer than 8 h. This can be an important cause of superparasitism when a high parasitoids/hosts ratio occurs in the field.

Of the egg distributions listed in table V, 15 are significantly more regular than a Poisson distribution and confirm the conclusion from Chapter 1, that *A. tabida* possesses an ability to avoid superparasitism. However, 5 distributions did not differ from a random distribution. For experiment nr. 2 (table V) we explain this as follows: via a crevice in the agar bottom, a number of larvae had escaped from the yeast patch and found a refuge under the agar, where the parasitoid could not reach them. This explains the high number of unparasitized larvae in this experiment. The other random egg distributions occurred in experiments in which the mean number of eggs per host larva was high ( $2.2 < \bar{m} < 3.4$ ; experiment nr. 15–20), but not all experiments with a high  $\bar{m}$  showed random distributions. To explain why experiments with a lower mean number of eggs per host ( $0.9 < \bar{m} < 2.0$ ) have regular distributions and why random distributions are found in experiments with high values of  $\bar{m}$  we must first consider the results of BAKKER *et al.* (1972) and VAN LENTEREN (1976) with *L. heterotoma*. BAKKER *et al.* (1972) found that the distribution of eggs of *L. heterotoma* was also regular when a high mean number of eggs per host was found. A model which assumed that the chance of an egg being laid in a host decreased when the number of eggs laid in a host increased. It gave a better fit than one which assumed equal chances for each supernumerary egg laid in a larva. Therefore they concluded that *L. heterotoma* can discriminate between hosts with different number of eggs. VAN LENTEREN's (1976) data confirmed this conclusion.

Our data suggest that *A. tabida* is only able to discriminate between parasitized and unparasitized hosts, but that it cannot discriminate



TABLE V

The distribution of *A. tabida* eggs over the host larvae when experienced females are confined with 32 during 20 h. The distributions are compared with Poisson distributions. Probabilities marked with \* indicate significant differences from Poisson distributions.

| Number of <i>A. tabida</i> eggs per larva: |    |     |      |     |      |     |     |     |     |     |     |     |     |     |     |          |          |           |
|--|----|-----|------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------|----------|-----------|
| exp.                                       | N  | 0   |      | 1   |      | 2   |     | 3   |     | 4   |     | 5   |     | 6   |     | $\chi^2$ | P        | $\bar{m}$ |
|  |    | obs | exp  | obs | exp  | obs | exp | obs | exp | obs | exp | obs | exp | obs | exp |          |          |           |
| 1  | 32 | 6   | 13.0 | 23  | 11.7 | 3   | 5.3 | 0   | 1.6 | 0   | 0.4 | 0   | 0   | 0   | 0   | 17.2     | < 0.001* | 0.9       |
| 2  | 32 | 9   | 11.8 | 16  | 11.8 | 4   | 5.9 | 3   | 2.0 | 0   | 0.5 | 0   | 0   | 0   | 0   | 2.4      | > 0.1    | 1.0       |
| 3  | 31 | 0   | 10.3 | 29  | 11.4 | 2   | 6.2 | 0   | 2.3 | 0   | 0.6 | 0   | 0.2 | 0   | 0   | 43.4     | < 0.001* | 1.1       |
| 4  | 32 | 6   | 10.7 | 18  | 11.7 | 8   | 6.4 | 0   | 2.4 | 0   | 0.6 | 0   | 0.2 | 0   | 0   | 5.8      | < 0.05 * | 1.1       |
| 5  | 32 | 5   | 10.7 | 19  | 11.7 | 8   | 6.4 | 0   | 2.4 | 0   | 0.6 | 0   | 0.2 | 0   | 0   | 11.2     | < 0.01 * | 1.1       |
| 6  | 32 | 1   | 9.6  | 23  | 11.7 | 8   | 6.9 | 0   | 2.8 | 0   | 0.8 | 0   | 0.2 | 0   | 0   | 22.6     | < 0.001* | 1.2       |
| 7  | 32 | 2   | 9.6  | 22  | 11.7 | 7   | 6.9 | 1   | 2.8 | 0   | 0.8 | 0   | 0.2 | 0   | 0   | 17.2     | < 0.001* | 1.2       |
| 8  | 32 | 4   | 9.6  | 17  | 11.7 | 11  | 6.9 | 0   | 2.8 | 0   | 0.8 | 0   | 0.2 | 0   | 0   | 11.9     | < 0.01 * | 1.2       |
| 9  | 32 | 2   | 8.7  | 21  | 11.3 | 7   | 7.4 | 1   | 3.2 | 0   | 1.0 | 1   | 0.4 | 0   | 0   | 15.0     | < 0.001* | 1.3       |
| 10   | 30 | 1   | 7.4  | 17  | 10.4 | 12  | 7.2 | 0   | 3.4 | 0   | 1.2 | 0   | 0.4 | 0   | 0   | 17.9     | < 0.001* | 1.3       |
| 11   | 32 | 1   | 6.5  | 15  | 10.3 | 12  | 8.3 | 3   | 4.4 | 1   | 1.8 | 0   | 0.7 | 0   | 0   | 9.6      | < 0.01 * | 1.6       |
| 12   | 32 | 2   | 6.5  | 12  | 10.3 | 15  | 8.3 | 2   | 4.4 | 1   | 1.8 | 0   | 0.7 | 0   | 0   | 11.0     | < 0.01 * | 1.6       |
| 13   | 31 | 1   | 5.7  | 15  | 9.6  | 10  | 8.4 | 3   | 4.6 | 1   | 2.0 | 1   | 0.7 | 0   | 0   | 7.9      | < 0.02 * | 1.7       |
| 14   | 30 | 0   | 4.5  | 9   | 8.5  | 16  | 8.1 | 5   | 5.1 | 0   | 2.4 | 0   | 1.4 | 0   | 0   | 16.0     | < 0.01 * | 1.9       |
| 15   | 32 | 1   | 3.5  | 8   | 7.8  | 11  | 8.6 | 10  | 6.3 | 2   | 3.5 | 0   | 1.5 | 0   | 0.6 | 7.2      | > 0.05   | 2.2       |
| 16   | 32 | 1   | 3.2  | 7   | 7.4  | 11  | 8.5 | 9   | 6.5 | 2   | 3.7 | 1   | 1.7 | 1   | 0.7 | 4.1      | > 0.30   | 2.3       |
| 17   | 32 | 0   | 3.2  | 10  | 7.4  | 10  | 8.5 | 7   | 6.5 | 3   | 3.7 | 1   | 1.7 | 1   | 0.7 | 4.7      | > 0.10   | 2.3       |
| 18   | 27 | 0   | 1.6  | 2   | 4.6  | 8   | 6.4 | 10  | 6.0 | 7   | 4.2 | 0   | 2.4 | 0   | 1.1 | 6.0      | = 0.05 * | 2.8       |
| 19   | 32 | 0   | 1.2  | 1   | 3.9  | 8   | 6.4 | 13  | 7.1 | 7   | 5.8 | 2   | 3.8 | 1   | 2.1 | 10.3     | < 0.05 * | 3.3       |
| 20   | 31 | 0   | 1.0  | 3   | 3.5  | 7   | 6.0 | 6   | 6.8 | 7   | 5.8 | 7   | 3.9 | 1   | 2.2 | 1.6      | > 0.50   | 3.4       |

between hosts with different numbers of eggs. Thus, *A. tabida* will first distribute its eggs regularly among the hosts, until all (or nearly all) contain one egg (table II). When the restraint to oviposit in parasitized hosts breaks down and *A. tabida* begins to superparasitize, the supernumerary eggs will be distributed randomly among the parasitized hosts. This explains why egg distributions with lower values for  $\bar{m}$  will be regular while those with high  $\bar{m}$  values may be random. If this is true for *A. tabida* we would expect the distribution of supernumerary eggs over the parasitized hosts to be random in all our experiments. We tested this hypothesis using data in which the mean ( $\bar{m}$ ) exceeded 1.7 eggs/host (experiments 13–20, table VI). At lower values of  $\bar{m}$  too few hosts contain supernumerary eggs to allow a statistical analysis of their distribution. Indeed all distributions do not differ from Poisson distributions. Hence, our data are consistent with the notion that *A. tabida* can discriminate between unparasitized and parasitized hosts, but cannot discriminate between hosts with different numbers of eggs.

TABLE VI

The distribution of supernumerary *A. tabida* eggs over the parasitized hosts. The distributions do not differ from the calculated Poisson distributions.

| exp<br>nr | N<br>N | Number of supernumerary <i>A. tabida</i> eggs per parasitized larva: |      |     |      |     |     |     |     |     |     |     |     | $\chi^2$ | P      | $\bar{m}$ |
|-----------|--------|--|------|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|----------|--------|-----------|
|           |        | 0  |      | 1   |      | 2   |     | 3   |     | 4   |     | 5   |     |          |        |           |
|           |        | obs  | exp  | obs | exp  | obs | exp | obs | exp | obs | exp | obs | exp |          |        |           |
| 13        | 30     | 15   | 13.5 | 10  | 10.8 | 3   | 4.3 | 1   | 1.2 | 1   | 0.2 | 0   | 0   | 0.3      | > 0.5  | 0.8       |
| 14        | 30     | 9  | 12.2 | 16  | 11.0 | 5   | 4.9 | 0   | 1.5 | 0   | 0.4 | 0   | 0   | 3.6      | > 0.05 | 0.9       |
| 15        | 31     | 8  | 9.3  | 11  | 11.2 | 10  | 6.7 | 2   | 2.7 | 0   | 0.8 | 0   | 0.2 | 2.6      | > 0.2  | 1.2       |
| 16        | 31     | 7  | 8.4  | 11  | 11.0 | 9   | 7.1 | 2   | 3.1 | 1   | 1.0 | 1   | 0.3 | 0.8      | > 0.9  | 1.4       |
| 17        | 32     | 10   | 8.7  | 10  | 11.3 | 7   | 7.4 | 3   | 3.2 | 1   | 1.0 | 1   | 0.3 | 0.4      | > 0.8  | 1.3       |
| 18        | 27     | 2  | 7.2  | 8   | 8.0  | 10  | 7.2 | 7   | 4.3 | 0   | 2.0 | 0   | 0.7 | 4.9      | > 0.05 | 1.8       |
| 19        | 32     | 1  | 3.2  | 8   | 7.4  | 13  | 8.5 | 7   | 6.5 | 2   | 3.7 | 1   | 1.7 | 4.8      | > 0.1  | 2.3       |
| 20        | 31     | 3  | 2.8  | 7   | 6.7  | 6   | 8.1 | 7   | 6.5 | 7   | 3.9 | 1   | 1.7 | 1.6      | > 0.8  | 2.4       |

Model I of BAKKER *et al.* (1972) should give a good description of the egg distributions of *A. tabida*. In this model it is assumed that the probability that an egg will be laid in an unparasitized host, when such a host is encountered is equal to 1, while the chance that an egg is laid in a host containing 1, 2, 3, ..., n ( $= \delta_1, \delta_2, \delta_3, \dots, \delta_n$ ) is such that  $\delta_1 = \delta_2 = \delta_3 = \dots = \delta_n$ . Because we did not observe the wasps in our experiments, we cannot directly estimate the  $\delta$ 's. Furthermore we believe that our own analysis of the data is sufficient. Therefore we did not calculate the goodness of fit of our data with model I of BAKKER *et al.* (1972).

VAN LENTEREN *et al.* (1978) presented several reasons why it may be dangerous to conclude from random egg distributions in samples of field collected hosts that a given parasitoid species lacks the ability to discriminate between parasitized and unparasitized hosts. Our experiments add an extra reason to theirs: individual wasps, well able to discriminate between parasitized and unparasitized hosts, may produce random egg distributions because they do not discriminate between hosts with different numbers of eggs.

#### 6. The Acceptance of Parasitized Hosts by Females inexperienced with Unparasitized Hosts

The acceptance of parasitized hosts by females of *L. heterotoma* which had no previous experience with unparasitized hosts can be an important cause of superparasitism (VAN LENTEREN, 1976). The question whether *A. tabida* females which had no experience with unparasitized hosts, also show no restraint to oviposit in parasitized hosts was investigated in the following way.

*Preliminary experiments*

*Material and technique.* During preliminary experiments with inexperienced *A. tabida* females we found that these wasps sometimes lay 2 eggs per oviposition (see section 1). We also observed that inexperienced, as well as experienced, wasps sometimes showed the normal oviposition behaviour without laying eggs. In these preliminary experiments we offered hosts parasitized by experienced females to an "inexperienced" female. (the latter as a shorthand for a female which has never oviposited in an unparasitized host). We observed the behaviour of the inexperienced female and removed each larva which appeared to have been parasitized by her, using the behaviour of the parasitoid as criterium for oviposition. However, when we dissected these hosts after the experiment, they contained 1, 2 or 3 eggs. Of the hosts containing one egg we were unable to say whether the egg had been laid by the first (experienced) or the second (inexperienced) female. Likewise we were unable to say whether hosts with 2 eggs contained one egg of each wasp or 2 eggs laid by the inexperienced wasp. When an inexperienced wasp finds an unparasitized host of which we erroneously believed to be parasitized, its behaviour may change in an unexpected way. The inconsistent results of the preliminary experiments indicated that this kind of errors indeed occurred. To circumvent this problem we chose a procedure for the first series of experiments, which enabled us to check whether an egg was from the first or from the second female.

Freshly laid *A. tabida* eggs are thinly spindle shaped, but once in the host they immediately start to swell. We can use this change in shape to distinguish a freshly laid egg from an egg which has been laid 30 min or longer before the host is dissected (fig. 2). The difference between eggs laid 30 min and 90 min before dissection is only slight. Hence, it is not possible to be sure whether an egg is laid by the first or by the second wasp when the hosts are dissected after the experiment. Therefore, our first series of experiments was done as follows: 25 parasitized hosts were offered to an *A. tabida* female which had never oviposited before. As soon as she had finished her first oviposition, the experiment was interrupted, the wasp was gently driven onto the lid of the petridish and the

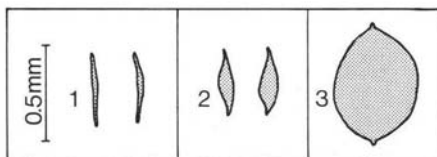


Fig. 2. The outlines of *A. tabida* eggs: 1. freshly laid eggs; 2. 30 min after oviposition; 3. after 3 days at 20°C.

lid with the wasp was removed and placed on the table so that the wasp was confined under the lid. The larva that had been parasitized by the inexperienced wasp was replaced by a once-parasitized one. The removed larva was dissected. When it contained 1 swollen and 1 or 2 slender, freshly laid eggs, we concluded that the inexperienced wasp had superparasitized the host. In this case, the wasp was placed back into the petridish with parasitized hosts and the whole procedure was repeated until the wasp apparently had parasitized in a host without a swollen egg. If we found only one swollen egg when the first larva was dissected, we discarded the wasp and took another inexperienced female. When we only found a slender egg in the host, we concluded that the wasp had now experienced an unparasitized host. We then allowed this female to parasitize some more unparasitized hosts before we returned it to the petridish containing parasitized hosts (in 3 experiments it was directly placed back in the petridish with parasitized hosts). When a host larva was rejected, we also interrupted the experiment and replaced the rejected larva. The removed larva was dissected. In this way we obtained sequences of contacts with parasitized hosts, alternated with sequences of contacts with unparasitized hosts.

*Results and discussion.* Table VII shows the number of hosts accepted and rejected in the alternating sessions with parasitized and unparasitized hosts. In the first series of contacts with parasitized hosts, when the females have no experience with unparasitized hosts, all the parasitized hosts were accepted for oviposition. After the wasp has had experience with unparasitized hosts in the second series of contacts with parasitized hosts, parasitized hosts are rejected in the majority of contacts. This is also the case in the third and fourth sessions with parasitized hosts.

These results suggest that inexperienced *A. tabida* females show no restraint to oviposit in parasitized hosts. However, the number of contacts with parasitized hosts is rather low ( $<8$ ) and the unnatural procedure, to disturb the wasp after each oviposition may have affected the wasp's behaviour.

Following this first series of experiments it was discovered that experienced females which showed oviposition behaviour without laying eggs could be detected by dissecting 5 hosts on which oviposition behaviour by a female had been observed. When an egg was found in each of these 5 hosts, the chance was negligible that such a female would later show oviposition behaviour without egg laying. When less than 5 eggs were found, the female was discarded. This knowledge allowed us to do experiments in which the wasp could remain on the patch after an oviposition.

TABLE VII

Ovipositions in and rejections of parasitized and unparasitized hosts of females which had never oviposited the beginning of the experiment: first series of experiments.

| exp | par |     | unpar |     | par |     | unpar |     | par |     | unpar |     | par |     | unpar |     |  |
|-----|-----|-----|-------|-----|-----|-----|-------|-----|-----|-----|-------|-----|-----|-----|-------|-----|--|
|     | acc | rej | acc   | rej | acc | rej | acc   | rej | acc | rej | acc   | rej | acc | rej | acc   | rej |  |
| 1   | 6   | 0   | 5     | 0   | 2   | 25  | 8     | 0   |     |     |       |     |     |     |       |     |  |
| 2   | 6   | 0   | 8     | 0   | 0   | 15  | 5     | 0   |     |     |       |     |     |     |       |     |  |
| 3   | 2   | 0   | 2     | 0   | 1   | 0   | 1     | 0   | 1   | 0   | 8     | 0   | 0   | 7   | 5     | 0   |  |
| 4   | 6   | 0   | 6     | 0   | 1   | 18  | 3     | 0   |     |     |       |     |     |     |       |     |  |
| 5   | 4   | 0   | 1     | 0   | 0   | 4   | 2     | 0   | 0   | 8   | 1     | 0   |     |     |       |     |  |
| 6   | 5   | 0   | 4     | 0   | 0   | 8   | 1     | 0   |     |     |       |     |     |     |       |     |  |
| 7   | 7   | 0   | 6     | 0   | 0   | 10  | 1     | 0   | 0   | 12  | 5     | 0   |     |     |       |     |  |
| 8   | 5   | 0   | 4     | 0   | 0   | 5   | 8     | 0   | 0   | 11  | 7     | 0   |     |     |       |     |  |
| 9   | 7   | 0   | 9     | 0   | 2   | 9   | 8     | 0   | 1   | 6   | 5     | 0   |     |     |       |     |  |
| 10  | 4   | 0   | 1     | 0   |     |     |       |     |     |     |       |     |     |     |       |     |  |
| 11  | 7   | 0   | 1     | 0   | 1   | 2   | 6     | 0   | 1   | 18  | 3     | 0   |     |     |       |     |  |
| 12  | 8   | 0   | 8     | 0   | 0   | 7   | 5     | 0   |     |     |       |     |     |     |       |     |  |
| 13  | 5   | 0   | 5     | 0   | 1   | 2   | 2     | 0   | 2   | 6   | 8     | 0   | 0   | 3   | 1     | 0   |  |

#### *Experiments in which the wasp remains on the patch*

*Material and technique.* In the five experiments of this series an inexperienced female was alternately offered hosts parasitized by selected experienced females and unparasitized hosts. The first exposure to parasitized hosts lasted 45 min; the subsequent exposures to parasitized or to unparasitized hosts 20 min. The behaviour of the wasps was observed and each larva parasitized or rejected was replaced by a larva of the same category. The removed hosts were dissected at the end of the day on which the experiment was done.

*Results and discussion.* Table VIII gives the numbers of accepted and rejected hosts for each session. Fig. 3 is a graphical representation of the sequence of acceptance and rejection in each experiment.

Again the results show that inexperienced parasitoids accept parasitized hosts for oviposition more often than experienced wasps. Yet there is a substantial proportion of rejections even in the first exposures to parasitized hosts. Our dissections showed that all the hosts we offered as parasitized indeed contained an egg. Therefore we are sure that these rejections do not occur after experience with an unparasitized host.

*D. melanogaster* larvae show reduced activity, the first hours following parasitization by *A. tabida* (VAN ALPHEN & GALIS, in prep.). It is possible that the parasitized larvae, which are rejected by the inexperienced

TABLE VIII

The numbers of ovipositions in and rejections of parasitized and unparasitized hosts of females which had never oviposited at the beginning of the experiment: second series.

| exp. nr. | par. |     | unpar. |     | par. |     | unpar. |     | par. |     | unpar. |     |
|----------|------|-----|--------|-----|------|-----|--------|-----|------|-----|--------|-----|
|          | acc  | rej | acc    | rej | acc  | rej | acc    | rej | acc  | rej | acc    | rej |
| 1        | 21   | 17  | 15     | 0   | 0    | 18  | 14     | 0   |      |     |        |     |
| 2        | 16   | 4   | 10     | 0   | 1    | 19  | 8      | 0   |      |     |        |     |
| 3        | 11   | 9   | 15     | 0   | 0    | 16  | 10     | 0   |      |     |        |     |
| 4        | 22   | 1   | 5      | 1   | 6    | 9   | 5      | 0   | 1    | 7   | 5      | 0   |
| 5        | 11   | 15  | 13     | 3   | 0    | 7   | 11     | 0   |      |     |        |     |
| tot.     | 81   | 46  | 58     | 4   | 7    | 69  | 48     | 0   | 1    | 7   | 5      | 0   |

wasps, are not rejected because they are recognized as being parasitized, but because of their unattractive physical state.

Alternatively they may recognize the hosts as being parasitized, but do not refrain from oviposition because their tendency to oviposit is too

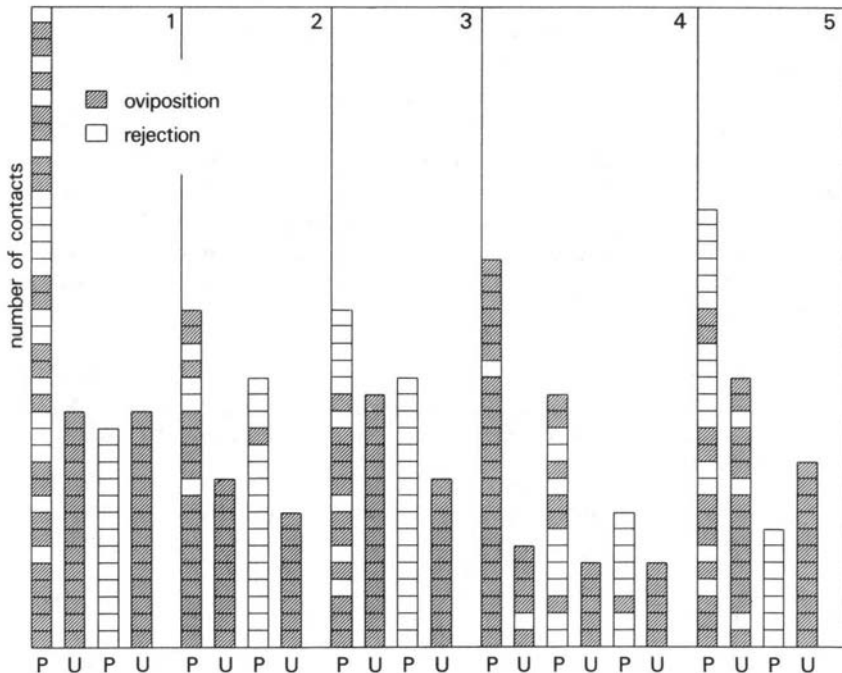


Fig. 3. The sequence of oviposition in and of rejection of parasitized hosts (P) and unparasitized hosts (U) of 5 *A. tabida* females, which had never oviposited at the beginning of the experiment.

high. This is suggested by experiments 3 and 5 in which the wasps seem to develop a higher restraint to oviposit in parasitized hosts by the end of their first exposure to such hosts. To test this last hypothesis, 2 series of control experiments were done.

*A test of the "restraint" hypothesis*

*Material and technique.* In the five experiments of a third series, a female which had never oviposited before was first exposed to 25 unparasitized hosts, and then alternately to 25 parasitized and 25 unparasitized. Each exposure lasted 30 min. The wasp's behaviour was observed and each parasitized or rejected larva was immediately replaced by one of the same category. The removed larvae were dissected.

The fourth series of experiments was identical to the third one, except the first exposure to unparasitized larvae: a wasp which had never oviposited before was allowed to parasitize only one unparasitized larva and then placed in the petridish with parasitized hosts.

*Results and discussion.* Table IX and table X present the results of the third and the fourth series of experiments. If the wasps in series 2 accepted a number of parasitized hosts for oviposition because of a strong tendency to oviposit, we would also have expected the wasps in series 4 to accept a number of them during their first exposure to parasitized larvae, because only one oviposition in an unparasitized host is probably not enough to lower their high tendency to oviposit. We expected the wasps in series 3 to reject all parasitized hosts, because after half an hour exposure to unparasitized larvae their tendency to oviposit will be reduced. However, in both series of experiments all parasitized hosts are

TABLE IX

The numbers of ovipositions in and rejections of unparasitized and parasitized hosts of females which had never oviposited at the beginning of the experiment: third series.

| exp.<br>nr. | unpsr. |     | par. |     | unpar. |     | par. |     | unpar. |     |
|-------------|--------|-----|------|-----|--------|-----|------|-----|--------|-----|
|             | acc    | rej | acc  | rej | acc    | rej | acc  | rej | acc    | rej |
| 1           | 15     | 11  | 0    | 26  | 17     | 12  | 0    | 6   | 11     | 22  |
| 2           | 23     | 1   | 0    | 13  | 24     | 0   | 0    | 2   | 26     | 4   |
| 3           | 2      | 17  | 0    | 7   | 22     | 16  | 0    | 15  | 23     | 29  |
| 4           | 18     | 13  | 0    | 17  | 28     | 2   | 0    | 2   | 28     | 4   |
| 5           | 19     | 2   | 0    | 16  | 27     | 1   | 0    | 17  | 26     | 2   |
| tot.        | 77     | 44  | 0    | 79  | 118    | 31  | 0    | 42  | 114    | 41  |

TABLE X

The numbers of ovipositions in and rejections of unparasitized and parasitized gosts of females which had never oviposited at the beginning of the experiment: fourth series.

| exp<br>nr | unpar. |     | par. |     | unpar. |     | par |     | unpar |     |
|-----------|--------|-----|------|-----|--------|-----|-----|-----|-------|-----|
|           | acc    | rej | acc  | rej | acc    | rej | acc | rej | acc   | rej |
| 1         | 1      | 0   | 0    | 14  | 9      | 2   | 0   | 7   | 24    | 5   |
| 2         | 1      | 0   | 0    | 13  | 20     | 5   | 0   | 25  | 23    |     |
| 3         | 1      | 0   | 0    | 6   | 12     | 1   | 0   | 13  | 17    | 6   |
| 4         | 1      | 0   | 0    | 4   | 21     | 11  | 0   | 13  | 30    | 11  |
| 5         | 1      | 0   | 0    | 5   | 10     | 20  | 0   | 6   | 16    | 9   |
| tot.      | 5      | 0   | 0    | 42  | 72     | 39  | 0   | 64  | 110   | 32  |

rejected. The wasps in series 4 reject the parasitized hosts after one oviposition in an unparasitized one.

Hence, a strong tendency to oviposit is not the cause of the superparasitization that occurs in series 2.

Therefore, it is more likely that the proportion of parasitized hosts that were rejected in the first exposure to parasitized hosts in series 2 can be explained as rejection in response to their unattractive physical state. The occurrence of more rejections towards the end of the first exposure to parasitized hosts may then be explained as follows: *A. tabida* finds its hosts by reacting to their movements (VAN ALPHEN & DRIJVER, 1982). Active larvae are therefore more easily found. Towards the end of the exposure period the proportion of larvae with a low activity must have increased, because proportionally more active larvae have been found by the parasitoid and are subsequently removed from the petri-dish (this holds even though the removed larvae are replaced). The results of the experiments of series 4 show that the rejection of parasitized hosts is not a foraging decision based on information, obtained by the parasitoid after a period of sampling, about the ratio unparasitized/parasitized hosts. The parasitoid was able to discriminate between parasitized and unparasitized hosts after laying one egg in an unparasitized host. This can be interpreted as evidence that discrimination has to be learnt by *A. tabida* in the sense of learning as used by VAN LENTEREN (1976) and KLOMP *et al.* (1980). Yet, we cannot discard the possibility that the unexperienced wasps recognize parasitized hosts as such, but accept them for oviposition because it is a good strategy to do so as long as there is no evidence that unparasitized hosts are available.



## GENERAL DISCUSSION AND CONCLUSIONS

When we compare our results on *A. tabida*, with those of VAN LENTEREN (1976) on *L. heterotoma*, we see several similarities in the host discrimination behaviour of the 2 species: (1) individuals of both species are able to distinguish unparasitized hosts from those previously parasitized by themselves or by a conspecific; (2) some superparasitism in both species may occur because a female sometimes lays a second egg in a host within the time needed for building up the factor which causes avoidance of superparasitism; (3) a more important cause of superparasitism in both species is that females oviposit in parasitized hosts when they have not encountered unparasitized hosts for a long period; (4) superparasitism in both species may also occur because inexperienced females do not refrain from oviposition in parasitized hosts.

There is also a number of differences between the two species with respect to the occurrence of superparasitism: (1) inexperienced *A. tabida* females lay sometimes 2 eggs during one oviposition. This was never observed for *L. heterotoma* (2) *L. heterotoma* females have been observed to oviposit simultaneously in the same host. This is very unlikely to occur with *A. tabida*, because it has a rigid ovipositor and stands over its host during oviposition. (3) An intriguing difference between the 2 species is that *L. heterotoma* is able to distinguish between hosts with different numbers of eggs, whereas *A. tabida* apparently can only distinguish unparasitized hosts from parasitized hosts. BAKKER *et al.* (1972) and VAN LENTEREN (1976) do not give a satisfactory functional explanation for this faculty of *L. heterotoma*. The fact that *A. tabida* lacks this ability makes the question whether this ability in *L. heterotoma* is functional all the more intriguing. Of course, not every behavioural trait needs to be adaptive, but, in our opinion, the main task of the behavioural ecologist is to provide functional explanations for an animal's behaviour. The search for functional explanations is the only way in which the question whether a trait is functional or not can be answered.

A possible function of the ability of *L. heterotoma* to distinguish hosts with different numbers of eggs could be the following: when the parasitoid/host ratio in the field is high and *L. heterotoma* finds only parasitized hosts, it will start to superparasitize. A second egg laid in a host has some probability to survive, and therefore superparasitism can be functional when only parasitized hosts are available. It is likely that the survival chance of an egg laid in a parasitized host is smaller, the more eggs this host already contains. Therefore it will be functional to discriminate between hosts with different numbers of eggs and oviposit only in the hosts with the lowest numbers of eggs. An additional advantage of this ability is that females which have never oviposited in

unparasitized hosts, are able to distinguish hosts in which they already laid an egg from hosts containing an egg of a conspecific. When this ability is indeed adaptive in *L. heterotoma*, why is it lacking in *A. tabida*?

Another question which is difficult to answer is whether it is adaptive for inexperienced wasps to lay eggs in parasitized hosts. When an inexperienced female arrives on a patch already exploited by conspecifics, two strategies are possible: (1) reject the parasitized hosts until an unparasitized host is found or until the patch is left after a number of contacts with parasitized hosts in search of a better patch, or (2) to oviposit in parasitized hosts. Whether it is a better strategy to do the latter depends on the survival chance for the female's eggs in parasitized hosts versus the chance of finding unparasitized hosts. At present we do not know the survival chance for supernumerary eggs in a host. The question whether the acceptance of parasitized hosts by inexperienced wasps has some survival value can only be answered after detailed field research, and a study of the survival chances of eggs laid in parasitized hosts.

Inexperienced females of *Caraphractus cinctus* show no restraint to oviposit in hosts parasitized less than 42 hours before, but after a number of ovipositions in parasitized hosts they will start to reject parasitized hosts at subsequent encounters. No experience with unparasitized hosts at subsequent encounters. No experience with unparasitized hosts is necessary to make them refrain from ovipositing in parasitized hosts (JACKSON, 1966). JACKSON also found evidence that the additional eggs laid by the inexperienced wasps in a parasitized host were not fertilized. Therefore it is likely that the oviposition in parasitized hosts by inexperienced wasps of *C. cinctus* is part of a sex ratio game as explained in the introduction.

The strain of *Trichogramma embryophagum* used by KLOMP *et al.* (1980) was thelytokous. Inexperienced females of this strain accepted parasitized hosts for oviposition. For this strain oviposition in a parasitized host cannot be part of a sex ratio game. *T. embryophagum* usually lays only one egg in eggs of *Ephesttia kuehniella*, but when superparasitism occurs within a short period after the first oviposition, up to 3 wasps may survive till the adult stage in one host egg (KLOMP & TEERINK, 1978). Hence supernumerary eggs have a substantial survival chance. It is unknown whether the restraint to oviposit in parasitized hosts can, as in *C. cinctus*, manifest itself after contacts with parasitized hosts and without experience with unparasitized hosts; KLOMP *et al.* (1980) never offered more than 2 parasitized hosts to an inexperienced female.

One oviposition in an unparasitized host by an inexperienced female of *C. cinctus* (JACKSON, 1966), *T. embryophagum* (KLOMP *et al.*, 1980), *Telonomus sphingus* (RABB & BRADLEY, 1978) and *A. tabida* is sufficient to

make the restraint to oviposit in parasitized hosts manifest. This implies that ovipositing in parasitized hosts does not occur because of a strong tendency to oviposit. However, the observation by JACKSON (1966), that the restraint to oviposit in parasitized hosts by *C. cinctus* becomes manifest after a number of ovipositions in parasitized hosts suggests that inexperienced females may recognize a parasitized host in which they oviposit, but lay eggs in them because this is a good strategy. The fact that inexperienced females of *Pachycrepoideus vindemiae* refrain from ovipositing in parasitized hosts indicates that it is not a general phenomenon that this restraint only becomes manifest after experience. Whether the differences in discrimination behaviour between parasitoid species as discussed in this paper are functional adaptations of the parasitoids to their different ecologies remains an open question.

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