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Consequences of infanticide for a gregarious ectoparasitoid of leafroller larvae

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Abstract. 1. In this laboratory study, the clutch size and handling time of *Goniozus jacintae* were investigated, a comparison of its life-history performance between primary and secondary (laid after infanticide events) broods was carried out, and the lipid and protein concentrations in the haemolymph of non-parasitised and parasitised hosts were estimated.

2. It was found that *G. jacintae* temporarily paralysed its host larvae for 66 min and briefly guarded its brood for 66 min. The clutch size of *G. jacintae* increased from two to seven with increasing larval fresh weight of its host, and both ovicide and larvicide of primary clutches occurred in 81% of encounters.

3. Secondary clutches of *G. jacintae* were significantly larger than primary clutches in two of three ovicide treatments for the same host individuals. Secondary clutches also experienced greater brood survivorship than primary clutches.

4. Lipid concentrations were consistently higher in the haemolymph of parasitised hosts, and protein concentrations were initially higher (until egg hatch), but increased at a lower rate in parasitised hosts than in non-parasitised hosts.

5. This study is the first to provide evidence that improved nutritional quality could be an important benefit of infanticide for an insect parasitoid, allowing for larger clutch size and improved brood survivorship among secondary broods.

Key words. Clutch size, competition, *Epiphyas postvittana*, *Goniozus jacintae*, handling time, macronutrients, nutritional quality.

Introduction

Exploitative competition within the broods of gregarious parasitoids has often been minimised through the evolution of exact clutch sizes, allowing parent females to accurately assess the quality of a host and adapt their clutch size to match the availability of resources for offspring development. Clutch size in gregarious parasitoids is also known to be influenced by other factors, such as host density, host quality, energetic reserves, and the presence of competitors (Godfray, 1994). For example, superparasitism by gregarious parasitoids frequently results in the oviposition of a smaller secondary clutch, and in most cases the resultant offspring develop to become smaller adults as a consequence of resource sharing (Godfray, 1994; Visser & Rosenheim, 1998). In some cases, however, the size of a secondary clutch may not be greatly reduced in the presence of competitors if the parent female has the ability to successfully secure the host resources through infanticide of the primary clutch.

Infanticide occurs when a parent female parasitoid either removes or kills the eggs or larvae of competitors to eliminate or at least reduce competition for the remaining host resources for its own offspring (van Alphen & Visser, 1990). Infanticide is known to occur among parasitoids in the Aphelinidae (Netting & Hunter, 2000), Bethylidae, Braconidae, and Pteromalidae (Godfray, 1994; Mayhew, 1997), Dryinidae (Yamada & Kitashiro, 2002; Ito & Yamada, 2005), Encyrtidae (Tena *et al.*, 2008), Eupelmidae (Rojas-Rousse *et al.*, 2005), and Ichneumonidae (Takasuka & Matsumoto, 2011). In addition to infanticide, parasitoids can respond to previously parasitised, but otherwise suitable, hosts by rejecting them and searching for a non-parasitised host (Mayhew, 1997), by host feeding on their haemolymph to gain additional resources for search and/or egg production (Tena *et al.*, 2008), or by superparasitizing (intraspecific) or multiparasitizing (interspecific) them by laying additional eggs (van Alphen & Visser, 1990).

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Based on an evolutionary model of infanticide, Strand and Godfray (1989) suggested that ovicide is more likely to occur in parasitoids when: (i) the first clutch has a competitive advantage for superparasitised hosts; (ii) non-parasitised hosts are rare; (iii) time taken to perform ovicide is minimal; and (iv) the risk of removing an individual's own clutch is low. In support of their model, Strand and Godfray (1989) found that ovicide by the gregarious ectoparasitoid, *Bracon hebetor*, occurred more frequently when either the time to encounter unparasitised hosts or the frequency of encountering previously parasitised hosts increased. In support of this evolutionary model, experiments have shown that ovicide is more frequent when the first clutch has a competitive advantage, and when the time taken to perform ovicide was minimal (Mayhew, 1997; Netting & Hunter, 2000; Yamada & Kitashiro, 2002). Although Tena *et al.* (2008) found ovicide to be more likely when the first clutch had a competitive advantage, there was a substantial increase in the handling time (time from first encounter to departure from a host) required for secondary clutches on previously parasitised hosts compared with primary clutches on non-parasitised hosts.

The fitness consequences of infanticide for a secondary clutch can be a reduction in offspring size, as found by Mayhew (1997) for the gregarious ectoparasitoid, *Laelius pedatus* (Say). For example, a previously parasitised host may be a lower-quality host for parasitoid offspring compared with a non-parasitised host if it deteriorates post-parasitism (Strand & Godfray, 1989; Mayhew, 1997). Alternatively, a previously parasitised host could be a better-quality resource than a non-parasitised host if the host encapsulation response is completely or partially suppressed (van Alphen & Visser, 1990; Tena *et al.*, 2008) or if the nutritional quality is increased (Harvey *et al.*, 2013). Host nutritional quality can be increased if injection of parasitoid venom (Asgari & Rivers, 2011) elevates lipid levels in the haemolymph (e.g. Nakamatsu & Tanaka, 2004), if parasitic castration of the host occurs, as this has been linked to an increase in protein and triacylglycerol concentration in the haemolymph (Pennacchio *et al.*, 1995), if polydnaviruses initially increase trehalose levels in the haemolymph (e.g. Nakamatsu *et al.*, 2001), or if polydnaviruses or symbionts redirect the allocation of metabolic resources from host metabolism and reproduction to juvenile parasitoid growth (Rahbe *et al.*, 2002; Thompson *et al.*, 2005).

In this study, we investigate the occurrence and fitness consequences of parasitism and infanticide for *Goniozus jacintae* Farrugia, a gregarious larval ectoparasitoid of the Light Brown Apple Moth, *Epiphyas postvittana* (Walker). Bethylid parasitoids, such as *G. jacintae*, have long been recognised for their aggressive interspecific and intraspecific competitive interactions (Hardy & Blackburn, 1991) and their use of ovicide and/or larvicide (Mayhew, 1997). Larvae of *E. postvittana* are susceptible to parasitism by *G. jacintae* from the third to the fifth instar and also during the extended sixth instar, but parasitism primarily occurs during the fourth and fifth instars (Danthanarayana, 1980). Females of *G. jacintae* host feed on individuals selected for oviposition, temporarily paralyse them with venom, and partially guard their brood. We compare the life-history performance of parasitoid offspring from primary clutches with those from secondary clutches laid after infanticide events. We also compare the concentration of proteins and lipids in the haemolymph of non-parasitised and parasitised hosts to evaluate the potential nutritional benefits of infanticide for *G. jacintae*.

In general, clutch size for gregarious ectoparasitoids increases with host size (Godfray, 1994; Harvey, 2005), as has been documented for the congeneric parasitoid, *Goniozus nephantidis* (Muesebeck) (Hardy *et al.*, 1992). In this context, we hypothesise that *G. jacintae* females should match their clutch size to host size for both primary and secondary clutches. We also hypothesise that the size of a secondary clutch should be greater than or equal to that of a primary clutch for cases of ovicide in which the eggs of the primary clutch had not yet hatched into larvae. As eggs of most ectoparasitoids do not increase in size before larval hatch, no host resources are used, and thus host quality should remain the same for secondary clutches as it was for primary clutches (Goubault *et al.*, 2007). Moreover, if previous parasitism does increase the nutritional quality of a host through the action of venom, polydnavirus, or symbiont, and can be detected by parasitoid females, a secondary clutch might even be expected to be larger in size than a primary clutch. For example, an increase in clutch size was observed for the gregarious ectoparasitoid, *Hyssops pallidus* (Askew) ovipositing on hosts previously parasitised by the solitary endoparasitoid *Ascogaster quadridentata* Wesmael (Zaviezo & Mills, 2001). However, once host resources begin to be extracted by larvae from a primary clutch, host quality would decline and, consequently, secondary clutches that result from larvicide would be expected to be smaller than the preceding primary clutches. The consequences of parasitism on the metabolic processes and nutritional quality of parasitised hosts remain poorly understood, particularly in the context of parasitoids that exhibit infanticide. Nonetheless, we anticipate that protein and lipid concentrations in the haemolymph of hosts with a primary clutch should be as high as for non-parasitised hosts, provided that the parasitoid eggs have not yet hatched. This study aims to contribute to our knowledge of parasitoid behavioural ecology and clutch-size decisions in the context of infanticide and host quality.

Materials and methods

Host and parasitoid colonies and experimental conditions

Host and parasitoid colonies and all experimental observations were made at 25–27 °C and with an LD 16:8 h photoperiod in a quarantine facility at the University of California, Berkeley. A laboratory colony of *E. postvittana* was established from individuals collected in 2007 from an invasive population in Santa Cruz, California. Prior to experimentation, we reared the host larvae on an artificial bean-based diet developed by Cunningham (2007). Upon pupation, *E. postvittana* pupae were transferred to ventilated 956-ml transparent polypropylene deli containers (Fabri-Kal, Kalamazoo, Michigan) and provided with 10% honey-water with 0.1% sorbic acid via a 4-cm cotton wick in a 22-ml translucent polystyrene cup (SOLO, Highland Park, Illinois). Following oviposition, freshly laid egg masses were sterilised following Singh *et al.* (1985) in a 5% formaldehyde solution for 20 min, soaked in water for 20 min, and air-dried

before being transferred to 95-ml translucent polystyrene cups (SOLO) filled one-third with artificial diet.

A laboratory colony of *G. jacintae* was established from individuals collected in 2008 from the Yarra Valley, Australia, where it is an indigenous parasitoid of *E. postvittana.* It was maintained on *E. postvittana* larvae fed on leaves of plantain (*Plantago lanceolata* L.). Mated *G. jacintae* females were kept individually in ventilated 500-ml transparent polypropylene deli containers (Pactiv, Lake Forest, Illinois) with streaks of a 50 : 50 honey-water solution applied to the sides twice each week. Five fourth- to fifth-instar host larvae were provided weekly on excised plantain leaves with their stems inserted into 7-ml glass vials (Fisher Scientific, Pittsburg, Pennsylvania) of water plugged with cotton wool. Individual parasitised larvae were transferred to 7 ml glass vials plugged with cotton wool to await adult parasitoid emergence. For experiments, naïve, mated *G. jacintae* females were kept individually in 35-ml glass vials (Fisher Scientific) and provided with streaks of honey-water solution until they were 2–3 days old.

Influence of host size on parasitoid handling time, clutch size, and brood performance

As in other clutch-size studies (Godfray, 1994), to determine the influence of host size on the handling time, clutch size, and fitness consequences of *G. jacintae*, we selected host larvae with as wide a range of fresh weights from among the instars that are susceptible to parasitism to avoid a consistent linkage between host size and age. We measured their fresh weight on a Sartorius GD503 microbalance (± 0.1 mg). Following Zaviezo and Mills (2001), host larvae were exposed to *G. jacintae* females individually in arenas consisting of two 50-ml glass vials (2.6×9.5 cm^2) connected by a copper tube (1.5 mm diameter and 40 mm in length) that was inserted through their plastic caps. Both host and parasitoid were placed into one glass vial and a honey-water solution was streaked onto the sides of both glass vials. The double glass vial arena reduced the likelihood of self-superparasitism by allowing a female parasitoid to move to the second empty glass vial once it had completed handling the host larva in the first vial. We prepared digital images of each experimental female to estimate hind tibia length (mm) using ImageJ, as a measure of parent female size (Godfray, 1994).

For each experimental female parasitoid, we made observations every 5 min for a 3-h period to record time to host encounter, duration of attack, oviposition time, guarding time, total host handling time, and duration of host paralysis (all in min). We analysed handling times for females that successfully oviposited eggs on a host. We defined time to host encounter as the time it took a female to find and initiate attack of a host larva. The duration of attack began with host encounter and lasted until oviposition, and included the time taken to temporarily paralyse a host and to host feed before oviposition. Oviposition time included the time it took a female parasitoid to mount a paralysed host larva, lay a full clutch of eggs, and dismount the host. Guarding time began once a female dismounted a host larva on which she had oviposited, and ended when she moved through the copper tubing into the second glass vial. As our observations lasted only 3 h, the guarding time continued beyond the observation period in some cases and so may represent an underestimate of the true guarding time. Total host handling time began at host encounter and was completed when a female exited the first glass vial through the copper tube and represents the sum of the duration of attack, oviposition time, and guarding time. The duration of host paralysis began with probing of a host larva by a female parasitoid to initiate paralysis, and ended at the first sign of host larval movement.

Parasitoid oviposition success was measured as a binary outcome, based on whether a female that encountered a host larva also oviposited within the 3-h observation period. All parasitised host larvae derived from the handling time observations were subsequently kept in 50-ml glass vials with fresh excised plantain leaves until the brood had completed their development and adult offspring had emerged. As measures of parasitoid performance, we noted the clutch size (number of eggs laid by the parent female), development time from egg to adult emergence (in days), brood survivorship (from egg to adult emergence), and offspring sex ratio (percentage female). A total of 93 parasitoid females were observed to encounter a host larva within the 3-h observation period, but levels of replication declined for subsequent behavioural events and measures of parasitoid performance; and are included in the results section.

Occurrence and consequences of infanticide

To examine infanticide in *G. jacintae*, individual naïve secondary females were exposed to host larvae representing different infanticide treatments in 50-ml glass vials with an excised plantain leaf and streaks of honey-water solution. To generate the infanticide treatments, we exposed fourth- and fifth-instar host larvae individually to a first female parasitoid in glass vials with streaks of honey-water solution and checked every 24 h for the presence of a clutch of eggs. After oviposition was observed, these first females were removed from the vials. Parasitoid eggs hatched on day 3 under the experimental conditions used, and so parasitised host larvae were maintained on excised plantain leaves for either 2 days for the ovicide treatments or 4–5 days for the larvicide treatment. For the ovicide treatments, parasitised host larvae supporting natural primary clutch sizes of either one to two, three to four, or five to seven eggs were used. By contrast, only one clutch size (one to two larvae) was used for the larvicide treatment, and this was generated by removing any extra parasitoid larvae from natural primary clutches to limit the variance in host feeding. In addition, non-parasitised host larvae of the same age without a primary clutch were used as controls. For all treatments, the host larvae were then exposed to a second female parasitoid for 24 h to monitor the occurrence and consequences of infanticide.

We verified the occurrence of infanticide and the laying of a secondary clutch of eggs by using the visual remains of removed larvae and eggs and digital images of the host larvae before and after exposure to the second female parasitoid. The consequence of infanticide on the clutch size and performance of secondary broods was monitored as in the previous experiment,

with average hind tibia length of female offspring from 19 broods as an additional performance measurement. The level of replication for each of the infanticide treatments was as follows: one to two eggs ($n = 20$), three to four eggs ($n = 27$), five to seven eggs ($n = 17$), one to two larvae ($n = 16$), control ($n = 35$). As in the previous experiment, levels of replication declined for successive measures of brood performance and are presented in the results section.

Influence of host size and parasitism on host macronutrients

To examine the influence of host size and parasitism on the nutritional quality of *E. postvittana* larvae, protein and lipid concentrations of the haemolymph were analysed using fifth-instar larvae. To determine the effect of host size alone on protein and lipid concentrations in *E. postvittana* larvae, we selected a range of sizes (based on fresh weight) of non-parasitised larvae, and used 38 and 11 individuals for the protein and lipid analyses, respectively. As the duration of the fifth larval instar is 8–9 days, we determined macronutrient concentration 4 days post-moult to ensure that the moulting process would not confound the relationship between host size and macronutrient concentration.

To examine the effect of parasitism by *G. jacintae* over time on protein and lipid concentrations in fifth-instar host larvae, we used newly moulted larvae for both non-parasitised (control) and parasitised treatments. Host larvae were placed individually in 50-ml glass vials with a fresh excised plantain leaf and a mated, naïve *G. jacintae* female and some streaks of honey-water solution were added to the parasitised treatment only. After 24 h the parasitoid females were removed and both parasitised and non-parasitised host larvae were maintained on excised plantain leaves in the glass vials. We monitored larval fresh weight and changes in protein and lipid concentrations in the haemolymph of host larvae at 1, 4, and 7 days post-treatment. These time intervals were selected because parasitoid egg hatch occurred on day 3, parasitoid larvae pupated within 9–10 days, and non-parasitised fifth-instar host larvae pupated within 8–9 days under the experimental conditions used. A total of 125 larvae (91 non-parasitised and 34 parasitised) were used for this experiment and 40, 53, and 32 of them were analysed on days 1, 4, and 7 post-treatment. As not all larvae were used for analysis of both macronutrients, specific levels of replication for each are included in the results.

For both the host size and parasitism experiments, host larvae were first weighed using a Sartorius GD503 microbalance, and then surface-sterilised by wiping with a 90% ethanol-soaked tissue prior to severing a proleg with a needle to collect haemolymph. We collected 2 µl of haemolymph with a microcapillary tube and placed it into 20 µl of ice-cold anticoagulant buffer (98 mM NaOH; 0.19 M NaCl; 1.7 mM ethylenediaminetetraacetic acid; 41 mM citric acid, pH 4.5) (Strand *et al.*, 1997). We centrifuged each sample (1000 ***g*** for 2 min) to remove unwanted tissues and stored the supernatant at −20 °C for further analysis (Salvador & Cônsoli, 2008). We modified the macronutrient analysis protocols of Salvador and Cônsoli (2008) to accommodate preparation of samples in 96 flat-well polystyrene microplates (BD FALCON Becton, Dickinson and Company, Franklin Lakes, New Jersey) and estimation of colorimetric absorbance in a microplate reader (iMark, BioRad, Hercules, California). We determined total protein concentration in the haemolymph samples using the Coomassie Plus reagent (Coomassie Plus Protein, Pierce Biotechnology, Inc., Rockford, Illinois) and bovine serum albumin (A3803, Sigma-Aldrich, St. Louis, Missouri) as a standard, and measured absorbance at 595 nm. We determined the lipid concentration in the haemolymph using the vanillin reagent and vegetable oil as a standard. Haemolymph supernatant and standards were first transferred to glass vials and evaporated at 100 °C along with 200 µl chloroform : methanol (1 : 1). Subsequently we added 100 µl sulphuric acid and incubated the samples at 100 °C for 10 min. Absorbance was read at 590 nm both before and 5 min after adding 100 µl vanillin reagent, and lipid concentration was determined from the difference in the two absorbance readings. We used duplicates for all samples and standards in the microplates to control for any pipetting error.

Statistical analysis

All statistical analyses were carried out using R version 3.0.2 (R Development Core Team, 2013) and were primarily based on generalised linear models (GLMs). To analyse the influence of host size on parasitoid handling time, clutch size, and brood performance, host larval fresh weight and female parasitoid size (hind tibia length) were included as fixed factors in GLM models for each measurement variable. For the consequences of infanticide experiment, primary and secondary clutch sizes from the same host larvae were first compared using a paired Wilcoxon signed-rank test. The various measures of brood performance of secondary clutches were then analysed using GLMs with infanticide treatment, host larval fresh weight, and parent parasitoid female size as fixed factors. For the experiment on macronutrient concentrations in host larvae, we first analysed protein and lipid concentrations in non-parasitised host larvae using general linear models (LMs) with larval fresh weight as the only fixed factor. Subsequently, ANCOVA was used to examine differences in the responses of macronutrient concentrations to treatment (control versus parasitised larvae) and to time (days post-treatment) and host larval fresh weight ($\log_e$-transformed) as continuous explanatory variables.

Generalised linear model error distributions were selected to best represent the measurement variables analysed (Gaussian for continuous variables, Poisson for counts, binomial for proportions), quasi-distributions were used when overdispersion occurred, and standard link functions were used in each case. Full models that included all explanatory variables and two-way interactions were used initially, and stepwise model simplification was performed manually using likelihood-ratio tests (χ^2) in the absence of overdispersion and F-tests to incorporate an empirical scale parameter in the presence of overdispersion (Crawley, 2013). We ensured that error distributions and homogeneity were appropriate by inspecting residual, standardised deviance, and normal quantile plots (Crawley, 2013). As two-way interactions did not make a significant contribution to any of the GLMs, only the individual fixed factors are considered in the results, whereas interactions are presented in addition

Table 1. Mean time (min) spent by *Goniozus jacintae* in encountering and handling larvae of *Epiphyas postvittana*, duration of host paralysis, and results of generalized linear models (Gaussian errors) to test for the effects of host larval fresh weight and parasitoid size.

Model/factor	Mean ± SD (min)	*F*	d.f.	*P*
Time to host encounter	28.85 ± 39.39			
Host weight		0.51	1, 58	0.48
Parasitoid size		0.48	1, 57	0.49
Duration of attack	28.80 ± 19.99			
Host weight		1.11	1, 49	0.30
Parasitoid size		0.01	1, 48	0.93
Oviposition time	47.20 ± 38.05			
Host weight		5.33	1, 38	**0.03**
Parasitoid size		1.99	1, 37	0.17
Guarding time	66.05 ± 49.17			
Host weight		0.00	1, 18	0.95
Parasitoid size		0.00	1, 17	0.97
Total handling time	118.60 ± 62.39			
Host weight		0.05	1, 17	0.82
Parasitoid size		0.49	1, 18	0.49
Duration of paralysis	65.72 ± 27.15			
Host weight		0.27	1, 8	0.62
Parasitoid size		5.08	1, 9	**0.05**

Significant effects are shown in bold.

to the fixed factors for ANCOVA analyses of the effects of treatment, time post-treatment, and host larval fresh weight on host macronutrients.

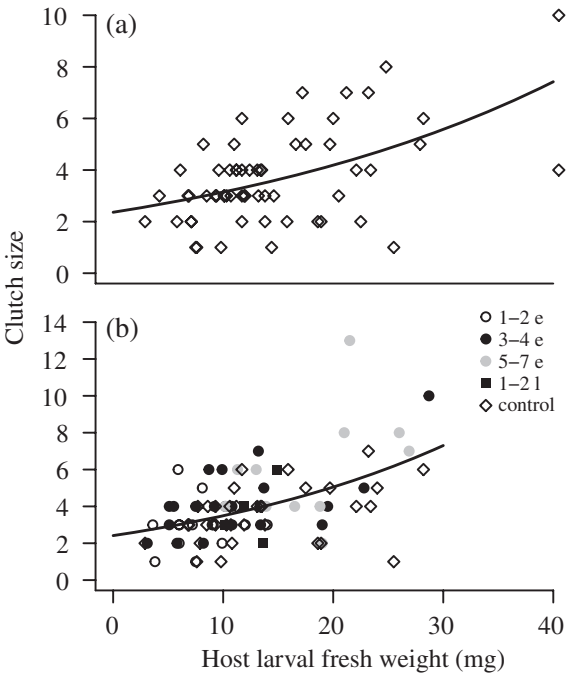

Fig. 1. Clutch size response of *Goniozus jacintae* in relation to larval fresh weight of the host, *Epiphyas postvittana* for: (a) primary clutches of first females from the host size experiment [$y = \exp(0.03x + 0.86)$]; (b) secondary clutches of second females from the infanticide experiment [$y = \exp(0.04x + 0.88)$]. All successful ovipositions by the second female took place after complete removal of eggs (ovicide) or larva (larvicide) from the first female. Legend: e, eggs; l, larvae.

Results

Influence of host size on parasitoid handling time, clutch size, and brood performance

The mean time spent by *G. jacintae* on each of the successive host larval handling events, and the effects of host larval fresh weight and female parasitoid size on these events are presented in Table 1. Oviposition and guarding times contributed the most to the total handling time, and guarding times increased significantly with host larval fresh weight. Only 15% of the individuals that were monitored for total handling time took longer to exit the glass vial than the 3-h observation period. There was no effect of host larval fresh weight or parasitoid size on the duration of these events, with the exception of oviposition time, which increased with host larval fresh weight, and the duration of host paralysis, which decreased with parasitoid size (Table 1).

Oviposition success (65%) did not vary significantly with host larval fresh weight or parasitoid size (GLM binomial, $n = 93$, $\chi_1^2 = 0.26$, $P = 0.61$; $\chi_1^2 = 0.22$, $P = 0.65$ respectively). Clutch size was not affected by parasitoid size, but did increase significantly with host larval fresh weight (Fig. 1a, Table 2). For subsequent brood performance, mean survivorship was 68% (± 31% SD), mean sex ratio was 79% female (± 21% SD), and neither was significantly influenced by host larval fresh weight or by parasitoid size (Table 2). The mean development time (oviposition to adult emergence) was 18.3 days (± 1.2 SD) and decreased significantly with an increase in both host larval fresh weight and parasitoid size (Table 2).

Occurrence and consequences of infanticide

Of the 80 *G. jacintae* presented with hosts previously parasitised with a primary clutch, 81% successfully removed all eggs or larvae from the first parasitoid and oviposited their own secondary clutch of eggs. Removal of eggs from their own secondary clutch was very rare for parasitoids committing infanticide, and only occurred when left with a host for more than 24 h. Of the 15 parasitoids that did not oviposit on a previously parasitised host, five partially removed the eggs or larvae from the primary brood. These parasitoids were not used for further analysis, but it was noted that the remaining eggs and larvae from the primary broods survived to adult emergence.

In comparing primary and secondary clutch sizes from the same individual host larvae (excluding the control treatment), second females oviposited a significantly larger clutch of eggs than first parasitoids for infanticide treatments with one to two eggs or three to four eggs (paired Wilcoxon signed-rank test, $V = 88.5$, $n = 15$, $P = 0.02$ and $V = 94.5$, $n = 22$, $P = 0.05$, respectively). However, there was no significant difference between the clutch sizes of first and second parasitoids for infanticide treatments with five to seven eggs or one to two

Table 2. Statistical analysis of the brood performance characteristics of primary clutches of *Goniozus jacintae* from the host size experiment, and of secondary clutches from the infanticide experiment.

	Primary clutch				Secondary clutch			
Model/factor	*n*	GLM family	d.f.	*P*	*n*	GLM family	d.f	*P*
Clutch size	60	Poisson, χ^2			87	Poisson, χ^2		
Host weight		12.94	1	**< 0.001**		19.29	1	**< 0.001**
Parasitoid size		0.61	1	0.44		0.58	1	0.45
Infanticide treatment		–	–	–		6.62	4	0.16
Brood survivorship	38	Quasi-binomial, *F*			87	Quasi-binomial, *F*		
Host weight		0.68	1, 34	0.53		2.73	1, 81	0.1
Parasitoid size		0.90	1, 35	0.35		2.21	1, 80	0.31
Infanticide treatment		–	–	–		23.84	4, 82	**0.03**
Development time	37	Gaussian (log), *F*			67	Gaussian (log), *F*		
Host weight		7.18	1, 34	**0.01**		3.82	1, 60	0.06
Parasitoid size		6.65	1, 34	**0.01**		7.66	1, 60	**0.01**
Infanticide treatment		–	–	–		3.84	4, 60	**0.01**
Offspring sex ratio	26	Binomial, χ^2			47	Binomial, χ^2		
Host weight		0.12	1	0.73		0.01	1	0.93
Parasitoid size		0.00	1	0.98		0.39	1	0.53
Infanticide treatment		–	–	–		0.69	4	0.95
Offspring size	–	–			19	Gaussian (log), *F*		
Host weight		–	–	–		1.11	1, 18	0.31
Parasitoid size		–	–	–		0.06	1, 14	0.82
Infanticide treatment		–	–	–		0.42	1, 15	0.74

Significant effects are shown in bold.

larvae ($V = 46.5$, $n = 15$, $P = 0.46$ and $V = 22.0$, $n = 12$, $P = 1.0$, respectively).

When analysed alone, the secondary clutch size dataset showed a significant increase in clutch size with host larval fresh weight (Fig. 1b) that was not affected by infanticide treatment (including the control treatment) or the size of the second parasitoid (Table 2). Thus, the clutch size response of *G. jacintae* to host size was the same for both primary (Fig. 1a) and secondary (Fig. 1b) clutches. Brood survivorship from secondary clutches was not significantly influenced by host larval fresh weight or parasitoid size, but varied significantly with infanticide treatment (Table 2). Brood survivorship was greater for secondary clutches in the treatments involving one to two and three to four eggs compared with controls (a primary clutch on host larvae of the same age), with the treatments with five to seven eggs and one to two larvae being intermediate in brood survivorship (Fig. 2). As found from the initial experiment on primary clutches, development time of secondary clutches decreased with increasing parasitoid size and host larval fresh weight (marginal effect), but was also influenced by infanticide treatment (Table 2). Development time was shorter for the treatment with five to seven eggs (16.00 ± 2.20 SD) than for all other treatments (18.27 ± 1.47 SD) with the exception of the treatment with one to two larvae, where it was also slightly shorter (17.00 ± 1.22 SD) [stepwise deletion, GLM, Gaussian (log), $F_{3,60} = 29.89$, $P = 0.004$ and $F_{1,60} = 2.43$, $P = 0.28$ respectively]. The sex ratio ($77 \pm 22\%$ SD) and size of female offspring (0.42 ± 0.07 SD) from secondary clutches were not significantly influenced by parasitoid size, host larval fresh weight, or infanticide treatment (Table 2).

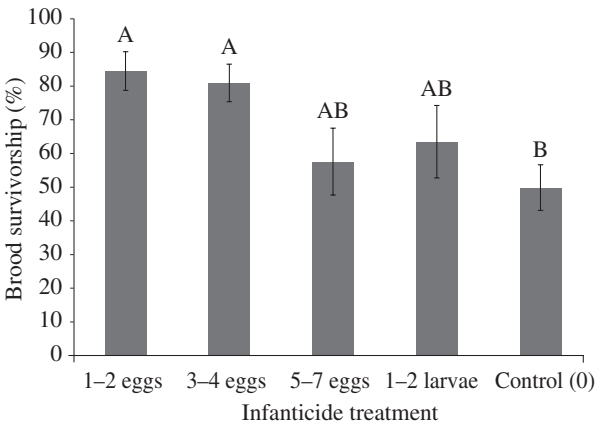

Fig. 2. Mean (± SE) brood survivorship of secondary clutches laid by *Goniozus jacintae* in relation to infanticide treatment; treatments with different letters are significantly different using a generalised linear model (quasi-binomial) with stepwise deletion.

Influence of host size and parasitism on host macronutrients

To determine whether host size alone can influence the nutritional quality of *E. postvittana* larvae for *G. jacintae* we first analysed non-parasitised fifth-instar larvae. Both protein (LM, $F_{1,37} = 21.27$, $P < 0.001$) and lipid (LM, $F_{1,10} = 9.27$, $P = 0.01$) concentrations increased significantly with host larval fresh weight for non-parasitised larvae 4 days post-moult (Fig. 3). We then compared the nutritional quality of similar-sized control and parasitised fifth-instar host larvae over a period of 7 days. A significant interaction between treatment and time for host

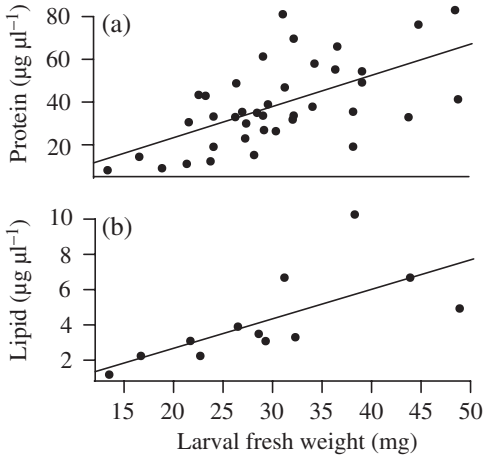

Fig. 3. Protein (a) and lipid (b) concentrations ($\mu g\,\mu l^{-1}$) in the haemolymph of non-parasitized fifth-instar larvae of *Epiphyas postvittana* (4 days post-moult) in relation to fresh weight (protein, $y = 1.46x - 6.08$; lipid, $y = 0.17x - 0.67$).

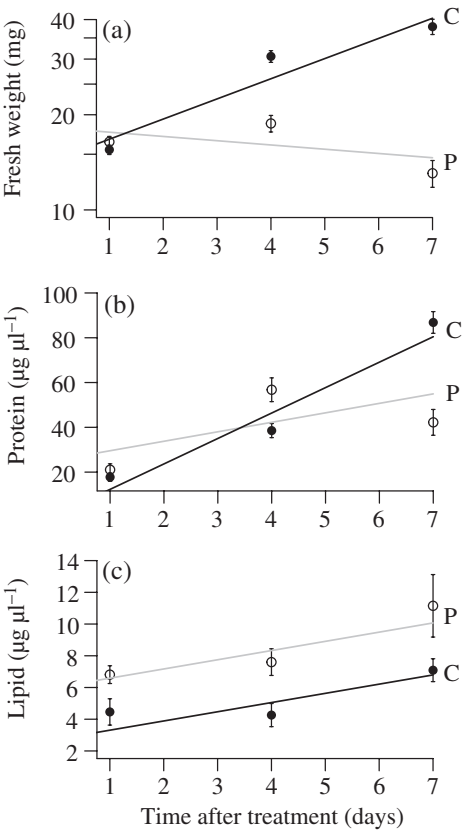

Fig. 4. Mean (± SE) larval fresh weight (a), and protein (b) and lipid (c) concentrations in the haemolymph of fifth-instar *Epiphyas postvittana* as non-parasitized controls (C) and as hosts parasitized by *Goniozus jacintae* (P) in relation to time after treatment (days). Fitted lines are as follows: (a) control, $y = \exp(0.147x + 2.67)$, parasitized, $y = \exp(-0.031x + 2.89)$; (b) control, $y = 11.36x + 0.94$, parasitized, $y = 4.23x + 25.31$; and (c) control, $y = 0.58x + 2.73$, parasitized, $y = 0.58x + 6.01$.

larval fresh weight (LM, $F_{1,123} = 54.8$, $P < 0.001$) indicated that the two treatments differed in slope; control larvae continued to grow and gain weight, whereas following parasitism, parasitised larvae declined in fresh weight (Fig. 4a). For the analysis of protein concentration in larval haemolymph, there were significant interactions between host larval fresh weight and both time ($F_{1,117} = 15.82$, $P < 0.001$) and treatment ($F_{1,117} = 6.81$, $P = 0.01$). These interactions resulted from a strong increase in protein concentration over time as control larvae grew in fresh weight (Figs 3a and 4a,b), together with a weak increase in protein concentration over time despite a decline in the fresh weight of parasitised larvae (Fig. 4a,b). Consequently, while protein concentration was initially higher in parasitised larvae, it dropped below that of control larvae soon after the timing of parasitoid egg hatch (day 3). For the analysis of lipid concentration in larval haemolymph, there were no significant interactions among host larval fresh weight, time, and treatment ($F_{3,47} = 0.65$, $P = 0.59$) and no effect of host larval fresh weight ($F_{1,51} = 0.20$, $P = 0.66$), but there were significant effects of both treatment ($F_{1,52} = 18.81$, $P < 0.001$) and time ($F_{1,52} = 11.96$, $P = 0.001$). The absence of an interaction between treatment and time indicated that lipids increased at the same rate (no difference in slope) for both control and parasitised larvae, and the significant effect of treatment indicated that lipid concentration in parasitised larvae was higher over the entire post-treatment period than was the case for control larvae (Fig. 4c).

Discussion

Clutch size, and consequently oviposition time, of the gregarious ectoparasitoid *G. jacintae* was found to increase with larval fresh weight of its host *E. postvittana* in a similar way to that described for the related species *G. nephantidis* (Muesebeck) (Hardy *et al.*, 1992). The allocation of more eggs to larger hosts is a common pattern that has been observed repeatedly for gregarious ectoparasitoids (Godfray, 1994; Zaviezo & Mills, 2000; Wang *et al.*, 2008; Koppik *et al.*, 2014). In addition, our observations indicated that the host handling time for *G. jacintae* was short and that parent females readily committed infanticide of conspecific broods, laying larger secondary clutches in two out of three ovicide treatments, which subsequently experienced greater survivorship and, in some cases, a shorter development time than primary clutches. From our analysis of macronutrient concentrations in parasitised and non-parasitised host larvae, it appears that the difference in performance of primary and secondary clutches may be related to an increase in the protein and lipid concentration of the host larval haemolymph following parasitism. In addition, some anomalous effects of parasitoid female size on the duration of host larval paralysis and the development time of both primary and secondary parasitoid broods were observed during the study, but we are currently unable to explain the significance of these observations.

From our direct observations of host handling by *G. jacintae*, adult females guarded their brood for an average of only 66 min post-oviposition, a brief period that seems unlikely to provide much protection for the developing brood from either

intraspecific or interspecific competitors. While this represents a slight underestimate of the true guarding time, due to the 3-h limit of our experimental observations, the majority of female parasitoids left their hosts before the end of the observation period and so the degree of underestimation is probably small. Variation in the duration of brood guarding and propensity for infanticide among bethylid parasitoids may be influenced by life-history strategies linked to the rarity of their hosts (Godfray, 1994) or by the likelihood of interspecific competition (Hardy & Blackburn, 1991). The fitness costs of brood guarding may be related to the rarity of hosts, such that species with a greater lifetime fecundity use hosts that are typically more common and spend less time guarding individual broods (Mayhew, 1997), but readily commit infanticide under conditions when hosts are less abundant. However, *Goniozus legneri* Gordh has a high fecundity of 156 eggs (Laumann *et al.*, 2000), but guards its larval brood for 5–6 days, and commits both ovicide and larvicide (Bentley *et al.*, 2009). Similarly, *G. nephantidis* has a fecundity of 117 eggs (Sreekanth & Muralimohan, 2013), guards its brood until pupation (Hardy & Blackburn, 1991; Goubault *et al.*, 2007), and yet commits ovicide only (Hardy & Blackburn, 1991). By contrast, *G. indicus* Ashmead has a low fecundity of 30 eggs, but guards its brood only until egg hatch and commits ovicide only (Takasu & Overholt, 1998). In this context, *G. jacintae* has an intermediate fecundity of 64 eggs (N. J. Mills, unpublished), a minimal level of brood guarding, and readily commits both ovicide and larvicide. Thus brood guarding and infanticide vary widely among bethylid species and appear not to be influenced by rarity of hosts (fecundity) or intraspecific competition (vulnerability to infanticide). In contrast, brood guarding may be more important for protection against interspecific rather than intraspecific competitors, as appears to be the case for *G. nephantidis* (Hardy & Blackburn, 1991; Venkatesan *et al.*, 2009).

Infanticide may also be adaptive for *G. jacintae* for reasons other than competition and the potential rarity of non-parasitised hosts. For example, encapsulation rates for secondary clutches were found to be lower than those for primary clutches of *Metaphycus flavus* (Howard) in soft scale hosts (Tena *et al.*, 2008). In addition, Takasu and Hirose (1991) proposed that parasitised hosts were preferred over non-parasitised hosts by *Ooencyrtus nezarae* Ishii due to the presence of pre-drilled oviposition holes in host eggs that reduced the handling time for a second female. While a reduction in encapsulation or host handling time would not be applicable to *G. jacintae*, other potential advantages of infanticide include nutrients gained from consumption of eggs or larvae from a primary clutch (Goubault *et al.*, 2007) and improved nutritional quality from previous parasitism.

One of the important findings from this study was that for the same host individuals, secondary clutches of *G. jacintae* were significantly larger than primary clutches for the ovicide treatments with one to two eggs and three to four eggs. In addition, these secondary broods experienced greater survivorship and, in some cases, a shorter development time, although other aspects of performance, such as sex ratio and offspring size, did not differ from that of primary broods. This contrasts with a study of the bethylid *L. pedatus*, which showed that there was no difference between primary and secondary clutch sizes and that offspring size was smaller from secondary than from primary broods (Mayhew, 1997). However, a study by Zaviezo and Mills (2001) demonstrated that clutch size of the eulophid ectoparasitoid *H. pallidus* was twice as large, with twice as many females of typical size, on hosts previously parasitised by the braconid endoparasitoid *A. quadidentata*. This latter study suggested that previous parasitism might have improved the nutritional status of host larvae for *H. pallidus*. Our analysis of protein and lipid concentrations in *E. postvittana* larvae parasitised by *G. jacintae* also suggests that the mechanisms permitting greater clutch size and brood survivorship for secondary clutches could be nutritional.

Protein concentrations in the haemolymph of parasitised *E. postvittana* larvae continued to increase at a slow rate over time despite a reduction in host larval fresh weight. However, the rate of increase was less than observed for non-parasitised host larvae that grew substantially in fresh weight such that protein concentrations in parasitised larvae fell below that of non-parasitised larvae after day 3. A similar increase in protein concentration of the haemolymph over time has been observed for parasitised aphids (Pennacchio *et al.*, 1995). In addition, lipid concentrations in the haemolymph of parasitised larvae were both consistently higher than in non-parasitised larvae and increased over time at the same rate as in non-parasitised larvae. The elevated lipid concentrations are consistent with similar findings by Nakamatsu and Tanaka (2004) for the gregarious ectoparasitoid, *Euplectrus separatae* Kamijo. In this case, both natural and artificial injection of parasitoid venom increased lipid concentrations in the larval haemolymph of *Pseudaletia separata* Walker. As the majority of adult parasitoids are incapable of synthesizing lipids (Visser *et al.*, 2010), their acquisition from hosts during larval development is extremely important for the realised fecundity of adult females. Thus the greater protein and lipid concentrations in the haemolymph of previously parasitised *E. postvittana* larvae may provide a valuable advantage to *G. jacintae* in enhancing the life-history performance of secondary clutches derived from ovicide events. As primary clutches were removed by second females before any of the eggs had hatched, first females appear to have primed the host nutritionally during host handling, perhaps through the use of venom for temporary host paralysis. If detectable by an infanticidal female, the improved nutritional status of previously parasitised hosts could provide the cue for laying a larger secondary clutch on the same host individual than the first female. Whether a second parasitism event via infanticide leads to a further additive effect on lipid and protein concentrations in host haemolymph, beyond that observed in response to a first parasitism event, was not tested in the current study. Thus it remains unknown whether the increased survivorship of secondary broods was due to nutritional priming of the host by the first female or to additional priming during the second parasitism event.

In conclusion, as a gregarious ectoparasitoid, *G. jacintae* appears to base its clutch size decisions on both the nutritional quality and size of its host. This study is the first to provide evidence that improved nutritional quality could be an important benefit of infanticide for a gregarious ectoparasitoid, and one

that may be responsible for the larger clutch size and enhanced performance observed among secondary ovicidal clutches of *G. jacintae*.

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