



Benefits of repeated mine trackings by a parasitoid when the host leafminer has a tortuous feeding pattern

YOSHIKO AYABE*, MIDORI TUDA† & ATSUSHI MOCHIZUKI*

*Division of Theoretical Biology, National Institute for Basic Biology

†Institute of Biological Control, Faculty of Agriculture, Kyushu University

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The complex pattern of mines made by leafminers is postulated to hinder the host-searching behaviour of parasitoids, and if this is true, parasitoids should have evolved strategies of searching mines in a way that will achieve the highest efficiency. We investigated the possible deterrent effect of mine pattern complexity on a parasitoid searching for a host within a patch, and the searching behaviour used by the parasitoid to shorten search time. For this, we used the leafminer *Liriomyza trifolii* (Diptera: Agromyzidae) and its generalist parasitoid *Hemiptarsenus varicornis* (Hymenoptera: Eulophidae). Observations of the parasitoids showed that searching time increased on mines with complex patterns and crosses. We also found that the parasitoids used a specific searching strategy: they made multiple trackings to find the host and each tracking was terminated with a constant mine-leaving rate per unit time irrespective of mine complexity. With an individual-based model, we also found that while search time to host encounter increased with mine complexity, the optimal mine-leaving rate that attained the most efficient search did not vary with mine complexity, indicating that making multiple trackings with an optimal constant rate was advantageous in terms of search time for any search on a mine over a range of levels of complexity. Furthermore, the mine-leaving rate estimated from the behavioural observations was consistent with the optimal rate obtained in the simulations. Multiple-tracking behaviour by *H. varicornis* is the best way for foraging for hosts whose feeding pattern varies from simple to more tortuous.

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A host and a parasitoid are in an antagonistic interaction. On the one hand, parasitoids need to search for their hosts efficiently because finding hosts to deposit their eggs is directly linked with fitness gain (van Alphen et al. 2003). On the other, various host defence mechanisms often spoil parasitoids' searching attempts (Waage 1983; Lederhouse 1990; Wang & Keller 2002, 2003; Outreman et al.

2005). When a host species has a characteristic that has an adverse effect on parasitoids' search by decreasing their time efficiency, parasitoids would be expected to respond by allocating their search time in a way that minimizes their efficiency losses. The length of time parasitoids should allocate to searching for hosts is one of the key factors determining their foraging success (Boivin et al. 2004; Mills & Wajnberg 2008). With this perspective, many studies have focused on how long parasitoids should remain searching in one patch to maximise their long-term rate of gain among several patches in a habitat (Mills & Wajnberg 2008). However, little attention has been paid to how long they require to handle each host efficiently within a patch. For parasitoids confronted with a variety of hosts that differ in handling characteristics, determining the shortest time necessary to search efficiently for

Correspondence: Y. Ayabe, Laboratory of Forest Protection, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan (email: ayabe@agr.nagoya-u.ac.jp). M. Tuda is at the Institute of Biological control, Agriculture, Kyushu University, Fukuoka 812-8581, Japan. A. Mochizuki is at the Theoretical Biology Laboratory, Advanced Science Institute, Riken, Hirosawa, Wako, Saitama 351-0198, Japan.

their hosts that are most difficult to handle must be one of the essential components for attaining optimal foraging behaviour.

Leafminer larvae consume leaf mesophyll while leaving the epidermis intact and form species-specific complex feeding patterns called mines on plant tissues. Mines may serve not only as a cue but also as a deterrent against searching parasitoids (Kato 1984, 1985; Ayabe & Ueno 2004). Several researchers have studied the possibility that a mine itself functions as a defence (e.g. Kato 1984, 1985; Brandl & Vidal 1987; Casas 1989; Djemai et al. 2000; Salvo & Valladares 2004). For example, Kato (1985) showed theoretically that the branching and crossing structures of mines could lead to an unsuccessful search by misleading tracking parasitoids towards an incorrect direction away from hosts and thus increasing their foraging costs.

Tracking a mine while recognizing the direction of the occupant host is a tactic for efficient search on a mine, and, indeed, Ayabe & Ueno (2004) have shown that the leafminer parasitoid *Hemiptarsenus varicornis* has this ability. It can correctly track a mine towards the host immediately after encountering a mine and even when selecting an incorrect direction it can later turn to the correct direction during tracking. This suggests that a vibratory cue from the eating activity of host larvae is involved in directional recognition, as is found in eulophid parasitoids of leafminers (Sugimoto 1977; Djemai et al. 2004). However, parasitoids cannot always recognize the host direction correctly, and as an explanation for this, Ayabe & Ueno (2004) suggested that the geometrical complexity of mines such as multiple branching, crossing and double-turn structures may lead a parasitoid to track a mine in an incorrect direction that is closer to the vector from the parasitoid to the source of host vibrations than the correct direction is. With such complex patterns of mines, parasitoids that use a new search tactic to increase the success of host encounter by increasing search efficiency will be selected for.

We first aimed to determine how the complexity of mining patterns of a leafminer affects parasitoid success for within-patch foraging using the American serpentine leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) and its parasitoid *H. varicornis* (Girault) (Hymenoptera: Eulophidae), which is one of the major native parasitoids of *L. trifolii* in Japan (Saito et al. 1997; Arakaki & Kinjo 1998). It is a solitary ectoparasitoid (Saito et al. 1997), attacking a number of dipteran leafminers (Lin & Wang 1992; Bordat et al. 1995). We also aimed to examine how the parasitoid maximizes the efficiency of searching complex mine patterns that appear to be difficult to track to find the host larvae. It is frequently observed that *H. varicornis* leaves a mine, retracks the same mine and repeats such leaving and retracking procedures until it encounters a host (Ayabe & Ueno 2004). Thus, *H. varicornis* needs to determine each tracking time to maximize its gain in a searching bout, that is, to encounter the host within the shortest time. We hypothesized that retracking by the parasitoids is an advantageous searching strategy ensuring an efficient search on a variety of mines that differ in handling from simple to complex. To test the

hypothesis, we used both observational and theoretical approaches. In behavioural observations, we investigated how each tracking time was affected by the behaviour of parasitoids during tracking and by the complexity of mines searched, together with descriptions of parasitoid search behaviour. Then, we used an individual-based simulation model to clarify the significance of repeated mine tracking by comparing search time until the host is found between a nonrepeated tracking strategy and a repeated tracking strategy on mines with different degrees of complexity. Thus, the optimal searching behaviour by *H. varicornis* was determined from both observation and simulation results.

METHODS

Observations of Parasitoid Behaviour

Host leafminers

Female *L. trifolii* deposit eggs in host leaf tissues; the larvae tunnel within the leaf and feed on the leaf mesophyll. Since mine width increases with larval growth (Fagoonee & Toory 1984; see also Fig. 1a), the width of a particular mine section indicates which larval instar (first–third) has made it (Ayabe & Ueno 2004). Developing larvae are mostly situated at the end of the mines. After spending their larval stage inside a leaf, mature larvae emerge from the mine to pupate in the soil.

The leafminer culture used in the present study originated from Fukuoka, Japan (Ohno et al. 1999) and was maintained on the kidney bean, *Phaseolus vulgaris* (L.), under a constant light at 25 ± 1 °C. To obtain leaves with one host, several leafminer adults were randomly collected from the culture and were transferred into a plastic cage with two kidney bean plants for 1–2 h. The plants were then removed and were kept at 20 ± 0.5 °C. Mined leaves containing one third-instar host were used for the behavioural observations.

Parasitoids

Hemiptarsenus varicornis typically attacks *Liriomyza* species that form tortuous mines (Shepard et al. 1998; Bjorksten et al. 2005). An *H. varicornis* female locates infested plants by flight and lands on a leaf containing mines. She then searches for host mines by walking over the leaf. When she finds a mine, she starts to track it by drumming on it with her antennae. When she finds the occupant host larva, she deposits a single egg on it or pierces it with her ovipositor to feed (Saito et al. 1997).

The *H. varicornis* population used in our study was derived from the culture reared in Shizuoka Agricultural Experiment Station, Japan (Saito et al. 1996) and was kept on *L. trifolii* at 25 ± 1 °C under a constant light, following basic rearing procedures described by Saito et al. (1997). Newly emerged females were placed in a plastic cup (8 cm in diameter and 4 cm high) together with males and a honey solution for 1 day, allowing all females to be inseminated. On the second day, the females were transferred into rearing boxes where two kidney bean plants infested by several hosts were placed for host-feeding experiences. This

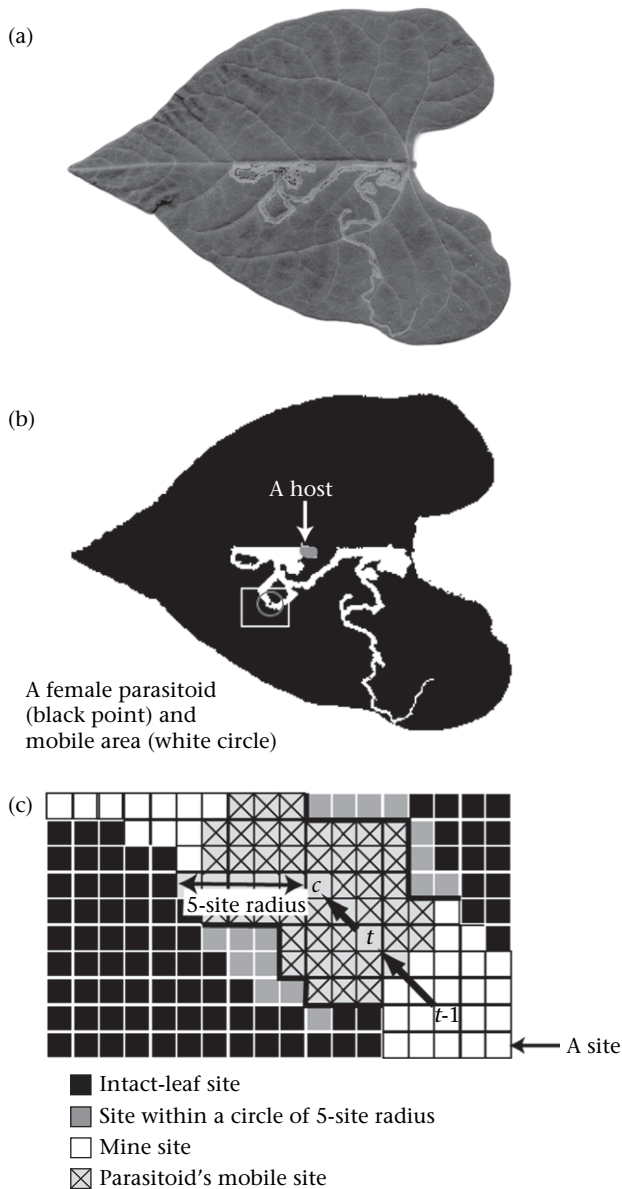


Figure 1. (a) Photograph of a leafmine of *L. trifolii* and (b and c) lattices for computer simulations. (b) A lattice based on an actual leaf photograph. In the computer simulations, a model parasitoid (the black point within the white circle) was allowed to search on the leaf area. (c) An enlarged figure of the white square in (b). The model parasitoid had oriented movement on the mine sites. The position at $t + 1$ was randomly selected from the mine sites within a circle of radius five sites, the centre (c) of which was selected on the vector extended from the site at $t - 1$ towards the site at t and $5/4$ -sites (see Appendix) forward from the position at t .

procedure is essential for *H. varicornis* because it is synovigenic; it carries only a few or no mature eggs at emergence, and requires nutrients from the host to mature the eggs (Ho & Ueno 2002). The females were then collected in a plastic cup (8 cm in diameter and 4 cm high) with honey solution and kept in an incubator (20 ± 0.5 °C) for 1 additional day, and, thus, 4-day-old females carrying matured eggs and motivated to oviposit (Ho & Ueno 2002) were used for behavioural observations.

Observations

A petri dish (15 cm in diameter and 3 cm high) covered with a glass plate was used as an experimental arena. A test female parasitoid was released inside the dish containing a leaf with a single host mine. Observation was started when she landed on the leaf and was continued until she found a host larva with a time limit of 1 h. A total of 24 *H. varicornis* females were used for observations, and their behaviour was recorded with a CCD camera attached to a VCR. The parasitoid behavioural components recorded were: (1) number of mine trackings until host detection; (2) total searching time from the landing on a leaf until host detection (hereafter, total searching time; Fig. 2a); and (3) Time spent on each mine tracking (hereafter, tracking time). The female occasionally rested for a moment during mine tracking, and this was excluded from the tracking time. We also recorded the (3a) direction towards/away from the host selected upon encountering a mine; (3b) the change in direction during mine tracking; and (3c) the point of encounter on a mine. On the basis of mine width, we could recognize three types of encounter on mines: encounters with the first, second and third instars. None of the mine trackings was started at mine crosses. Accordingly, we could estimate the distance along the mine from contact points to the occupant hosts (see Ayabe & Ueno 2004 for details). We noted (3d) whether a female encountered a cross in a mine during mine tracking and (3e) its behaviour on a mine cross. Two types of behaviour were observed: first, passing a cross and continuing the tracking and, second, terminating tracking and leaving the mine by walking away, the latter behaviour leading to retracing the same mine. Finally, we noted whether a mine had at least one cross. A cross was defined as a branching or crossing structure of a mine. Several mines were highly tortuous with multiple crosses, and the number of crosses was not accurately estimated (see below for our approach using fractal dimensions).

Data analyses

Numbers of mine-tracking events were tested using Poisson regressions. The total searching and tracking times were subjected to survival analysis. We first tested whether total searching time differed between mines with and without crosses, using a log-rank test. Total searching time had no censored data since no female failed to find a host.

We estimated mean tracking time and tested whether tracking time was affected by the following parasitoid behavioural events (3a-d): tracking in the wrong direction (opposite to the direction of hosts), changing direction, beginning a mine tracking from three different contact locations and/or encountering a cross. The tracking time data were pseudoreplicated because of multiple mine trackings. Therefore, we used a Cox regression-type generalized linear mixed model (GLMM) with Poisson family and log link function (i.e. mine tracking times were log transformed and assumed to follow a Poisson distribution) with a random intercept and included wasp individuals as a random factor and each of the above parasitoid behavioural components as a fixed factor. The model was fitted

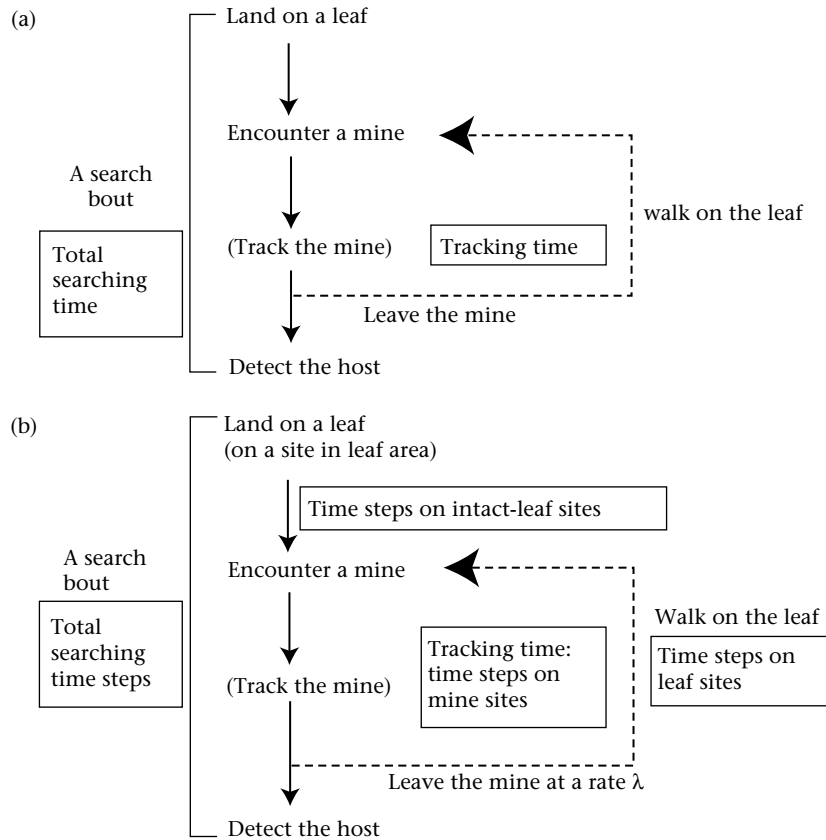


Figure 2. The behavioural sequence of (a) female parasitoid *Hemiptarsenus varicornis* and (b) the model parasitoid in the computer simulations. (a): Parasitoids frequently showed the specific behaviour indicated by the dashed arrow; they left the mine and returned to the same one, resulting in multiple mine trackings. (b): Model parasitoids with the mine-leaving rate $\lambda = 0$ continued a mine tracking until they encountered a host, while those with $\lambda > 0$ sometimes repeated mine trackings (dashed arrow).

using the maximum likelihood method. With the same model framework, we tested whether behaviour on a mine cross (3e) affected tracking times, using only the data in which parasitoids had actually encountered a cross. We also tested whether tracking time was affected by the presence of cross structures of mines. In these GLMMs, the mine trackings that resulted in successful host encounter were considered as censored data. We excluded from the analyses mine trackings in which females directly encountered a host without tracking the mine (however, direct encounters were counted as one visit event in the observation of the number of mine trackings). For statistical computations we used Stata 9.0 (StataCorp 2005).

Computer Simulation Model

We carried out computer simulations to understand why female *H. varicornis* used multiple mine trackings to find a host, in terms of searching efficiency. Our simple assumption was that parasitoids leave a mine at every n (= 25 in the Results) time steps with a mine-leaving rate λ which took the values 0–0.3. In the simulation program, we generated a uniform random number between 0 and 1 at every n time step and assumed that a parasitoid left a mine when the value of the generated random number was below λ , following Rubinstein (1981). With this

Monte Carlo method, the probability of parasitoids terminating tracking by time t approximates a simple cumulative distribution function for event data, $1 - \exp(-\lambda T)$ (Haccou & Meelis 1994), where T is the integer division, $[t/n]$ (see Appendix). When $\lambda = 0$, parasitoids continue a single tracking until they find a host, that is, they use a nonrepeated tracking strategy. As λ increases, parasitoids tend to terminate each mine tracking in a shorter time. The model parasitoids were allowed to search for hosts on scanned images of 22 actual leaves, each containing a mine of different tortuosity (Fig. 1b). Using a Cox regression model, we compared total searching time until host detection among different λ s on three different degrees of mine tortuosity: low, moderate and high, classified according to fractal dimensions. Fractal dimension is a description of complexity (tortuosity) of a geometric object (Mandelbrot 1983) and allows us to evaluate mine tortuosity as a quantitative character objectively. Fractal value can take not only integer but also noninteger values. Leafmines, which are two-dimensional objects, can take a fractal value in the range from 1 to 2. A high fractal value of a leafmining pattern indicates that the mine is highly tortuous. We used the box-counting method to estimate fractal dimensions of the scanned mine images (for details, see Mandelbrot 1983; Falconer & Falconer 2003). Mean \pm SD fractal values were 1.24 ± 0.03 , 1.33 ± 0.03 and 1.41 ± 0.02 for low ($N = 8$),

medium ($N = 7$) and high ($N = 7$) tortuousness groups, respectively. A positive correlation between the occurrence of crosses in mines and high fractal values is expected because the scanned mines in the high-tortuousness group had crosses and tangles, whereas those in the low-tortuousness group were plainer in pattern and had no more than one cross.

RESULTS

Searching Behaviour of Parasitoids

Number of mine trackings and total searching time

Female *H. varicornis* tracked on average \pm SE 3.50 ± 0.86 times per mine and all finally encountered a host ($N = 24$). The number of mine trackings until they found a host was significantly larger when they encountered crosses during the searching bouts ($\bar{X} \pm$ SD = 4.9 ± 4.3 , $N = 10$) than when they encountered no crosses (2.1 ± 1.2 , $N = 14$; Poisson regression: coefficient = 0.86, $Z = 3.67$, $P < 0.001$). The presence of cross structures affected total searching time as well. Females spent significantly more time on mines with crosses (log-rank test for equality of survivor functions: $N = 24$, $\chi^2_1 = 4.22$, $P = 0.04$; Fig. 3). Thus, mine tortuousness had an adverse effect on parasitoid searching time.

Tracking time and tracking behaviours

Mean tracking time \pm SE was 16.4 ± 2.0 s ($N = 72$). In most the cases, *H. varicornis* started to track a mine from a section made by second- or third-instar hosts (38.9% and 50.0% of the trackings observed, respectively). Only 11.1% of mine tracking started from a mine section made by the first instars.

None of the behavioural events carried out by *H. varicornis* during tracking affected the tendency to stop tracking (GLMM: N of level 1 and 2 units = 756 and 24, respectively, variance and covariance of random effect = 1.20×10^{-21} and 2.75×10^{-12} ; Table 1). When *H. varicornis* encountered a cross, it did not vary its tracking time according to the behaviour on the cross (GLMM: N of level 1 and 2 units = 307 and 10, respectively, variance and covariance of random effect = 4.18×10^{-25} and 7.86×10^{-14} , hazard ratio

Table 1. Results of a Cox regression on tracking time of *Hemiptarse-nus varicornis*

Effects	Exp(b)	Z	P
Direction towards/away from host	0.98	-0.23	0.82
Cross encounter	0.94	-0.72	0.47
Change in direction	1.08	0.98	0.33
Locations of mine encounter	1.06	0.89	0.37

Exp(b) refers to conditional hazard ratios.

\pm SE = 1.07 ± 0.15 , $Z = 0.5$, $P = 0.62$). Unlike total searching time, tracking time was not affected by the presence of cross structures (GLMM: N of level 1 and 2 units = 756 and 24, respectively, variance and covariance of random effect = 6.07×10^{-19} and 6.28×10^{-11} , hazard ratio \pm SE = 0.97 ± 0.12 , $Z = -0.25$, $P = 0.80$). Therefore, the tracking time of *H. varicornis* females was correlated neither with their behaviour during mine tracking nor with mine complexity.

Computer Simulation Model

The behavioural observations demonstrated that mine-tracking time was independent of behavioural events and the presence and experiences of mine crosses. This supports our simple assumption in computer simulations that tracking time was governed only by a mine-leaving rate λ .

There was a significant difference between the relative hazards with different λ values (Cox regression-based test for equality of survival curves: $N = 14\ 300$, likelihood ratio, LR $\chi^2_{12} = 208.62$, $P < 0.0001$). The relative hazard was highest at $\lambda = 0.05$, indicating that simulated parasitoids that repeated mine trackings with the termination rate $\lambda = 0.05$ were the most efficient at finding a host. The relative hazard at $\lambda = 0$ was below 1, indicating that parasitoids with the nonrepeated tracking strategy took longer to find hosts than the average of all λ values. Thus, the repeated mine-tracking strategy is advantageous for parasitoids in terms of searching efficiency. The smallest relative hazard occurred at $\lambda = 0.275$.

With respect to mine tortuousness, relative hazards were also significantly different between λ s for all of the three classes of tortuousness (Cox regression-based test for equality of survival curves: 5191 runs, LR $\chi^2_{12} = 112.71$, $P < 0.0001$ for the simple mines; 4549 runs, LR $\chi^2_{12} = 63.26$, $P < 0.0001$ for the moderately tortuous mines; 4512 runs, LR $\chi^2_{12} = 82.05$, $P < 0.0001$ for the highly tortuous mines). The relative hazard was highest at $\lambda = 0.05$ for simple and highly tortuous mines and 0.025–0.075 for moderately tortuous mines, and these optimal λ values did not vary with the tortuousness of mines searched (Fig. 4). In spite of the constancy of the optimal λ value with mine tortuousness, the mean total searching time of parasitoids increased significantly with mine tortuousness (14 300 total runs, hazard ratio = 0.892 ± 0.009 , $Z = -11.21$, $P < 0.0001$; Fig. 4). These were qualitatively consistent with the results of the behavioural observations in which mines with crosses did not affect the tracking time of each mine but increased the total searching time

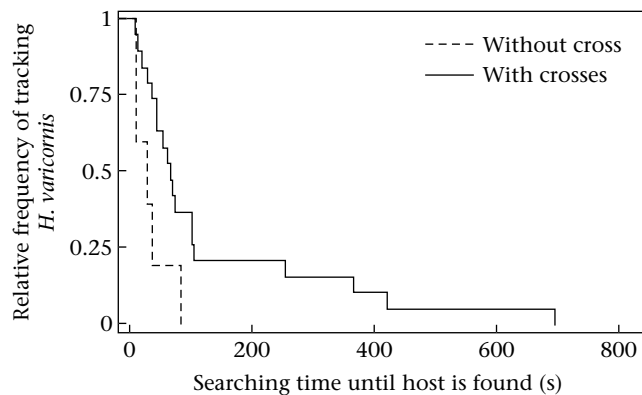


Figure 3. Relative frequency of *H. varicornis* tracking mines with and without crosses.

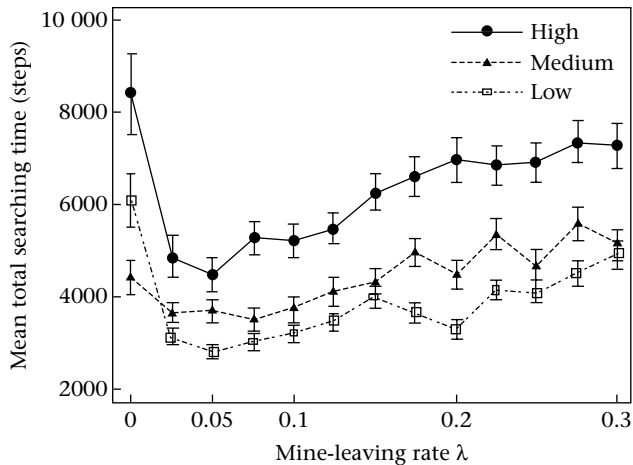


Figure 4. Mean \pm SE total searching time (steps) of different λ s on mines with different degrees of tortuosity.

of parasitoids. The difference in total searching time between $\lambda = 0$ and 0.05 was equally large on simple and highly tortuous mines, indicating that the retracking strategy at the optimal λ was highly advantageous for parasitoids whether they searched either highly tortuous or simple mines.

Parasitoid tracking strategies affected the frequency of unsuccessful searches. Forty-eight unsuccessful searches all occurred at $\lambda = 0$ and with significantly different frequencies in the three classes of mine tortuosity; 38 unsuccessful searches occurred on highly tortuous mines, and nine and one unsuccessful searches on simple and moderately tortuous mines, respectively (14 300 total runs, $\chi^2_2 = 51.43$, $P < 0.0001$).

The number of mine trackings until host finding at the optimal $\lambda = 0.05$ was 3.17 ± 0.13 ($\bar{X} \pm \text{SE}$) for the whole of the sample tested and increased significantly with mine tortuosity (Poisson regression analysis: $N = 1100$ runs, coefficient = 0.12, LR $\chi^2_2 = 30.4$, $P < 0.0001$).

As has been shown above, both the observations and our simple simulation model show qualitatively and quantitatively similar results: tracking time was invariable with mine tortuosity, the tendencies of total search time and number of trackings until host finding increased with mine tortuosity, and number of mine trackings until host finding in the observations (3.5) and in the simulations (3.2, at $\lambda = 0.05$) were in agreement. We confirmed that the optimal λ value (0.05) expected from the simulation results was consistent with that of the observations. We estimated track termination rate per unit time from the observational data and found it to be 0.058 (95% confidence interval: 0.045–0.076; $N = 72$).

DISCUSSION

The present study demonstrated the adverse effect of mining patterns of *L. trifolii* on parasitoid searching efficiency and the importance of the retracking strategy adopted by *H. varicornis* in terms of searching time. Behavioural observations of *H. varicornis* showed that, first, mines with crosses increased the number of mine trackings and

consequently increased total searching time of the parasitoid, and, second, each mine-tracking time was independent of both behavioural events during tracking and the complexity of mines. Furthermore, the computer simulations indicated that the retracking of a mine with a constant mine-leaving rate seems advantageous in terms of total searching time.

Mines of *L. trifolii* with crosses prolonged the total searching time of *H. varicornis*. This is the first demonstration of an adverse effect of a tortuous mining pattern on within-patch searching by a single parasitoid species. However, a tortuous mining pattern functions only partially as a defence against parasitoid attack because it does not lead to an unsuccessful search. Our work therefore only partly supports the hypothesis that complexity of mining patterns may function to defend leafminers from parasitoid attack (cf. Kato 1985; Hespeneide 1991). Alternative hypotheses for the adaptive significance of mining patterns include selective feeding on the leaf tissues with a high nutrient content and avoidance of tissues with a high structural and/or chemical defensive content (Hespeneide 1991; Connor & Taverner 1997; Scheirs et al. 1997, 2001; Y. Ayabe, unpublished data). We suspect that the tortuous feeding pattern of *L. trifolii* may in fact be the direct result of selective feeding on more nutritious or less defensive tissue, with the beneficial side-effect of protecting against searching parasitoids. Such bottom-up selection is known to alter host–parasitoid dynamics dramatically (Tuda 1998; Tuda & Iwasa 1998; Tuda & Shimada 2005). Despite the incomplete defence provided by the tortuous mining pattern, the increase in parasitoid search cost through mine complexity might be an important component influencing antagonistic interactions between leafminers and their parasitoids, or parasitoid search behaviour.

The present study shows that *H. varicornis* repeats mine trackings with a fixed termination rate until it finds a host. Previous studies showed experimentally that a variety of parasitoid species can flexibly change patch residence time in response to host density, experience and information acquired while foraging (e.g. Hemerik et al. 1993; van Alphen 1993; Wajnberg et al. 1999, 2003, 2004; Outreman et al. 2005; Tentelier et al. 2005; Burger et al. 2006; Darrouzet-Nardi et al. 2006; Schreiber & Vejdani 2006). Leafminer parasitoids can also flexibly change their searching time in response to host density. The hymenopteran leafminer parasitoid *Opius dimidiatus* increases searching time with increasing host density in the patch and modifies giving-up time in response to experience of successful oviposition and encountering parasitized hosts (Nelson & Roitberg 1995). Other leafminer parasitoids give up a patch search (on a leaf) when a marking pheromone accumulates on the leaf over a triggering threshold that increases with host density in the patch (Sugimoto et al. 1987; Sugimoto & Tsujimoto 1988). In contrast with previous studies which emphasized foraging flexibility by parasitoids in response to foraging situations, our study shows that *H. varicornis* did not vary tracking time even though it experienced some behavioural events while tracking as well as a range of mine complexity that caused differences in handling.

Our computer simulation models, in which repeats of mine tracking that ended with an advantageous λ shortened the total search time compared to nonrepeated tracking, may explain this inflexible tracking time. The optimal λ s are all nonzero positive values for different levels of mine tortuosity. The optimal λ on all three classes of mine tortuosity lay between 0.025 and 0.075, and therefore the optimal λ is independent of mine complexity. Thus, we can hypothesize that a parasitoid using a retracking strategy with a constant track termination rate may be favoured irrespective of selection acting on the leaf-mining hosts to develop more tortuous mines to elongate its parasitoid searching time.

Following the optimal foraging concept that foragers should search at the best achievable balance of costs and benefits (Krebs & Kacelnik 1978), *H. varicornis* should ideally search for hosts with the most advantageous track termination rate to save foraging costs. The optimal track termination rate in the simulations was $\lambda = 0.05$, and this value was consistent with the termination rate we observed. In addition, the observation and the simulation had qualitatively and quantitatively similar results. Our primary aim in the simulation models was not to verify the consistency of the actual track termination rate in the behavioural observations and the optimal λ predicted by the simulations, but to explain the significance of repeated mine trackings in host search. The consistencies in the results between the observations and the simulations further confirm the validity of our simulation model, namely the advantage of the repeated mine trackings by *H. varicornis*.

Reduction of searching time by retracking seems to be equally significant in simple and in highly tortuous mines compared with moderately tortuous mines. For simple mines, retracking would be advantageous because parasitoids can avoid a lengthy tracking towards the wrong end of a mine by leaving the mine once, as well as by turning during the tracking (Ayabe & Ueno 2004). For highly tortuous mines, the retracking strategy could prevent parasitoids from wasting time searching crosses; this hypothesis was supported by the results showing that tracking time was invariable whether or not the parasitoids passed crosses. The retracking strategy contributed equally to searching simple and highly tortuous mines in terms of total searching time. However, it had an additional contribution to the success of encounters on highly tortuous mines (which caused more unsuccessful searches than simple mines), compared to the one-tracking strategy. Thus, retracking contributes most to searches on highly tortuous mines. Other studies concerning searching time of parasitoids against defensive hosts have shown that host defence influences the tendency of the parasitoids to leave a patch, that is, it may increase (Outreman et al. 2005) or decrease (Wang & Keller 2002, 2003) the tendency to leave. In *H. varicornis*, the retracking strategy might have been developed as a result of giving up a single-tracking search on a highly tortuous mine.

Coevolution of a pair of species that are specialized with each other must be very rare (Thompson 1994), and both *L. trifolii* and *H. varicornis* interact with several other counterpart species. Although the present study concerns the adverse effect of *L. trifolii* mines on only one species, *H.*

varicornis, among several associated parasitoids, crossing and branching structures of mines may prolong the search time of other parasitoid species as well, and the retracking strategy may be common among leafminer parasitoids foraging on linear mines. In fact, we observed several leafminer parasitoids retracking linear mines. As our results have shown, repeated mine tracking is advantageous in terms of search time for any mines in the natural variation of mine complexity in *L. trifolii*; such advantages can be extended to the mining-pattern variation found in different host species within the host range of *H. varicornis*.

There have been several studies on the defences of several leafminers making different mine types and the foraging behaviours of leafminer parasitoids (e.g. Kato 1984, 1985; Brandl & Vidal 1987; Meyhofer et al. 1994, 1997; Bacher et al. 1997; Djemai et al. 2000, 2004). The present study demonstrates an antagonistic interaction in the context of searching time through the investigation of strategies of both leafminers and their parasitoids. The leafminer parasitoid *Closterocerus tricinctus* of a blotch leafminer searches a blotch mine repeatedly (Connor & Cargain 1994). In contrast to the present study, the repeated search (revisiting a mine) in *C. tricinctus* is reported to be time consuming. The advantage of retracking may depend on the type of mines searched, and leafminer parasitoids may develop searching strategies adapted to the type of mines they habitually search. Study of searching strategies of parasitoids confronting types of mine other than linear ones would provide a greater understanding of leafminer–parasitoid behavioural interactions.

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Appendix: Computer Simulation Model

Descriptions of parasitoid behaviour

We defined a two-dimensional lattice map of 320×240 sites as a field for parasitoid search. The size of each site was about $0.3 \times 0.3 \text{ mm}^2$. Lattice maps were prepared by processing the scanned images of 22 actual leaves containing an empty mine from which the occupant host had emerged for pupation and which was used to control the mine stage (Fig. 1b). The leaves were scanned with an EPSON PM-2800 scanner, and the scanned images were transformed into digital forms using NIH image 1.63 (<http://rsbweb.nih.gov/ni-image/>).

Each site of the lattice was assigned one of the following states: the mine, host, intact-leaf and off-leaf (Fig. 1b, c). A clump of approximately 70 host sites was located in the region of an emerging hole at the end of a mine and was equivalent to about 5% of the total number of mine sites. A simulation run began with randomly selecting a leaf or mine site as a parasitoid positional site (i.e. landing on a leaf); it continued with a series of positional site changes on the leaf (intact-leaf, mine and host sites) at each time step (i.e. moving on the leaf), and terminated after the positional site was selected on a host site (i.e. host finding). A parasitoid's positional change was made randomly or directionally, depending on the site it was visiting. A random movement was made on a leaf site, and a directional movement was made on a mine site to mimic mine-tracking behaviour. In random movement, a new positional site at step $t + 1$ was selected randomly within the circle with a radius of five sites from the position at step t (see Fig. 1c). In directional movement on a mine, the centre of the circle with a radius of five sites was set on the extension of the vector of the last movement.

Then, a new site was selected randomly within this circle (Fig. 1c). The size of the circle was almost the same as that of *H. varicornis*. We attempted using a circle of size 10 sites, which proved so large that parasitoids jumped to a point distant along the mine in areas where different parts of a mine were close together. The length of the movement vector was varied from 5/5 to 5/2 sites. The numerator 5 is the same size as the radius of the circle determining the mobile area of a parasitoid. As the denominator increases (i.e. vector length decreases), the probability that a parasitoid will change its tracking direction also increases. As the vector length decreases, the centre of the circle for determining the positional site at $t + 1$ becomes closer to the positional site at t . This increases the number of within-circle sites that make the parasitoid move from t to $t + 1$ in the opposite direction to the previous movement from $t - 1$ to t . Since changes in vector length affected the frequency of turning (i.e. change in directions) but not the overall results substantially, we used a movement of 5/4-sites vector length. With this value, simulated parasitoids are expected to turn in the closest frequency to that of *H. varicornis* which changes its direction with the probability 42.3% (newly estimated from Ayabe & Ueno 2004). Whether a simulated parasitoid terminates tracking is determined at every n time steps. In more detail, a mine tracking was terminated when the value of the random number generated at every n time steps was below λ . After that, a new positional site was randomly selected from leaf sites near the last position, and then random movement on leaf sites followed until a mine site was selected again (i.e. retracking). This procedure was repeated until a host site was selected (Fig. 2b). For $10 \leq n \leq 100$, mine-tracking times did not differ dramatically depending on whether λ was 0. Therefore, we used a moderate value $n = 25$ to mimic the retracking behaviour of *H. varicornis*. Similar results were obtained for $10 \leq n \leq 100$.

Calculations of total searching time

We compared total searching time until host detection obtained with different λ s. Total searching time was determined by the total number of time steps spent on both mine and intact-leaf sites (Fig. 2b). We defined 50 000 steps as the maximum for a single mine-tracking bout to prevent parasitoids with $\lambda = 0$ searching the host for further time steps.

Fifty runs were performed for each latticed mine with each of 13 different λ values. Of 14 300 runs, 48 were compulsorily terminated because the parasitoids failed to find host leafminers within 50 000 time steps. Terminated runs were termed 'unsuccessful'. These unsuccessful runs all occurred with $\lambda = 0$, at which parasitoids explored a mine only once before finding a host. Total searching times were compared among the searching strategies with different λ s using a log-rank test and Cox regression models. In the analyses, unsuccessful searches were treated as censored data.