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Superparasitism, immune response and optimum progeny yield in the gregarious parasitoid *Palmistichus elaeisis*

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Abstract

BACKGROUND: The subsequent deposition of an egg clutch by a female parasitoid into a host already parasitised either by itself or a conspecific (i.e. superparasitism) is a counterintuitive adaptive strategy, particularly considering the female parasitoid's ability to recognise the parasitised hosts. Such a scenario suggests that the adaptive value of superparasitism depends on the number of clutches laid in the same host, with consequences for parasitoid progeny yield. Here, we tested whether such is the case for the gregarious parasitoid *Palmistichus elaeisis* and explored its underlying basis.

RESULTS: Allowing female parasitoids to lay multiple egg clutches in a single melonworm host pupa, parasitoid progeny and fitness exhibited a peak or optimum at three egg clutches laid per host pupa. In addition, haemocyte count, encapsulation and melanisation decreased with the number of egg clutches laid per host pupa.

DISCUSSION: An optimum number of three clutches laid per host pupa was detected for *P. elaeisis*. As immune response via haemocyte production, encapsulation and melanisation decreased with the number of clutches laid per host, the higher parasitoid yield and fitness observed is the likely consequence of a compromised immune response coupled with an accommodative (i.e. scramble) larval competitive strategy allowing enough resources for optimum balance of parasitoid number and quality produced.

Keywords: larval competition; intrinsic parasitoid competition; idiobiont parasitoid; immunity; haemocyte; encapsulation

1 INTRODUCTION

Resource competition is a determinant of animal behaviour and life history where two alternative (intraspecific) strategies may take place – contest and scramble competition.[1–3] While in contest competition there is direct interference among competing individuals, with the successful competitor securing much of the governing resources needed for its development and reproduction, in scramble competition there is resource sharing and accommodation of all competitors, favouring their survival, but at the expense of their individual fitness.[4–6] Competition is a particular problem for insect endoparasitoids, the larval stages of which are spent within a single host, where intrinsic competition takes place (i.e. among immature parasitoids in a host), with potential implications for their rearing and use as bicontrol agents in pest management programmes.[7–9]

Most endoparasitoids seem able to recognise and reject parasitised hosts, preventing potential larval competition.[1–13] This is commonly observed among solitary parasitoids, where only a single larva is able to complete its development within a host and direct larval interference (i.e. aggression) is expected,[14,15] as also observed in some stored-grain beetles.[16–18] Nonetheless, gregarious parasitoids provide a different context in which two or more offspring complete development in a single host, favouring a scramble (or accommodation) type of competition.[19,20] However, even among gregarious parasitoids, the rejection of already parasitised hosts is expected, favouring progeny development and survival, but several species do superparasitise their hosts.[10–12,21]

Superparasitism takes place when a female parasitoid lays a clutch of eggs on or in a host already parasitised by the female itself (i.e. self-superparsitism) or a conspecific.[10,19] This phenomenon is counterintuitive and was considered maladaptive until eventually recognised as adaptive in some situations by the 1980s.[10] Curiously, superparasitism is frequent in several hymenopteran species and remains a study challenge where the host and the parasitoid need to be considered, as well as the underlying mechanisms involved.[9,20,21] Therefore, not only is the output of superparasitism important but also the process involved. For instance,

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although avoiding superparasitism will potentially benefit the siblings from a egg clutch, host (or resource) use likely improves under such conditions, suggesting that superparasitism should prevail with host scarcity.[9,20,21] Nonetheless, multiple ovipositions may take place in different species without apparent host scarcity, indicating a more complex scenario[21,22] and the possibility of a density-dependent optimum number of egg clutches laid, allowing for maximum parasitoid larval fitness and host use.

Infanticide and haemocyte-mediated encapsulation are two non-exclusive alternative strategies that may favour superparasitism.[23,24] Infanticide (including ovicide) takes place when the superparasitising female increases the likelihood of her offspring's survival by killing at least some individuals of the previous egg clutch laid in the same host.[11,14] Regarding encapsulation, the immune response of the parasitoid host is also potentially compromised, with superparasitism favouring this outcome because multiple egg clutches within the host are likely to overwhelm the host's defences.[19,22]

Both phenomena, infanticide and encapsulation, may take place simultaneously, enhancing the odds in favour of superparasitism, although the development of mixed broods within a host may allow for indirect benefits and/or brother–sister matings advantageous for some species,[12] and still benefit from compromising the host immune response.[22] However, the host immune response and particularly the cellular immune response (or haemocyte-mediated encapsulation) have been characterised in few insect species other than traditional model insects, and merit further attention.[25,26] The same argument is valid for the larva-competition-oriented studies of superparasitism, where the process of competition is usually inferred from the outcome of competition without direct assessment,[11,27–31] which may result in misleading conclusions, as observed in grain weevils.[18]

The gregarious endoparasitoid *Palmistichus elaeisis* Delvare & LaSalle (Hymenoptera: Eulophidae) provides an opportunity to assess larval competition with its consequences and underlying mechanisms. This is so because this parasitoid is a generalist species with potential economic importance as a biocontrol agent of several arthropod pest species, including the melonworm *Diaphania hyalinata* (L.) (Lepidoptera: Crambidae), the tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) and the sugarcane borer *Diatraea saccharalis* (F.), among others.[22,32,33] Furthermore, the species undergo superparasitism, particularly self-superparasitism, but also conspecific superparasitism overcoming its host cellular immune response.[22]

Here, we investigated the mechanism of larval competition with increasing levels of self-superparasitism of melonworm pupae by *P. elaeisis*, using digital X-ray imaging to follow the parasitoid development within its host as a direct method of investigation, and also using the outcome of superparasitism for indirect assessment of the process of competition and the fitness of the progeny produced. The strategy potentially allows the recognition of the eventual optimum level of superparasitism, measured as the number of ovipositions per host (or number of egg clutches laid per host), which was one of the aims of our study, which also attempted to recognise the potential density-dependent relationship between the level of superparasitism and the host immune response. Therefore, the immune response of the parasitised host pupae was also recorded under increasing superparasitism to recognise its potential relevance in determining the optimum level of parasitism, if any density-dependent relationship exists in such a phenomenon. A scramble type of competition was expected, with the accommodation of multiple larvae within a single host pupa, based on previous reports of frequent superparasitism in this species.[22,31–33] However, we also expected an optimum level of superparasitism, as detected in grain beetles[16,18,34] but not yet explored in endoparasitoids in the context of larval competition and host immune response.

2 MATERIALS AND METHODS

2.1 Host insect

The pupae of melonworm were from a laboratory colony maintained on chayote leaves [*Sechium edule* (Jacq.) Sw.], which were daily provided until the prepupal stage in 3 L plastic containers with a perforated lid covered with organza tissue. The prepupae were transferred to clean 3 L plastic containers lined with paper towel until eventual pupation, and then the pupae were gathered and transferred to wooden-framed cages of organza (33 × 33 × 33 cm) until adult emergence. Sucrose solution (60 g sucrose, 10.5 g honey, 1.05 g nipagin and ascorbic acid diluted in 1.05 L of water) was provided in imbibed cotton balls placed over a petri dish in each cage.[35] Pumpkin leaves were placed within the cage for the adult females of the melonworm to lay their eggs, which were subsequently collected and placed in 3 L plastic containers for rearing.[36] The insects were reared in a controlled environment room at 25 ± 2 °C, 70 ± 10% relative humidity and a 12:12 h (L:D) photoperiod.

2.2 Parasitoid

The parasitoids were also from a laboratory colony maintained at 25 ± 2 °C, 70 ± 10% relative humidity and a 14:10 h (L:D) photoperiod, the same conditions in which the entire study was carried out. The parasitoids were reared in glass tubes (14 cm length × 2.2 cm diameter) with a drop of honey provided on a cotton ball.[37] Females and males of *P. elaeisis* were paired between 24 and 36 h after emergence, and mealworm pupae (24–72 h old) were individualised with six parasitoid females each; the emerging adult parasitoids were subsequently used in the study.

2.3 Parasitoid larval competition

Each melonworm pupa (24 h old; 114 ± 0.08 mg) was exposed to one ($n = 34$), two ($n = 27$), three ($n = 27$), four ($n = 24$) or five ovipositions ($n = 21$) by the same female parasitoid of *P. elaeisis* (76–96 h old, when their oocytes are mature and suitable for egg laying).[22,23] This was done on plastic cylinder arenas (2.2 cm diameter × 1.8 cm height) after sexing the females using morphological characteristics of their abdomen.[38] The parasitism was monitored through recording, using a camera (charge coupled device, CCD) coupled to a stereomicroscope (Stemi 2000; Zeiss, Göttingen, Germany). The parasitised pupae were individualised in glass tubes (14 cm length × 2.2 cm diameter) and maintained under controlled conditions, as previously detailed for parasitoids.

The parasitoid development within each host was directly followed through daily inspections of the host pupae using a LX-60 specimen radiography system equipped with a digital camera (Faxitron X-Ray Corp., Wheeling, IL). Therefore, the locations and eventual interaction among larvae within the host, as well as their developmental stage and overall health condition, were digitally recorded throughout their development. Parasitoid developmental time, emergence, sex and body mass were all recorded, and the progeny fitness was estimated as the biomass of progeny produced per host pupa.[3,6,39] As fecundity and longevity are associated with body mass and larval survival,[20] and adult body mass is

affected by larval competition, these parameters are included in measures of larval fitness. This was achieved by determining the parasitoid biomass (mg) produced per host, generating larval fitness curves.[6]

2.4 Host immune response

Host immune response was recorded for all levels of superparasitism and included haemocyte counts and determination of the rates of encapsulation and melanisation, as detailed below.

2.4.1 Haemocyte count and characterisation

Unparasitised and parasitised pupae of the same age (24 h after parasitism) were rinsed with sodium hypochloride solution (1%) for 5 s and subsequently with distilled water. Host insect haemolymph (4 µL) was collected with micropipettes from a small head incision and transferred to a 20 µL solution of anticoagulant (98 mM of NaOH, 186 mM of NaCl, 17 mM of EDTA-Na_2 and 41 mM of citric acid; pH 4.5) to prevent haemocyte aggregation.[27] The haemolymph samples were stained (4 µL of Giemsa), and two 8 µL aliquots of this solution were added at each side of a Neubauer haemocytometer with a 40× objective lens (Bright-Line; New Optics, Gyeibggi-do, South Korea) to recognise and quantify the haemocyte types.[22,27,40] Ten host pupae at each level of superparasitism were subject to such characterisation and quantification.

2.4.2 Encapsulation and melanisation

The encapsulation defence response by the host pupa was assessed through the haemocyte adherence to nylon filaments (2 × 0.2 mm) implanted into individuals subjected to the range of parasitoid ovipositions scrutinised. The nylon filaments were initially sterilised with sodium hypochloride (1%) and subsequently washed with distilled water before being implanted into the melonworm pupae (24 h old; 109 ± 0.01 mg of fresh body mass). Between ten and 13 pupae at each level of superparasitism (1–5 parasitoid ovipositions) were implanted with nylon filaments. Their immune response was recorded 48 h after implant insertion while removing them from the pupae under dissection. The nylon implants removed were mounted on slides and digitally photographed with a AxioCam MRc5 camera coupled to a stereomicroscope (Zeiss) and processed with AxioVision software (Zeiss).

The rate of encapsulation was determined on the basis of the haemocyte cell layer covering the implanted nylon filaments and estimating the encapsulated area of the implant.[22,41,42] The digitalised figures of the nylon implants were reversed to greyscale with a range variation from 0 (black) to 255 (white) to determine the rate of melanisation, the average level of which within the greyscale was determined using ImageJ 1.48 software (US National Institutes of Health, http://imagej.nih.gov/ij/download.html). The values were standardised by the darkest value obtained in the implants after adjustment by extracting the background value.[22]

2.5 Statistical analyses

Parasitoid survival results were subjected to survival analyses using Kaplan–Meier estimators (PROC LIFETEST, SAS software; SAS Institute, Cary, NC). A (partial) canonical correlation analysis was performed to test the contribution of each haemocyte type to the total haemocyte count, using the CANCORR procedure from SAS (SAS Institute). All of the remaining results

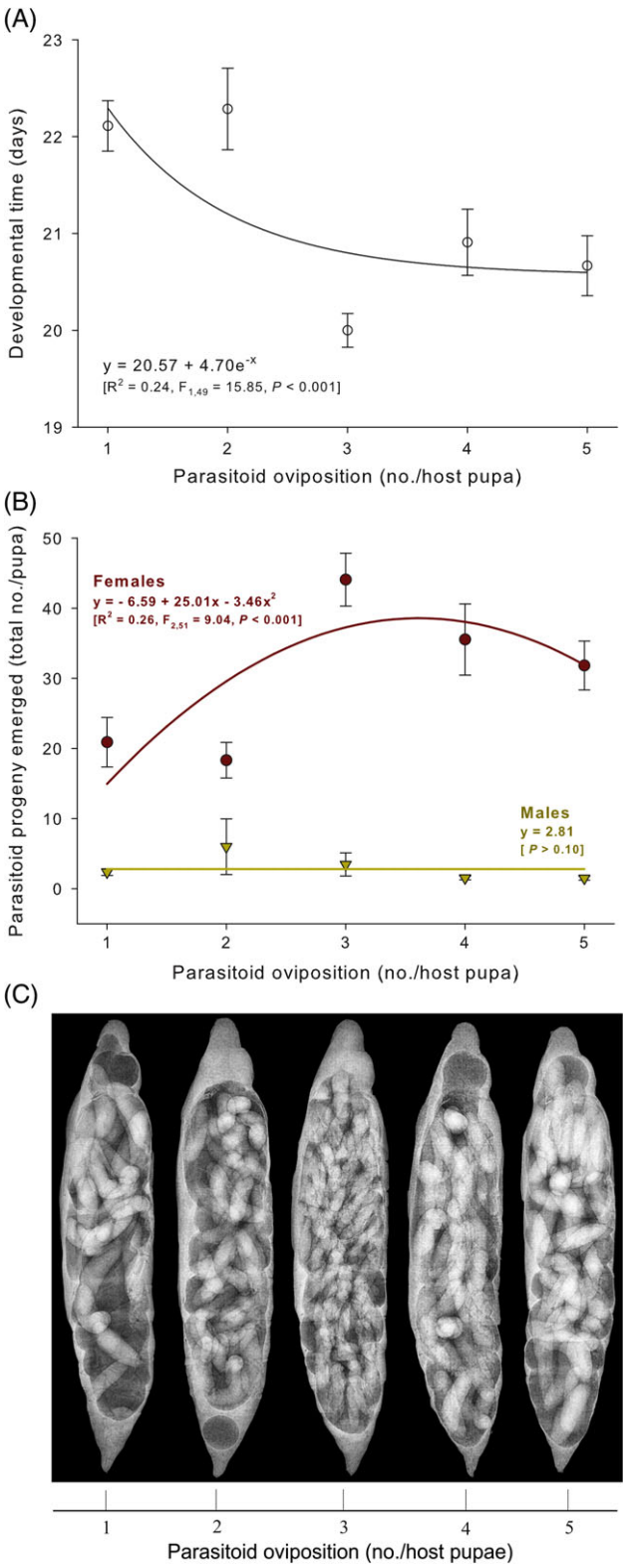

Figure 1. Developmental time (A), total progeny emerged per host pupa (B) and sequential X-ray digital images (C) showing a representative sequence of developing parasitoid larvae of *P. elaeisis* within the same host pupa of the melonworm *D. hyalinata*. Each symbol in plots (A) and (B) represents the mean values of 24–34 replicates (i.e. pupae), and the vertical bars indicate the standard error of the means.

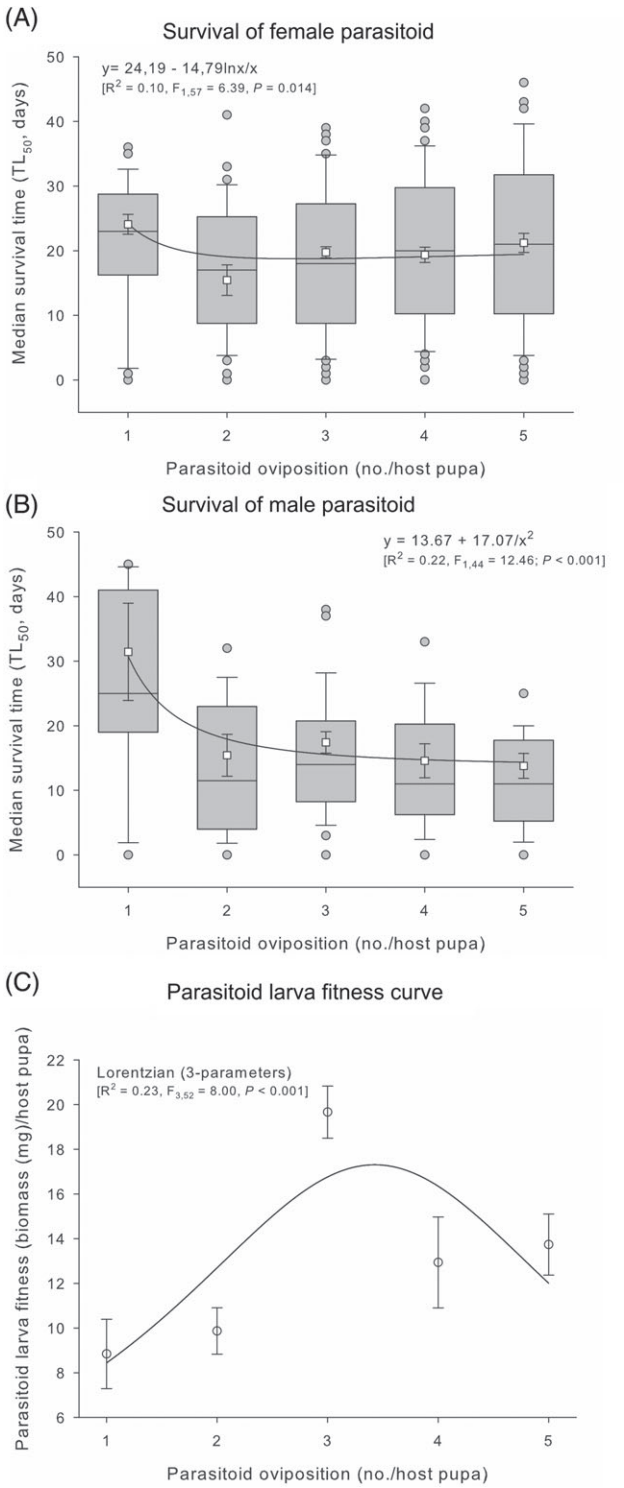

Figure 2. Median survival time (TL_{50}) of female (A) and male (B) parasitoids, and larva fitness curves (C) of the gregarious hymenopteran parasitoid *P. elaeisis* reared at increasing levels of superparasitism in pupae of the melonworm *D. hyalinata*. Box plots (A, B) indicate the range of data dispersion (lower and upper quartiles and extreme values), median and outliers (symbols). Each symbol in (C) represents the mean values of 24–34 replicates (i.e. pupae), and the vertical bars indicate the standard error of the means.

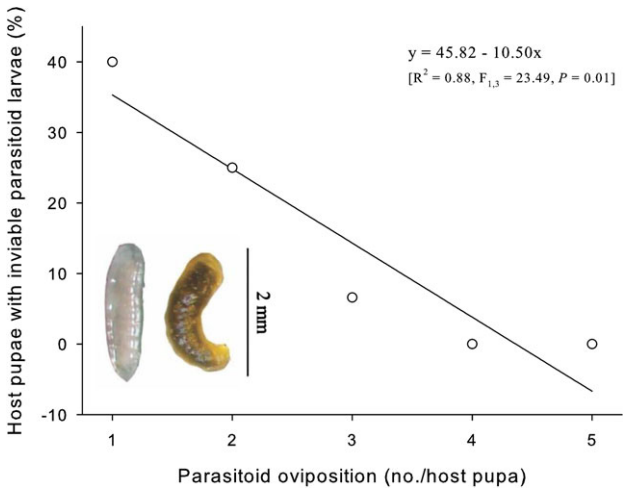

Figure 3. Host pupae of the melonworm *D. hyalinata* containing inviable parasitoids (%) of the gregarious species *P. elaeisis* reared at increasing levels of superparasitism. The insets are representative pictures of a healthy and an inviable larva of the parasitoid *P. elaeises*. Each symbol represents the observed data points used in the regression estimate.

obtained were subjected to regression analysis, with the number of parasitoid ovipositions as the independent variable using the curve-fitting procedure from TableCurve 2D (Systat, San Jose, CA); the significant regression models ($P < 0.05$) were tested from the simplest (linear and quadratic) to more complex peak models, and model selection was based on parsimony, high F-values (and mean squares) and steep increases in R^2 with model complexity.

3 RESULTS

3.1 Parasitoid larval competition

The developmental time of the parasitoid larvae varied significantly with the number of parasitoid egg clutches laid in the host pupae, exponentially decreasing with the number of ovipositions, reaching a plateau around three parasitoid ovipositions per pupa (Fig. 1A). Parasitoid female progeny emergence also varied with level of superparasitism, reaching a peak of female emergence at around three ovipositions per pupa (Fig. 1B). Curiously though, male parasitoid emergence was not affected by the level of superparasitism (Fig. 1B), and the female-biased sex ratio in this parasitoid species also remained unaffected by parasitoid load (number of females/number in total = 0.93 ± 0.02; $F_{1,55} = 3.11$, $P = 0.08$). The X-ray imaging analyses confirmed these results, indicating a larger number of developing parasitoids with three ovipositions per pupa and a quicker development at the higher levels of superparasitism. At these high levels of superparasitism, the parasitoid pupa is already visible within the host, unlike at lower numbers of ovipositions per host pupa (i.e. <3) (Fig. 1C).

The level of superparasitism also negatively affected parasitoid survival, with median survival times (TL_{50}) successfully estimated through survival analysis ($P < 0.01$) and exhibiting extended survival with a sole oviposition per pupa, both for female (Fig. 2A) and male (Fig. 2B) parasitoid progenies. The individual body mass of the emerging adult female and male parasitoids was not significantly affected by the level of superparasitism ($P < 0.05$), but female body mass was always higher than that of the male parasitoids (female: 0.42 ± 0.001 mg; male: 0.17 ± 0.004 mg). The parasitoid progeny biomass produced per pupa at each level of

superparasitism provided a bell-shaped curve exhibiting a peak in parasitoid progeny yield at around three ovipositions per host pupa (Fig. 2C), following a trend similar to that obtained with female progeny emergence.

3.2 Host immune response

Inviability of parasitoid larva owing to the host immune response was also affected by the level of superparasitism, with a linear decrease in inviability with an increasing number of parasitoid egg clutches laid per pupa (Fig. 3). The host immune response did not prevent pupal death, but it did compromise parasitoid emergence by also killing the developing parasitoid progeny within the host pupa, as illustrated in the sequential X-ray images in Fig. 4. The cellular or haemocyte-mediated encapsulation of the developing parasitoids was also recorded for the melonworm pupa–*P. elaeisis* system.

The level of superparasitism linearly compromised the total haemocyte count in the host pupa (Fig. 5A). Five different haemocyte types were observed – granulocytes, oenocytes, plasmatocytes, prohaemocytes and spherulocytes. All of these haemocyte types were correlated with the total haemocyte count using (partial) canonical correlation ($r = 0.99$, $P < 0.001$), with the highest contributions from oenocytes and prohaemocytes, but with a significant contribution from all types (Table 1 and Fig. 5B).

Encapsulation and melanisation were clearly observed in the implanted nylon filaments (Fig. 6A). Haemocyte adherence and aggregation in the nylon filaments, leading to encapsulation, was negatively affected by the level of superparasitism, following a linear trend (Fig. 6B). Melanisation by the host pupa was also compromised by the level of superparasitism, although an exponential rather than a linear trend was observed, with the melanisation decline accentuated at three ovipositions per pupa (Fig. 6C).

4 DISCUSSION

Superparasitism and particularly self-superparasitism are currently recognised as a potentially adaptive strategy frequently occurring among hymenopteran parasitoids, with potential consequences for biological control, and thus for insect pest management.[9,10,20] Curiously, the mechanism of larval competition in such species and the potential fitness consequences of increasing superparasitism remain poorly investigated, although they are potentially important for optimising the mass rearing and field use of the biocontrol species. They were the target of our study, where scramble competition among parasitoid larvae was expected, based on the incidence of superparasitism in *P. elaeisis*.[22,32,33] We suggested the potential existence of an optimum level of superparasitism, which had not yet been explored in endoparasitoids in the context of larval competition and host immune response. Our findings provide support for these stated expectations.

Increasing levels of superparasitism of melonworm pupae by *P. elaeisis* are associated with decreased larval developmental time and (female and male) longevity, which suggests a scramble strategy of larval competition, with larval accommodation within the host without aggressive interference among them.[3,6,17,18] In contrast, the level of superparasitism did not affect male and female (fresh) body mass, nor the highly female-biased sex ratio and male emergence, which would also be expected under scramble competition.[6,18] Nonetheless, males are not as frequent within this parasitoid species and exhibit lower body mass, indicating only a minor contribution for the parasitoid biomass produced per parasitised pupa. Therefore, as the body mass was not substantially

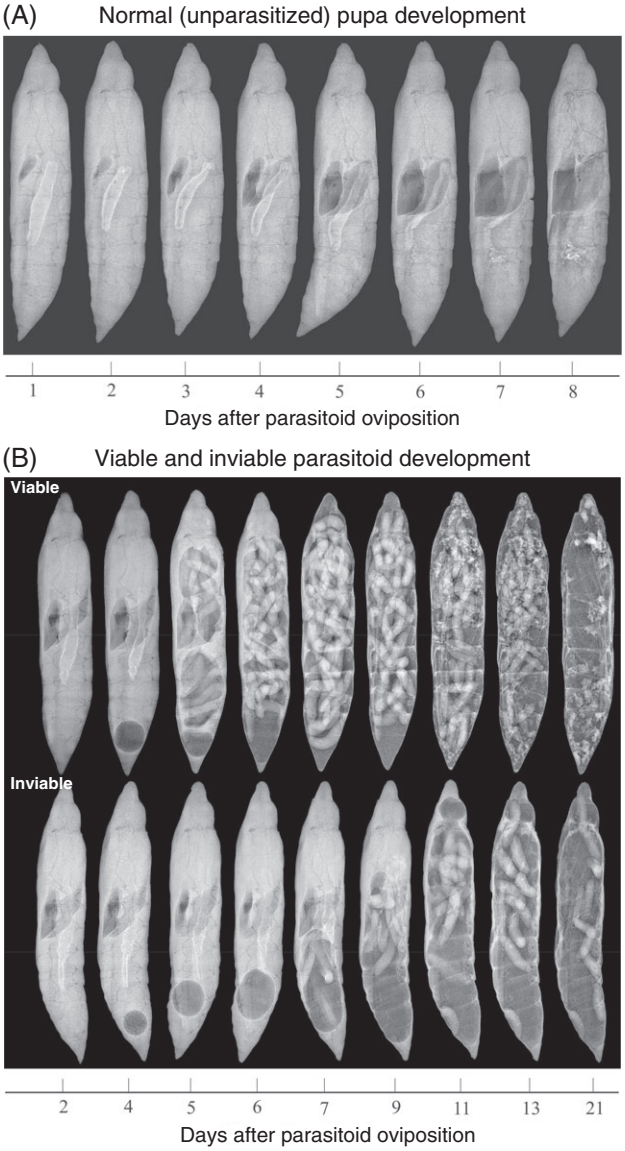

Figure 4. Digitalised X-ray images of the host pupae of the melonworm *D. hyalinata* undergoing normal development until exhibiting the pharate adult stage (A), superparasitised by *P. elaeisis* with parasitoids fully completing their development and starting to emerge as adults (B), and with inviable parasitoids undergoing encapsulation (B).

influenced by the level of superparasitism, female emergence is the key determinant of larval fitness. Indeed, the peak of female emergence observed with three ovipositions per pupa was translated into a peak and thus an optimum larval fitness curve at a similar level of superparasitism, an expected outcome of a scramble process of competition.[6,18]

The outcome reported above is consistent with a scramble process of competition, but indirectly inferred. X-ray imaging allows larval development within the host pupa to be followed, and thus the occurrence of direct aggressive interference or larval accommodation to be recognised. The larvae of *P. elaeisis* did not exhibit aggressive interference with one another, but spaced themselves within the host pupa, allowing accommodation and resource sharing, expected among endoparasitoids,[21] without any evidence of infanticide (by the adult female) or siblicide (among larvae), with an apparent slower development

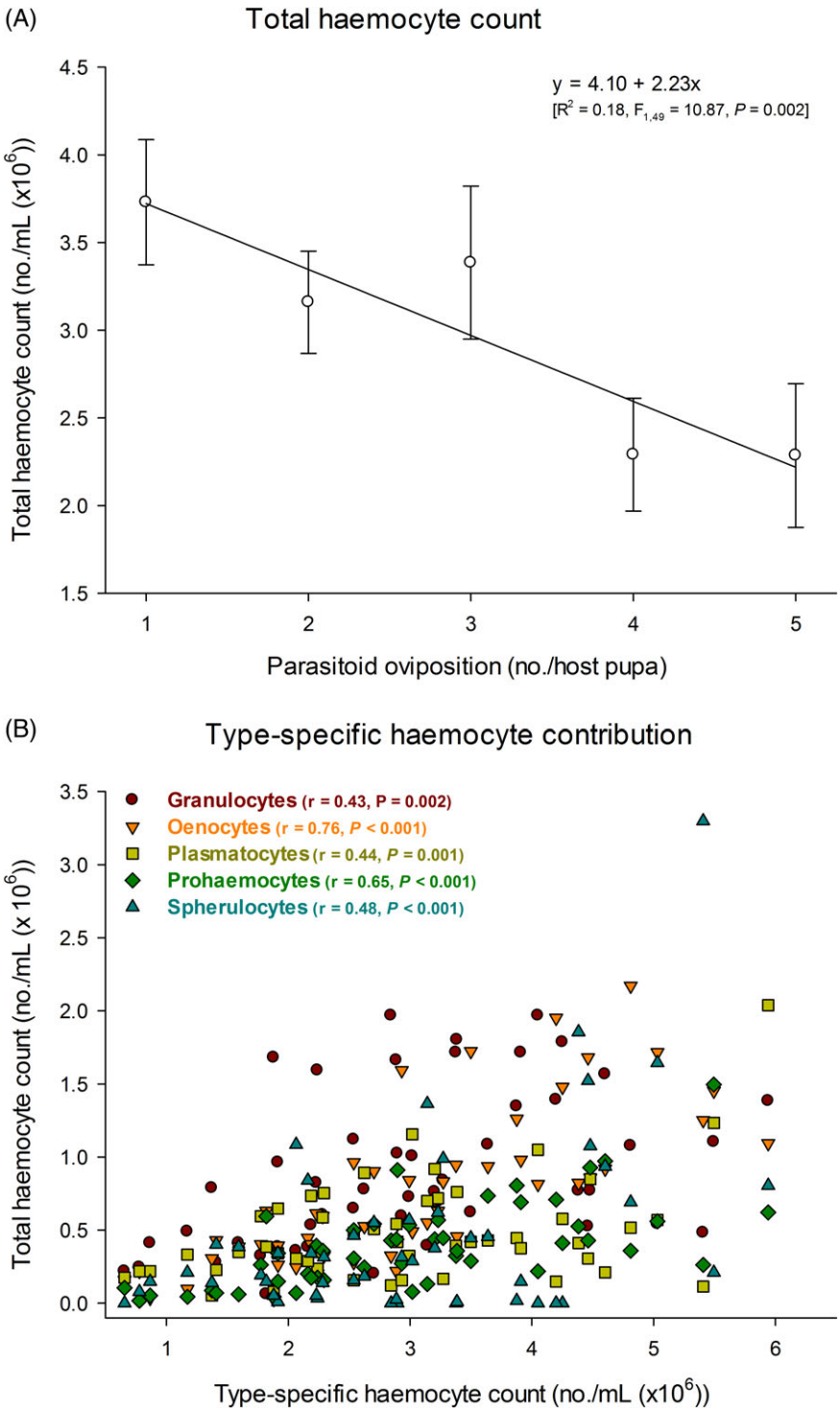

Figure 5. Total haemocyte counts (A) and relationship between total haemocyte counts and counts of each haemocyte type (B) from parasitised (and superparasitised) pupae of the melonworm *D. hyalinata*. Each symbol represents the observed values (A: means ± SEM; B: individual non-averaged values) of haemocyte counts.

under low superparasitism. Infanticide and siblicide are strategies that allegedly favour superparasitism,[11,24–26] but here we demonstrated that superparasitism may take place without them. These reported findings provide further and direct evidence of a scramble type of competition among parasitoid larvae within the host pupa. Such an outcome and process, though, may differ if the superparasitic egg clutches are from different conspecific females rather than from the same female (i.e. self-superparasitism), as explored in our study. However, previous findings by Andrade *et al.*[22] suggest that the outcome and process are likely the same for *P. elaeisis*.

High levels of oviposition (at least four per host pupa) seem to compromise resource acquisition by the developing parasitoid larvae, thus reducing their individual body mass and longevity,

Table 1. Canonical pairs and canonical loadings of a (partial) canonical correlation between total haemocytes and specific haemocyte types, induced by parasitism of *P. elaeisis* in pupae of *D. hyalinata* (the main contributors are indicated in bold)

	Canonical pair	
Traits	Coefficient	Correlation
First set of traits		
Total haemocytes (number mL^{-1}) ($\times 10^6$)	1.00	**1.00**
Second set of traits		
Granulocytes (number mL^{-1}) ($\times 10^6$)	**0.41**	0.43
Oenocytes (number mL^{-1}) ($\times 10^6$)	**0.41**	**0.76**
Plasmatocytes (number mL^{-1}) ($\times 10^6$)	0.28	0.44
Prohaemocytes (number mL^{-1}) ($\times 10^6$)	0.23	**0.65**
Spherulocytes (number mL^{-1}) ($\times 10^6$)	**0.48**	0.48
r	0.99	
F_{appr}	∞	
$df_{num/den}$	5/45	
P	<0.0001	

as reported on other parasitoid species.[43–46] In contrast, low levels of oviposition (i.e. no more than two per host pupa) seem to favour longer development and individual parasitoid longevity, but with suboptimal resource use and lower progeny yield per host. Although the level of superparasitism may sometimes affect the sex ratio,[20,28,47,48] this did not take place with melonworm pupae and *P. elaeisis*. Thus, an optimum parasitoid progeny outcome of *P. elaeisis* in pupae of melonworm was apparent at three ovipositions per host pupa, but besides of an optimum resource use within the level of superparasitism, such an outcome is dependent on the host immune response, particularly its cellular or haemocyte-mediated response.

The encapsulation activity of parasitised melonworm pupae was directly recorded by X-ray imaging progressing with time and leading to inviability of parasitoid larvae. Such a response was particularly strong with a single parasitoid egg clutch, and thus without superparasitism. Superparasitism compromised the inviability of parasitoid larvae, and higher levels of superparasitism further compromised not only haemocyte counts but also both encapsulation and melanisation response. Such effects on haemocyte counts and encapsulation were reported earlier,[22] but the former encompassed counts of all five haemocyte types observed in the melonworm immune reponse, with particularly higher contributions from oenocytes and prohaemocytes. Although granulocytes and plasmatocytes are reported as the main haemocytes in lepidopteran immune response,[24–26,40] melonworm pupae exhibit a more even count of the different types of haemocyte, with granulocyte counts prevailing only in parasitised, but not superparasitised, pupae.

Plasmatocytes are important in encapsulation, while granulocytes are usually associated with phagocytic activity in Lepidoptera.[24,25,40] Thus, plasmatocyte counts should have more influence on encapsulation response in the melonworm pupa against *P. elaeisis*, but the prohaemocyte response was even higher, which is still consistent with expectations as this type of haemocyte is a precursor of the others,[40] including plasmatocytes and oenocytes. Oenocyte response to increased superparasitism was the strongest and was closely related to total haemocyte response, declining with increasing number of parasitoid ovipositions. As oenocytes are involved in melanisation during

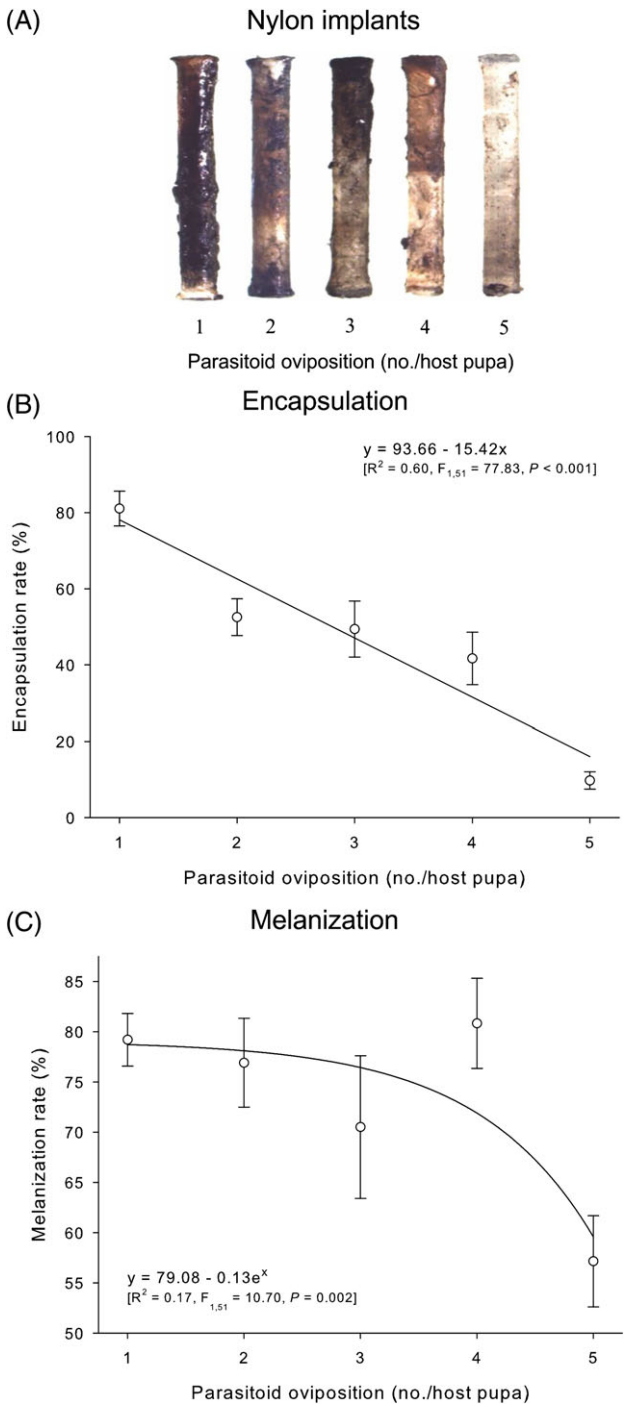

Figure 6. Implanted nylon filaments (A) and rates of encapsulation (B) and melanisation (C) recorded from pupae of the melonworm *D. hyalinata* subjected to increasing levels of superparasitism by *P. elaeisis*. Each symbol in (B) and (C) represents the mean observed values, and the vertical bars indicate the standard error of the respective means.

encapsulation owing to their production of phenoloxidases,[40] the response is consistent with the patterns of immune response observed, particularly encapsulation and melanisation. The functions of spherulocytes are not well known, but their response followed those of the other haemocyte types, with the total haemocyte count declining with increased superparasitism in pupae of the melonworm.

Higher parasitoid oviposition favoured exhaustion of the host immune response in melonworm pupae, likely owing to the injection of symbiotic virus, teratocytes and/or ovary proteins from the parasitoids into the host, as reported in other parasitoid species.[21,49–51] Haemocyte exhaustion compromised encapsulation activity (i.e. the binding of haemocytes forming a sheath around the targeted invader), a relationship also observed in our study, as in different parasitoids.[11,21,25] Melanisation, resulting from phenoloxidase activity in the encapsulated invader, was also compromised with superparasitism, as would be expected based on previous findings.[22,25] Although the parasitised host was unable to survive parasitism by even a single parasitoid egg clutch, parasitoid inviability was higher in such conditions, while a higher number of egg clutches favoured parasitoid yield, in addition to their fitness, benefiting their role as biocontrol agents.

Altogether, increased superparasitism by *P. elaeisis* compromises the host immune response, as reflected by a reduction in haemocyte counts, encapsulation and melanisation. Therefore, such a trend, combined with an optimum parasitoid larval fitness at three ovipositions per host, indicated that host resource use is optimum at this level of superparasitism, allowing a peak in parasitoid progeny yield without compromising progeny body mass and thus its subsequent performance. In summary, optimum parasitoid yield is due to the scramble process of larval competition with maximum host resource sharing and depletion of the host immune response. Host pupal survival is prevented with a single oviposition by *P. elaeisis*, but maximum parasitoid yield is relevant for their mass rearing and the optimum release rate as a biological control agent to be considered in insect pest management programmes. Eventual mass rearing of *P. elaeisis* should consider the optimum clutch size per host pupa to maximise parasitoid yield and quality production, which also needs to be considered when estimating the rate of field release of the parasitoid for purposes of biological control.

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