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Superparasitism acceptance and patch-leaving mechanisms in parasitoids: a comparison between two sympatric wasps

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Parasitoids often exploit hosts that have a clumped distribution. To divide their foraging effort between patches, females use encounters with unparasitized hosts as a source of information regarding local host density. Theory predicts that depending on host distribution, ovipositions should either increase (if the distribution is aggregated) or decrease (if the distribution is regular) the females' tendency to remain on the patch. However, patch time allocation theories usually ignore the possibility of superparasitism, even though it can change the predictions. We compared patch exploitation strategies of two related and sympatric *Drosophila* parasitoids. We investigated, with the Cox model, how females divide their foraging effort between patches of different densities and what proximal leaving mechanism they use. The species differed in the information input they used to adjust their patch time. Ovipositions decreased the patch-leaving tendency of *Leptopilina heterotoma*, which seldom superparasitized; this is consistent with previous results and with the aggregated distribution of *Drosophila* larvae. In contrast, no effect of oviposition was found on *L. boulardi*, which accepted most already parasitized hosts. Rejection of parasitized hosts increased the leaving tendency of *L. heterotoma*, but had no effect on *L. boulardi*. These wasps have thus evolved very different patch-leaving rules, possibly in response to differences in their superparasitism behaviour.

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Extensive theoretical and experimental investigations have focused on how animals exploit resources that are patchily distributed, especially in the context of optimal-foraging theory (reviewed in Stephens & Krebs 1986). Parasitoid insects often exploit hosts that are arranged in clumps, and females have thus to divide their foraging effort among patches. Patch residence time is predicted to depend both on the rate of fitness gain in that patch and on the mean rate of fitness gain expected from the whole environment (Charnov 1976). The ability of a parasitoid to achieve optimal patch residence time thus strongly depends on its ability to get reliable information on the profitability of the environment.

Several proximal mechanisms allowing the parasitoid to obtain such information and to approximate patch

residence time to the optimum have been proposed. They include use of host-associated chemical cues (Vet et al. 1998), and the experience acquired during patch exploitation (Waage 1979; Driessen & Bernstein 1999).

The best-documented patch-leaving rule considers that, when entering a patch, a female has an initial responsiveness to host cues that declines as long as no host is encountered (Waage 1979). Then, responsiveness is modified by each oviposition which represents an information input (reviewed in Van Alphen et al. 2003). Depending on species-specific and individual characteristics, ability to process information and habitat quality, each oviposition either increases (incremental mechanism) or decreases (count down or decremental mechanism) the responsiveness of the female and, accordingly, its patch residence time. When hosts are distributed in patches of very different densities, and when females are not able to estimate the initial patch quality accurately, the incremental mechanism is expected to be favoured by natural selection. In contrast, when females are able to estimate the initial patch quality, or when patch quality is regular, ovipositions should decrease patch residence time (Iwasa et al. 1981). In parasitoid species, both mechanisms have

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been reported (reviewed in Van Alphen et al. 2003). Predictably, encounter with an already parasitized host (either by the female herself or by conspecifics), which means that the patch is going to depletion, may also have a decremental effect (Hemerik et al. 1993; Wajnberg et al. 1999, 2000).

In most theoretical models developed so far, females are considered to reject already parasitized hosts systematically, and thus to lay their eggs only in unparasitized hosts. However, it has been repeatedly observed that parasitoid females sometimes superparasitize. Two alternative hypotheses have been put forward to explain such behaviour. The constraint hypothesis states that superparasitism is caused simply by a breakdown in females' discrimination ability (ability to distinguish between parasitized and unparasitized hosts, Van Lenteren 1981; Outreman et al. 2001), whereas according to the adaptive hypothesis, females do recognize encountered hosts as being parasitized, but actively decide to accept them (Van Alphen & Visser 1990). This last interpretation states that superparasitism is the best strategy when unparasitized hosts are scarce, given that the offspring of a superparasitizing female still has a chance of completing development in a superparasitized host. Whatever its origin (constraint or adaptive decision), superparasitism can have a strong influence on optimal patch-leaving strategies. If superparasitism is due to a constraint, this behaviour leads, in most cases, to egg and time wastage, and also to a biased information about the actual profitability of the patch. Overestimating the quality of the patch will extend the patch residence time, reinforcing the probability of the foraging female re-encountering a parasitized host without further fitness gain. Since females suffering such constraint cannot properly perceive the depletion of the patch of hosts, selection pressures are expected to promote early patch leaving (Rosenheim & Mangel 1994). In contrast, if superparasitism is an adaptive decision, females are predicted to invest in this activity, thus increasing their patch time (Van Alphen & Visser 1990; Haccou et al. 2003).

Parasitoids that attack *Drosophila* larvae have been extensively studied with regard to their patch-leaving mechanisms (reviewed in Van Alphen et al. 2003). In all species studied, ovipositions increase patch residence time (incremental mechanism). Since convergence on an incremental mechanism occurs among both related species (the Figitidae *Leptopilina heterotoma* Thompson and *L. clavipes* Hartig) and unrelated species (*L. heterotoma* and the braconid *Asobara tabida* Nees, for example), it has been interpreted as an adaptation to the aggregative distribution of *Drosophila* sp. larvae, consistent with theoretical prediction. Furthermore, two of these solitary species have been studied with regard to their superparasitism strategies, and the results appeared to be in qualitative agreement with predictions of optimal-foraging models, reinforcing the adaptive interpretation of this behaviour in these species (*L. heterotoma*: Bakker et al. 1990; *A. tabida*: Van Alphen & Nell 1982).

In the south of France, *L. heterotoma* females compete for the same hosts with the closely related species *L. boulandi* Barbotin et al. (Carton et al. 1986; Allemand

et al. 1999, 2002; Fleury et al. 2004). In this geographical area, *L. boulandi* females harbour infectious particles (presumably viral) that strongly increase their tendency to accept superparasitism, compared with uninfected females (experimental demonstration in Varaldi et al. 2003). Since these particles benefit from horizontal transmission across parasite larvae within superparasitized hosts (in addition to vertical transmission, Varaldi et al. 2003), we hypothesized that this behavioural alteration corresponds to a manipulation of the wasp behaviour by infectious particles. In the present study, we quantified the differences in superparasitism behaviour expressed by *L. boulandi* and *L. heterotoma* from the south of France, then used the observed difference in superparasitism behaviour to test the hypothesis that superparasitism should influence the patch-leaving strategy. We first compared the ability of *L. heterotoma* and *L. boulandi* to adjust their time and egg budgets to various host densities. We then compared proximal mechanisms responsible for patch time adjustment by the two parasitoids while exploiting two host species (*D. melanogaster* and *D. subobscura*). Experimental data were analysed using the Cox proportional hazards rate model, which has been extensively used to study animal decision rules, and particularly patch-leaving decisions by parasitoids (Van Alphen et al. 2003). This statistical model allowed us to evaluate the effect of time-dependent variables, such as the occurrence of ovipositions, on the instantaneous probability of leaving the patch.

METHODS

Strains and Experimental Conditions

Wasps

In the south of France, *L. heterotoma* and *L. boulandi* are the most common parasitoids of *Drosophila* larvae living in fermenting substrates. Dozens of wild females of the two species were caught in Antibes, south of France, in May 2000, and used to found laboratory strains. They were reared under a 12:12 h light:dark regime at 25°C. Virgin parasitoid females were isolated at the pupal stage of the host, and they were 2 or 3 days old at the time of the experiments.

Drosophila

Drosophila melanogaster (strain from Ste Foy-lès-Lyon, France) was used as the rearing host. For testing parasitoid females, host larvae were 1 day old (time since egg hatching) for *D. melanogaster* and 2 days old for *D. subobscura* (at 21°C), since at these ages, larvae of the two species have the same estimated volume (larvae were measured under a stereomicroscope and assumed to be cylinders, $\bar{X} \pm \text{SE} = 0.032 \pm 0.001 \text{ mm}^3$; $t_{41} = 1.63$, $P = 0.11$). Both species are suitable hosts for *L. heterotoma*, whereas only *D. melanogaster* is suitable for *L. boulandi* (Carton et al. 1986; unpublished data). We used these two host species to test the ability of *L. boulandi* females to adjust their patch residence time to host suitability.

Preliminary Experiment

Ten females of each wasp species were individually kept enclosed within a petri dish (diameter 5 cm) with 10 unparasitized *D. melanogaster* larvae from 1700 to 1000 hours, the period known to cover activity peaks of wasps (Fleury et al. 2000). This set-up ensures that females will re-encounter parasitized larvae. After exploitation, hosts were killed by freezing and dissected to determine the distribution of parasitoid eggs.

Twenty-five newly emerged (<24 h) females of each species were killed by freezing and dissected in a drop of physiological saline to determine their egg load: one ovary was transferred into disodic eosin (1%) and eggs were carefully scattered between slide and cover glass. Coloured eggs were counted under the microscope with the help of a video system.

Experimental Arena and Patch-leaving Criteria

Wasps were observed in open petri dishes (diameter 15 cm) with agar layer and *Drosophila* larvae (number and species depending on experiments) while foraging on a circular patch of yeast (diameter 2 cm). Both *Leptopilina* species localize their hosts through systematically probing the substrate, and they usually make short excursions outside the yeast patch (Van Alphen & Vet 1986). These short excursions constitute patch-leaving events and delimit successive visits to the patch. The patch was considered definitively abandoned either when the female stayed out of it for more than 3 min, or when she crossed the edge of the petri dish either by walking or by flying. Experiments were conducted in a climate room at 23°C.

Experiment 1: Host Density

In experiment 1, we investigated how *L. boulardi* and *L. heterotoma* invest time and eggs on host patches of different densities. Experienced females (each provided with 10 *D. melanogaster* larvae the day before) were released on a patch of either 0, 4, 8, 16 or 32 unparasitized *D. melanogaster* larvae. We measured the time from the moment the female first inserted its ovipositor in the yeast to definitive departure. We then killed the larvae by freezing and dissected them to assess the number and distribution of parasitoid eggs. Ten replicates were carried out with each parasitoid species and each host density.

Experiment 2: Within-patch Experience

In experiment 1, we did not record timing of oviposition. Therefore, in experiment 2, we timed behavioural events on a patch at a single host density, to determine their effect on the patch-leaving decision. We observed females either on a *D. melanogaster* patch as in the previous experiment, or on *D. subobscura*.

The day before the experiment, females had oviposition experience on a patch of four *D. melanogaster* and four *D. subobscura*. During the experiment, females were

observed on a patch of either eight *D. melanogaster* or eight *D. subobscura* larvae. We recorded the behaviour of each female under a stereomicroscope until the patch was definitively left. Each female was used only once and was free to leave the patch. Infested hosts were not replaced during the experiment, so the females experienced patch depletion. Numbers of replicates varied between 10 and 17.

Using an event recorder (accuracy: 1 s), we recorded time and duration of the following behavioural events: (1) entering or leaving the patch; (2) oviposition (stinging a host with egg deposition); (3) rejection (stinging a host without egg deposition); (4) preening; (5) standing still. After the experiment, we dissected the larvae to determine the distribution of parasitoid eggs. In the two wasp species, distribution of duration of stings was clearly bimodal and we thus defined, as did Haccou et al. (1991), a threshold under which a sting is considered a rejection. Threshold values (17 s for *L. heterotoma* and 13 s for *L. boulardi*) were chosen so as to maximize the correlation between estimated and actual numbers of eggs laid (Pearson correlation: *L. heterotoma*: $r_{20} = 0.99$, $P < 0.01$; *L. boulardi*: $r_{30} = 0.85$, $P < 0.01$). Data were used to infer patch-leaving decision rules of the two wasp species on the two *Drosophila* hosts (see below).

Statistical Procedure

The collected data set of experiment 2 was analysed with the Cox proportional hazards model. This model allowed us to evaluate the effect of variables (called covariates) on the probability of occurrence of a particular event (here patch departure), without any assumption about its distribution in time (Kalbfleisch & Prentice 1980). Furthermore, cutting observation data into fragments delimited by the different behavioural items (ovipositions, rejections, etc.) allowed us to investigate their effect at the precise moment they occurred. Leaving tendency is formulated as the instantaneous rate of patch leaving at time $T = t$ conditional upon being in the patch at that moment:

$$\lambda(t) = \lim_{\Delta t \rightarrow 0^+} \frac{P(t \leq T < t + \Delta t | T \geq t)}{\Delta t} \quad (1)$$

where $\lambda(t)$ is leaving tendency, t the time since the first entry, $P(t \leq T < t + \Delta t | T \geq t)$ is the probability of leaving the patch between t and $t + \Delta t$, given that the female is in the patch at time t . Leaving tendency of a given wasp at a given time is assumed to be the product of a basic tendency to leave the patch (baseline hazard) and a hazard ratio corresponding to the joint effects of all explanatory variables (the covariates) according to the following equation:

$$\lambda(t) = \lambda_0(t) \times \exp\left(\sum_{i=1}^p \beta_i Z_i(t)\right) \quad (2)$$

where $\lambda_0(t)$ is the basic leaving tendency (left unspecified), β the regression coefficient that gives the relative contributions of p covariates $Z_i(t)$. Covariates can be either fixed or time dependent. The exponential term

corresponds to the hazard ratio. If this term is below unity, basic leaving tendency decreases, whereas if it is above it increases. Change in leaving tendency is assessed with reference to a group whose leaving tendency is $\lambda_0(t)$, characterized by $Z_i(i:1 \rightarrow p)(t) = 0$.

In experiment 2, continuous observation of females allowed definition of time-dependent variables. Six covariates were included in the model: (1) parasitoid species, (2) host species, (3) rank of patch visit, (4) number of ovipositions during the previous visits, (5) number of ovipositions in the current visit and (6) number of rejections during the current patch visit. In addition to these main suspected factors, we chose to incorporate interactions between them, taking into account only those that are biologically meaningful. We included interactions 2×5 and 2×6 to see whether oviposition and host rejection have the same effect whatever the host species. Together with the obvious factor 'parasitoid species' (1), we thus considered 15 parameters in the model.

The first two (time-independent) factors are necessarily incorporated in the model since we studied patch-leaving tendency of two wasps on two hosts. Distinction between ovipositions that occurred during either current or previous visits of the patch allowed us to evaluate the proper effect of oviposition, apart from the potential effect of the initial patch quality (degree of patch exploitation at the beginning of a given visit). Contrary to covariate 5, covariate 4 does not change at each oviposition, but only at a new entrance into the patch. The beta values associated with each covariate were estimated by maximizing the partial likelihood function (see Kalbfleisch & Prentice 1980 for details) using the S-Plus package (Venables & Ripley 1994). This procedure gives estimates of the beta values and the associated matrix of variance-covariance. This allowed us to test the significance of coefficients, using the classical Wald test.

The name 'proportional hazards model' stems from the assumption that hazard rates of the different groups studied are proportional. This means that for the different groups studied (*L. heterotoma* versus *L. boulardi* for instance) the cumulative basic leaving tendencies ($= -\log(s(t))$, where $s(t)$ is the survival estimate) have to be approximately parallel when plotted against time (on a log-scale plot, see Wajnberg et al. 1999 for such a plot). An efficient way to test this hypothesis is proposed in the Venables & Ripley (1994) package of S-Plus. The proportional hazards assumption is cast in terms of a time-varying coefficients model. It is assumed that:

$$\lambda(t) = \lambda_0(t) \times \exp\left(\sum \beta_i(t) \times Z_i(t)\right) \quad (3)$$

The proportional hazards assumption is then a test for $\beta(t) = \beta$, which is a test for zero slope in the appropriate plot of $\beta(t)$ on t (MathSoft 1995). This procedure provides a quantitative test for each covariate included in the model (with a P value). In our case, these tests led to the conclusion that proportional assumption was not justified for the wasp species factor ($P < 0.05$). In this case, the model can still be fitted by means of stratification. This procedure allows each group (*L. heterotoma* and *L. boulardi*) to have its own basic leaving tendency

($\lambda_0(t)$). Nevertheless, for this reason, the wasp species factor could not be directly tested (df of the model is thus decreased by one).

Finally, following Wajnberg et al. (1999), we assessed the adequacy of the fitted model by making residuals plots and using deviance residuals that have the same properties as residuals used in linear models. If the model describes the data correctly, residuals should be uncorrelated and symmetrically distributed around zero. This was the case.

RESULTS

Superparasitism Behaviour

When females were constrained to stay several hours confined with a patch of 10 hosts, both wasp species parasitized on average 8.9 larvae (Wilcoxon rank-sum test: $W = 56.5$, $N_1 = N_2 = 10$, $P = 0.62$), but the distribution of eggs differed strongly: the mean number of eggs per parasitized host \pm SE was 3.56 ± 0.84 for *L. boulardi* and 1.04 ± 0.03 for *L. heterotoma* ($W = 1$, $N_1 = N_2 = 10$, $P < 0.001$; Fig. 1). This difference was confirmed in experiments 1 and 2 where females were free to leave the patch. In experiment 1, *L. heterotoma* hardly ever superparasitized (only one case at density 32), whereas *L. boulardi* laid a mean \pm SE of 1.51 ± 0.83 , 1.44 ± 0.41 , 1.40 ± 0.26 and 1.11 ± 0.04 eggs per parasitized host at densities 4, 8, 16 and 32, respectively. Data from experiment 2 on *D. melanogaster* (this host is suitable for both parasitoids) confirm the difference: *L. boulardi* laid a mean \pm SE of 1.41 ± 0.09 eggs per parasitized host, whereas *L. heterotoma* always laid a single egg in each host (1.00 ± 0.00). Regardless of the number of stings, *L. heterotoma* females never deposited more than eight eggs (i.e. the number of available host larvae), whereas the number of eggs laid by *L. boulardi* females increased linearly with the number of host encounters (Fig. 2). This suggests that the difference in the pattern of egg distribution by *L. heterotoma* and *L. boulardi* females does not result from differences in their searching efficiency, but

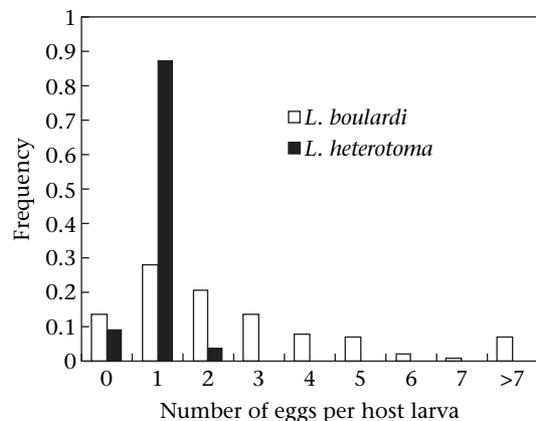


Figure 1. Distribution frequency of wasp eggs when females were constrained to stay on a patch of 10 *D. melanogaster* larvae. Individual distributions were pooled ($N = 10$ for both).

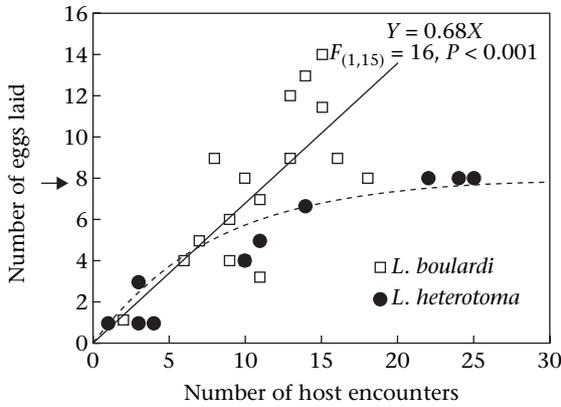


Figure 2. Relation between numbers of host encounters (N_e) and eggs laid (N) by the two wasps (on *D. melanogaster*). Arrow indicates the number of hosts present on patches (8). Broken line indicates expected values under assumptions of random search and total superparasitism avoidance ($N = 8 \times (1 - \exp(-N_e/8))$).

rather from differences in their acceptance of parasitized hosts: *L. heterotoma* rejected almost every parasitized host, whereas *L. boulandi* accepted some of them.

In both species, the rate of acceptance of unparasitized hosts was 1.0, since during observation in experiment 2, each encounter with the first host, obviously unparasitized, always led to an oviposition ($N = 12$, $N = 16$ for *L. boulandi* and *L. heterotoma*, respectively).

To quantify the rate of superparasitism acceptance, we built a simple model using data collected in experiment 2 on *D. melanogaster*. We assumed that (1) females lay only one egg per oviposition (based on the good correspondence between the numbers of observed ovipositions and the numbers of eggs found at dissection), and (2) each encounter with an unparasitized host is followed by an oviposition. Knowing the number of host encounters (N_e), the number of parasitized hosts (n_p) and the total number of eggs laid by each female (n_o), we can estimate for each female a mean rate of superparasitism acceptance as: $r = (n_o - n_p) / (N_e - n_p)$. The numerator corresponds to supernumerary eggs and the denominator to the number of encounters with parasitized hosts. The mean rate \pm SE was 0.41 ± 0.08 ($N = 16$) for *L. boulandi* and 0.00 ± 0.00 ($N = 12$) for *L. heterotoma*. In both species, these values are lower than rates of acceptance of unparasitized hosts (1.0), thus indicating that both of them are able to discriminate between unparasitized and parasitized hosts, although *L. boulandi* engaged in superparasitism much more often than *L. heterotoma*.

This difference in superparasitism behaviour of wasp species is not a consequence of their difference in egg load, which was lower in *L. boulandi* ($\bar{X} \pm SE = 189 \pm 7$) than in *L. heterotoma* (297 ± 8 ; Student's *t* test: $t_{47} = -6.82$, $P < 0.001$).

Experiment 1: Host Density

As predicted by optimal patch use theory, there was a clear positive relation between the richness of the patch and the time females of both species spent on it

(*L. boulandi*: $Y = 52.6X + 353.9$, $R^2 = 0.65$, $F_{1,48} = 88.3$, $P < 0.0001$; *L. heterotoma*: $Y = 67.2X + 343.8$, $R^2 = 0.82$, $F_{1,47} = 229$, $P < 0.0001$). An increase in patch residence time was associated with an increase in the number of parasitized hosts (Fig. 3). Numbers of hosts parasitized by the two species were the same at densities 4, 8 and 16, but not at density 32 where *L. boulandi* parasitized fewer hosts than *L. heterotoma* (Student's *t* test: $t_{17} = 3.70$, $P = 0.001$). No significant difference in patch times was observed between wasp species (log-rank test: all NS). Both parasitoid species were thus able to adapt their patch residence time to the quality of the patch.

Experiment 2: Within-patch Experience

The model including all the covariates was highly significant (Table 1, likelihood ratio test₁₄ = 71.2, $P < 10^{-8}$). As mentioned above, we had to stratify on the wasp species covariate which implies that we cannot test the effect of such a factor on leaving tendency. We can only estimate the effect of other covariates on the leaving tendency of each wasp species. Coefficients presented in Table 1 were arbitrarily fitted for a *D. melanogaster* patch since, in both parasitic wasps, effects of ovipositions and rejections during the current patch visit did not depend on the host species.

In *L. heterotoma*, each oviposition of the female during the current patch visit significantly decreased her patch-leaving tendency by 0.50 ($\exp(-0.70)$). We also found a strong effect of each host rejection on patch-leaving tendency ($\times 1.67$). Rejections concerned only already parasitized hosts, since every first contact with a host was followed by oviposition ($N = 22$). Ovipositions during previous visit(s) did not influence the duration of the female's next visit on the patch. Taken together, our results indicate that *L. heterotoma* females use only encounters with hosts (resulting in either oviposition or host rejection) to track the degree of patch exploitation. Neither rank of patch visit nor host species significantly influenced patch residence time. *Leptopilina heterotoma*

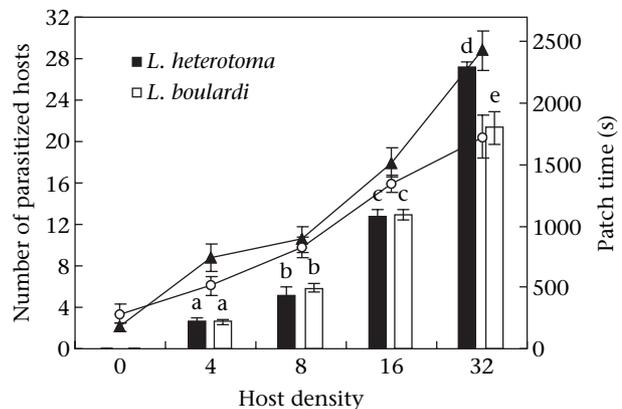


Figure 3. Effect of host density on patch time and number of parasitized hosts. Patch times are plotted as lines (right axis) and numbers of parasitized hosts as bars (left axis). Means are given \pm SE. Numbers of parasitized hosts with the same letter are not significantly different (Wilcoxon rank-sum test).

Table 1. Estimated regression coefficients (beta), SE, hazard ratio (exp(beta)) and level of significance (Wald test, $df = 1$) of the covariates included in the Cox regression

	<i>L. heterotoma</i>					<i>L. boulardi</i>				
	Beta	SE (beta)	exp(beta)	<i>P</i>	Effect	Beta	SE (beta)	exp(beta)	<i>P</i>	Effect
Ovipositions during current patch visit	-0.70	0.15	0.50	<0.001	↓	0.01	0.07	1.01	0.83	=
Rejections during current patch visit	0.51	0.11	1.67	<0.001	↑	0.27	0.26	1.30	0.31	=
Ovipositions during previous visit(s)	-0.02	0.11	0.98	0.87	=	0.14	0.05	1.15	<0.01	↑
Visits	0.19	0.12	1.21	0.12	=	-0.12	0.07	0.89	0.10	=
Host species (<i>D. subobscura</i>)	-0.49	0.32	0.61	0.12	=	0.76	0.28	2.12	<0.01	↑

Coefficients correspond to a *D. melanogaster* patch (reference level). Interaction terms with host species were all nonsignificant and led to the same effects of ovipositions and rejections. Arrows represent the effect on leaving tendency. All coefficients were computed with all other terms of the model.

parasitized the same numbers of larvae of the two host species before leaving (*D. melanogaster*: $\bar{X} \pm SE = 5.13 \pm 0.87$; *D. subobscura*: 5.74 ± 0.40 ; Student's *t* test: $t_{20} = 0.59$, NS), consistent with its ability to develop equally well in both host species (Carton et al. 1986; unpublished data).

The patch-leaving strategy of *L. boulardi* was very different. Neither oviposition nor rejection during the current patch visit had any influence on patch-leaving tendency (Table 1). In contrast to *L. heterotoma*, the number of ovipositions during previous visits to the patch had a strong influence on the current patch-leaving tendency. The more eggs laid during previous visits, the higher the leaving tendency during the current patch visit ($\times 1.15$). Thus, *L. boulardi* females modified their patch-leaving tendency according to events that occurred in the course of previous visit(s). As for *L. heterotoma*, rank of patch visit did not influence patch-leaving tendency. Nevertheless, we found in *L. boulardi* a strong effect of host species on patch-leaving tendency, which was 2.12 times as great on *D. subobscura* as on *D. melanogaster*. This difference in leaving tendency resulted in fewer parasitized hosts in *D. subobscura* patches (*D. melanogaster*: $\bar{X} \pm SE = 5.16 \pm 0.45$; *D. subobscura*: 3.03 ± 0.65 ; Student's *t* test: $t_{30} = 2.76$, $P < 0.01$), consistent with the lower suitability of *D. subobscura* for this wasp species (Carton et al. 1986; unpublished data).

DISCUSSION

The comparison of patch exploitation strategies of two sympatric parasitoids (*L. heterotoma* and *L. boulardi*) revealed a clear difference: *L. heterotoma* almost never superparasitized, whereas *L. boulardi* frequently did. The two species proved able to adjust their residence time to the richness of the patch of hosts, but they used different leaving decision rules.

First, our results confirm our report (Varaldi et al. 2003) that *L. boulardi* from the south of France express a strong superparasitism tendency. This superparasitism could result from a defect in the way the female perceives cues

signalling a previous infestation (a reduction in discrimination abilities). However, since females proved at least partly able to distinguish between parasitized and unparasitized hosts, their superparasitism behaviour could also result from a modification of their acceptance decision after signal perception (for instance a lower acceptance threshold). Whether this superparasitism behaviour is advantageous for the parasitoid in natural conditions cannot be determined at present. However, several arguments favour the hypothesis of nonadaptive superparasitism. First, the agent responsible for superparasitism is vertically transmitted through the maternal lineage, and also benefits from contagious horizontal transmission across parasitoid larvae within superparasitized hosts (Varaldi et al. 2003). Thus, an increase in superparasitism tendency enhances the horizontal transmission of infectious particles, allowing colonization of new parasitoid lineages. As a consequence, our hypothesis is that superparasitism behaviour in the Antibes strain of *L. boulardi* could reflect the strategy of the infectious element itself, rather than that of the parasitoid. Furthermore, the game theory model of Visser et al. (1992a) showed that when searching alone on a patch of healthy hosts, a female should never self-superparasitize (acceptance of hosts parasitized by herself), suggesting that the behaviour observed in *L. boulardi* does not result from an adaptive decision of the parasitoid. An alternative adaptive interpretation for self-superparasitism would consider the increased ability to overcome the *Drosophila* larva's immune reaction (encapsulation). However, encapsulation of *L. boulardi* eggs by sympatric *D. melanogaster* larvae is extremely low in the Mediterranean basin (Dupas et al. 2003) and could hardly account for this behaviour. For all these reasons, we hypothesize that self-superparasitism in the Antibes strain of *L. boulardi* is maladaptive to the wasp.

In contrast to *L. boulardi*, females of the Antibes strain of *L. heterotoma* seldom superparasitized during our experiment where they were foraging alone. They engage in superparasitism only when there is competition for hosts (Visser et al. 1992a, b, c; Visser 1995), which is expected under adaptive superparasitism assumptions (Van Alphen & Visser 1990).

According to theoretical prediction (Rosenheim & Mangel 1994), when a parasitoid expresses nonadaptive superparasitism, it should be selected to promote early patch departure, thus reducing risks of self-superparasitism and associated costs. Furthermore, optimal patch time is sensitive to even low rates of superparasitism acceptance (<0.1). Under our assumption that superparasitism of *L. boulandi* is nonadaptive, this prediction was even more likely to be met, since this rate equals 0.41 in this strain. However, our results do not support this prediction: despite their different superparasitism behaviour, the two *Leptopilina* species invested the same time and parasitized the same number of hosts (except at density 32). The only experimental support of this theory comes from Outreman et al. (2001), who found that female *Aphidius rhopalosiphi*, parasitoids of the grain aphid, do not discriminate freshly parasitized hosts (<3 h) and reject some healthy hosts, resulting in incomplete patch exploitation, which is thought to be consequence of imperfect host discrimination. Since the cost of self-superparasitism encompasses the cost of both supernumerary eggs and the extra time allocated to already parasitized hosts, self-superparasitism is probably especially costly to parasitoids with a limited egg load ('egg limited'). It is plausible that *A. rhopalosiphi* is much more egg limited than *L. boulandi*, accounting for the difference between the two species. Of course, an alternative explanation would be that our hypothesis that *L. boulandi* expresses nonadaptive superparasitism is invalid.

The Cox regression allowed us to study the proximal mechanisms involved in the patch-leaving decision. *Leptopilina heterotoma* females used contacts with hosts to adjust patch residence time and, consistent with previous results (Haccou et al. 1991), ovipositions increased patch time. This suggests that *L. heterotoma* females use an incremental mechanism, which is well suited to aggregated host distribution, with females having poor prior information about host density (Iwasa et al. 1981). Host rejections proved efficient in increasing the leaving tendency of *L. heterotoma*. This effect was also documented in a closely related species (Hemerik et al. 1993) and in two egg parasitoids (Wajnberg et al. 1999, 2000), but it was never clearly shown in *L. heterotoma* (but see Van Alphen & Galis 1983; Van Lenteren 1991; Van Alphen 1993). Rejections occurred more and more frequently in the course of patch exploitation, thus keeping the foraging female informed about progress in patch depletion. From this effect we can infer strong consequences at the population level, since when arriving on a patch already attacked by conspecific(s), a foraging female will leave it quickly and search for other unparasitized patches, thus reducing intraspecific competition on the patch and enhancing the parasitization rate.

In contrast to *L. heterotoma*, *L. boulandi* females did not vary their leaving tendency after oviposition or rejection. The only covariate clearly influencing patch departure was the number of egg(s) laid during previous visit(s) to the patch. This covariate corresponds to the degree of patch exploitation at the time the female re-enters the patch, and should not be taken as the 'proper' effect of oviposition (see Methods). This means that the more the female

exploited the patch in previous visits, the higher its leaving tendency at the next visit. Adjustment of total patch time should be achieved through progressively shorter visits on the patch, each of them being negatively influenced by the number of ovipositions during the previous visit. Different hypotheses could explain such a pattern, such as patch marking, a decrease in motivation caused by egg depletion, habituation to host kairomones, or tiredness. Thus, our results suggest that *L. boulandi* females use an indifferent mechanism (no effect of ovipositions, Driessen & Bernstein 1999) which has never before been documented in parasitoids.

How can one explain the difference in the effect of oviposition in *L. heterotoma* and *L. boulandi*? First, for *L. boulandi*, the effect of oviposition we measured corresponds to the mean effect of oviposition in unparasitized and parasitized hosts, since we could not distinguish between them. Thus, we cannot rule out the possibility that the effects of ovipositions in both host types are significant but act in an opposite way (for instance incremental in unparasitized and decremental in parasitized), thus cancelling each other out. This scenario fits the case where females correctly perceive the parasitized status of the host but decide to superparasitize. In contrast, if ovipositions in unparasitized and parasitized hosts have no influence on *L. boulandi* patch time, what is the adaptive significance of the differences between *L. heterotoma* and *L. boulandi*? Since both related and unrelated *Drosophila* parasitoids reveal incremental mechanisms (*L. heterotoma*, *L. clavipes* and *A. tabida*, Van Alphen et al. 2003), we can speculate on the ancestral effect of oviposition in *L. boulandi* as being incremental. The evolution of *L. boulandi* to an indifferent mechanism is consistent with the hypothesis that superparasitism is caused by a reduction in (but not loss of) the ability of the female to recognize parasitized hosts. Indeed, when ovipositions do not bring the female reliable information on patch quality, alternative patch-leaving rules that do not make use of oviposition information can be favoured by natural selection. Clearly, further investigations are required to evaluate the different hypotheses considered here. Whatever the hypothesis explaining such a pattern, these results show that the patch exploitation strategy of parasitoids exploiting mostly the same hosts can vary between species.

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