Tendency and consequences of superparasitism for the parasitoid *Ooencyrtus pityocampae* (Hymenoptera: Encyrtidae) in parasitizing a new laboratory host, *Philosamia ricini* (Lepidoptera: Saturniidae)

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**Abstract.** The tendency for self-superparasitism and its effects on the quality of the parasitoid *Ooencyrtus pityocampae* (Mercet) (Hymenoptera: Encyrtidae) in parasitizing a new laboratory host, *Philosamia ricini* (Danovan) (Lepidoptera: Saturniidae), were investigated. In this study, female parasitoids of various ages (1-, 3- and 5-day-old) were tested individually. Parasitoids were provided with 1-day-old *P. ricini* eggs at ratios of 5, 10, 20, 30 and 40 host eggs per wasp. The tendency to superparasitize was dependent on the female’s age and host density. Five-day-old females showed a strong tendency to superparasitize at low host densities. The development time of wasps in superparasitized eggs was longer than that of wasps in singly parasitized eggs. The size and longevity of adult parasitoids decreased significantly with superparasitism. This work contributes to the development of an efficient mass rearing and laboratory rearing of the parasitoid *O. pityocampae* using a new host.

**INTRODUCTION**

*Ooencyrtus* is a genus of solitary polyphagous egg parasitoids, which attacks many of the insect pests of agriculture and forestry. Ten species of *Ooencyrtus* are used in biological control programs (Noyes & Hayat, 1984; Huang & Noyes, 1994). *Ooencyrtus pityocampae* (Mercet) (Hymenoptera: Encyrtidae) is the most effective parasitoid of the pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Thaumetopoidea) and is used in inundative biocontrol programs aimed at controlling this forest pest (Battisti et al., 1990; Masutti et al., 1993; Tiberi et al., 1994; Zhang et al., 2005; Binazzi et al., 2013; Samra et al., 2015).

The improvement of biocontrol programs depends on the successful mass rearing of beneficial insects. Successful mass rearing is defined as producing high quality insects at low cost (Norlund, 1998). To produce large numbers of high quality parasitoids in a laboratory or insectary, rearing methods need to be automated and environmental conditions need to be optimal, during the production process. A high quality parasitoid can be obtained by optimising their life history parameters, such as growth, development, longevity, body size, fecundity, fertility, sex ratio and generation time (Bratti & Costantini, 1991; Messing et al., 1993; Morales Ramos et al., 1998; Gandolfi, 2002; Wajnberg et al., 2008; Consoli et al., 2010).

These parameters are very important for producing parasitoids that perform well both in a laboratory and the field. However, superparasitism can adversely affect the quality of the parasitoid. Superparasitism refers to the oviposition behaviour of parasitoid females that lay eggs in previously parasitized hosts (Gu et al., 2003; Gandolfi et al., 2006; Dorn & Beckage, 2007). Superparasitism can adversely affect offspring fitness as they have to compete for resources (van Alphen & Visser, 1990). Superparasitism, however, is recorded in certain situations such as (i) when two or more females search together in a patch, (ii) when unparasitized hosts are rare (egg-limited parasitoid model) and (iii) when females have many mature eggs (time limited model) (Iwasa et al., 1984; van der Hoeven & Hemerik, 1990; Visser et al., 1992; Godfray, 1994).

Superparasitism is categorized into self- and conspecific superparasitism: Self-superparasitism occurs when a female parasitoid attacks a host that has already been attacked and exploited by herself (Waage, 1986), whereas in conspecific superparasitism, a female attacks a host that has been previously attacked by a conspecific (Waage, 1986; van Dijken & Waage, 1987). Moreover, self-super-
parasitism can result in host-sharing by solitary endoparasitoids, leading eventually to the evolution of gregariousness (Riddick, 2002; Paxton & Mayhew, 2005; Khafagi & Hegazi, 2008). Many biological factors affect the incidence of superparasitism, including the biological properties of female parasitoids (e.g., age, mating status, egg load, oviposition period, density), host species, host size, host density and exposure time (Brodeur & Boivin, 2006; Shoeb & El-Heneidy, 2010). In this study, we focused on the effects of female age and host density.

Hymenopteran parasitoids are classified as either proovigenic or synovigenic (Flanders, 1950; Quicke, 1997). Proovigenic females complete oogenesis prior to emergence and lay their eggs over a relatively short period of time. In synovigenic parasitoids, however, females emerge with no or few eggs and produce eggs throughout their lifetime. Egg production and mode of parasitism are also related to female age (Jervis & Kidd, 1986; Jervis et al., 1996; Quicke, 1997). Ueno (1999) and Sirot et al. (1997) show that oviposition decisions depend upon the egg load of the female parasitoid. A higher egg load may result in parasitoids laying eggs in parasitized hosts, and in this case the probability of superparasitism increases (Kearsa et al., 2006). *O. pityocampae* is also a synovigenic parasitoid and superparasitism by this species may be associated with female age.

Selection of a potential host for oviposition has a major role in determining the fitness of the parasitoid’s offspring (Doutt, 1959; Vinson, 1976; Hassell, 2000). The offspring of parasitoids that oviposit in high-quality hosts (i.e. which provide sufficient food resources) are more likely to survive and be more fecund than those of parasitoids that oviposit in low-quality hosts (Bernal et al., 1999; King, 2000).

When high-quality hosts are scarce, a parasitoid may accept poor-quality hosts (Van Alphen & Vet, 1986). Hosts that are already parasitized (self or conspecific superparasitism) are generally of lower quality, as embryos developing within them have to compete for food resources (Godfray, 1994). For successful parasitism, parasitoid females must oviposit and their progeny develop in individual hosts. However, egg load, longevity of the female parasitoid and host density all affect the acceptance of both unparasitized and parasitized hosts (Roitberg et al., 1992, 1993; Fletcher et al., 1994; Hughes et al., 1994; Sirot et al., 1997; Ueno, 1999; Islam & Copland, 2000; Hopper et al., 2013).

In this study, self-superparasitism by the solitary synovigenic parasitoid *O. pityocampae* was first tested using a new host *Philosamia ricini* (Donovan) (Lepidoptera: Saturniidae). The aim of this study was to determine whether the tendency of female parasitoids to superparasitize depended both on female age and host density. In addition, we investigated the effects of superparasitism on parasitoid progeny quality.

### MATERIAL AND METHODS

This study was conducted at the INRA-PACA Mediterranean Forest and Entomology Unit, Laboratory of Biological Control, Antibes, France. All experiments were performed under controlled conditions of 25 ± 1°C, an RH of 65% ± 5% and a 16L : 8D photoperiod.

**Study species**

The *O. pityocampae* used in this study came from a stock culture established from field-collected parasitized eggs of *T. pityocampa* collected in the Bouches du Rhone province and reared on *P. ricini* eggs. Two females were isolated in glass tubes (7 × 1 cm) containing approximately 70–80 fresh *P. ricini* egg masses and a drop of bio-honey for feeding. After parasitism, the female parasitoids were removed and the tubes were maintained in an incubator (25 ± 1°C, RH 65% ± 5% and 16L : 8D h photoperiod). After emergence, adult female parasitoids were used for subsequent experiments and to initiate parasitoid rearing. *O. pityocampae* was reared for over 9 generations in eggs of *P. ricini*.

Large numbers of *P. ricini* can be easily reared on privet foliage under laboratory conditions of 25 ± 1°C, RH 65% ± 5% and a 16L : 8D h photoperiod. *P. ricini* eggs were collected daily and kept in an incubator. Upon hatching, the neonates were placed in plastic containers (26 × 12 × 7 cm) and fed privet foliage. Fresh foliage was provided every day, and separate containers were used for the different larval stages. At pupation, individual pupae were transferred into adult rearing cages (30 × 39 × 30 cm). This process was repeated daily.

**Experimental procedure**

To quantify the tendency to superparasitize, recently emerged females were transferred individually to glass tubes (1 × 7 cm)

| Table 1. Results of the GLM analysis of the percentage emergence of parasitoids, percentage of superparasitized eggs and percentage of single-parasitized eggs. |
|-----------------|--------|--------|--------|--------|
| Source of variation | DF    | SS     | F     | P value |
| Parasitoid emergence |       |        |       |        |
| Parasitoid age | 2      | 0.53889 | 11.40 | <0.001 |
| Host egg number | 4      | 0.80056 | 8.47  | <0.001 |
| Parasitoid age × Host egg number | 8      | 0.10080 | 0.53  | 0.822 |
| Error | 30 | 0.70907 |        |        |
| Superparasitized eggs |       |        |       |        |
| Parasitoid age | 2      | 0.20936 | 4.10  | 0.027  |
| Host egg number | 4      | 0.50517 | 4.94  | 0.004  |
| Parasitoid age × Host egg number | 8      | 0.11046 | 0.54  | 0.817  |
| Error | 30 | 0.76660 |        |        |
| Single parasitized eggs |       |        |       |        |
| Parasitoid age | 2      | 0.02225 | 0.38  | 0.690  |
| Host egg number | 4      | 0.45745 | 3.87  | 0.012  |
| Parasitoid age × Host egg number | 8      | 0.03941 | 0.17  | 0.994  |
| Error | 30 | 0.88680 |        |        |
and fed bio-honey prior to experiments. These females were separated into three groups (1-, 3 and 5-day old) and were tested individually. One-day-old *P. ricini* eggs were exposed to inexperienced 1-, 3- and 5-day-old females at ratios of 5, 10, 20, 30 and 40 host eggs per wasp. At the end of the 14th day of exposure, the female parasitoid was removed and parasitized eggs were placed individually in glass tubes (7 × 1 cm). These tubes, each containing one parasitized egg, were incubated at 25 ± 1°C, RH 65% ± 5% and a 16L : 8D photoperiod until the parasitoid progeny emerged. In this study, 45 females and 315 host eggs were used. The number of individual parasitoids per host egg was evaluated by counting how many emerged from each host egg. If self-superparasitism occurs in *O. pityocampae*, it is possible to obtain two parasitoids from each *P. ricini* egg. The parasitoid emergence and frequency singly or superparasitized eggs were calculated, and percentage data on parasitoid emergence >100% exists due to superparasitism. In addition, we compared the development time, longevity and body size (weight) of the progeny that emerged from superparasitized and singly parasitized hosts.

Data analysis

Percentage data relating to “parasitoid age” and “host egg number” was analyzed using a General Linear Model. Development time, longevity and body size (weight) were analyzed using a simple t-test (Minitab Release 14, McKenzie & Goldman, 2005; SAS Institute, 2000). Means were separated using Duncan’s test at a significant level of α = 0.05 (SAS Institute, 2003).

### RESULTS

The statistical results are shown in Table 1. There was a significant effect of both female age and number of host eggs on parasitoid emergence and number of eggs superparasitized. Single-parasitized eggs were affected only by the number of host eggs, but there was no significant interaction between female age and number of host eggs (GLM; PEmergence Rate = 0.822, PSuperparasitized Egg = 0.817, PSingle Parasitized Egg = 0.994) (Table 1). The highest percentage parasitoid emergence was recorded for 5 day old parasitoids (126%) and 5 host eggs (135.55%). The highest percentage of superparasitized eggs was recorded for 3–5 day old parasitoids (22.16%–30.44%) and 5–10 host eggs (42.22%–28.88%). The highest percentage of singly parasitized egg was recorded for 1 day old parasitoids (70.28%) and 30–40 host eggs (77.03%–75.83%) (Figs 1 and 2).

In addition, there were significant differences in the development time, longevity and size of *O. pityocampae* progeny that developed in superparasitized and singly parasitized eggs (t1993 = 10.33, P < 0.000; t82 = –9.15, P < 0.000; t308 = –15.65, P < 0.000) (Table 2). Development time increased and parasitoid size and longevity decreased with superparasitism. Therefore, self-superparasitism had a negative effect on these parameters of parasitoid progeny.

### DISCUSSION AND CONCLUSIONS

*T. pityocampa* is one of the pine defoliators of high economic importance, especially in forests in the Mediterranean area. Various species of *Pinus* serve as food plants for this polyphagous forest pest (Devkota & Schmidt, 1990). Ecologically based integrated pest management strategies are very important for controlling these and other forest pests (Lieutier & Ghaioule, 2005) This strategy is a broad-based approach that coordinates multiple tactics for ecologically and economically controlling pests of agro and forest ecosystems (Ehler, 2006). Biological control is a sustainable and environmentally friendly way of controlling insect pests.

Among the biological control approaches, augmentation of natural enemies has been suggested, and is considered safe and efficacious. In this process, natural enemies are reared in an insectary and released at target sites in large.
numbers for suppression and reduction of damaging pest populations (Orr, 2009; Perera & Hemachandra, 2014). Among the parasitoids, egg parasitoids have great advantages over larval or pupal parasitoids, because egg parasitoids destroy the pest before they attack the crop. Parasitoid fitness is also very important for biological control programs. The fitness of females is mainly dependent on their ability to find hosts, and evaluating their life-history entails examining traits such as the percentage of eggs parasitized and percentage parasitoid emergence, development time, sex ratio and longevity (Bigler et al., 1991; Fournet et al., 2001; Perera & Hemachandra, 2014).

The solitary synovigenic egg parasitoid Ooencyrtus pityocampae can be utilized in the biological control of the pine processionary moth due to its biological characteristics, which are as follows: it is successful in parasitizing this host both in the laboratory and the field, has a short development time, long adult longevity, is able to successfully overwinter as a diapausing female and can locate its host by responding to its sex pheromone (Biliotti, 1958; Battisti et al., 1990; Tiberi, 1990; Tsankov et al., 1996, 1999; Schmidt et al., 1997, 1999; Mirchev et al., 2004). For this reason, successful mass and laboratory rearing of this parasitoid is very important. However, mass or laboratory rearing can have negative effects on parasitoid performance. One of the major problems encountered in the rearing of parasitoids is superparasitism (van Lenteren & Bigler, 2010).

Superparasitism is recorded for many species of wasp. It occurs both in nature and the laboratory, and occurs when an individual host is attacked by one or several females of the same species. Especially in nature, superparasitism is mainly recorded under certain specific conditions such as when parasitoids are unable to distinguish between previously parasitized and unparasitized hosts. Superparasitism occurs when unparasitized hosts are scarce and females have a high egg load (Salt, 1934; van Alphen & Visser, 1990; Godfray, 1994; Wanberg et al., 2008). All these situations may also occur under laboratory conditions. Self-superparasitism by solitary parasitoids requires the most rigorous conditions to be favoured by natural selection, since it inevitably results in the elimination of supernumerary larvae (Rosenheim & Hongkham, 1996). The conditions that favour self-superparasitism are: (1) when high quality hosts are rare or the risk of adult parasitoid mortality is great and (2) when parasitoids are abundant.

However, under certain conditions, the evolutionary stable strategy predicts that many species of parasitoids are able to detect hosts that have already been parasitized by conspecifics or by themselves and avoid ovipositing eggs in these hosts (van Dijken & Waage, 1987; van Alphen & Visser, 1990; Visser et al., 1992; Metcalf & Luckmann, 1994). The avoidance of superparasitism could work in two ways; the wasp might recognize a parasitized host or the patch it occupies. For example Venturia canescens (Hymenoptera: Ichneumonidae) (Hubbard et al., 1987) Epidinocarisis lopezi (Hymenoptera: Encyrtidae) (van Dijken et al., 1991) and Leptopilina heterotoma (Hymenoptera: Eucoilidae) (Visser, 1993) can recognize parasitized hosts. Strand (1986) reports that Telenomus heliotidis (Hymenoptera: Scelionidae), which attacks the eggs of Heliothis virescens (Lepidoptera: Noctuidae), does not superparasitize a host after the egg of the first female has hatched.

The simplest models of superparasitism in solitary wasps depend on the type of host acceptance. Females are assumed to maximize their rate of fitness gain and previously parasitized host are treated simply as hosts of low quality (Harvey et al., 1987; Janssen, 1989; van Alphen & Visser, 1990). In solitary parasitoid species, normally only one progeny per host survives. Parasitism by more than one egg laid by the same female results in sibling competition, which results in small offspring or the death of some or all of the offspring, and a long development time (Godfray, 1987; Rosenheim, 1993; Vet et al., 1994, Potting et al., 1997; Ode & Rosenheim, 1998; Jones et al., 1999, Mackauer & Chau, 2001). Therefore, superparasitism is an important factor in parasitoid population dynamics (Salt, 1934). However, the outcome depends on host quality, which for parasitoids is associated with the following features of the host: species, shape, size, movement, sound, chemical cues (Visser, 1976) and age (Colinet et al., 2005). Generally large and young insects are the best hosts for wasps (Da Rocha et al., 2006; Liu et al., 2011). Parasitoids prefer hosts that are the best sources of nutrients for their offspring, and hymenopteran wasps adjust their sex ratios according to host quality in a way that maximizes the benefits. Host size is an indication of quality with larger hosts providing more resources. Charnov et al. (1981) found that sex ratios vary with host size given that host size affects parasitoid size and fitness.

P. ricini eggs are larger than those of the other hosts (Aelia rostrata, Carposcoris sp., Nezara viridula, Dolycoris baccarum, Rhaphigaster nebulosa, Eurydema ventralis [E. ventralis], E. olaracea, Eurygaster maura, Graphosoma lineatum italicum) of O. pityocampae (Halperin, 1990; Tiberi et al., 1991, 1993). In this study we tested 1-day-old P. ricini eggs and our results indicate that two egg (O. pityocampae) can successfully complete development and emerge from one host egg (P. ricini). Thus the nutritional resources in an egg of P. ricini is sufficient to support self-superparasitism by O. pityocampae. More parasitoid progeny can emerge from a large than from a small host egg (Andrade et al., 2011). Mackauer et al. (1997) note that parasitoid growth and development varies with the amount and type of host resources available. For the parasitoid Diachasmimorpha longicaudata (Hymenoptera: Braconidae), the large host Anastrepha fraterculus (Diptera: Tephritidae) contains more resources which support the development of larger and more competitive parasitoids with a greater reproductive potential (Chau & Mackauer, 2001). According to López et al. (2009), D. longicaudata more frequently superparasitizes when reared in large hosts. Mayhew & van Alphen (1999) report that the solitary parasitoid Aphaejeta genevensis (Hymenoptera: Braconidae) normally lays one egg per host, but two or more offspring can successfully complete their development when superparasitism occurs.
On the other hand, Vinson (1984), van Alphen & Visser (1990) and Godfray (1994) report that, the survival of only one egg can be adversely affected by the host’s immune response. The presence of two or more eggs in one host may enable a parasitoid to maximize the utilization of the host, in particular it could represent a strategy for overcoming the host’s immune response and thus increase the probability of the offspring surviving. Similarly, a study on *Metaphycus flavus* (Hymenoptera: Encyrtidae) has demonstrated that laying several eggs in a single host suppresses the host’s immune defences and reduces egg encapsulation (Kapranas et al., 2012). This would make self-superparasitism advantageous (Puttler, 1959, 1967; Streams, 1971; Blamberg & Luck, 1990; Quicke, 1997; Montoya et al., 2000; Keinan et al., 2012).

Synovigenic parasitoids are egg-limited and thus their fitness is very dependent on the number of additional eggs they can produce during their adult life (Jervis & Kidd 1996; Rosenheim, 1996). Synovigenic species can experience short-term egg limitation (Heimpel & Rosenheim, 1998; Rosenheim, 1999). The incidence of egg limitation in these species is even lower than in pro-ovigenic parasitoids. For this reason, synovigenic parasitoids can lay more eggs per host and are not selective when deciding whether to parasitize or superparasitize. In the synovigenic parasitoid *O. pityocampae*, the tendency to superparasitize increased with the age of the parasitoid females. The highest percentage was recorded for 5-day-old females (Fig. 1). The physiological basis of the two egg laying strategy may be in differences in the egg loads (number) of the different females depending on their age.

Physiological suppression associated with egg load of females of different ages is very important for superparasitism. Carbone & Rivera (2003) report a similar result for the synovigenic parasitoid *Anaphes niten* (Hymenoptera: Mymaridae). The high egg loads of *Diaeretiella rapae* (Hymenoptera: Aphidiidae) could have resulted in them ovipositing repeatedly in the available hosts, as high egg loads in parasitoids encourage superparasitism (Keasar et al., 2006; Silva-Torres et al., 2009).

In addition, the results of this study demonstrate that host density affects the incidence of superparasitism by *O. pityocampae*, for example, a low host density was associated with high superparasitism (Fig. 2). Therefore, even the parasitoid *O. pityocampae* could find sufficient hosts to parasitize, and thus superparasitism can be seen. This could be due to the large size of the eggs of *P. ricini*.

Kraft & van Nouhuys (2013) report that low percentages of superparasitism by the parasitoid *Pteromalus apum* (Hymenoptera: Pteromalidae) of its hosts *Melitaea cinxia* and *Melitaea athalia* (Lepidoptera: Nymphalidae) are recorded in high-host density treatments.

Similarly, Lester & Holtzer (2002) report that superparasitism by *Diaeretiella rapae* (Hymenoptera: Aphidiidae) occurs more frequently at low host densities. Hanan et al. (2015) report that, with increase in host density from 20 to 140, the percentage superparasitism by *Eretmocerus variae* (Hymenoptera: Aphelinidae) decreases significantly.

Our experiments indicate that superparasitism by *O. pityocampae* negatively affects the development of their offspring, because it results in an increase in their development time and the production of small short lived adults. Wylie (1965) reports that, superparasitism affects the size of the parasitoid *Nasonia vitripennis* (Hymenoptera: Pteromalidae). Santolamazza Carbone & Cordero Rivera (2003) and González et al. (2006) report that superparasitism decreases the percentage emergence of the parasitoid *Dia-chasmimorpha longicu data* (Hymenoptera: Braconidae) and *Anaphes niten* (Hymenoptera: Mymaridae), respectively. Keasar et al. (2006) report that, superparasitism also reduces the quality of emerging parasitoids, which are small and short lived. Superparasitism increases the development time of *Venturia canecens* (Hymenoptera: Ichneumonidae) reared from third (L3) and fifth (L5) instar *Plodia interpunctella* (Lepidoptera: Pyralidae). The size of *V. canecens* emerging from L3 hosts was unaffected by superparasitism, but parasitoids from superparasitized L5 were significantly smaller than those from singly parasitized hosts (Harvey et al., 1993). Tunca & Kilnçer (2009) report that the percentage emergence and size of *Chelonus oculator* (Hymenoptera: Braconidae) decreases with increase in parasitism, but development time of the parasitoid increases with increase in superparasitism. These experimental results are supported by many previous studies (e.g., Simmonds, 1943; Gerling, 1972; Vinson & Sroka, 1978; Wylie, 1983; Eller et al., 1990; Potting et al., 1997, Hegazi & Khafagi, 2005; Chau & Maeto, 2008).

This work provides clear evidence that old females of *O. pityocampae* show a strong tendency to superparasitize when host densities are low. We also recorded that the size of the host can affect their decision to superparasitize. In addition, laying more than one egg in a host could be adaptive as it enables *O. pityocampae* to prevent the induction of the defence system in large hosts. Self-superparasitism may provide extra nutrition for the surviving parasitoid larva when host density is low. This research indicates that host density could be integrated with female age, by offering more hosts to older females. Our results may provide helpful information for improving mass and laboratory rearing of *O. pityocampae*.

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