

Searching Speed of *Trichogramma minutum* and Its Potential as a Measure of Parasitoid Quality

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Searching speed has been proposed as a quality criterion for the prediction of field efficacy of mass-reared *Trichogramma* spp. The searching speed, walking speed, and activity of female *T. minutum* Riley were measured using an automated video tracking system. Two- and 3-day-old thelytokous females searched twice as fast as 1-day-old females, whereas the searching speed of a strain of arrhenotokous females was similar at all ages. When reared on *Actebia fennica* Tauscher, the resulting wide variation in wasp size was not related to searching speed or walking speed. Both strains of *T. minutum* searched faster later in the day. In a second laboratory experiment, the proportion of hosts parasitized, the proportion of hosts accepted, and searching speeds of parasitoids were measured simultaneously for groups of females of both strains. Percentage parasitism was related to host acceptance but not searching speed. Searching speed was unrelated to the estimated number of host contacts. These results suggest that searching speed is not a useful measure of parasitoid efficacy for *T. minutum*. © 2000 Academic Press

Key Words: Insecta; *Trichogramma minutum* Riley; *Ephesttia kuehniella* Zell.; *Actebia fennica* Tauscher; biological control; mass rearing; searching speed; host acceptance; parasitoid quality.

INTRODUCTION

The starting point for many inundative release programs using insect parasitoids is to demonstrate the potential of the natural enemy to suppress a pest population. This potential has been demonstrated for egg parasitoids in the genus *Trichogramma* in both agriculture (Stinner, 1977) and forestry (Smith *et al.*, 1990). Subsequent requirements for successful inundative release programs include the determination of the

factors that contribute to successful parasitism (Bergeijk *et al.*, 1989; Cerutti and Bigler, 1994; Bouchier *et al.*, 1993; Bouchier and Smith, 1996), the development of cost-effective methods of parasitoid production (Bigler, 1986; Laing and Eden, 1990), and the development of methods to maintain parasitoid quality (Bigler, 1989). Parasitoid quality measures that have been proposed and tested for *Trichogramma brassicae* Bezdenko (= *maidis* Pint. et Voegelé) released against the European corn borer (*Ostrinia nubilalis* Hübner) include parasitoid size, rearing host, fecundity, host acceptance behavior, and searching speed (Bigler *et al.*, 1991).

Searching speed has been adopted as a quality measure for the mass production of *T. brassicae* (Bigler, 1989; Cerutti and Bigler, 1994) because of its theoretical link to host finding (parasitoids that move faster should find more hosts) and because of its demonstrated correlation with parasitism in the field (Bigler *et al.*, 1988). Similar quality factors have been suggested for the use of *T. minutum* against forest pests (Laing and Bigler, 1991); size, rearing host, host acceptance, and fecundity have been tested as predictors of parasitism (Bouchier *et al.*, 1993, 1994; Bouchier and Smith, 1996). The factors affecting searching speed and the relationship between searching speed, host finding, and subsequent parasitism have not been investigated in *T. minutum*.

The objective of this investigation was to assess the feasibility of using searching speed as a measure of parasitoid quality within and between strains of mass-reared *T. minutum* by (1) examining factors (strain, age, size, and time of day) that might affect searching speed and walking speed and (2) determining if variability in searching speed relates to the host-finding ability of the parasitoid. Variation in searching speed within and between two strains was examined to obtain a wider range of values for this potential quality attribute.

MATERIALS AND METHODS

Definitions

The terms searching speed and walking speed have often been used interchangeably, since *Trichogramma*

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are generally believed to contact hosts by walking rather than flying. For this study, searching speed describes the average velocity of a walking insect over a given observation time and includes time spent immobile. Walking speed denotes the average velocity of an insect only while walking. These two values are related in that searching speed is equal to the walking speed multiplied by the fraction of the observation period spent walking. Searching speed is usually the variable of interest because it is a quantitative measure of how far a parasitoid searches in a given time. There is however, a more intuitive link between walking speed and factors such as parasitoid size or ambient temperature. Knowing which factors affect walking speed, the proportion of time spent walking, and how these contribute to the variation in searching speed is important for designing an efficient monitoring system in a mass-rearing facility.

Parasitoid Rearing

In each of the experiments described below, except when stated otherwise, the parasitoids were reared on eggs of the Mediterranean flour moth (MFM), *Ephestia kuehniella* Zell., obtained from Beneficial Insectary, Guelph, Ontario. MFM eggs were stored at 4°C in a ventilated container for up to 14 days. Parasitoids used to examine the effect of wasp size on searching speed were reared on eggs of the black army cutworm (BAC), *Actebia fennica* Tausher, obtained from the Canadian Forest Service, Sault Ste. Marie, Ontario.

Parasitoids were reared in shell vials (95 mm long by 22 mm diameter) closed with screen-vented corks. Fresh host eggs were glued to one side of a 70 × 20-mm piece of light cardboard and presented to the parasitoids. All parasitoid cultures were reared at 25°C and 16L:8D photoperiod, the light period beginning at 0600 h Eastern Standard Time.

Three strains of *T. minutum* were selected for these experiments. Strain-a, an arrhenotokous form of *T. minutum*, originated from parasitized spruce budworm (*Choristoneura fumiferana* (Clemens)) egg masses collected in Vermillion Bay, Ontario, Canada (49° 55' N, 93° 55' W) in 1990. This strain was reared for more than 100 generations on MFM. Strain-t, a thelytokous form of *T. minutum*, originated from parasitized spruce budmoth (*Zeiraphera canadensis* Mut. and Free.) eggs collected in 1992 in northern New Brunswick near St. Leonard (47° 15' N, 67° 30' W). This strain was reared on MFM eggs at 15°C for approximately 28 generations and at 25°C for another 5 generations. These strains had been shown to have significantly different biological attributes (Wang and Smith, 1996) and were chosen to provide a range of values for the potential parasitoid quality attributes being measured. Strain-t94, also thelytokous, was collected from the St. Leonard site in June 1994 using sentinel MFM egg masses. This strain

was reared on MFM at 15°C and then reared on BAC eggs at 25°C for 5 generations prior to experimentation. In November 1994 and January 1995, strain-a and strain-t were divided into 10 and 5 separate populations, respectively. Phenology was staggered to provide a consistent supply of newly emerged parasitoids for experimentation.

One day before scheduled emergence, parasitized egg cards were cut into 10 × 20-mm pieces and placed individually into 15 × 50-mm shell vials. One hour after the onset of the light period on the day of emergence, egg cards were inspected under a binocular microscope and any emerged females removed. All females emerging during the second hour of the light period (hour of peak emergence) were collected in a clean shell vial and fed 50% honey-water solution. These females were kept at 25°C 16L:8D for 24 h. This method ensured that almost all females were mated, fed, and of approximately equal age.

Measurement of Searching Speed

An automated system for measuring the movement of individual parasitoids was developed using software originally written by C. Ramcharan (Ramcharan and Sprules, 1989). A transparent arena was constructed using two square pieces of clear acetate (120 × 120 × 2 mm) in one of which a circular depression 4–5 mm deep and 35 mm in diameter was formed by bending the plastic while heating. When clamped together a space was created between the two sheets in which a parasitoid was placed. To provide sufficient contrast and prevent any phototactic response, the insect was lit from below using a light-box equipped with four, 60-cm, 20-W cool white fluorescent bulbs.

The behavior of each parasitoid was monitored using a video camera, equipped with a macro lens, mounted 100 mm above the plane of the arena. A video digitizing board (Oculus 200A; CORECO Inc., Montreal) converted the video image to black and white for use by a computer. Tracking software then interpreted the image and identified the insect as any three adjacent black pixels. The X,Y coordinates of this location were then stored in a file, 15 times per second, using an IBM-PC 386 20 MHz. If the program failed to identify the location of the insect during a time interval, its location was noted as missing in the file.

The X,Y coordinate data were analyzed using a IBM-BASIC program written by B.V.H. which computed the total distance traveled, total time tracked, number of intervals in which the insect was not located, time spent moving, searching speed, and walking speed. To compensate for spurious movement caused by small vibrations and voltage fluctuations, the X,Y coordinate data were first smoothed using a four-point moving average of the values from times $t - 2$ to $t + 1$ and then rounded to the nearest integer. To test the accuracy and

precision of the system, a black dot on white paper was tracked while it was moved a known distance of 50.0 mm diagonally across the field of view. The average estimated distance using the tracking system was 50.5 mm \pm 0.008 (mean \pm SEM) after 10 runs.

Experiment 1: Factors Affecting Searching Speed

The searching speed of two different strains of *T. minutum* and three different ages were compared. The searching speed of individual female wasps was measured using the video tracking system. Parasitoids were moved to an environmentally controlled room (20 \pm 1°C, \approx 65% R.H.), placed individually into 10 \times 25-mm shell vials, and allowed to acclimate for 30 min. An equal number of females from each strain was randomly selected and their searching speed measured for approximately 120 s. To control for environmental factors, females from each strain were tested in an alternating sequence. All measurements were recorded between 1300 and 1700 h. Differences in mean searching speed between the two strains and three ages were analyzed as a two-way ANOVA with strain and age as factors. The total sample sizes for each strain were 28 1-day-old females, 49 2-day-old females, and 14 3-day-old females. A *post hoc* test for all pair-wise comparisons between means was conducted using the Tukey-Kramer HSD method (Wilkinson, 1990). To stabilize the variances between groups and normalize group distributions, variables were transformed using the equation $\ln(Y + 0.1)$. Using probability plots of the data (Sokal and Rohlf, 1981), it was found that adding 0.1 to each variate produced the most symmetrical distributions with the least number of outliers.

To examine the effect of wasp size independent of host species, wasps from strain t-94 were reared on black-army cutworm eggs, which are large enough to allow for the successful development of one to four wasps per egg, producing a wide range of parasitoid sizes.

The searching speed of 102 female wasps was measured for a 120-s period using the video tracking system. All measurements of searching speed were made between 1100 and 1700 h. After tracking, parasitoids were frozen. Wing lengths were later measured according to the procedure described by Bouchier *et al.* (1993). The searching speed of individual females was compared to time of day and size of each female using multiple regression analysis (Wilkinson, 1990). The searching speed data were first transformed using the equation $\ln(Y + 0.1)$.

Experiment 2: The Relationships of Host Acceptance and Searching Speed to Parasitism

This experiment was designed to measure the degree to which variation in searching speed and host accep-

tance explained variation in parasitism. To include a greater amount of variation in searching speed than would be present within one strain, both the thelytokous and the arrhenotokous strains of *T. minutum* were used in this experiment. A population from each strain was divided into three groups such that the variables searching speed, host acceptance, and the proportion of hosts parasitized could be measured simultaneously for the populations. This allowed us to determine the relationships between these three variables at the group level without the confounding effects of changes in time of day or parasitoid age (as found in Experiment 1).

All parasitoids were mated, fed, naïve with respect to hosts, at least 24 h old, reared, and handled under the conditions outlined above.

Trials were conducted on 5 separate days with test periods in both the morning and the afternoon. During each test period three measurements were taken for each strain: (1) average searching speed of 21 females, (2) percentage host acceptance of 20 females, and (3) proportion of 11 females which found and parasitized hosts. In some test periods sample sizes were smaller due to missing data.

The ability of female wasps to find and parasitize hosts was assessed using a clean 4-L glass pickle jar laid on its side which contained a single patch of host eggs. The patch consisted of MFM eggs on the sticky surface of a 5 \times 5-mm square of Post-it Note, backed with two-sided tape. Single females were released from a 10 \times 25-mm shell vial at the bottom of the jar. The host patch was located half way up the side of the jar and 12 cm toward the opening. After 3 h, the female wasp was removed and scored as either "on" or "off" the host patch. The host patch was then incubated at 25°C 16L:8D. After a minimum of 7 days, the host patches were scored as either parasitized (at least one black egg) or not parasitized. Females were scored as having parasitized the host patch by either being found on the eggs with the ovipositor inserted in an egg and/or producing black eggs. This assay was conducted on females from both strains in one test period between 0830 and 1130 h and another test period between 1330 and 1630 h. In each period 11 females from each strain were tested and the proportion of females that parasitized the host was recorded. This value will be referred to as the parasitism rate.

At the same time, the mean searching speed was measured using the video tracking system. Twenty-one females from each strain were tracked for approximately 120 s in an alternating sequence between 0900 and 1130 h and again between 1400 and 1630 h using different females each time. The mean searching speed was calculated for each strain and each test period.

Simultaneously, the host acceptance behavior of females from each strain was measured by observing

their behavior upon first contact with a potential host. Females were placed in the center of four 5×5 -mm MFM egg patches glued in a 2×2 -cm grid on a piece of white paper (Fig. 1). Around each egg patch, a circle with a 1-cm radius was drawn, indicating the maximum distance of perception (Pak *et al.*, 1990). Parasitoid behavior was observed at $10\times$ magnification with a binocular microscope equipped with a glass fiber light source. Time to first contact and the onset of drilling was measured to the nearest second. Host acceptance behavior of the wasp was categorized as either "accepted," "rejected," or "did not find." A female "accepted" the host if she contacted an egg patch and did not leave before beginning to drill. A female "rejected" the host if she walked outside of the circle after contacting an egg patch without drilling. A female "did not find" if she did not contact an egg patch within 3 min. If females wandered out of the grid of egg patches, they were returned to the center of the grid and remeasured. This assay was conducted alternately on females from a strain-a and strain-t, in one test period between 0900 and 1130 h and another test period between 1400 and 1630 h. In each period, approximately 20 females from each strain were tested. For each strain, the proportion of females that accepted the host was calculated. This value will be referred to as the host-acceptance rate.

To compare the relative utility of searching speed and host acceptance for predicting parasitism, the data were modeled using logistic regression with binomial errors since the response variable, parasitism, is bino-

mial (Crawley, 1993). The initial model included both searching speed and host acceptance as predictors and test day and time of day as blocking variables. Variables were systematically removed until the simplest best-fitting model was found.

To determine if searching speed was related to probability of a female finding a host, an estimate of the proportion of females which found a host (referred to as the host-finding rate) was calculated by dividing the parasitism rate by the host-acceptance rate. Data from the morning and afternoon test periods were pooled to ensure nonzero host-acceptance rates. The relationship between the host-finding rate and the back-transformed mean searching speed was assessed using linear regression (Wilkinson, 1990).

If searching speed is related to the host-finding rate, then according to the model by Doult (1959) proposing that the parasitism rate should be a function of a parasitoid's ability to find a host and its rate of host acceptance, the parasitism rate should also then be a function of a parasitoid's searching speed and its host-acceptance rate. To test this, we calculated a predicted parasitism rate for each trial by multiplying the average searching speed by the host-acceptance rate of females in that trial and then compared these predicted values to the observed rates of parasitism for each trial. For ease of comparison, the predicted parasitism values were scaled to lie between zero and 100. These data were then analyzed using linear regression.

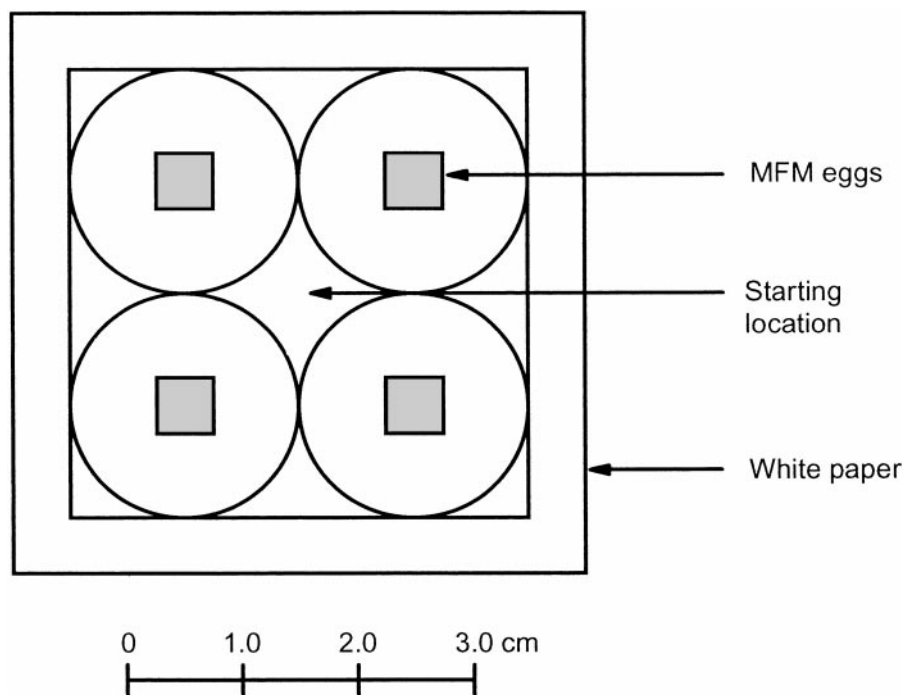


FIG. 1. Host-acceptance test arena. Female *T. minutum* were placed in the center of the arena and their behavior was observed until they contacted an egg mass. Females were classified as rejecters if they left the egg patch and as acceptors if they began oviposition.

RESULTS

Experiment 1: Factors Affecting Searching Speed

Both wasp strain ($df = 1,173$; $F = 17.135$; $P < 0.001$) and wasp age ($df = 2,173$; $F = 5.372$; $P = 0.005$) had a significant effect on searching speed. There was no interaction between the two main effects ($df = 2,173$; $F = 1.451$; $P = 0.237$) (Fig. 2). Strain-t, the thelytokous form of *T. minutum*, had a searching speed approximately twice as fast as strain-a, the arrhenotokous form, averaged over all ages. The searching speeds of both strains were relatively low at 1 day old and not significantly different (Tukey's HSD test, $P = 0.789$) (Fig. 2). While the searching speed of strain-a remained low at 2 and 3 days old (Tukey's HSD test, $P = 0.896$ and $P = 0.955$, respectively), the searching speed of strain-t was significantly higher than strain-a at 2 days old (Tukey's HSD test, $P = 0.009$) and remained higher than strain-a at 3 days old.

Size was not a significant factor affecting either searching speed or walking speed (Table 1) despite the large range of wing lengths of parasitoids emerging from BAC eggs (0.435 to 0.661 mm). Time of day had a significant positive effect on both searching speed and walking speed with faster speeds later in the day.

Experiment 2: The Relationships of Host Acceptance and Searching Speed to Parasitism

The simplest best-fitting model for predicting parasitism included host acceptance and test day, with a

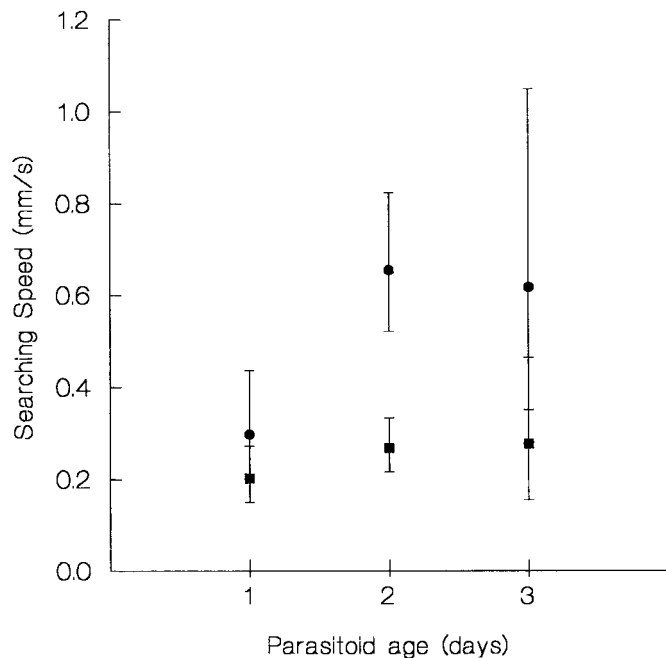


FIG. 2. Mean searching speeds of arrhenotokous (squares) and thelytokous (circles) *T. minutum* at different ages. Error bars indicate 95% confidence intervals.

TABLE 1

Least-Squares Multiple Regression Analysis of the Effects of Size, Time of Day, and Age on Mean Searching Speed and Walking Speed ($N = 102$ Females)

Model	Coef.	SE ^a	P ^b	R ^{2c}	P (overall) ^d
Searching speed ^e					
= Time of day	0.217	0.048	<0.001	0.175	<0.001
+ Size	0.57	1.974	0.774		
+ Constant	-4.336	1.372	0.002		
Walking speed					
= Time of day	0.14	0.034	<0.001	0.167	<0.001
+ Size	1.363	1.42	0.34		
+ Constant	-0.251	0.987	0.8		

^a SE, standard error of the coefficient (Coef.).

^b P values shown are for two-tailed *t* tests.

^c R², proportion of total variance explained by the model.

^d P (overall) is for the test of the significance of the model using ANOVA.

^e Searching speeds were first transformed using the equation $Y' = \ln(Y + 0.1)$.

residual deviance of 12.579 with 14 *df*. This model explained 89.6% of the total deviance in the odds of an egg mass being parasitized. Removing searching speed or time of day from the full model did not significantly increase the residual deviance ($df = 1$, $\chi^2 = 0.069$, $P = 0.792$ and $df = 1$, $\chi^2 = 0.351$, $P = 0.554$, respectively). Host acceptance and test day were left in the model because their removal resulted in a significant increase in the residual deviance ($df = 1$, $\chi^2 = 84.689$, $P < 0.001$ and $df = 1$, $\chi^2 = 11.066$, $P = 0.025$, respectively). There was no significant interaction between host acceptance and test day ($df = 4$, $\chi^2 = 2.679$, $P = 0.613$).

There was no relationship between the mean searching speed for parasitoids in a trial, and the estimated number of host contacts (slope = -0.233 , $P = 0.854$).

When the predicted parasitism rate (based on searching speed multiplied by host-acceptance rate) was compared to the observed parasitism rate (Fig. 3a) we found that there was a significant positive relationship and the slope did not differ significantly from unity (slope = 0.812 , $t = 1.191$, $P = 0.249$) nor did the intercept differ significantly from zero (intercept = 5.234 , $t = 0.693$, $P = 0.497$). When host-acceptance rate and searching speed were examined separately for their ability to predict observed rates of parasitism, searching speed was unrelated to observed parasitism rate ($F = 0.055$, $P = 0.816$) (Fig. 3b) but host acceptance was significantly related to the observed parasitism rate ($F = 73.327$, $P < 0.001$) (Fig. 3c).

DISCUSSION AND CONCLUSIONS

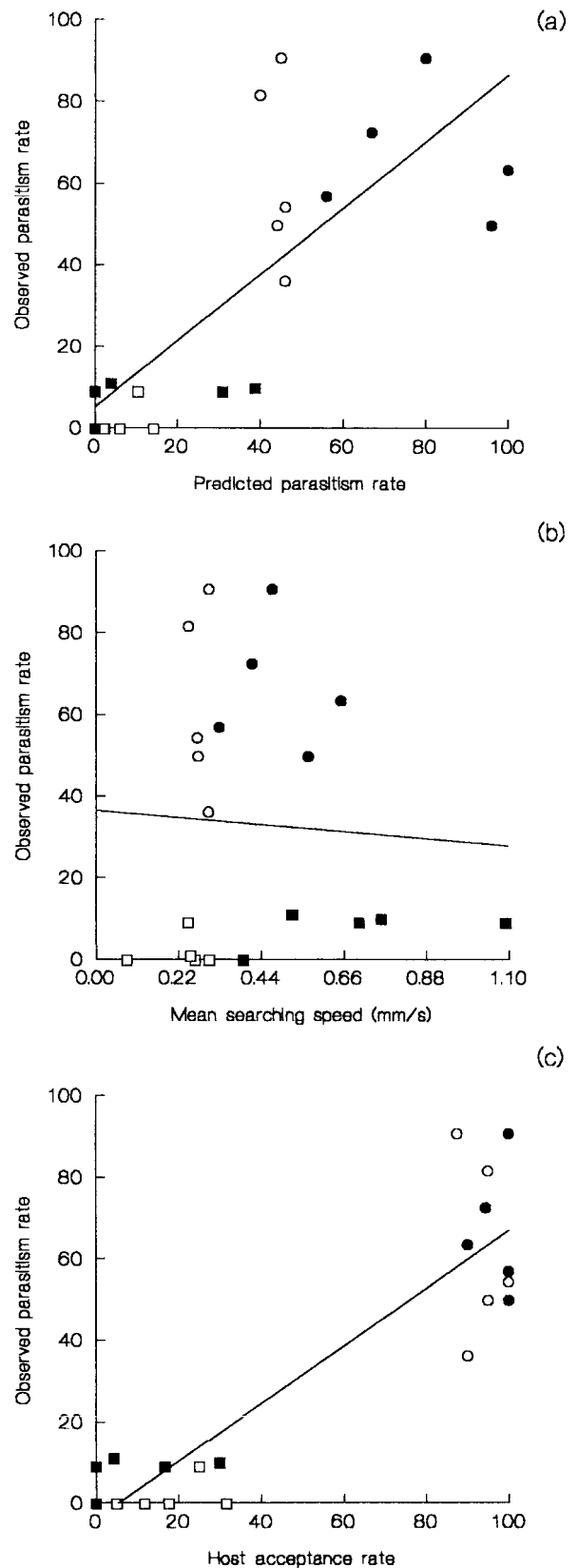
The effect of parasitoid age on searching rate has been documented for a variety of *Trichogramma* species

(Marston and Ertle, 1973; Lukianchuk, 1992; Pompanon *et al.*, 1993). These researchers found that older females (24–36 h) search faster and more actively than young females (0–8 h). We found that strain-t females displayed higher searching speeds with age but that the searching speed of strain-a females was similar across the three ages. The behavior of strain-t is consistent with the finding of Wang and Smith (1996) in which the ovipositional rate of thelytokous females increased during the 3 days after emergence. Many authors have reported behavioral traits in hymenopteran parasitoids progressively declining or being entirely lost during artificial rearing (Ashley *et al.*, 1973; Boller, 1972; Geden *et al.*, 1992). Mackauer (1976) has outlined the genetic problems associated with mass rearing, and Pompanon *et al.* (1994) has shown that there is a genetic basis for variation in locomotion in *Trichogramma*. Our finding that the searching speed of strain-a did not increase with age is possibly a result of this trait being lost during more than 100 generations in culture. Although strain-t has been reared for approximately 40 generations, heritable traits for locomotion may have been preserved due to its parthenogenic mode of reproduction. Our findings show that there is a significant amount of variation between strains and within strains over time (Fig. 2). The design of monitoring programs in a quality control system should consider this variation.

A significant amount of the variation in walking and searching speed can be explained by the time of day at which these variables were measured. The average searching speed and average walking speed of the thelytokous form of *T. minutum* increased linearly between 5 and 11 h after the onset of the light period. Pompanon *et al.* (1994) report a similar increasing trend for *Trichogramma cacoeciae* Marchall, another thelytokous species, but in *Trichogramma brassicae* Bezdenko linear speed and percentage of females active peak early in the photoperiod and decline thereafter (Pompanon *et al.*, 1993, 1994). The ecological implications of this activity pattern are unknown; the applied implication is that the timing of quality control assays must be standardized to ensure comparable results. Furthermore, it would be useful to know how the searching speed of a given strain changes over longer periods of time (days) to predict how the wasps will behave after an inundative release.

Previous work by Marston and Ertle (1973), Boldt

FIG. 3. Relationships between the observed parasitism rates and (a) predicted parasitism rates (host-acceptance rate \times mean searching speed), (b) mean searching speeds, and (c) host-acceptance rates. All rates are expressed as percentages. Circles represent measurements on thelytokous females; squares represent arrhenotokous females. Open symbols are morning measurements; closed symbols are afternoon measurements.



and Marston (1974), and Boldt (1974) has shown that larger parasitoids emerging from larger host eggs walked significantly faster than smaller parasitoids emerging from smaller hosts. Based on these results, it has been hypothesized that size-related differences in searching speed may be an important quality trait in the field (Kazmer and Luck, 1995). It has not, however, been established whether these differences in searching speed were caused by larger parasitoid size or by other differences in the rearing host. Pavlik (1993) found no relationship between size and searching speed when parasitoids were reared on MFM but this host produced wasps of a very limited size range. Bigler *et al.* (1987) found no difference in searching speed in *T. maidis* reared on MFM and *Sitotroga cerealella* Olivier but these two hosts are very similar in size. In our experiment in which a wide range of wasp sizes was obtained from the same host species, no relationship was found between parasitoid size and searching speed. This result is important for research purposes because size differences can be eliminated as a possible cause of observed difference in searching speed in *T. minutum*. Differences in searching speed previously attributed to the effects of host volume on wasp size may actually be due to other differences between host species, such as nutritional value. Although size does not appear to affect searching speed, it may still be related to other quality parameters. Kazmer and Luck (1995) showed that larger *T. pretiosum* females find more egg masses in the field. This may be caused by increased longevity, flight propensity, or predator avoidance by larger wasps and not necessarily increased searching speed.

The Relationship of Host Acceptance and Searching Speed to Parasitism

Doutt (1959) provided a generalized model of parasitoid behavior in which host habitat location, host location, host recognition and acceptance, and host suitability lead sequentially to parasitism. Each of the steps in Doutt's model is required for achieving successful parasitism but the efficiency with which each step is performed will dictate the overall degree of success. In Experiment 2, we measured the rates of host acceptance and searching speed (hypothesized to be related to host location) in an attempt to predict rates of parasitism in a standardized assay. According to Doutt's model, the proportion of parasitoids finding the host, multiplied by the proportion of parasitoids accepting the host once found, should be related to the proportion of hosts parasitized. We found that for *T. minutum* this was the case (Fig. 3a), although as shown by the results of the logistic regression, the relationship was entirely due to the host-acceptance factor. Searching speed likely contributed little to the prediction of parasitism rates, since it was also found to be unrelated to the host-finding rate.

Our experiments show that host acceptance is a more useful predictor of parasitism than a short-term measure of searching speed in *T. minutum* under controlled laboratory conditions. Although searching speed can be rapidly measured, it is a complex response that is affected by parasitoid age, strain, and the time of day. Variation in this trait does not seem to be related to rates of parasitism or host finding. These results suggest that searching speed as measured here is an unsuitable trait for estimating the quality of mass-reared *T. minutum*.

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