Journal of Animal Ecology 2004
73, 1179–1189

Genetic variation in the mechanisms of direct mutual interference in a parasitic wasp: consequences in terms of patch-time allocation

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Summary

1. Direct mutual interference between insect parasitoids has always been considered to have major consequences for the population dynamics of parasitoid–host systems. Furthermore, patch time allocation has also always been presented as an important behavioural component that is closely involved in the demographic features of host–parasitoid interactions.
2. Based on a detailed analysis of within-patch interactions between *Trissolcus basalis* (Hymenoptera: Scelionidae) females, the present study aimed to understand accurately how direct mutual interference can influence patch time allocation in this species.
3. The genetic variation in the behavioural mechanisms involved in the interaction between the females was also quantified in the study population using the isofemale strains method.
4. Behavioural interactions between the females were analysed using Cox's proportional hazards model.
5. Results indicated that the competitive interaction (through contacts and fights) between females strongly influenced their patch residence time.
6. Corresponding patch-leaving decision rules appeared to be under significant genetic variation. Such genetic variability is likely to correspond to intra-population genetic variation in susceptibility of females to interference.
7. The population dynamics and evolutionary consequences of these results are discussed.

Key-words: Direct mutual interference, path time allocation, genetic variability, *Trissolcus basalis*.

Journal of Animal Ecology (2004) **73**, 1179–1189

Introduction

The question of how animals distribute their foraging time among the different patches where resources are located has probably been the most studied problem in behavioural ecology over the last decades (van Alphen, Bernstein & Driessen 2003). The central issue is usually an understanding of how animals optimally allocate their foraging time in each patch that they exploit in order to maximize their rate of progeny production (Charnov 1976; Stephens & Krebs 1986).

When the distribution of resources in the environment is known by a foraging animal, the Marginal Value Theorem states that patches should be left the instant that resource consumption drops below the average rate that can be achieved in the environment (Charnov 1976). However, most animal species cannot be considered smart enough to achieve this, and a large number of both theoretical and experimental works have tried to discover what proximate behavioural patch-leaving decision rules animals are using to optimally adjust their foraging time (Green 1984; van Alphen & Vet 1986; Wajnberg, Fauvergue & Pons 2000). An important part of the published results were obtained from insect parasitoids foraging for hosts distributed in patches, and Waage (1978, 1979), working on the larval parasitoid *Venturia canescens* (Gravenhorst), proposed a behavioural mechanism that females seem to use to manage their patch residence time. Upon entering a patch, the female will have

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a certain motivation level to search for hosts, or a tendency to remain in the patch, which is thought to be related to the concentration of kairomones left by the hosts. This level then decreases over time until a threshold is reached, causing the wasp to leave the patch. Each time a host is attacked, the tendency to remain increases, thereby increasing the total patch residence time. This behavioural rule is therefore called an 'incremental' patch-leaving decision rule (van Alphen *et al*. 2003). More recently, it has been shown for some species that attacking a host could also have a decremental influence on the female's tendency to remain on the patch. Such a decremental effect is also called a 'count-down' mechanism (Driessen *et al*. 1995; Driessen & Bernstein 1999; van Alphen *et al*. 2003; Wajnberg *et al*. 2003).

As pointed out by Yamamura & Tsuji (1987), all these studies were developed by considering a single animal foraging alone on a patch. However, it is quite common to find several parasitoid females exploiting simultaneously the same patch under natural conditions (Godfray 1994). For a foraging animal using an incremental or a decremental patch-leaving mechanism, the use of kairomone concentration to determine the initial level of host searching motivation would probably be an unreliable prediction of patch quality if competitors are also exploiting the same patch (van Alphen *et al*. 2003). The optimal patch residence time of an animal foraging simultaneously with competitors will depend on the patch time that others are willing to invest (van Alphen 1988). Using game theory (Maynard Smith 1982), Sjerps & Haccou (1994), Haccou, Sjerps & van der Meijden (1999) and Haccou, Glaizot & Cannings (2003) developed an elegant theoretical approach demonstrating that, in most cases, animals should enter a 'war of attrition' when foraging simultaneously on a patch. More accurately, these authors demonstrated that, when animals interfere with each other, the predictions of the Marginal Value Theorem will no longer hold (Sjerps & Haccou 1994; Haccou *et al*. 2003).

Interference, that can be broadly defined as a decrease in host searching efficiency with an increase in parasitoid density (Hassell & Huffaker 1969; Hassell 1978; van Alphen & Vet 1986), thus appears to be an important component of the distribution of animals in patchy environments (Sutherland 1983, 1996; Bernstein 2000) and, since the pioneering work of Hassell & Varley (1969), several forms of interference were successively defined and studied. 'Direct mutual interference' refers to direct, within-patch interactions between foraging parasitoids, leading to a reduction in searching efficiency with an increase in parasitoid density (Rogers & Hassell 1974). Several theoretical works conducted to analyse this form of interference explicitly took into account the time 'wasted' (through contacts or even fights) by a foraging animal at each encounter with a conspecific (Beddington 1975; Holmgren 1995; Stillman, Goss-Custard & Caldow 1997). 'Pseudo-interference' refers to a decrease in overall search rate with increasing parasitoid density as a consequence of a nonuniform distribution of parasitoids across host patches (Free, Beddington & Lawton 1977). Finally, 'indirect mutual interference' refers to a decrease in searching efficiency that cannot be detected at the patch level, but only at the population level; for example, when foraging animals spend more time travelling between patches at higher parasitoid densities (Visser & Driessen 1991; Driessen & Visser 1997; Visser, Jones & Driessen 1999).

The influence of direct mutual interference on patch time allocation by foraging insect parasitoids, the associated proximate behavioural mechanisms involved, and their source of variation were never accurately described (van Alphen & Vet 1986; Godfray 1994). Therefore, through an accurate description of the within-patch fighting behaviours between two females of the parasitic wasp *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), the present paper aims to describe accurately how direct mutual interference might influence patch time allocation. The genetic variation of the behaviours involved was also quantified in the study population using the isofemale strains method (Parsons 1980; Hoffmann & Parsons 1988; Wajnberg 2004). The scelionid egg parasitoid *T. basalis* was chosen because it is known to show strong fighting behaviours when foraging simultaneously on a host patch with competitors (Field & Calbert 1998; Field, Calbert & Keller 1998; Field & Calbert 1999).

Results provided an accurate understanding of the behavioural mechanisms by which direct mutual interference might influence patch time allocation in the study species. By means of Cox's (1972) proportional hazards model, the effect of direct competitive interactions between females on a host patch were analysed in terms of patch-leaving decision rules. Finally, significant genetic variation was observed in these patch-leaving rules in the population analysed. This genetic variation is likely to correspond to a variation in the susceptibility of females to interference. The possible consequences of these results are discussed, both from a population dynamics and an evolutionary point of view.

Materials and methods

INSECTS

Trissolcus basalis is a minute solitary egg parasitoid exploiting egg masses of several Pentatomidae species, but its primary host is the southern green stink bug *Nezara viridula* (Linnaeus) (Heteroptera: Pentatomidae) (Jones 1988; Colazza, Salerno & Wajnberg 1999). The strain used in our experiments originated from nine females collected in weeds and field crops near Palermo, Italy, in Spring 2000. It was reared on freshly laid *N. viridula* egg masses under standard laboratory condition (25 ± 1 °C, 70 ± 10% RH and 16L : 8D).

Nezara viridula is a serious pest attacking a large variety of plants and showing a large geographical distribution world wide. Eggs are glued together on a plant leaf by the laying females, producing egg masses that can vary greatly in size, ranging from about 40 to more than 130 eggs. They constitute discrete patches for the parasitoids, and several *T. basalis* females have been regularly observed simultaneously exploiting the same egg mass (Colazza 1993). The *N. viridula* strain used here was started from adults also collected near Palermo about 2 years before the experiment. From the time of capture onwards, it was reared on beans and cabbage under the same standard laboratory conditions as those used for *T. basalis*. At regular intervals, the strain was refreshed by wild material collected in the same location.

EXPERIMENTAL SET-UP

During the F1 generation following collection of the *T. basalis* strain from the field, 13 mated females, taken at random from the mass-reared population, were used to establish 13 isofemale lines (i.e. families). Experiments were performed during the next generation. On average, 9·31 (range: 6–11) replicates were made per family and were distributed randomly over all the days of the experiment. Therefore, a total of 121 replicates were analysed. The statistical test of the variation observed among families provided a way to analyse the intra-population genetic variation in the different behavioural traits quantified for the females measured (Parsons 1980; Hoffmann & Parsons 1988; Wajnberg, Rosi & Colazza 1999; Wajnberg 2004).

In order to standardize as much as possible the conditions of the experiment, females were individually isolated at emergence and kept with one male only for 48 h for mating. They were then kept individually under standard laboratory conditions with a drop of honey solution and were used when they were 3–4 days old.

Each replicate consisted of observing two *T. basalis* females simultaneously exploiting the same host patch. The behaviour of one, so-called 'focal', female was accurately recorded and analysed, the other female was there just to generate the competitive interaction. The focal female originated from a clearly identified family, the other was chosen randomly from one of the remaining families, so the two females cannot be sisters. In order to distinguish the two females, the non-focal female was marked with a small dot of water-based white paint on the thorax between the wing bases, the focal female remained unmarked. The mark did not influenced the issue of the competitive interaction (two-sided Fisher's exact test, $P = 0{\cdot}700$).

Field & Calbert (1998, 1999) demonstrated that when there is a substantial difference in arrival time, the first *T. basalis* female arriving on a patch takes the initiative in the first fight in the majority of the cases, and usually wins the interaction. In the present study, because we wanted to analyse the genetic variation of the behaviours involved, we decided to use a symmetrical situation both in resource value and ownership status between the two females. However, it was technically almost impossible to release the two females on the host patch simultaneously. Therefore, the non-focal female was always released first and the focal female was then released as soon as the first female appeared interested in attacking a first host. The average (± SE) difference in arrival time was 323·9 ± 15·6 s. Such a time difference is far smaller that those tested by other authors, and did not influenced the outcome of the fights because, as stated above, the two females had an equal chance to win the competitive interaction.

Females were introduced into a closed vertical, cylindrical arena (diameter: 1·8 cm, height: 5·0 cm) with a patch of five freshly laid (24–48 h-old) *N. viridula* eggs placed in the middle of the bottom side. Such an arena was used to avoid the females being confined and constrained to the patch. The patch contained two rows of two and three eggs. Females were not allowed to be in contact with any hosts before the experiment (i.e., they were inexperienced females) and were used only once. Attacked hosts were not replaced, so the patch suffered a continuous depletion. Experiments were carried out during the daytime at 25 ± 1 °C and 60 ± 5% RH.

As soon as the female wasps were released into the arena, they exhibited a succession of path-entering and patch-leaving behaviours. While on the patch and when a suitable host was encountered, they started to drum with their antennae on the host surface and then adopted a characteristic oviposition posture, starting to drill the egg chorion with their ovipositor (Bin *et al*. 1993). When the host was successfully attacked, females exhibited a typical marking behaviour, sweeping the host surface several times with their ovipositor. Such a behaviour is known to correspond to the deposition of a chemical mark indicating to all future females that the host has already been attacked (Wilson 1961; Colazza *et al*. 1996; Field 1998; Field *et al*. 1998). When an unsuitable host was encountered, the oviposition posture was adopted but no marking behaviour was observed thereafter. Such an event was considered to be a so-called host rejection behaviour (Colazza *et al*. 1996).

In order to reduce the duration of the experiment, six replicates, each on a separate arena, were simultaneously videotaped using a camera mounted overheard. The video tapes were then analysed by two separate observers, each following one female, with two event recorders running on two different computers. Sometimes, especially when escalated fights occurred, tapes were re-played to observe details of rapid behaviours in slow motion. For the focal female, the beginning and the end of the following behaviours were recorded with an accuracy of 0·1 s: (i) entering or (ii) leaving the patch, (iii) antennal drumming on a host, (iv) adopting an oviposition posture, and (v) showing a marking behaviour. For the non-focal female, only the timings of patch entering and patch leaving, and the marking behaviours indicating successful ovipositions were recorded. The location of the hosts on which behaviours iii–v were observed for the focal female and on which the non-focal female exhibited a marking behaviour was also recorded. Patch entering was considered the instant a female had at least four legs on the egg patch, and patch leaving when a female had all her legs on the substrate. These different behaviours were observed for the two females during initial patch exploitation. Then, as the patch became progressively depleted, behavioural interaction between the two females started to appear (Miura 1992; Field & Calbert 1998; Field *et al*. 1998). Here, we distinguished simple, brief contacts between the two females from real, longer-lasting fights, and, for these two types of interactions, we also distinguished whether the focal female was active, running to the other female, or passive if the non-focal female triggered the interaction. The timings of the corresponding four types of interaction were also recorded for the focal female. Observation started as soon as the focal female entered the patch for the first time and stopped either when she left the patch for more than 60 s or when the non-focal female left the patch for more than 5 min.

The ability of an animal to win a contest is usually considered to be related to difference in body size between the two competitors, the larger animal having a higher ability to win the interaction (Petersen & Hardy 1996). We thus also decided to measure the size of the two females for each replicate. For this, the two females were collected after each replicate and were frozen and stored at −40 °C. Thereafter, one hind tibia of each female was mounted on a slide and its size was measured with an eyepiece micrometer mounted on a standard microscope to an accuracy of 0·5 µm. The measure served as an estimation of adult size.

STATISTICAL ANALYSIS

After analysing all the video tapes, the outcome of the contest between the two females in each replicate was scored as 'won' by the focal female when she triggered more than 50% of the fights (and conversely 'lost' when less than 50% of fights she triggered). This corresponds to the definition used by Pérez-Lachaud, Hardy & Lachaud (2002), working on interspecific competition between bethylid wasps. However, for some replicates there were either no fights at all or the proportion of active fights was exactly 50 : 50. Such an outcome was considered an egalitarian relationship between the females (Hand 1986) and was scored as 'none'. This way of coding the global outcome of the contest corresponds to a way of quantifying the dominance of the focal female over the non-focal female (Drew 1993).

Variation in patch-time allocation and the patch-leaving decision rules of the focal females were analysed by means of survival analysis methods that appear to be particularly appropriate for studying time duration when there is competition between conspecifics (Haccou *et al*. 1999; Moya-Laraño & Wise 2000). More accurately, the experimental data were analysed using Cox's proportional hazards model (Cox 1972). Such a model has been used successfully on several occasions to identify patch-leaving mechanisms used by parasitoid females foraging alone on a patch (e.g., Haccou *et al*. 1991; Hemerik, Driessen & Haccou 1993; Wajnberg *et al*. 1999, 2000, 2003; Boivin, Fauvergue & Wajnberg 2004). A detailed description of this model can be found in the literature dealing with survival analysis (e.g., Collett 1994). This model enables the correct handling of censored data (see Bressers *et al*. 1991). In our case, the patch time duration of the focal female was right-censored when the end of the replicate was triggered by the non-focal female. This happened for 22 replicates (i.e. 18·2% of the total number of replicates).

Briefly, the model is formulated in terms of patch-leaving tendency (i.e., hazard rate), which is the probability per unit of time that the focal female leaves the patch, given that she is still on it. Such a leaving tendency is expressed as the product between an innate leaving tendency (i.e., baseline hazard) and an exponential factor (i.e., hazard ratio) that gives the joint effect of a set of explanatory variables (i.e., covariates). The general equation of the model is:

$$h(t) = h_0(t)\exp\left\{\sum_{i=1}^{p}\beta_i z_i\right\} \qquad \text{eqn 1}$$

where $h(t)$ is the hazard rate, $h_0(t)$ the baseline hazard, t the time passed since the focal female entered the patch, and β_i the regression coefficients that give the relative contribution of p covariates z_i. A hazard ratio greater than one indicates an increasing effect of the covariates on the females' patch-leaving tendency, while a hazard ratio lower that one is interpreted in the opposite way. Covariates can be time-dependent or fixed. The baseline hazard, which is the leaving tendency when all covariates are equal to zero, is left unspecified.

Concerning the behaviour of the focal female only, and as performed in other studies (Wajnberg *et al*. 2000, 2003; Boivin *et al*. 2004), the number of successful ovipositions and host rejections of a healthy host or of a host attacked previously by either of the two females were used as covariates in order to understand their influence on the focal females' patch-leaving tendency. The family to which each focal female belonged was also considered. For the effect of the behavioural interactions between the two females, we considered the number of active and passive contacts and fights. Hughes, Harvey & Hubbard (1994) demonstrated that the behaviour of a wasp at the time of the encounter can influence the outcome of the interaction. Thus, we decided to distinguish between two types of passive encounters: (i) those that occurred while the focal female was ovipositing in a host, and (ii) those that occurred while the focal female was performing another behaviour. Moreover, all these behavioural interactions were observed either in isolation or in series, the last case corresponding to escalated contests. We thus also distinguished these two types of situations. The time co-ordinate of a series was set as the midpoint between the times of the first and the last corresponding behaviours.

Because hosts attacked during the experiment were not replaced, each of the two females suffered from direct mutual interference through contacts and fights, but might also, at the same time, experience other kinds of interference through the competition for hosts and the effect of encounters with hosts already attacked by the other female. However, because the covariates used to describe the behavioural competition between the females (e.g., numbers of direct contacts and fights) were explicitly linked to direct mutual interference only, direct mutual interference and any other kinds of interference were easily disentangled without ambiguity.

Taking into account the different competitive behaviours provides another way to quantify the dominance relationship between the two females. Moreover, because patch residence time is usually linked to total host searching efficiency of a parasitoid (Galis & van Alphen 1981; van Alphen & Galis 1983), their effect on females' patch-leaving tendency is likely to correspond to a quantification of females' susceptibility to interference.

Finally, the difference between the hind tibia length of the two females and the global outcome of the fights were also added as fixed covariates. In the latter case, the outcome 'won' was assumed arbitrarily to be the reference level corresponding to the baseline hazard with a parameter set to zero (see Collett 1994; Wajnberg *et al*. 1999 for a detailed explanation). Table 1 gives a detailed list of all these covariates.

In order to quantify the genetic variation in each of the behavioural mechanisms studied, the interaction between the 'family' effect and all the relevant covariates was also considered. Regression parameters and their variance-covariance matrix were estimated from the data by means of partial likelihood maximization (Cox 1975). The significant effects of the covariates were tested with standard likelihood ratio tests using the iterative procedure described in Wajnberg *et al.* (1999) to find the most significant parameters. The adequacy of the final model was assessed by making residual plots (see Wajnberg *et al*. 1999; for an example of such a plot). All computations were done in S-Plus (Venables & Ripley 1994).

Table 1. List of the explanatory covariates used in the Cox's proportional hazards model. Covariates 1–5 concerned the behaviour of the focal female only, whereas covariates 6–19 considered the interactions between the two females. Covariates 5, 18 and 19 were fixed, all others were time-dependant

No.	Covariates
1	Number of ovipositions in a healthy host
2	Number of ovipositions in a previously attacked host
3	Number of rejections of a healthy host
4	Number of rejections of a previously attacked host
5	Family each focal female belongs to
6	Number of active contacts
7	Number of passive contacts while the focal female was ovipositing
8	Number of passive contacts while the focal female was not ovipositing
9	Number of active fights
10	Number of passive fights while the focal female was ovipositing
11	Number of passive fights while the focal female was not ovipositing
12	Number of series of active contacts
13	Number of series of passive contacts while the focal female was ovipositing
14	Number of series of passive contacts while the focal female was not ovipositing
15	Number of series of active fights
16	Number of series of passive fights while the focal female was ovipositing
17	Number of series of passive fights while the focal female was not ovipositing
18	Difference between the hind tibia length of the two females
19	Outcome of the fights

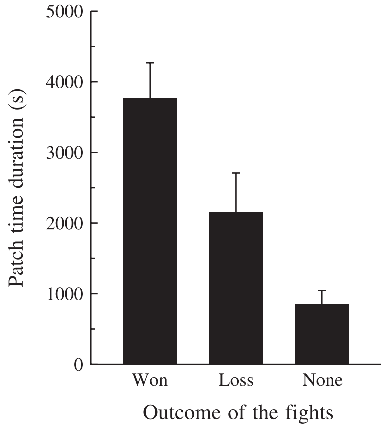

Fig. 1. Average (+ SE) patch residence time for the focal females in relation to the outcome of the fights between the two females in each replicate. Both averages and standard errors are computed from the Kaplan–Meier estimator of the corresponding survivor functions.

Results

As previously demonstrated by Field & Calbert (1999), the difference in size between the two females did not influence the outcome of the fights (generalized linear model with multinomial error: $\chi^2 = 1·07$, d.f. = 1, $P = 0·301$). Moreover, as can be seen in Fig. 1, the average total time the focal females remained on the patch strongly depends on the outcome of the fights between the two females in each replicate (Log-rank test: $\chi^2 = 32·93$, d.f. = 2, $P < 0·001$). Winning females

Table 2. Estimated regression coefficients (β), standard errors (SE) and hazard ratios (exp(β)) for only the covariates having a significant effect ($P < 0{\cdot}05$) on the patch leaving tendency of focal females. χ^2 correspond to the likelihood ratio tests. The fight outcome 'won' was assumed to be the reference level (i.e. baseline hazard with a coefficient set to zero). For each test including the 'isofemale lines' effect, more than one parameter was estimated. They are not shown here

	β	SE	exp(β)	χ^2 (d.f)	*P*-value
Rejection of a healthy host (1)	0·575	0·116	1·78	21·28 (1)	< 0·001
Series of active contacts (2)	0·099	0·046	1·10	4·99 (1)	0·025
Series of passive fights while the female was not ovipositing (3)	0·482	0·114	1·62	15·95 (1)	< 0·001
The fights' outcome is 'won'	0·000	–	1·00	44·13 (2)	< 0·001
The fights' outcome is 'loss'	0·526	0·370	1·69		
The fights' outcome is 'none'	1·818	0·319	6·16		
Interaction 'Isofemale lines' × (1)	–	–	–	25·56 (12)	0·012
Interaction 'Isofemale lines' × (2)	–	–	–	25·73 (12)	0·012
Interaction 'Isofemale lines' × (3)	–	–	–	22·67 (12)	0·031
Interaction 'Isofemale lines' × 'outcome of the fights'	–	–	–	49·37 (33)	0·033

remained on the patch on average almost twice as long as females loosing the competitive interaction. Even on a patch of only five hosts, the average patch residence time of the winning females was more than 1 hour. Such a residence time was almost 3·5 times longer than the average time necessary for the patches to be fully exploited (i.e., 1101·98 ± 65·11 s). In this case, most of the patch residence time is spent both in competitive interaction with the other female, and in re-attacking (i.e., superparasitizing) the available hosts several times. When none of the females won the contest, the time allocated by the focal females to the patch was strongly reduced. In order to understand such a result, the total number of ovipositions performed by the two females was computed. When none of the females won the competitive interaction, this total number of ovipositions was 2·40 ± 0·65, whereas it rose to 21·13 ± 3·45 when the focal females won the fights, and to 10·13 ± 2·96 when fights were lost. Such a difference, which is statistically highly significant (log-linear model with Poisson error: $\chi^2 = 47{\cdot}04$, d.f. = 2, $P < 0{\cdot}001$), seems to simply indicate that, in the case of no outcome of the fights, none of the females were interested in the patch and both decided to leave it earlier.

The patch-leaving mechanisms used by the focal females were identified by means of a Cox's proportional hazards model and Table 2 gives the estimated effect of all the covariates having a significant influence on the females' patch-leaving tendency. The tendency of the focal females to leave the patch significantly increased by a factor of 1·78 each time she rejected an unparasitized host. Such a host rejection type has thus a decremental influence on the time focal females allocated to the host patch. In contrast, none of the other behaviours concerning the focal females only (i.e., successfully attacking a healthy ($\chi^2 = 0{\cdot}02$, d.f. = 1, $P = 0{\cdot}880$) or a previously attacked host ($\chi^2 = 0{\cdot}39$, d.f. = 1, $P = 0{\cdot}533$) or rejecting an already parasitized host ($\chi^2 = 0{\cdot}23$, d.f. = 1, $P = 0{\cdot}631$)) showed any influence on her patch-leaving tendency. There was also no significant 'family' effect indicating no differences in the average patch residence time of the focal females among the different families compared ($\chi^2 = 14{\cdot}69$, d.f. = 12, $P = 0{\cdot}259$).

Considering the behavioural interaction between the two females, the patch-leaving tendency of the focal female clearly depended on the outcome of the fight. The loosing female had a leaving tendency 1·69 times higher than the female winning the contest. When none of the females won the interaction, the hazard ratio was raised to 6·16. Moreover, every series of active contacts with the other female or passive fights while the focal female was not ovipositing had a significant decremental influence on the focal females' patch residence time. However, any isolated behavioural interaction between the females or any sort of passive contacts or fights while the focal female was ovipositing did not have a significant effect on the tendency of the focal females to leave the patch (all χ^2 at $P > 0{\cdot}05$). There was also no significant influence of series of active fights ($\chi^2 = 0{\cdot}00$, d.f. = 1, $P = 0{\cdot}983$) and passive contacts ($\chi^2 = 0{\cdot}26$, d.f. = 1, $P = 0{\cdot}609$) while the focal female was not ovipositing. Finally, the difference in size between the two females also did not have any influence on the females patch-leaving tendency ($\chi^2 = 0{\cdot}34$, d.f. = 1, $P = 0{\cdot}559$).

Although there was no significant global variation among the different families compared, there were significant interactions among the isofemale lines and all the significant patch-leaving rules described so far (see Table 2). Figure 2 gives a graphical representation of these significant interactions between the 'family' effect and all the patch-leaving rules described with quantitative covariates. These results suggest that the different patch-leaving rules observed are family features, which in turn suggests that the phenotypic variation in the intensity of these different patch-leaving mechanisms may be under genetic control. The way the patch-leaving tendency was influenced by each rejection of a healthy host and by each series of active contacts and passive fights (i.e., escalated fights) while the focal female was not ovipositing thus appears to be

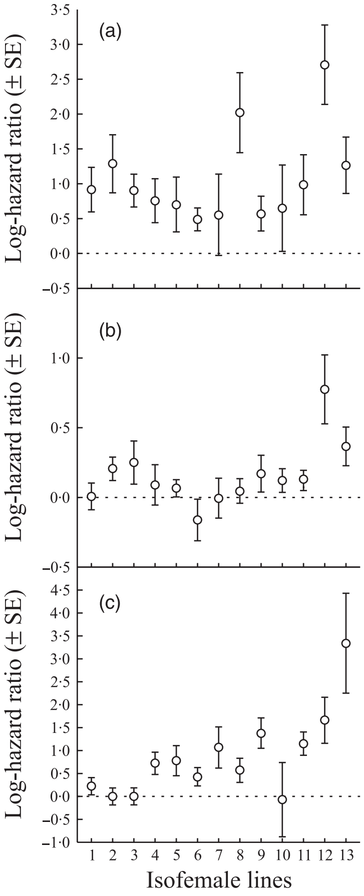

Fig. 2. Graphical representation of the interactions between the isofemale lines and the effect of (a) rejecting an unattacked host, (b) series of active contacts and (c) series of passive fights while the focal females was not ovipositing. Log-hazard ratios and their SE are computed from the fitted proportional hazards model. In all graphs, positive values correspond to a decremental influence of the covariates on female patch-leaving tendency. Negative values correspond to an incremental effect.

under significant genetic variation within the population studied. For the effect of the outcome of the fights, the significant interaction with the 'family' effect indicates that the average result shown in Fig. 1 differed among the isofemale lines compared. In this case also, this suggests the existence, within the population studied, of significant genetic variation. Finally, because most of these patch-leaving rules are describing the effect of direct mutual interference between the foraging females on the time they were willing to invest on the host patchs and thus on their total host searching efficiency, these results are likely to indicate the existence of a significant intrapopulation genetic variability in the susceptibility of females to interference.

Discussion

Using an accurate analysis of the within-patch behavioural interactions between *T. basalis* females, results presented first seem to indicate that direct mutual interference might have a strong effect on the time females are willing to allocate to patches of their hosts. Females winning fights remained a longer time on average on the patch, but spent an important part of their time interacting with the other female and re-attacking the available hosts several times. Hosts attacked by parasitoid females remained in the environment for hours or even days and thus are still vulnerable to further attacks by conspecifics. The decision about fighting for a parasitoid female can thus be an adaptive strategy both to defend resources in a host patch and also to defend the offspring she has already invested (Field & Calbert 1998, 1999). This is especially true for *T. basalis*, because for this species the risk of loosing offspring to a competitor through superparasitism was shown to be particularly high (Field, Keller & Calbert 1997). Such a competitive situation has been referred to as a 'war of attrition' (Maynard Smith 1974; Sjerps & Haccou 1994; Haccou *et al.* 1999; Haccou *et al.* 2003) in previous studies on *T. basalis* (Field *et al.* 1998).

As pointed out by van Alphen *et al.* (2003), the proximate behavioural mechanisms involved in the existence of a war of attrition in insect parasitoids still remained poorly described. At best, the presence of conspecifics has been suggested to influence female patch residence time (Visser, van Alphen & Nell 1990; Visser, van Alphen & Hemerik 1992) or, more accurately, to influence their patch-leaving tendency (Hemerik *et al.* 1993). This is one of the reasons why survival analysis methods were recommended as efficient tools to analyse modifications of the patch-leaving mechanisms of foraging animals in the presence of conspecifics (Sjerps & Haccou 1994; Haccou *et al.* 1999). Using Cox's proportional hazards model, the results presented enabled us to identify accurately the effect of several within-patch behaviours on females' patch-leaving strategies, and thus on their patch-residence time.

On average, each rejection of an unattacked host significantly increased the females' patch-leaving tendency. A similar result was observed on another egg parasitoid, *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), by Wajnberg *et al.* (2000). Any kind of host rejection has been repeatedly considered to provide the foraging females with some information regarding the decreasing value of the patch. Thus, a resulting increase in the tendency to leave the patch is usually considered to be adaptive (van Alphen & Vet 1986; Wajnberg *et al.* 1999).

According to Waage's (1979) model, the patch residence time of a single parasitoid female foraging alone is supposed to depend on (i) the initial motivation to remain on the patch, which should be related to the amount of kairomones left by the hosts, and (ii) on the decreasing rate of this motivation during foraging time. However, when more than one female is simultaneously exploiting the same patch, such a simple model, which is based mainly on information coming from the hosts, is likely to become inaccurate. In this case, information coming from interactions with conspecifics should be more reliable and should thus have a stronger effect on the way females manage their patch residence time (van Alphen *et al*. 2003). This is indeed what results of the present study demonstrated. Both series of contacts triggered by the focal female and series of fights (i.e., escalated fights) she underwent while she was not ovipositing significantly increased her tendency to leave the host patch. The two corresponding information types collected by the female might be that: (i) there is another female foraging simultaneously on the patch, and (ii) this other female is likely to be dominant with a higher fighting ability. In this case, it would be adaptive to leave earlier to find other patches to exploit in the environment. It is interesting to note that isolated behavioural interactions between the two females had no significant effect to trigger a patch-leaving decision. The triggering information seems to be only perceived and processed when the relevant behaviours appear in series, probably determining a threshold above which a decision can be taken. Other behavioural interactions tested did not show any influence on focal females' patch-leaving decisions. As a general rule, contacts with the other female, either isolated or in series, did not have any effect if they were initiated by the non-focal female, and fights also did not have any influence if they were actively triggered by the focal female. Finally, any kind of passive behaviours that the females underwent while they were ovipositing in a host did not influence their tendency to leave the patch. This seems to indicate that females can only modify their patch-leaving tendency when they are performing another foraging behaviour on the patch, such as walking or searching for hosts.

Direct mutual interference in insect parasitoids can have strong consequences on the population dynamics of parasitoid–host systems (Visser *et al*. 1999). In this context, it is interesting to note that all significant behavioural interactions between *T. basalis* females had the same global effect: they all led to an increase in females' patch-leaving tendency. Thus, results seem to indicate that, in the species studied here, direct mutual interference will tend to increase parasitoids' dispersal with an increase in within-patch parasitoid density, which is actually what is predicted by several theoretical approaches (e.g., Cook & Hubbard 1977). Such a trend should be adaptive because it will result in the dispersal of parasitoids from patches that are, or are likely to become, over-exploited, and this will lead to maintenance of individuals' host searching efficiency (Cook & Hubbard 1977; van Alphen & Vet 1986). Results could thus be viewed as an understanding of the proximate behavioural mechanisms involved in the spatial dispersion of parasitoids exploiting patchily distributed resources, for example within the context of the ideal free distribution (Fretwell & Lucas 1970; Sutherland 1983, 1996).

In a previous study, Wajnberg *et al*. (1999), studying patch time allocation of single *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) foraging alone on a patch, demonstrated the existence of significant intrapopulation genetic variation in the patch-leaving decision rules adopted by the females. Their results were based on the isofemale strains method (Parsons 1980; Hoffmann & Parsons 1988; Wajnberg 2004), as in the present study. Contrary to what they found, we did not observe global genetic variation in the average patch residence time of the focal females. However, the present results demonstrated, within the *T. basalis* population studied, the existence of significant genetic variability in the intensity of all the significant patch-leaving rules observed. First of all, there was significant genetic variation in the intensity of the decremental effect of each rejection of a healthy host on females' patch residence time (see Fig. 2). As stated in Wajnberg *et al*. (1999), such genetic variability could correspond to genetic variation in the quality of the information acquired by the foraging females on the decreasing value of the patch each time a host is rejected. More interestingly, results also demonstrated significant genetic variation in all patch-leaving decision rules involving behavioural interactions between the two females. According to Caldow *et al*. (1999), individual variation in the competitive ability of foraging animals should arise from variation in their intrinsic foraging efficiency and in their susceptibility to interference. In the present case, because we only studied variation in the effect of competitive interactions between parasitoid females on their patch residence time and thus on their total host searching efficiency, the inter-individual genetic variation observed should likely be interpreted as genetic variability in females' susceptibility to interference.

Several studies were conducted recently to describe inter-individual variation in susceptibility to interference between foraging animals and its theoretical consequence in terms of population dynamics (Parker & Sutherland 1986; Goss-Custard & Durell 1987; Sutherland & Parker 1992; Sutherland 1996; van der Meer 1997; Caldow *et al*. 1999). Theoretical developments were usually based on the ideal free distribution that initially considered that competitors have all an equal competitive ability (Fretwell & Lucas 1970; Sutherland 1996; van der Meer 1997). Experimental work was conducted almost exclusively on birds (e.g. Goss-Custard & Durell 1987; Caldow *et al*. 1999). Such inter-individual variation has been related to differences in age, sex, or even morphological or physiological factors (see Goss-Custard & Sutherland 1997 for a review). Working on insect parasitoids, the present study demonstrated for the first time that such inter-individual variation can also be genetically determined, and can thus be the target of strong selective pressures leading animals to adapt their foraging strategy to local environmental conditions.

It would be interesting to include such an inter-individual genetic variability in susceptibility to interference into a multi-generation theoretical model, for example within the framework of the ideal free distribution as suggested by Sutherland (1996). For this, the details of the behavioural mechanisms involved should probably be considered using an approach similar to the one used by Stillman *et al*. (1997). Such a theoretical approach will enable us to accurately understand both the population dynamics and evolutionary consequences of the genetic variation observed here, and how such genetic variation can be maintained in the populations.

The experimental work presented was based on a symmetrical situation between the two *T. basalis* females. Experiments are now being performed in order to understand accurately modifications in patch-leaving decision rules of competing females having different previous experience (i.e., difference in age, difference in number of patches already visited, etc.). The expected results will enable us to understand more accurately how direct mutual interference between parasitoid females might affect the time they are willing to invest on patches of their hosts.

Acknowledgements

We tank G. Salerno for his patience in measuring tibia length of the females used in this study. C. Bernstein, F. Dubois, G. Driessen, R.H. Messing and J.S. Pierre are thanked for their encouragement and their critical reading of the manuscript. The music of Carmen Consoli helped us during the many hours of experiments; we would also like to thank her. This work is part of GDR 2155 'Ecologie Comportementale' (CNRS commission 30) and OECD 2000 Fellowships.

References

van Alphen, J.J.M. (1988) Patch-time allocation by insect parasitoids: superparasitism and aggregation. *Population Genetics and Evolution* (ed. G. de Jong), pp. 216–221. Springer-Verlag.

van Alphen, J.J.M., Bernstein, C. & Driessen, G. (2003) Information acquisition and time allocation in insect parasitoids. *Trends in Ecology and Evolution*, **18**, 81–87.

van Alphen, J.J.M. & Galis, F. (1983) Patch time allocation and parasitization efficiency of *Asobara tabida*, a larval parasitoid of *Drosophila*. *Journal of Animal Ecology*, **52**, 937–952.

van Alphen, J.J.M. & Vet, L.E.M. (1986) An evolutionary approach to host finding and selection. *Insect Parasitoids* (eds J. Waage & D. Greathead), pp. 23–61. Academic Press.

Beddington, J.R. (1975) Mutual interference between parasites or predators and its effect on searching efficiency. *Journal of Animal Ecology*, **44**, 331–340.

Bernstein, C. (2000) Host-parasitoid models: the story of a successful failure. *Parasitoid Population Biology* (eds M.E. Hochberg & A.R. Ives), pp. 41–57. Princeton University Press.

Bin, F., Vinson, S.B., Strand, M.R., Colazza, S. & Jones, W.A.J. (1993) Source of an egg kairomone for *Trissolcus basalis*, a parasitoid of *Nezara viridula*. *Physiological Entomology*, **18**, 7–15.

Boivin, G., Fauvergue, X. & Wajnberg, E. (2004) Optimal patch residence time in egg parasitoids: innate versus learned estimate of patch quality. *Oecologia*, **138**, 640–647.

Bressers, M., Meelis, E., Haccou, P. & Kruk, M. (1991) When did it really start or stop: the impact of censored observations on the analysis of duration. *Behavioural Processes*, **23**, 1–20.

Caldow, R.W.G., Goss-Custard, J.D., Stillman, R.A., Durell, S.E.A. le V. dit, Swinfen, R. & Bregnballe, T. (1999) Individual variation in the competitive ability of interference-prone foragers: the relative importance of foraging efficiency and susceptibility to interference. *Journal of Animal Ecology*, **68**, 869–878.

Charnov, E.L. (1976) Optimal foraging: the marginal value theorem. *Theoretical Population Biology*, **9**, 129–136.

Colazza, S. (1993) Factors influencing brood sex ratio in the egg parasitoid *Trissolcus basalis* (Woll.) (Hymenoptera; Scelionidae). *Entomologica*, **27**, 211–219.

Colazza, S., Rosi, M.C., Sebastiani, P. & Ursini, M. (1996) Host acceptance behavior in the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae). *Acta Oecologica*, **17**, 109–125.

Colazza, S., Salerno, G. & Wajnberg, E. (1999) Volatile and contact chemicals released by *Nezara viridula* (Heteroptera: Pentatomidae) have a kairomonal effect on the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae). *Biological Control*, **16**, 310–317.

Collett, D. (1994) *Modelling Survival Data in Medical Research*. Chapman & Hall.

Cook, R.M. & Hubbard, S.F. (1977) Adaptive searching strategies in insect parasites. *Journal of Animal Ecology*, **46**, 115–125.

Cox, D.R. (1972) Regression models and life tables. *Biometrics*, **38**, 67–77.

Cox, D.R. (1975) Partial likelihood. *Biometrika*, **62**, 269–276.

Drew, C. (1993) The concept and definition of dominance in animal behaviour. *Behaviour*, **125**, 283–313.

Driessen, G. & Bernstein, C. (1999) Patch departure mechanisms and optimal host exploitation in an insect parasitoid. *Journal of Animal Ecology*, **68**, 445–459.

Driessen, G., Bernstein, C., van Alphen, J.J.M. & Kacelnik, A. (1995) A count-down mechanism for host search in the parasitoid *Venturia canescens*. *Journal of Animal Ecology*, **64**, 117–125.

Driessen, G. & Visser, M.E. (1997) Components of parasitoid interference. *Oikos*, **79**, 179–182.

Field, S.A. (1998) Patch exploitation, patch-leaving and preemptive patch defence in the parasitoid wasp *Trissolcus basalis* (Insecta: Scelionidae). *Ethology*, **104**, 323–338.

Field, S.A. & Calbert, G. (1998) Patch defence in the parasitoid wasp *Trissolcus basalis*: when to begin fighting? *Behaviour*, **135**, 629–642.

Field, S.A. & Calbert, G. (1999) Don't count your eggs before they're parasitized: contest resolution and the trade-offs during patch defense in a parasitoid wasp. *Behavioral Ecology*, **10**, 122–127.

Field, S.A., Calbert, G. & Keller, M.A. (1998) Patch defence in the parasitoid wasp *Trissolcus basalis* (Insecta: Scelionidae): the time structure of pairwise contests, and the 'waiting game'. *Ethology*, **104**, 821–840.

Field, S.A., Keller, M.A. & Calbert, G. (1997) The pay-off from superparasitism in the egg parasitoid *Trissolcus basalis*, in relation to patch defence. *Ecological Entomology*, **22**, 142–149.

Free, C.A., Beddington, J.R. & Lawton, J.H. (1977) On the inadequacy of simple models of mutual interference for parasitism and predation. *Journal of Animal Ecology*, **46**, 543–554.

Fretwell, S.D. & Lucas, H.L. (1970) On territorial behaviour and other factors influencing habitat distribution in birds. *Acta Biotheoretica*, **19**, 16–36.

Galis, F. & van Alphen, J.J.M. (1981) Patch time allocation and search intensity of *Asobara tabida* Nees (Braconidae), a larval parasitoid of *Drosophila*. *Netherlands Journal of Zoology*, **31**, 596–611.

Godfray, H.C.J. (1994) *Parasitoids Behavioral and Evolutionary Ecology*. Princeton University Press.

Goss-Custard, J.D., Durell, S.E.A. le V. dit (1987) Age-related effects in oystercatchers, *Haematopus ostralegus*, feeding on mussels, *Mytilus edulis*. I. Foraging efficiency and interference. *Journal of Animal Ecology*, **56**, 521–536.

Goss-Custard, J.D. & Sutherland, W.J. (1997) Individual behaviour, populations and conservation. *Behavioral Ecology*, 4th edn (eds J.R. Krebs & N.B. Davies), pp. 373–395. Blackwell Science.

Green, R.F. (1984) Stopping rules for optimal foragers. *American Naturalist*, **123**, 30–40.

Haccou, P., de Vlas, S.J., van Alphen, J.J.M. & Visser, M.E. (1991) Information processing by foragers: effects on intra-patch experience on the leaving tendency of *Leptopilina heterotoma*. *Journal of Animal Ecology*, **60**, 93–106.

Haccou, P., Glaizot, O. & Cannings, C. (2003) Patch leaving strategies and superparasitism: an asymmetric generalised war of attrition. *Journal of Theoretical Biology*, **225**, 77–89.

Haccou, P., Sjerps, M. & van der Meijden, E. (1999) To leave or to stay, that is the question: prediction from models of patch-leaving strategies. *Herbivores: Between Plants and Predators* (eds H. Olff, V.K. Brown & R.H. Drent), pp. 85–108. Blackwell Science.

Hand, J.L. (1986) Resolution of social conflicts: dominance, egalitarianism, spheres of dominance, and game theory. *Quarterly Review of Biology*, **61**, 201–220.

Hassell, M.P. (1978) *The Dynamics of Arthropod Predator–Prey Systems*. Princeton University Press.

Hassell, M.P. & Huffaker, C.B. (1969) Regulatory processes and population cyclicity in laboratory populations in *Anagasta kuehniella* (Zeller) (Lepidoptera: Phycitidae). III. The development of population models. *Researches in Population Ecology*, **11**, 186–210.

Hassell, M.P. & Varley, G.C. (1969) New inductive population model for insect parasites and its bearing on biological control. *Nature*, **223**, 1133–1136.

Hemerik, L., Driessen, G. & Haccou, P. (1993) Effects of intra-patch experiences on patch time, search time and searching efficiency of the parasitoid *Leptopilina clavipes*. *Journal of Animal Ecology*, **62**, 33–44.

Hoffmann, A.A. & Parsons, P.A. (1988) The analysis of quantitative variation in natural populations with isofemale strains. *Genetic Selection Evolution*, **20**, 87–98.

Holmgren, N. (1995) The ideal free distribution of unequal competitors: predictions from a behaviour-based functional response. *Journal of Animal Ecology*, **64**, 197–212.

Hughes, J.P., Harvey, I.F. & Hubbard, S.F. (1994) Host-searching behavior of *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae): Interference – the effect of mature egg load and prior behavior. *Journal of Insect Behavior*, **7**, 433–454.

Jones, W.A. (1988) World review of the parasitoids of the Southern Green Stink Bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). *Annals of the Entomological Society of America*, **81**, 262–273.

Maynard Smith, J. (1974) The theory of games and the evolution of animal conflicts. *Journal of Theoretical Biology*, **47**, 209–221.

Maynard Smith, J. (1982) *Evolution and the Theory of Games*. Cambridge University Press.

van der Meer, J. (1997) The ideal free distribution when predators differ in competitive abilities. *Oikos*, **80**, 301–310.

Miura, K. (1992) Aggressive behavior in *Paracentrobia andoi* (Hymenoptera, Trichogrammatidae), an egg parasitoid of the green rice leafhopper. *Japanese Journal of Entomology*, **60**, 103–107.

Moya-Laraño, J. & Wise, D.H. (2000) Survival analysis: a powerful tool for evaluating fighting and assessment. *Animal Behaviour*, **60**, 307–313.

Parker, G.A. & Sutherland, W.J. (1986) Ideal free distributions when individuals differ in competitive ability: phenotype-limited ideal free models. *Animal Behaviour*, **34**, 1222–1242.

Parsons, P.A. (1980) Isofemale strains and evolutionary strategies in natural populations. *Evolutionary Biology*, Vol. 13 (eds M. Hecht, W. Steere & B. Wallace), pp. 175–217. Plenum.

Pérez-Lachaud, G., Hardy, I.C.W. & Lachaud, J.-P. (2002) Insect gladiators: competitive interactions between three species of bethylid wasps attacking the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). *Biological Control*, **25**, 231–238.

Petersen, G. & Hardy, I.C.W. (1996) The importance of being larger: parasitoid intruder–owner contests and their implications for clutch size. *Animal Behaviour*, **51**, 1363–1373.

Rogers, D.J. & Hassell, M.P. (1974) General models for insect parasite and predator searching behaviour: interference. *Journal of Animal Ecology*, **43**, 239–253.

Sjerps, M. & Haccou, P. (1994) Effect of competition on optimal patch leaving: a war of attrition. *Theoretical Population Biology*, **46**, 300–318.

Stephens, D.W. & Krebs, J.R. (1986) *Foraging Theory*. Princeton University Press.

Stillman, R.A., Goss-Custard, J.D. & Caldow, W.G. (1997) Modelling interference from basic foraging behaviour. *Journal of Animal Ecology*, **66**, 692–703.

Sutherland, W.J. (1983) Aggregation and the 'ideal free' distribution. *Journal of Animal Ecology*, **52**, 821–828.

Sutherland, W.J. (1996) *From Individual Behaviour to Population Ecology*. Oxford University Press.

Sutherland, W.J. & Parker, G.A. (1992) The relationship between continuous input and interference models of ideal free distributions with unequal competitors. *Animal Behaviour*, **44**, 345–355.

Venables, W.N. & Ripley, B.D. (1994) *Modern Applied Statistics with S-Plus*. Springer-Verlag.

Visser, M.E. & Driessen, G. (1991) Indirect mutual interference in parasitoids. *Netherlands Journal of Zoology*, **41**, 214–227.

Visser, M.E., Jones, T.H. & Driessen, G. (1999) Interference among insect parasitoids: a multi-patch experiment. *Journal of Animal Ecology*, **68**, 108–120.

Visser, M.E., van Alphen, J.J.M. & Hemerik, L. (1992) Adaptive superparasitism and patch time allocation in solitary parasitoids: an ESS model. *Journal of Animal Ecology*, **61**, 93–101.

Visser, M.E., van Alphen, J.J.M. & Nell, H.W. (1990) Adaptive superparasitism and patch time allocation in solitary parasitoids: the influence of the number of parasitoids depleting a patch. *Behaviour*, **114**, 31–36.

Waage, J.K. (1978) Arrestment responses of the parasitoid, *Nemeritis canescens*, to a contact chemical produced by its host, *Plodia interpunctella*. *Physiological Entomology*, **3**, 135–146.

Waage, J.K. (1979) Foraging for patchily-distributed hosts by the parasitoid, *Nemeritis canescens*. *Journal of Animal Ecology*, **48**, 353–371.

Wajnberg, E. (2004) Measuring genetic variation in natural enemies used for biological control. Why and How? *Genetics, Evolution and Biological Control* (eds L. Ehler, R. Sforza, T. Mateille), pp. 19–37. CAB International.

Wajnberg, E., Fauvergue, X. & Pons, O. (2000) Patch leaving decision rules and the Marginal Value Theorem: an experimental analysis and a simulation model. *Behavioral Ecology*, **11**, 577–586.

Wajnberg, E., Gonsard, P.A., Tabone, E., Curty, C., Lezcano, N. & Colazza, S. (2003) A comparative analysis of patch-leaving decision rules in a parasitoid family. *Journal of Animal Ecology*, **72**, 618–626.

Wajnberg, E., Rosi, M.C. & Colazza, S. (1999) Genetic variation in patch-time allocation in a parasitic wasp. *Journal of Animal Ecology*, **68**, 121–133.

Wilson, F. (1961) Adult reproductive behaviour in *Asolcus basalis* (Hymenoptera: Scelionidae). *Australian Journal of Zoology*, **9**, 739–751.

Yamamura, N. & Tsuji, N. (1987) Optimal patch time under exploitative competition. *American Naturalist*, **129**, 553–567.

Received 26 January 2004; accepted 26 April 2004