

Adaptive preferential selection of female coccinellid hosts by the parasitoid wasp *Dinocampus coccinellae* (Hymenoptera: Braconidae)

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Abstract. Females of the parasitoid wasp *Dinocampus coccinellae* are known to parasitise both male and female coccinellid hosts. It is suggested that female hosts provide more resources for developing wasp larvae because they tend to be larger than male hosts, and female coccinellids have a much greater food intake than males. Thus the wasp's lifetime reproductive success should be increased by ovipositing preferentially in female rather than male hosts when given a choice. Laboratory experiments, using *Coccinella septempunctata* as a host, show that such a preference does exist. Wasps preferentially oviposit in females, and this preference is not simply a result of the larger mean size of females compared to males. These results corroborate higher rates of prevalence in female compared to male hosts reported previously.

INTRODUCTION

Parasitoids are known to assess the quality of potential hosts using a number of host criteria. Empirical studies have shown an ability to differentiate between high and low quality hosts on the basis of species (van Alphen & Vet, 1986), size (Salt, 1941), life history stage (Luck et al., 1982), parasitisation status (Pijls et al., 1995), and age within a life history stage (Hurst et al., 1998). In some cases the factors leading to increased host quality are complex. Thus, the decision to lay in a host may depend on either the nutrients available in the host, the efficiency of its internal or external defence, the probability that it has been parasitised previously, its accessibility to the parasitoid or any combination of these (see Godfray, 1994 for review).

The braconid wasp, *Dinocampus coccinellae* (Schrank) is a parasitoid of coccinellids of the sub-family Coccinellinae (Ceryngier & Hodek, 1996). The wasp shows thelytokous parthenogenesis, female offspring resulting from unfertilised eggs, with only a handful of male specimens recorded (Shaw et al., 1999). In Britain, *D. coccinellae* almost exclusively parasitises adult beetles (Geoghegan et al., 1998). Considerable attention has been paid to this wasp, largely because of the economic importance of its aphidophagous hosts. Its life history has been well documented (Ogloblin, 1924; Balduf, 1926; Bryden & Bishop, 1945; Walker, 1962; Sluss, 1968; Maeta, 1969; Hodek, 1973; Obrycki et al., 1985; Majerus, 1991, 1994). In brief, *D. coccinellae* females lay a single egg into a host. Although hosts may be super-parasitised, only a single larva develops per host, first instar larvae being equipped with large mandibles that are used to kill other eggs and

larvae in their host. The larva initially feeds on trophic cells (teratocytes) that erupt into the host body when the egg hatches and swell, absorbing nutrients from the host. Later the larva may feed directly on the host's fat bodies and gonads (Ogloblin, 1924; Sluss, 1968; Kadono-Okuda et al., 1995; Geoghegan et al., 2000). Once fully developed, the larva exits, and spins a silken cocoon between the legs of its host. In Britain, *D. coccinellae* has two or three generations per year and usually overwinters as a first instar larva within its host (Majerus, pers. obs.).

Optimal oviposition theory predicts that *D. coccinellae* should exhibit mechanisms to select the most suitable host. Suitability here is influenced by two factors: first the ease of oviposition within the host, and second the suitability of the host for larval development (Godfray, 1994). Other parasitoids that attack coccinellids have been shown to optimise oviposition strategies. For example, the scuttle-fly *Phalacrotophora fasciata* (Fallén) (Diptera: Phoridae) selectively chooses older pre-pupae, so that the wait until the optimum time to oviposit, when the new pupal skin is softest, is minimised (Hurst et al., 1998). Here, efficient use of time is optimised. In the case of *D. coccinellae*, previous work has shown that prevalence of parasitism varies between host species (Majerus, 1997), and that within a particular host species, wasps preferentially attack adults rather than immature stages (Geoghegan et al., 1998) and young rather than old adults (Majerus et al., 2000), both these preferences being adaptive.

Coccinella septempunctata Linnaeus shows one of the highest prevalence levels for *D. coccinellae* in Britain (Majerus, 1997). Previously, wild samples have shown

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that prevalences are higher in female *C. septempunctata* than in males (Geoghegan et al., 1997). Furthermore, in previous experiments investigating whether *D. coccinellae* shows a preference for young or old adult *C. septempunctata*, both young and old females had, on average, more eggs laid within them (Majerus et al., 2000). In these tests, *D. coccinellae* females were not offered male and female *C. septempunctata* together in choice tests. Thus, while there is some suggestion that *D. coccinellae* may preferentially oviposit in female, rather than male hosts, this should be ascertained by direct choice tests.

There are several reasons why, theoretically, female *C. septempunctata* might be expected to be more suitable hosts for *D. coccinellae* than are male *C. septempunctata*. First, female ladybirds tend to be larger and heavier than males, thus providing greater resources for the parasitoid larvae (Barron & Wilson, 1998). Second, food consumption is greater in females than in males during the reproductive period (Hodek, 1996; Dixon, 2000). Third, *D. coccinellae* causes ovarian retardation in female ladybirds, but no significant retardation in the testes within males (Balduf, 1926; Smith, 1960, Geoghegan et al., 2000). Thus it appears that *D. coccinellae* larvae, when in female hosts, may be able to utilise resources from two sources, the ovaries and fat body, while those in male may only use resources from the fat body (Geoghegan et al., 1997). Fourth, ovarian retardation caused by *D. coccinellae* in pre-winter female ladybirds may increase the resources laid down in the fat body, thus increasing the likelihood that ladybird and host will survive through the winter.

Here we investigate the hypothesis that the wasps show preference for female over male *C. septempunctata* using direct choice tests. Further, we assess whether the non-random rates of oviposition found are purely a function of the larger average size of female compared to male *C. septempunctata*.

MATERIAL AND METHODS

Stocks

Samples of *C. septempunctata* were taken from overwintering sites around Cambridge, Thetford Forest and North Staffordshire, between 19/10/2003 and 07/11/2003. Ladybirds were sexed (Randall et al., 1992) and the maximal width of the pronotum was measured under carbon dioxide anaesthetic using a dissecting microscope. They were maintained at 21°C, 16L : 8D, on a diet of pea aphids, *Acyrtosiphon pisum* (Harris) with a once weekly provision of artificial food as described by Geoghegan et al. (2000). All *D. coccinellae* adults were from infected *C. septempunctata*. They were fed on an agar based artificial diet (Geoghegan et al., 2000)

Test procedures

A single *D. coccinellae* was placed in a 14 cm Petri dish with one male and one female *C. septempunctata*, at least 10 min after the ladybirds had been placed in the dish. The ladybirds used in a test were collected on the same day from the same overwintering site. The male and female used in each test were chosen randomly from a sample: they were not match-paired for size or other phenotypic traits. Each ladybird was only used in a single test.

All interactions between wasp and ladybirds were recorded over a 20 min period, directly following the introduction of the wasp. There tended to be a decline in activity over time and this time length was thus deemed the most useful in terms of maximising the data recorded. Behavioural observations were categorised as: pursuance and investigation of the host (PI); ovipositional stance (OS); or ovipositional attack (OA) (after Richerson & DeLoach, 1972). The time elapsed since the start of the test, and the sex of the ladybird being approached was recorded at the start of each observed interaction. *Dinocampus coccinellae* does not exhibit the ability to avoid super-parasitism, with high numbers of eggs being laid in each host in enclosed choice tests (Geoghegan et al., 1998; Majerus et al., 2000). Thus comparative tests of total number of attacks on ladybirds could be used.

A total of 70 tests were carried out between 24/10/03 and 14/12/03. Wasps were labelled and were varied as much as possible. However, some wasps were used for more than one test. Trials in which no interaction was observed were omitted from the relevant statistical analyses.

Statistical analysis

As females in this species are often larger than males (see Results), it was necessary to test that a preference by the parasitoid for one sex was not simply an artefact of the weight difference. To do this, we fitted Generalised Linear Mixed Models, using each trial as our unit of observation. The Mixed Model framework also allowed us to account for the fact that some wasps were used more than once in the choice trials. To analyse the data referring to the total number of times a behaviour was performed by the parasitoid, we fitted a GLMM with a Gaussian error structure:

$$\Delta \text{Behaviour} \sim \text{Constant} + \Delta \text{Weight} + \text{Wasp ID}$$

where Δ Behaviour represents the number of times a behaviour (e.g. PI) was directed towards the female minus the number of times it was directed towards the male during a given trial; Δ Weight is the difference in weight between the female and male in each trial; and Wasp ID is a random factor that accounts for the multiple use of the same parasitoid in several trials. In this model, a significant deviation from zero for the constant reveals a preference by the parasitoid (positive constant = female preference for females, negative constant = preference for males).

We adopted a similar approach to investigate whether the first instance of a behaviour during each trial was biased towards either sex. We scored the first instance of a behaviour during a trial as 0 if it was directed towards a male and 1 if it was directed towards a female. A GLMM with a binomial error structure with a logit link was then fitted to the binary data:

$$\text{Behaviour} (0,1) \sim \text{Constant} + \Delta \text{Weight} + \text{Wasp ID}$$

Because of the properties of the logit link, a significant deviation from zero represents a preference for a sex (positive constant = female preference for females, negative constant = preference for males).

RESULTS

The results of the investigation, outlined in Table 1, show that the wasps exhibited PI, OS and OA towards females significantly more than towards males (paired t test: for PI, $t_{69} = 6.74$, $p < 0.001$; for OS, $t_{68} = 4.73$, $p < 0.001$; for OA, $t_{52} = 3.56$, $p = 0.001$). If just the first interaction per test run is considered, there was no significant host sex preference for PI or OS (one proportion binomial test: for PI, $p = 0.550$; for OS $p = 0.148$). However, the

TABLE 1. First and total interactions of *D. coccinellae* with 70 pairs of male and female *C. septempunctata*.

Host sex	First interaction			Total interactions		
	PI	OS	OA	PI	OS	OA
Male	32	28	18	219	142	51
Female	38	41	35	376	231	91
Total	70	69	53	595	373	142

Analysis of transitions between behavioural stages of the wasps yielded that wasps were far more likely to follow PI with OS for female hosts ($\chi^2 = 31.682$, d.f. = 1, $p < 0.001$), and the same was true for the transition OS to OA ($\chi^2 = 27.654$, d.f. = 1, $p < 0.001$).

first OA was directed significantly more towards females ($p = 0.0027$).

Fig. 1 shows a comparison of the size of the male and female *C. septempunctata* used in this experiment. Clearly the females tended to be larger than the males tested. A mixed effects model, with correction for multiple tests run with the same wasp, was fitted to test the individual effect of sex and size on the preference for female ladybirds. At all stages the wasps exhibited significant preference for female hosts, independent of size effects (significantly positive constants for PI, $F_{1,46} = 36.3$, $p < 0.001$; for OS, $F_{1,45} = 17.8$, $p < 0.001$; for OA $F_{1,31} = 11.3$, $p = 0.002$), and no significant preference for large hosts independent of sex (for PI, $F_{1,46} = 0.4$, $p = 0.5437$; for OS, $F_{1,45} = 0.1$, $p = 0.777$; for OA, $F_{1,46} = 0.4$, $p = 0.520$). The greater significance (lower p value) for PI than OS, and for OS than OA, may be attributed to the diminishing number of observations through the sequence of behaviours shown by wasps towards ladybirds. When looking at the first instance of each behaviour during each trial, we find that the wasp exhibited a preference for females only for OA ($F_{1,31} = 5.0$, $p = 0.0320$), but not for PI ($F_{1,45} = 0.5$, $p = 0.4838$) or OS ($F_{1,31} = 2.4$, $p = 0.1321$), again with no effect of body weight ($p > 0.7$ for all behaviours).

DISCUSSION

Our results appear to confirm the hypothesis that *D. coccinellae* preferentially interacts with female rather than male *C. septempunctata*. This extends from a higher rate of initial investigation through to ovipositional attack. On the basis of greater average size and higher food intake of female compared to male *C. septempunctata*, the preference shown by *D. coccinellae* is likely to be adaptive. In choosing to oviposit in female hosts, the wasp is providing a higher quality host for her progeny.

A simple test of how many interactions were directed towards each sex during a trial does not provide information as to when the wasp assesses the sex of the ladybird, as all interactions within the 20 min of each test are included in the analysis. If once a wasp has determined the sex of the ladybirds in a test, this determination influences subsequent decision making the assumption that all interactions during the 20 min test period were independent unsound. The possibility that each interaction

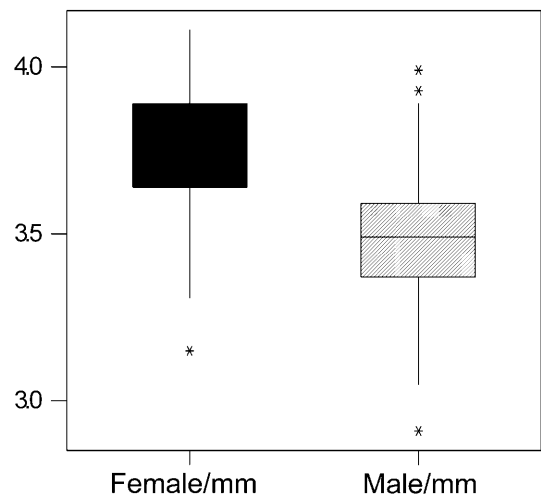


Fig. 1. Box/whiskers plot of maximum pronotal width of male and female *Coccinella septempunctata* used in the tests. Measurements are in millimetres. Outliers are marked with asterisks.

may have been affected by previous encounters in a test introduces potential pseudoreplication.

Indeed, testing the first of each type of interaction showed that the uptake of PI on novel ladybirds was random for the wasp. Adult ladybirds are known to secrete volatile chemicals (kairomones) and it is possible that *D. coccinellae* uses such olfactory signals to locate ladybirds in natural situations. Such host location mechanisms have previously been reported for hymenopteran parasitoids (Sternlicht, 1973; Majerus et al., 2000). However, our results suggest that the wasps initially encounter males and females randomly, so it is unlikely that they are using olfactory tracking to assess the sex of the ladybirds from a distance.

The fact the wasps were more likely to proceed to OS from PI, and then OA from OS when the host was female rather than male, suggests that the wasp was most likely to be assessing host sex using tactile cues. It is probable that the most important factor in the wasp's assessment of host sex was by sensing non-volatile surface chemicals on the ladybird. Arthur (1981) has reviewed examples of this sort of host assessment. Such sensing is likely to be through sensory cells on the wasps' antennae, and possibly the ovipositor sheath or abdomen (during OS, the wasp no longer keeps its antennae in contact with the ladybird).

The fact that in *D. coccinellae* the preference for female hosts was independent of size is noteworthy. It is possible that the sex has a considerably stronger influence on host discrimination for the wasp than do size differences, so that any influence of size on host selection was swamped by the influence of host sex. The implication of this is that the greater resources in female hosts, resulting simply from their larger mean size is not the main reason for wasps preferentially ovipositing in female *C. septempunctata*. Rather, the greater resources available to larvae of *D. coccinellae*, due to the greater food intake of female compared to male hosts and the potential in female hosts to utilise resources in the gonads, is probably more

important. A further benefit may accrue once resources have been diverted from the ovaries to the teratocytes that enter the ladybird's body when the wasp's egg hatches. Once a female host's ovaries have regressed, the high feeding rate characteristic of female ladybirds may continue, both before and after winter. This is likely to increase both the survival of the ladybird, and so the wasp larva within her, through the winter, and the development rate of the larva the following spring.

The adaptive nature of the wasp's preference for female rather than male hosts is based upon knowledge of the life-history of the wasp, but the evidence is circumstantial. More direct evidence, obtained by a comparative monitoring of the rate of larval development, larval survival and the fecundity of resulting adult wasps from both female and male *C. septempunctata*, using the non-invasive technique of sequential magnetic resonance imaging (Geoghegan et al., 2000) would be valuable. Here, as the state of the host's fat body and gonads can be monitored simultaneously, insights into the differences in resource utilisation of host resources may also be gained.

Many parasitoids show host discrimination after inserting their ovipositor (Godfray, 1994). Consequently some OA observations may not have resulted in oviposition. Some species only oviposit in the presence of certain chemical stimuli from the interior of the host (Hegdekar & Arthur, 1973). Additional experiments in which the success of an ovipositional attack was verified by dissecting all ladybirds subjected to OA and counting the number of wasp eggs present would increase the stringency of our findings.

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