Journal of Animal Ecology

British Ecological Society

Journal of Animal Ecology 2012, **81**, 503–511 doi: 10.1111/j.1365-2656.2011.01926.x

Multi-objective behavioural mechanisms are adopted by foraging animals to achieve several optimality goals simultaneously

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Summary

1. Animals foraging for resources are under a variety of selective pressures, and separate optimality models have been developed predicting the optimal reproductive strategies they should adopt.

2. In most cases, the proximate behavioural mechanisms adopted to achieve such optimality goals have been identified. This is the case, for example, for optimal patch time and sex allocation in insect parasitoids. However, behaviours modelled within this framework have mainly been studied separately, even though real animals have to optimize some behaviours simultaneously.

3. For this reason, it would be better if proximate behavioural rules were designed to attain several goals simultaneously. Despite their importance, such multi-objective proximate rules remain to be discovered.

4. Based on experiments on insect parasitoids that simultaneously examine their optimal patch time and sex allocation strategies, it is shown here that animals can adopt multi-objective behavioural mechanisms that appear consistent with the two optimal goals simultaneously.

5. Results of computer simulations demonstrate that these behavioural mechanisms are indeed consistent with optimal reproductive strategies and have thus been most likely selected over the course of the evolutionary time.

Key-words: insect parasitoids, Monte Carlo simulation, multi-objective, patch time allocation, sex allocation

Introduction

Animals are continuously under selection to adopt reproductive behaviours that lead them to maximize the number of offspring they produce during their life (Stephens & Krebs 1986). An important body of both theoretical and experimental studies has explored the foraging behaviour of different animal species, assuming that natural selection will favour individuals which exploit their resources most efficiently. One of the most important selective constraints faced by foraging animals concerns the way they optimize the time they allocate to resources exploitation (Wajnberg 2006). This is especially true for insect parasitoids that are time-limited, dying before they can deposit all their eggs (Rosenheim 1999), and that have to exploit resources (hosts) that typically occur in discrete, depletable patches in the environment (Godfray 1994; Wajnberg 2006). The optimal amount of time female parasitoids allocate to each patch of hosts they visit has been the subject of active research over the past few decades (Stephens & Krebs 1986; van Alphen, Bernstein & Driessen 2003; Wajnberg 2006). Several theoretical models have been developed to predict the optimal amount of time females parasitoid should remain on patches of their hosts. The most important is the Marginal Value Theorem (MVT; Charnov 1976), which states that females should leave a patch the instant their encounter rate with hosts in the patch drops below the average rate available in the environment. Among other things, this model and its subsequent modifications predict that females should stay longer on patches containing more hosts to attack (McNair 1982), and patches of different quality should be reduced to the same level of profitability before leaving (Cook & Hubbard 1977; Wajnberg 2006).

Besides optimizing their foraging time on host patches, animal species also face other selective constraints leading them, for example, to optimize the proportion of sons in their progeny (i.e. sex ratio) (Charnov 1982; West 2009). Most parasitoid species have a haplodiploid sex determination system under which males develop from unfertilized eggs and females from fertilized eggs. By controlling egg fertilization, females can accurately control the sex ratio of their offspring (Waage 1986; King 1987; West 2009). Several evolutionary models have shed light on the optimal sex allocation strategy female parasitoids should adopt. Among them, the theory of Local Mate Competition (LMC) has probably been the most important contribution (Hamilton 1967). This model, which

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applies to a common parasitoid life history, considers structured populations in which mated females colonize host patches on which their progeny develop and mate before dispersal to find new patches. The LMC model predicts that the optimal strategy for the mothers is to produce a sex ratio that depends on the number of females colonizing the patch (Hamilton 1967; Charnov 1982; Waage 1986; West 2009). More accurately, when n females colonize a patch of hosts, the optimal percentage of males they have to produce should be $(n-1)/2n$ (Hamilton 1967).

Both the MVT and the LMC models make theoretical predictions that are usually well matched by many animal species (Hardy 1994; Wajnberg 2006). To achieve this, individuals of many species have developed proximate, rule-based behavioural mechanisms that appear consistent with the goal-based optimal patch time and sex allocation predictions separately (Hardy 1992; Wajnberg, Fauvergue & Pons 2000; Wajnberg 2006). For example, considering the optimal residence time on host patches, Waage (1979) proposed a mechanism in which a parasitic wasp encountering a host patch starts to search for hosts with some level of motivation to remain. The motivation then decreases with time until a threshold is reached leading the wasp to leave. Each host found and attacked causes an increment in the motivation, leading the foraging animal to remain longer on patches of better quality. Iwasa, Higashi & Yamamura (1981) theoretically demonstrated that such a simple, so-called incremental rule can lead to patch residence times that closely approximate the optimal strategy when there is large variance in the number of hosts per patch and thus a low reliability in the expectation of host availability by the foraging animal. A decremental rule, in which each host attack causes a decrease in the motivation of the wasp to remain, is shown to be conversely adaptive when hosts are uniformly distributed among patches, and thus when there is a high reliability in the expectation of host availability by the foraging wasp (Iwasa, Higashi & Yamamura 1981; Wajnberg 2006). Similarly, female wasps are able to adjust offspring sex ratio according to the number of ovipositing females on a host patch using different proximate mechanisms. For example, females respond to the frequency of physical contacts with other females (Wylie 1976) or to the perception of chemical traces left by previous females on the host patch (Viktorov & Kochetova 1973). However, the most efficient and simple mechanism is for females to lay their sons and daughters in a particular order, usually starting with male eggs (Hardy 1992; Wajnberg 1993). Using a simulation model, Wajnberg (1994) demonstrated that such a 'male-first strategy' appears to be consistent with the prediction of the LMC model.

Optimal reproductive behaviours promoted by other selective constraints have also been studied (e.g. optimal number of eggs laid per host, see Parker & Courtney 1984; etc.). In all cases, animals were shown to adopt proximate behavioural rules consistent with predictions from optimality models. In most of the cases, each of the behaviours analysed within such a theoretical framework mainly has been studied separately, especially when the goal was to look for proximate behavioural mechanisms adopted by foraging animals. However, animals are continuously under a set of several selective pressures leading them to optimize all behaviours simultaneously. Some of these constraints can sometimes even be conflicting (Rothley, Schmitz & Cohon 1997), and it would thus be better if multi-objective proximate behavioural rules were designed to attain several goals simultaneously. Such multi-objective behavioural rules remain to be discovered.

Finding multi-objective solutions to optimization problems in engineering application has always been considered difficult (Deb 2001). It is thus of interest to determine how foraging animals have been selected to solve similar kinds of problems. In this study, I examined potential multi-objective behavioural rules adopted by parasitic wasps and that are consistent with patch time and sex allocation optimal predictions simultaneously. Experiments were conducted on mated females of the egg parasitoid *Trichogramma chilonis* Ishii (Hym.; Trichogrammatidae) to which patches of one of their hosts, eggs of the Mediterranean flour moth *Ephestia kuehniella* Zeller (Lep.; Pyralidae), were offered. Patches containing different numbers of hosts were used, and through an accurate analysis of females' patch time and sex allocation strategy, accurate potential multi-objective behavioural rules were identified in the light of the predictions of both the MVT and the LMC. A series of Monte Carlo simulations were then developed, demonstrating that the rules observed were indeed consistent with the two optimal goals simultaneously. These results are the first demonstrating that animals can develop multi-objective behavioural reproductive mechanisms leading them to approximate optimal reproductive strategies in complex, multi-demanding, real-world environments (Schmitz *et al*. 1998).

Materials and methods

INSECTS AND EXPERIMENTAL DESIGN

Trichogramma chilonis is a polyphagous, haplodiploid parasitic wasp that attacks eggs of several moth species that usually occur in discrete patches in the environment. Like all *Trichogramma* species, *T. chilonis* is known to adjust the number of eggs laid per host according to host size. On *E. kuehniella*, females usually lay one egg per host. The species is also known to adopt a sex allocation strategy that is consistent with the prediction of the LMC (Suzuki, Tsuji & Sasakawa 1984). The strain used in the experiment originated from a population that was collected several years ago and that has been maintained in the laboratory on *E. kuehniella* eggs at 25 °C, LD 12:12. Isolated females were offered a single patch of UV-killed *E. kuehniella* eggs arranged on a regular square grid pattern, with a distance of one mm between the hosts aligned in rows and columns. Three patch sizes were compared (number of replicates in parentheses): four (61), nine (62) or 16 (63) hosts. Wasp females were < 24 h old and honey-fed. They were kept individually with a male for mating and were not allowed to contact any host before the experiment.

All experiments were carried out during daytime at 25 ± 1 °C and 60 ± 5% RH. Hosts were not replaced during the observation, so patches suffered a continuous depletion. Females were free to leave the patch whenever they wanted, and their behaviour was observed continuously from the moment they entered the patch for the first time until they left the patch for more than 60 s. Using an event recorder, the time of entering and leaving the patch was recorded along with the time of each oviposition and the location of each host attacked. Among other things, this last information was used to identify superparasitized hosts.

The data were used to quantify females' patch residence time and the level of patch profitability before leaving. This last parameter, which corresponds to females' rate of reproductive success just before leaving, was estimated as the rate of hosts attacked during the last 5 min before the patch was abandoned (Wajnberg, Fauvergue & Pons 2000). Additional computations were performed estimating final rates of reproductive success over an interval of time ranging from 1 to 10 min. Results led quantitatively to the same conclusion in all cases. In principle, the sex of each egg laid can be determined by observing the abdominal movements of the female during the oviposition behaviour (Suzuki, Tsuji & Sasakawa 1984). In the present work, however, to obtain a large number of replicates, hosts attacked during the experiment were isolated and the sex of the emerging progeny was recorded after development at 25 °C. In turn, the information was used to quantify the sex-sequence pattern laid by the females observed in each case.

STATISTICAL ANALYSIS

Sequences in which male and female progeny were laid in each case were first described according to the recorded data (see Fig. 2). These sequences were then used in a Cox proportional hazards model to quantify the behavioural mechanisms used by the females to trigger their patch-leaving decision (Wajnberg 2006). Briefly, this special type of regression models the data in terms of leaving tendency (so-called hazard rate), which is the probability per unit of time that a female leaves the patch given that she is still on it. This leaving tendency can be modified by some predefined explanatory factors (i.e. covariates) according to the following equation:

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

in which $h(t)$ is the hazard rate, $h_0(t)$ is the innate patch-leaving tendency (so-called baseline hazard), t the time passed since the female entered the patch, and β_i the regression coefficients that give relative contributions of p covariates z_i. The relative contribution of the covariates can be interpreted through the exponential term (so-called hazard ratio). A hazard ratio greater than one indicates an increasing effect of the associated covariate on the females' patch-leaving tendency, while a hazard ratio lower than one will be interpreted in the opposite way. The effects of the number of sons and daughters laid by the females on their patch-leaving decision were tested in the model as two time-dependant covariates, while the initial size of patch was added as a fixed categorical covariate.

The experimental protocol used generated a small number of missing values due either to pre-adult mortality of the developing wasps or to superparasitism events (in 76 and 37 cases of the 1490 host attacks observed, respectively). In these cases, the sex of the missing progeny was estimated by the average sex ratio observed at the corresponding rank of oviposition. Hence, the Cox regression model used in this study was sometimes based on two events appearing simultaneously, coded as the probability of laying a son or a daughter, respectively. Regression coefficients of the model were estimated from the data by partial likelihood maximization, and the significance of the covariates was tested using standard likelihood ratio tests through an iterative procedure. Finally, the fit of the model was assessed by making residual plots (see Wajnberg, Rosi & Colazza 1999 for an example of such a plot). All computations were performed in S-Plus (Venables & Ripley 1994).

Results

Trichogramma chilonis females stayed longer on patches containing initially a higher number of hosts (Fig. 1a), but their final rates of progeny production remain statistically similar, whatever the initial quality of the patch they were offered (Fig. 1b). Thus, at first glance, their patch exploitation strategy seems to be consistent with the optimal predictions of the MVT.

Fitting a Cox regression model to the data revealed that the patch-leaving tendency of the females depends on the initial quality of the patch the females were foraging on (likelihood ratio test: χ^2 = 152·72, 2 d.f., P < 0·0001), with females leaving earlier from poorer quality patches. Furthermore, each daughter laid by the females increases their patch-leaving tendency by a factor of 1·199 (regression coefficient: 0·1811 ± 0·0416, χ^2 = 21·43, 1 d.f., P < 0·0001) but, although remaining always significant, such a decremental effect was less pronounced on higher quality patches (interaction 'patch quality' × 'laying a daughter': χ^2 = 11·59, 2 d.f., P = 0·003). On the other hand, laying a son did not appear to have a significant effect on the patch-leaving tendency of the females (regression coefficient: −0·0613 ± 0·0936, χ^2 = 0·4316, 1 d.f., P = 0·511) regardless of the initial qual-

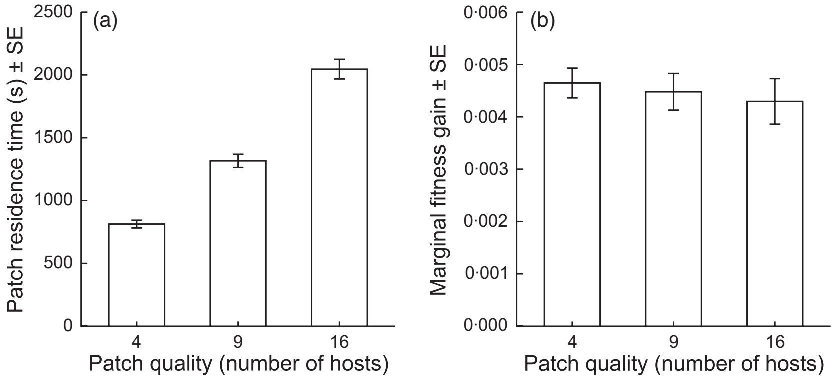

Fig. 1. Average (±SE) patch residence times (a) and marginal fitness gains (b) of *Trichogramma chilonis* females on patches containing different numbers of hosts. Patch residence times are strongly influenced by patch quality (Log-rank test: χ^2 = 185·88, 2 d.f., P < 0·0001). Marginal fitness gains are expressed as the number of progeny produced per second during the terminal 5-min period on the patch. Marginal fitness gains averages do not differ significantly between the three patch qualities (Kruskal–Wallis test: χ^2 = 0·789, 2 d.f., P = 0·674).

ity of the patch offered to the females (interaction 'patch quality' × 'laying a son': $\chi^2 = 3{\cdot}29$, 2 d.f., $P = 0{\cdot}193$).

Because daughters are laid preferentially later in oviposition bouts, it is possible that the decremental effect of laying a daughter might simply be the effect of patch depletion alone and thus might not really be related to the actual sex of the egg laid by the mother. Two approaches were used to determine whether there might be indeed a confounding effect between the sex of the progeny laid and patch depletion. First, the Cox regression model was fit again to the observed data after shuffling randomly the observed sequence in which sons and daughters were laid. Hence, in such a re-sampling procedure, observed patch residence times and numbers of sons and daughters actually produced remained the same, but the order in which they are laid was randomly drawn. Such a re-sampling procedure thus enables to test the effect of laying sons or daughters whatever their location in the egg-laying sequence, thus disentangling the effect of the sex of the progeny from patch depletion. The re-sampling procedure was repeated a thousand of times, and in all cases, laying a daughter still had a significant decremental influence on the patch residence time of the mothers, while laying a son still had no significant effect. This demonstrates that this is the number of sons and daughters laid that matters, not the actual times at which they are laid. Second, additional experiments were performed with virgin mothers that are constrained to lay sons only. These additional experiments were performed under exactly the same conditions and at the same time as the original one. Fitting a Cox regression model to these additional data revealed that laying a son never significantly influenced the patch-leaving decision of the mothers ($\chi^2 = 1{\cdot}36$, 1 d.f., $P = 0{\cdot}243$; on average 20 females measured for each of the three patch qualities) despite the fact that patches were depleted like in the original experiment. These two arguments demonstrate that the sex of the eggs laid significantly influences the patch-leaving decision of the mothers when daughters are laid, rather than patch depletion alone.

Concerning sex allocation, *T. chilonis* females lay male eggs early in their ovipositional bout (here, on average, at the second position) and then mostly lay female eggs (Fig. 2). Such a so-called male-first strategy is observed regardless of the initial quality of the patch females were foraging on. As was demonstrated theoretically (Wajnberg 1994), such a sex-sequence reproductive strategy enables the females to approach the predictions of LMC.

SIMULATION MODELS

Are the simple patch-leaving rules and sex-sequence patterns observed sufficient for the females to approach the predictions of optimal patch time and sex allocation simultaneously? At first glance, this could be the case. Indeed, males are laid preferentially early in the ovipositional bout. The number of daughters laid subsequently should correspond, at least under extreme LMC, to the mating capacity of the males previously laid (Waage 1986). Hence, it would not be adaptive for a female to leave the host patch too soon each time a son is laid lest too few daughters are subsequently laid to serve as mates for each son. Conversely, it would not also be adaptive for a female to leave the host patch too late each time a son is laid lest too many daughters are subsequently laid exceeding the mating capacity of the sons, leaving some daughters unmated. Such arguments would be even stronger under situations in which optimal sex ratio is less female-biased, e.g. under partial LMC, stochasticity in sex allocation, developmental mortality or limited insemination capacity of males (Hardy 1994; Hardy *et al.* 2000). This is likely the reason why laying a son did not significantly influence the patch-leaving decision of the females. On the other hand, ovipositing females should become progressively motivated to leave the patch as more daughters are laid. Ideally, the mother should leave the patch the moment she had laid the total number of daughters that can be mated by the sons previously laid. Therefore, laying daughters should have a decremental influence on the patch residence time of the ovipositing mother. Without such a decremental effect, too many daughters would be laid on a patch and some of them would remain unmated (Hardy & Cook 1995). When the number of hosts available increases, laying sons still did not influence the patch-leaving decision of the females, although they are laying an increasing number of male eggs (on aver-

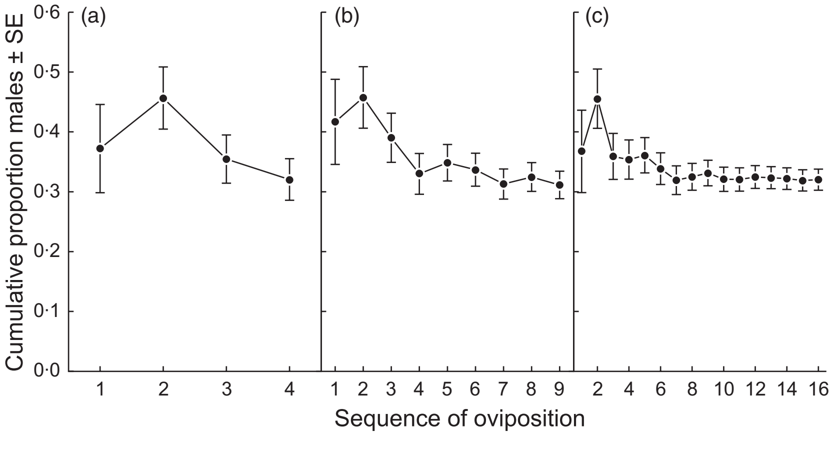

Fig. 2. Average (±SE) cumulative sex ratio produced by *Trichogramma chilonis* females as a function of the sequence of oviposition on patches containing either four (a), nine (b) or 16 (c) hosts.

age 1·25 ± 0·08, 2·55 ± 0·13 and 3·89 ± 0·19, on patches containing four, nine and 16 hosts, respectively). In this case, females should remain longer to attack more hosts and to lay more daughters to be mated. This should be the reason why the decremental influence of laying daughters on the females' patch residence time, although remaining significant, was observed to be less pronounced on patches containing more hosts.

To obtain an accurate quantitative understanding of the adaptive meaning of the patch time and sex allocation mechanisms observed, a series of Monte Carlo simulations was developed. The detailed procedure is described in the Appendix. Using this simulation procedure, two complementary questions were addressed: (i) Are the observed effects of laying a son and a daughter on the patch-leaving decision of the females leading them to control their patch residence time in a way that appears consistent with the predictions of the MVT? For this, simulations were run with different values of the Cox's regression parameters describing the effect of laying a son and a daughter, with an egg-laying sequence corresponding to the one observed experimentally. (ii) Conversely, given the effects of laying a son and a daughter on the patch-leaving decision of the females, does the observed sex-sequence pattern also enable females to organize their patch time allocation behaviour in a way that approaches the optimal predictions of the MVT? Here, simulations were carried out with different intensities of the male-first strategy, using the Cox's regression parameters estimated on the observed females.

Results of simulations testing the effects of laying a son and a daughter on the females' patch-leaving decision are shown Fig. 3. Kruskal–Wallis χ^2 tests have been used to compare the marginal fitness gains on the three patch qualities, and the values obtained are presented to describe the deviation between the reproductive strategy of the simulated females and the optimal predictions of the MVT. As can be seen on this figure, the strategy adopted by the observed *T. chilonis* females (i.e. not changing patch-leaving decision when a son is laid but increasing it when this is a daughter) corresponds with one of the lowest, non-significant values, suggesting that the females behave consistently with one of the predictions of the MVT (i.e. patches are reduced to the same level of profitability before leaving). Other possible behavioural strategies would also lead to patch time allocation approaching the predictions of the MVT. For example, an incremental effect associated with each son laid, instead of no effect, would probably also produce a patch time allocation consistent with the MVT (see the left side of the black dot on Fig. 3).

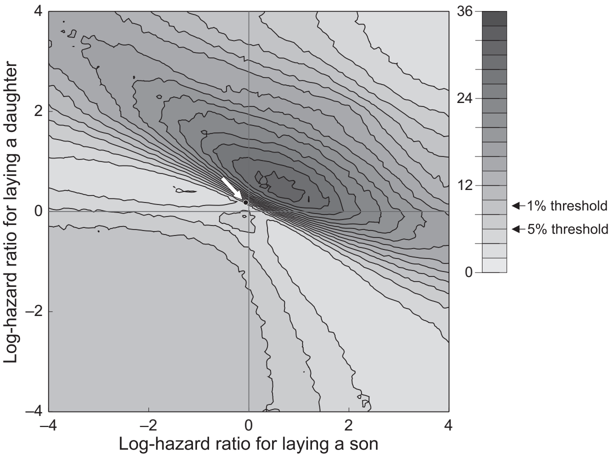

Fig. 3. Contour plot showing Kruskal–Wallis χ^2 test values (2 d.f.) comparing the marginal fitness gains (expressed as the number of progeny produced per second during the last 5 min before leaving the patch) of simulated wasp females foraging on patches containing initially either four, nine or 16 hosts, computed with different effects of laying a son (*x*-axis) and a daughter (*y*-axis) on their patch-leaving decision. Both axes represent regression parameters used in the Cox model for the simulation and values used ranged from −4·0 to 4·0, with a step of 0·1. On both axes, negative values correspond to an incremental influence on the females' patch-leaving tendency, while positive values correspond to a decremental effect. Each value represents the average of 1000 simulations. The black dot (white arrow) corresponds to the values estimated on real *Trichogramma chilonis* females (see text). Five per cent- and 1%-risk critical threshold values for a χ^2 test with 2 d.f. are indicated (i.e. 5·991 and 9·210, respectively). Lower Kruskal–Wallis χ^2 test values indicate higher ability of simulated females to follow one of the predictions of the Marginal Value Theorem.

Reciprocally, Fig. 4 gives the results of simulations testing the influence of different sex-sequence patterns on females' patch time allocation strategy, providing that effects of laying a son and a daughter on their patch-leaving decision are those observed on real *T. chilonis* females. Here again, Kruskal–Wallis χ^2 test values are used to see how accurately simulated females are following one of the predictions of the MVT. For given observed effects of laying a son and a daughter on females' patch-leaving decision, laying males early in their oviposition bout leads wasp females to behave progressively more and more consistently with one of the predictions of the MVT (i.e. equal patch profitability before leaving).

Discussion

When offered patches containing different initial numbers of hosts, *T. chilonis* females adopted a patch time allocation strategy that seems to be in agreement with the predictions of the MVT (i.e. longer patch residence times on better quality patches with a constant final rate of progeny production). A strict evaluation of the optimality of the patch time allocation strategy adopted by the females would imply a comparison of the observed final rate of progeny production with the long-term fitness gain rate achievable in the environment, but such information is not known. Hence, it remains possible that the final rate of progeny production observed, although not depending on patch quality, could be somewhat different from the real optimal gain rate females could achieve in their environment. An average of 0·37 ± 0·09, 1·63 ± 0·26 and 3·75 ± 0·39 hosts remained unattacked when the females left patches containing four, nine and 16 hosts, respectively. Therefore, the patch exploitation strategy adopted by

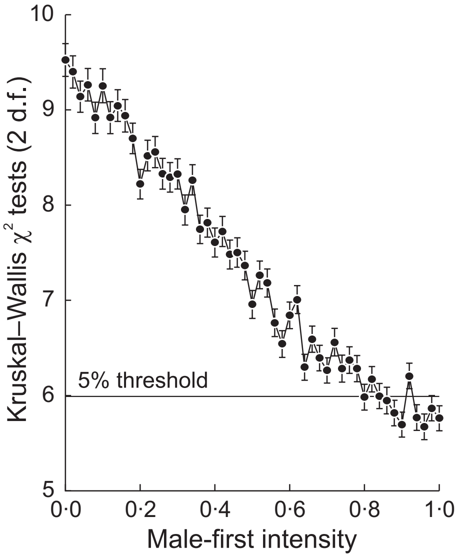

Fig. 4. Kruskal–Wallis χ^2 test values (2 d.f.) comparing the marginal fitness gains (expressed as the number of progeny produced per second during the last 5 min before leaving the patch) of simulated wasp females foraging on patches containing initially either four, nine or 16 hosts, computed for different intensity of the male-first strategy, from 0·0 to 1·0, with a step of 0·02. Small values on the x-axis correspond to sequences in which males are laid close to the end, while values close to 1·0 are describing sequences having males laid at the beginning. Each plotted value represents the average (±SE) of 1000 simulations. Five per cent-risk critical threshold value for a χ^2 test with 2 d.f. is indicated (i.e. 5·991). Lower Kruskal–Wallis χ^2 test values indicate higher ability of simulated females to follow one of the predictions of the Marginal Value Theorem.

T. chilonis females appears to be more complex than a simple complete exploitation before leaving.

It is hard to believe that foraging animals, and especially parasitoids exploiting hosts that are discrete resource items, are strictly estimating their marginal fitness gain rate to trigger a patch-leaving decision as suggested by the MVT. Hence, using both theoretical and experimental analyses, several authors hypothesized and sometimes verified that animals can base instead their leaving decisions on a so-called potential value rule that estimates patch quality as the expected number of remaining resource items a forager will obtain divided by the expected amount of time spent to find them (van Gils *et al.* 2003; Green 2006; Biernaskie, Walker & Gegear 2009). Although the potential value model can help identify optimal behaviours, it is considered less useful for identifying the actual proximate patch-leaving mechanisms adopted by foraging animals (Biernaskie, Walker & Gegear 2009). This is the reason why a Cox proportional hazards model was used here to analyse accurately the proximate patch-leaving rules used by *T. chilonis* females. Results revealed that laying a son did not influence females' patch-leaving decision, while laying a daughter was associated with a significant decremental mechanism whereby decreasing their patch residence time.

In a recent review, Wajnberg (2006) published a list of all studies describing the incremental/decremental influence of host encounter/attack on the patch-leaving decision of parasitic wasp females. Four more studies have been published since then (Lucchetta *et al.* 2007; Amat, Desouhant & Bernstein 2009; Jenner & Roitberg 2009; and Louâpre *et al.* 2011), representing a total of both 38 references and 37 different species. Among them, 17 species were haplodiploid, a reproductive system in which mated females produce both son and daughter eggs. In 14 species, experiments were performed with mated females. Then, for these species, the correct identification of an incremental or decremental effect of each host attack is most likely ambiguous because, in these studies, both male and female egg-laying events were pooled. More accurately, because the sex ratio is usually highly female-biased in parasitic wasps (Hamilton 1967), the effect measured in these studies was most likely the average effect of laying daughters only. In the present study, fitting a Cox's regression on pooled male and female egg-laying events leads indeed to a global significant decremental effect (likelihood ratio test: $\chi^2 = 25{\cdot}29$, 1 d.f., $P < 0{\cdot}0001$), and this is because of the laying of daughter eggs only with a global sex ratio that was indeed female-biased (Fig. 2).

In *Encarsia formosa*, the first egg laid in a host after entering a patch has a stronger influence on the patch-leaving decision of the females than the following eggs (van Roermund, Hemerik & van Lenteren 1994). Such a result could be a consequence of the fact that females of this species always begin their oviposition sequences by a particular sex which subsequently influences females' patch-leaving decision. Conversely, the sex of the following eggs laid might be either male or female, leading to non-significant effects on females' patch-leaving tendency. Finally, several studies demonstrate differences in patch time allocation strategy between virgin and mated females of haplodiploid wasp species (e.g. Michaud & Mackauer 1995; Kugimiya *et al.* 2010). Because only mated females are producing daughters, differences observed in patch exploitation strategy between virgin and mated females could be the result of the fact that laying a daughter might have an influence on females' patch-leaving decision, as this is the case here, modifying the patch residence time of mated females only.

Iwasa, Higashi & Yamamura (1981) demonstrated theoretically that an incremental patch-leaving mechanism associated with each host attacked can lead to optimal patch residence times when there is a large variance in the number of hosts per patch. Conversely, these authors also demonstrated that a decremental mechanism would be adaptive when hosts are uniformly distributed among patches. Thus, in this study, the decremental effect observed each time a daughter is laid should likely result from *T. chilonis* females exploiting hosts that are uniformly distributed or equivalently exploiting patches of small size. Unfortunately, the spatial distribution of potential hosts in natural patches is not accurately known, preventing confirmation of such a plausible hypothesis (Wajnberg *et al.* 2003). Results presented here demonstrated that the decremental effect observed is also most likely linked to the way females optimize their sex allocation strategy. Following Iwasa, Higashi

& Yamamura (1981), patch-leaving mechanisms adopted by foraging wasp females have been almost always interpreted as being adaptive answers to the distribution of their hosts (Wajnberg 2006; but see Lucchetta *et al.* 2007). Results presented here indicate that patch-leaving rules should also have additional adaptive meanings for animals facing selective constraints that are not necessary linked to their optimal patch time allocation strategy.

Trichogramma chilonis females are preferentially laying their male eggs early in their oviposition bouts (Suzuki, Tsuji & Sasakawa 1984). Such a so-called male-first strategy, also observed in several other wasp species (Hardy 1992; Godfray 1994), has always been presented as an elegant and efficient reproductive strategy enabling the females to follow the optimal predictions of the LMC, as has been shown on several *Trichogramma* species (Suzuki, Tsuji & Sasakawa 1984; Waage & Lane 1984; Waage 1986; Wajnberg 1994). Results presented here indicate that such a strategy would be even more elegant than this because, when combined with simple patch-leaving behavioural rules, it would enable females to follow simultaneously the predictions of the MVT.

Despite that, in real-world environments, animals have to face several selective constraints simultaneously, both optimization models and experimental studies are usually over-simplistic in that they typically focus on a single specific component of life history or behaviour (Schmitz *et al.* 1998). This is the case for optimal patch time and sex allocation problems that were addressed up to now largely independently. Foraging animals are usually supposed to be unable to reach an optimal compromise coping with several selective constraints because the information they have to process for this is most likely too complex (Ward 1993). Here, however, patch time and sex allocation strategies, two of the most important reproductive constraints faced by animal species, were studied, and in the case of the parasitoid species studied here, wasp females seemed to be able to set-up simple and elegant behavioural rules to optimize both strategies. For this, females should simply (i) lay their male eggs first, (ii) not change their patch-leaving tendency each time a son is laid, and (iii) increase their tendency to leave after laying each daughter.

As a general rule, although foraging animals are continuously under a set of several selective pressures leading them to adopt reproductive compromises, only a very small number of studies have tried to unravel the optimal, multi-objective decision-making processes they should adopt. On insect parasitoids, for example, studies have only addressed optimal sex allocation strategy simultaneously with (i) the decision to lay more than one egg per host (i.e. superparasitism) and the optimal number of eggs to lay (Suzuki & Iwasa 1980; Werren 1980; Waage & Ng 1984; Godfray 1986; Nagelkerke, van Baalen & Sabelis 1996; Mayhew & Godfray 1997; West, Flanagan & Godfray 1999; Abe *et al.* 2003), and (ii) the optimal number of host patches to visit (Nagelkerke 1994). The work presented here opens a new field, linking ultimate predictions with proximate behavioural rules in a multi-objective context. Mechanistic behavioural rules linking different optimal reproductive decisions of foraging animals should now be analysed (e.g. on optimal competing strategy or optimal diet choice, etc.).

In the present work, the multi-objective behavioural mechanisms observed were analysed mainly in the light of the predictions of the MVT. Simulation results were compared to the predictions of this theoretical model only (see Figs 3 and 4). The reason is that experiments presented were performed with isolated females, preventing to test the main prediction of the LMC, i.e. a change in the sex ratio produced as a function of the number of females exploiting a host patch. Therefore, experiments are now being performed by offering patches of hosts to different number of females, trying to identify their multi-objective behavioural decisions. Patch-leaving rules of several females competing together on a patch have already been accurately quantified (Wajnberg, Curty & Colazza 2004). Identifying their meaning in terms of optimal sex allocation would certainly lead to a better understanding of what sort of behavioural mechanisms are adopted by competing foraging animals to simultaneously attain several optimal goals.

Acknowledgements

C. Curty is thanked for her help in running the experiment. P. Coquillard, F. Kjelberg L. Mailleret and O. Pons are thanked for their expertise at different stages of development of the ideas presented in this work. P. Coquillard, I. Hardy and P. Ode are thanked for their comments on an earlier version of the manuscript. M. Jervis, P. Ode and P. Mayhew kindly read the English version of the manuscript. The code of the simulation kernel was developed thanks to GMP (http://gmplib.org) and MPFR (http://www.mpfr.org), two free GNU-based multiple precision libraries, and was run on the cluster of the INRA MIGALE bioinformatics platform (http://migale.jouy.inra.fr).

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Received 23 March 2011; accepted 4 October 2011
Handling Editor: Andre Gilburn

Appendix: details of the simulation procedure used

Wajnberg (1994) developed a simulation model based on randomly generated sequences of oviposition having predefined sex-sequence patterns. More recently, Wajnberg, Fauvergue & Pons (2000) developed another Monte Carlo procedure generating random patch residence times. This other procedure was based on a Cox regression model and was used to test the effect of pre-defined covariates. Here, a simulation model combining these two approaches (i.e. sequences of males and females and patch residence times) was developed. Such a procedure used randomly generated sequences of hosts attacked by simulated females foraging alone on a host patch without progeny mortality. Like in Wajnberg, Fauvergue & Pons (2000), this was performed by means of a Markov chain based on the time from the moment the patch is entered to the first attack or the time between two host attacks. These two times were drawn randomly from two-parameter exponential distributions (i.e. exponential distributions with a minimal time lag) whose features were estimated from the experimental data separately on the three different patch qualities. In each case, the time between two host attacks increased monotonically after each attack, taken thus into account patch depletion.

In the model, sex sequences were drawn randomly from the observed average sequence obtained by pooling sequences produced by all females observed on the three patch qualities (see Fig. 2). In turn, like in Wajnberg, Fauvergue & Pons (2000), the generated sequences and the Markov chains determining the times of host attacks were used to simulate patch residence times by means of a Cox model with regression parameters describing the effect of laying a son or a daughter, along with the effect of the quality of the patch the simulated female is foraging on. Using the same pooled sex sequence and the same regression parameters of a Cox model over the three patch qualities was justified because effects of laying a son and a daughter on mothers' patch residence times appeared to remain independent of patch quality. The only significant interaction observed between patch quality and laying a daughter did not lead to a qualitative change in the associated patch-leaving rule as a function of the number of hosts initially available on the patch. Furthermore, when such a simulation procedure is used with the Cox's regression parameters estimated on the observed females, the simulated patch residence times were in close agreement with the experimental observations (Log-rank tests: $\chi^2 = 0{\cdot}04$, 1 d.f., $P = 0{\cdot}844$; $\chi^2 = 0{\cdot}45$, 1 d.f., $P = 0{\cdot}500$; $\chi^2 = 0{\cdot}01$, 1 d.f., $P = 0{\cdot}927$; on patches containing four, nine and 16 hosts, respectively). Despite the fact this does not represent a real validation performed on independent data, it reveals that some important factors were not omitted.

Using the model, some simulations were performed with different sex-sequence patterns to test their effect on the reproduction efficiency of the foraging females. For this, the multivariate method proposed by Wajnberg (1994) was used. Briefly, after an overall sex ratio was drawn randomly from a normal distribution with average and standard error estimated from observed females on each patch quality separately, all the possible sequences having the maximal length observed in each case and leading to such a sex ratio were described by five non-parametric statistics qualifying their sequential features (see Wajnberg 1993). Among all the possible sequences, the one having the descriptive statistics closer to the one to be tested was used in the simulation. One of the five statistics used describes the intensity of the male-first strategy. It is based on the sum of the males' rank position, standardized to remain in the [0·0; 1·0] interval. A value of 0·0 corresponds to sequences in which all males are laid at the end, while a value of 1·0 describes sequences having all males laid at the beginning (see Wajnberg 1993, 1994 for a detailed explanation).

For each situation tested, and as performed on the experimental data (see Fig. 1), the marginal fitness gain of the simulated females was computed as the total number of progeny produced (both sons and daughters) per second during the last 5 min before leaving the patch. This was performed on a number of replicates corresponding to the number of females observed in the experiment. The marginal fitness gains obtained on the three patch qualities were then compared with a Kruskal–Wallis test as in Fig. 1b. This statistic was used to quantify how far the reproductive strategy of the simulated females was from the optimal predictions of the MVT. Each situation was simulated 1000 times, and average results are presented.