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Genetic variability in the reactive distance in *Trichogramma brassicae* after automatic tracking of the walking path

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Abstract

Efficiency of hymenopterous parasitoids to control pests in a biological control release program probably increases with increasing searching ability of the females, which, in return, likely depends on the distance from which females perceive their hosts (i.e., reactive distance). In this study, we first analyse this hypothesis with the help of a stochastic model simulating the walking path of isolated *Trichogramma* females during their searching behaviour. Then, this reactive distance is estimated using automatic recording and analysis of the walking path of female wasps. Finally, the genetic variability for this trait is analysed in a *Trichogramma brassicae* Bezdenko (Hym.; Trichogrammatidae) population. Three types of hosts were used: Eggs of *Ephestia kuehniella* Zeller (Lep.; Pyralidae), eggs of *Mamestra brassicae* L. (Lep.; Noctuidae) and 0.45 mm glass beads. *M. brassicae* eggs are perceived from 4.01 ± 0.15 mm, which is a significantly longer perceptive distance than for *E. kuehniella* eggs (3.69 ± 0.10 mm) and glass beads (3.67 ± 0.10 mm). Moreover, whatever the host tested, a significant genetic variation is observed in this trait in the population studied.

The ecological and evolutionary implications and the agronomical importance of these results are discussed.

Introduction

Parasitoid wasps of the genus *Trichogramma* are widely used for biological control by means of inundative releases (Stinner, 1977) against several lepidopteran pests in crops such as cabbage, corn or sugar cane. Their efficiency to control target pests, however, remains generally to be improved (Pak *et al.*, 1989).

Recently, a considerable amount of effort has been made to analyse the searching behaviour of *Trichogramma*. Indeed, a strain with a high searching efficiency will probably be more effective in a biological control release program. In this line, Laing (1937, 1938) was the first to estimate the maximal distance from which *Trichogramma evanescens* Westwood females perceive their hosts. According to an indirect method based on percentage parasitism, this author concluded that *T. evanescens* visually perceived eggs of *Sitotroga cerealella* Olivier from a distance of 1.8 mm. Then, other more accurate methods were developed, all in an indirect way, some using video equipment (Edwards, 1961; Yano, 1978; Glas *et al.*, 1981). Finally, Pak *et al.* (1991) described a direct way to estimate this trait.

Because of the fact that this maximal distance, now called 'reactive distance' (Roitberg, 1985; Pak *et al.*, 1991), is likely to be correlated with wasp searching efficiency (i.e., the bigger this distance, the shorter the time needed to find hosts), it is usually supposed to be an important factor determining parasitic wasps' success as biological control agents.

In this paper, we first decided to analyse such an hypothesis, with the help of a stochastic model, simulating the walking path of isolated *Trichogramma* females during their searching behaviour. The reactive distance of *T. brassicae* Bezdenko (= *T. maidis* Pintureau & Voegelé) was then estimated using an automatic analysis (Clément *et al.*, 1988; Chassain *et al.*, 1988) of video-recorded walking paths of isolated females in the presence of three different types of hosts (i.e., eggs of *Ephestia kuehniella* Zeller, eggs of *Mamestra brassicae* L., and glass beads). Finally, the genetic variability in this trait was analysed in the population studied. Such a study could provide a way to improve the wasps efficiency to control a target pest in inundative releases by means of genetic selection programs (Roush, 1979; Wajnberg & Pizzol, 1989). Moreover, it could contribute to understand better the adaptive and evolutionary mechanisms involved in host/parasitoid relationships (Boulétreau, 1986).

A simulation model

In order to estimate how much the distance from which females perceive their hosts is related to the number of hosts attacked per unit of time (i.e., wasp efficiency), we developed a stochastic model from the one described by Yano (1978) after an experimental analysis of *Trichogramma dendrolimi* Matsumura walking behaviour. In this model, a path is generated using the following assumptions (using Yano's notation):

- The path orientation of a female leaving the host she just attacked is drawn from a uniform distribution between $-\pi$ and π, the corresponding walking speed being fixed to its minimal value, *Vmin.*
- Then, the female's walking speed increases linearly up to its maximal value, *Vmax*, in relation to the distance to the last attacked host, the path direction for the nth step being drawn from a normal distribution with the direction in the n–1th step as mean and *SD* as standard deviation. This last procedure leads to an autocorrelation in the successive path directions, but this autocorrelation is not taken into account anymore if the absolute value of the last path direction is greater than a given value: *THmax* (see Yano, 1978, for a more detailed explanation).
- When the distance to the last attacked host gets greater than *RSA* (using Yano's notation), or if the time before this last attack become greater than *GT* (i.e., giving-up time), the female is supposed to walk more rapidly and straightly: the walking speed is turned to *Vmax*, and the successive directions of the path are drawn from a normal distribution with always the same mean and standard deviation.
- Finally, when the female enters within a circle of a certain radius *RD* (i.e., reactive distance; *RP* in Yano's notation) with any host as centre, she moves straight to this host and attacks it. Then, the simulation is resumed at the first step described above.

Table 1. Analysis of variance used to compare the average values of the wasp efficiency (estimated by the number of hosts attacked) obtained with the stochastic simulation of the walking path of isolated *Trichogramma* females during their searching behaviour. (**: P<0.01)

Source of variation	d.f.	variance	F
Host spatial pattern (1)	2	1003.39	71.37 **
Reactive distance (2)	7	50565.45	3596.41 **
Interaction (1)-(2)	14	276.58	19.67 **
Error	47976	14.06	
Total	47999	21.55	

In this procedure, hosts already attacked are not removed; they can be perceived as healthy hosts. However, when an already attacked host is touched by a female, it is not taken into account in the computation of the total number of hosts attacked in the whole simulation for a given female. The total walking behaviour simulated here consists thus of successive steps ending with the discovery of a host, each of it being generated with the same set of parameter's values.

These assumptions were used with hosts showing different spatial patterns from regular up to aggregated distributions. Fig. 1 gives some examples of the results obtained.

Whatever the host spatial distribution, an increase in the reactive distance leads to a strongly significant increase in the number of hosts attacked (Fig. 2, Table 1). This trait is thus likely correlated with the wasp efficiency in biological control release programs. Moreover, when hosts are regularly distributed, females that have to be close to them to perceive them (i.e., low reactive distance) seem to be more efficient then females showing a high reactive distance. On the other hand, with randomly distributed hosts, females that perceive them from a longer distance (i.e., high reactive distance) appear more efficient than the others.

The fact that the interaction between reactive distance and host spatial pattern is also strongly significant indicates that the increase in wasp efficiency on

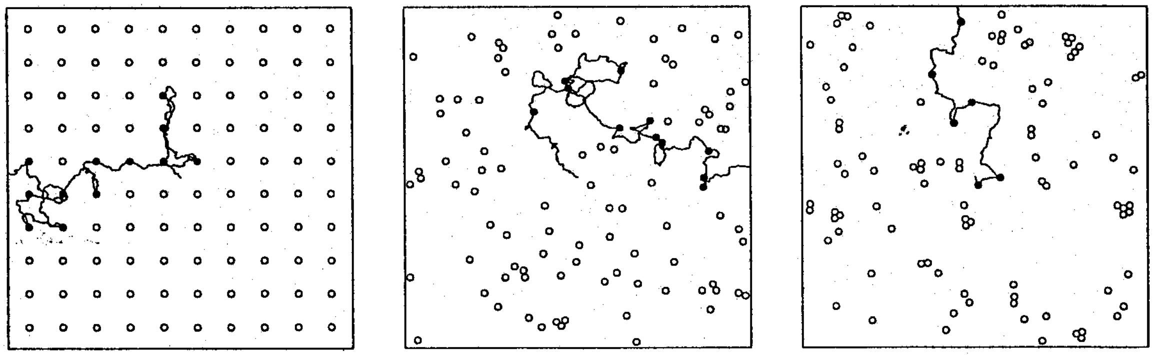

Fig. 1. Examples of the simulation of the walking behaviour of isolated *Trichogramma* females using the model published by Yano (1978). In each case there are 100 hosts, and their spatial distribution is either regular (left), random (middle) or aggregated (right). The aggregated spatial pattern of the hosts is simulated using a negative binomial distribution. The female is always released in the centre of the graph, and the simulation stopped as soon as the female reaches the outer square. In all cases, the parameters' value are: $SD = 40$ deg.; $THmax = 30$ deg.; $Vmin = 1.5$; $Vmax = 7.5$; $RSA = 80$; $RD = 30$; $GT = 300$ (arbitrary units).

randomly or aggregatedly distributed hosts is stronger than on hosts showing a regular spatial pattern (see Fig. 2 and Table 1). Therefore, an increase in the reactive distance, through natural selection, would likely provide, in the course of generations, a smaller payoff on regularly distributed hosts than on hosts showing a random or aggregated spatial pattern.

Trichogramma females are polyphagous and usually experience different types of hosts, with different spatial patterns. Therefore, according to the results obtained above, *Trichogramma* females are likely to be continuously under the pressure of opposite environmental constraints. In such a case, a genetic variability in reactive distance may be maintained in the wasp population. Such genetic variation was thus analysed experimentally.

Materials and methods

Because both olfactory and visual host cues are likely to be involved in the trait studied, we compared reactive distances obtained for real hosts (*Ephestia kuehniella* and *Mamestra brassicae*) and those obtained for a factitious host (glass beads). Eggs of *E. kuehniella* are faded white, oval shaped and about 0.5–0.6 mm long and 0.3–0.4 mm wide. Eggs of *M. brassicae* are about 0.60 mm in diameter, also faded white, spherical and have a ribbed surface. The glass beads are round, 0.15 mm in diameter, and transparent.

The *T. brassicae* population was collected from parasitized *Ostrinia nubilalis* Hübner eggs, in Alsace (France), during the summer of 1986. These strains 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) for this species. Since then, it was reared in the laboratory at 25 °C on UV-killed *E. kuehniella* eggs, L12:D12. This population was also used by Wajnberg (1989), Wajnberg & Pizzol (1989), Wajnberg *et al.* (1989), and Wajnberg (1991).

For each of the three hosts tested, the reactive distance of respectively 30, 19 and 29 unfed, less than 24 h old *Trichogramma* females, isolated at random from the mass-reared population, was measured. For all of them, the reactive distance of three of their daughters was also measured in the next generation. This experimental protocol allows an estimation of reactive distances for each female and for each host attacked. It also provides two ways to estimate the genetic part of the inter-female variability observed: the regression between the mothers and the average of the daughters gives an estimation of the genetic transmissibility of the reactive distance over two successive generations; the statistical test of the variation observed between families, in the daughter generation, also provides a way to quantify the genetic variation in the trait studied (i.e., isofemale lines method; Parsons, 1980).

About 30 min before the experiment, naive females (i.e., females that had not experienced previous presence of hosts) were offered a single host egg to motivate them for host searching. This host was of the same species as the one used in the experiment, except for females tested on glass beads that were offered a single *E. kuehniella* egg. For each experiment, an isolated female was released in an arena (9 cm diameter,

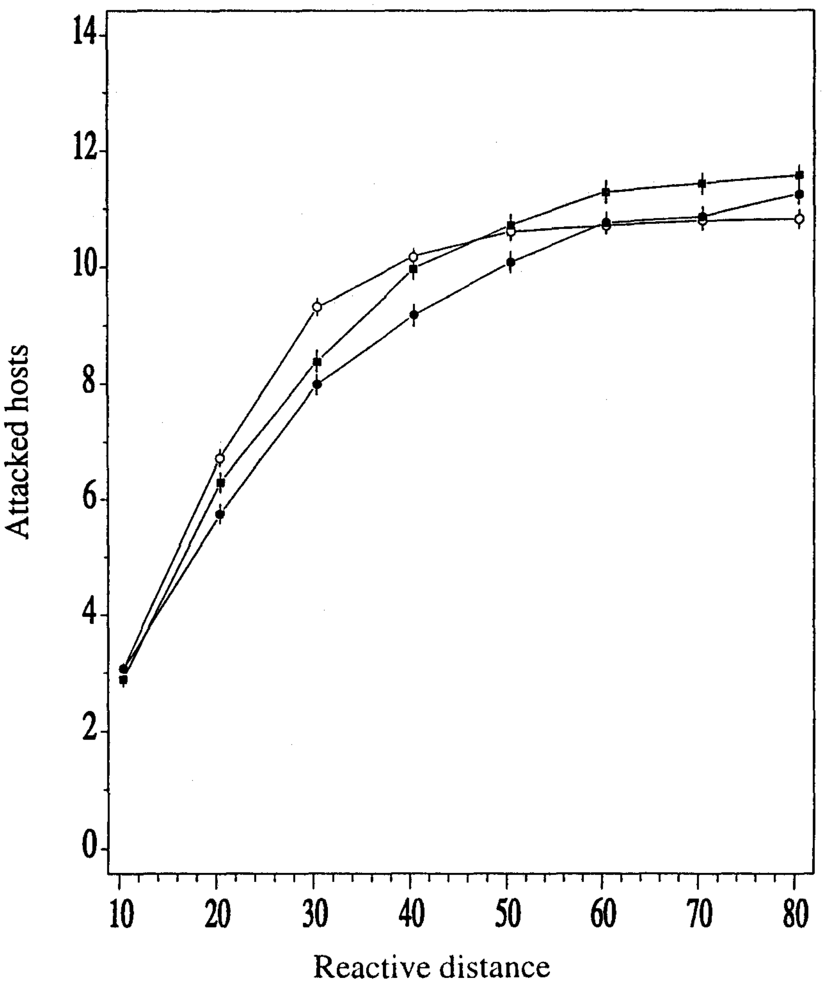

Fig. 2. Effect of a change in the reactive distance on wasp efficiency (estimated by the number of hosts attacked) obtained by simulation. Each point is the average (± 2 s.e.) of 2,000 simulations, each of it being run as explained in Fig. 1 with hosts showing regular (open circle), random (square) or aggregated (closed circle) spatial patterns.

without a lid) in which 12 hosts were placed in a regular pattern, the distance between two neighbour hosts being 15 mm in row and column. A strip of vaseline was placed around the hosts to prevent females walking out of the arena.

A video camera (RCA-TC 1005/U01X) with a 55 mm lens (diaphragm 5.6) was placed about 70 cm above the arena, and the walking path of the female was video-recorded up to the moment when the female touched a host. On average, about five measures were done for each female. Each experiment was carried out during daytime, under 6 white tubes of 36 Watt each (7,000 lux), at 23 ± 1 °C and 70% R.H.

From the video-recorded females' motions, Pak *et al.* (1991) drew each walking path manually in order to get the X-Y coordinates for each female in the course of time. Here, however, we used an automatic tracking computer device that automatically transformed each walking path into X-Y coordinates with an accuracy of 25 points per second (Clément *et al.*, 1988; Chassain *et al.*, 1988). This fully automatic procedure produces a much more accurate quantification of the walking features produced by each females. Finally,

Table 2. Overall average (± 1.96 s.e.) reactive distance (mm) of *T. brassicae* females for each host tested. Values followed by the same letter do not differ significantly at the 5% level

	n	Reactive distance	
E. kuehniella	422	3.69 ± 0.10	b
M. brassicae	291	4.01 ± 0.15	a
Glass beads	396	3.67 ± 0.10	b

least-significant difference tests were used to separate different means.

Results

The average reactive distances for the three types of host are given Table 2. *Trichogramma* females perceived the eggs of *M. brassicae* from a significantly larger distance than those of *E. kuehniella* or glass beads. The reactive distances for the latter two were not significantly different.

Fig. 3 presents mother/daughter regression analyses for the three hosts tested. With glass beads, a significant relationship can be observed between the values obtained for the mothers and their daughters. This suggests that, in this case, there is a significant genetic variability in this trait in the *T. brassicae* population analysed. For the two other hosts, however, no significant relationship can be observed.

Table 3 shows that, for both *M. brassicae* and *E. kuehniella* as hosts, there are significant differences between the average values of each family. Thus, for these two hosts, the reactive distance seems to be a family feature, which also suggest that the observed variability is under a genetic control. With glass beads, however, no family effect is observed.

These results suggest that, in the population analysed, there is a genetic variability in the maximal distance from which *T. brassicae* females perceive their hosts. Within this population, some females need genotypically to be close to their hosts to perceive them, others are able to identify their presence from a longer distance.

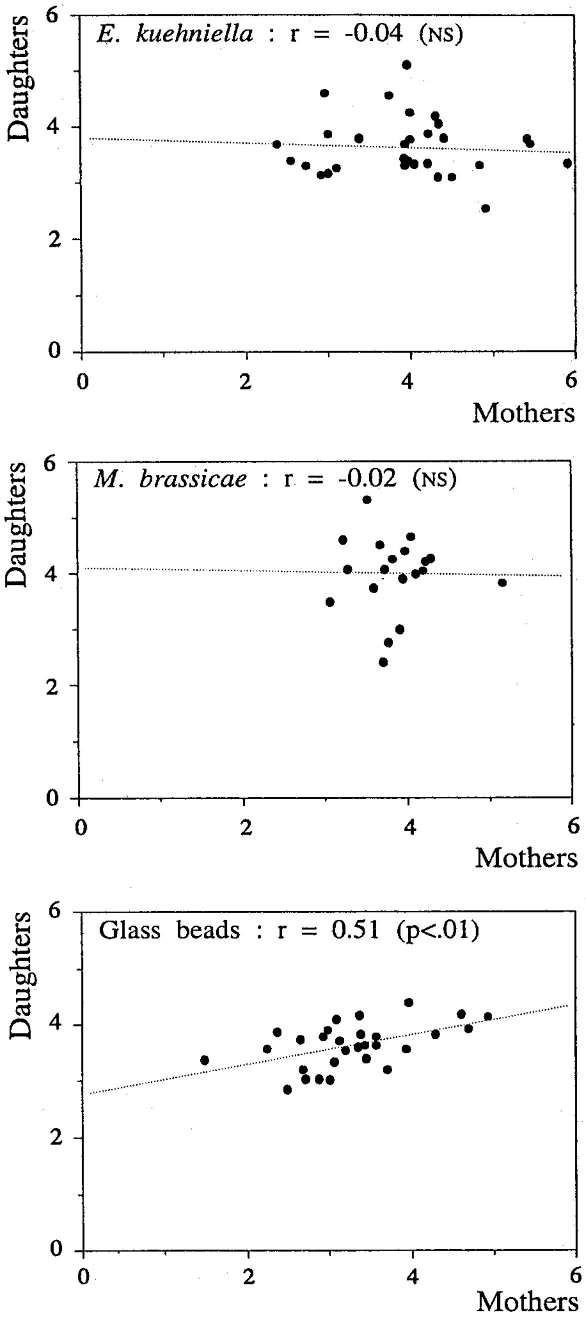

Fig. 3. Mother/daughter regression analysis of the reactive distance (mm) of *T. brassicae* females for three types of hosts (NS: non significant).

Discussion

Among the different behavioural characters involved in the efficiency of a biological control agent to control a target pest, the walking pattern of a wasp is proba-

Table 3. Analysis of variance used to compare the average values of the reactive distance to three types of hosts between different *T. brassicae* families, at the daughter generation (see text). The 'female' effect is nested within the 'family' effect. (NS: non significant; *: $P<0.05$; **: $P<0.01$)

	E. kuehniella		
Source of variation	d.f.	variance	F
Families	29	2.6056	1.65 *
Females.families	60	1.5751	2.54 **
Error	222	0.6208	
Total	311	0.9900	
	M. brassicae		
Source of variation	d.f.	variance	F
Families	18	4.5735	2.41 **
Females.families	37	1.8949	1.35 (NS)
Error	164	1.4009	
Total	219	1.7451	
	Glass beads		
Source of variation	d.f.	variance	F
Families	28	1.7138	1.15 (NS)
Females.families	58	1.4868	1.89 **
Error	224	0.7851	
Total	310	1.0003	

bly both the easiest one to quantify and one of the best indications for female searching efficiency. In the present study, such walking patterns have been analysed in order to quantify the maximal distance from which *Trichogramma brassicae* females perceive their hosts (i.e., reactive distance). According to the simulation model developed here, this behavioural trait appears to be strongly correlated with wasp efficiency as a biological control agent (see Fig. 2).

According to Pak *et al.* (1991), *Trichogramma* females most likely perceive their hosts both visually and chemically. These authors concluded, after comparing *M. brassicae* with glass bead results, that the odour of host eggs increases the reactive distance of the wasps by no more than 10 to 20%. In the present research, *M. brassicae* eggs are perceived from a longer distance than glass beads. Besides the odour, these hosts differ also in colour, light reflection and shape. Therefore, the difference cannot be attributed exclusively to the odour of *M. brassicae* eggs.

Moreover, *Trichogramma* females perceived the *M. brassicae* eggs from a longer distance than the *E. kuehniella* eggs. Although the odours of these two host species might be different, the difference in reactive distance could also be due to their difference in size and appearance: *M. brassicae* eggs are much bigger than *E. kuehniella* eggs.

The small, odorous *E. kuehniella* eggs are perceived from the same distance as the bigger, odourless glass beads. A possible chemical (i.e., olfactory) response of the wasps to these eggs may have compensated a reduced visual perception, compared with the glass beads. It is worth noting that the average reactive distance of *T. brassicae* to *Mamestra* eggs and glass beads obtained here are significantly greater ($P<0.05$) than those obtained by Pak *et al.* (1991) with *T. evanescens* and *T. dendrolimi* (on *M. brassicae* eggs: 3.27 ± 0.16 and 3.32 ± 0.10; on glass beads: 3.14 ± 0.38 and 2.64 ± 0.32, respectively). Such significant differences might be explained simply by the fact that the species studied by Pak *et al.* (1991) need to be closer to their host to perceive them. These differences may also be due to differences in experimental conditions (e.g., light intensity) and to the fact that the tracking system used here gives much more accurate results than the manual procedure used by these authors.

Pak *et al.* (1991) found a substantial variability in the reactive distance between *Trichogramma* females. Here, such a variability is also observed, and its genetic determinism has been analysed. Using mother/daughter regression analysis, a significant genetic variation is observed only in the glass bead experiment. The fact that odourless glass beads can only be perceived through visual cues suggests that this genetic variability concerns only visual host perception mechanisms. The addition of olfactory cues, when the two other hosts are used, may have masked such genetic variability. On the other hand, with *M. brassicae* and *E. kuehniella* as hosts, there are significant differences between the average values of each tested family. Thus, for these two hosts, the reactive distance seems to be a family feature, which also suggests that the observed variability is under a genetic control. The difference in the results obtained with these two analyses could be explained by a difference in their accuracy to quantify genetic variability. The family analysis compared the average values of different families and takes the 'female' effect into account, and this is not the case in the mother/daughter regression analysis.

Investigations are now being carried out in order to understand better the biological mechanisms involved in the observed genetic variability. Such studies, partly based on a comparison of the variations in the reactive distance between different *Trichogramma* species that are known to attack different host species, should lead to a better understanding of the evolution of parasitoid populations in response to variations in environmental conditions (i.e., variations in the size, odour, shape and spatial distribution of their hosts). The corresponding results will provide the basic information required to start the genetic selection of mass reared *Trichogramma* populations in order to increase their efficiency in biological control programs.

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