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ORIGINAL RESEARCH PAPER

Contrasting patterns of ovarian development and oogenesis in two sympatric host-feeding parasitoids, *Diglyphus isaea* and *Neochrysocharis formosa* (Hymenoptera: Eulophidae)

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Abstract *Diglyphus isaea* (Walker) and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) are common idiobiont parasitoids of leafminers attacking vegetable crops. They exhibit differing levels of synovigeny, and host feeding enhances their fecundity and longevity. The reproductive systems of these two parasitoids are typical of hymenopteran eulophids, consisting of two ovaries, each usually comprising three polytrophic meroistic ovarioles. *Diglyphus isaea* possesses two obvious oviduct accessory glands, which are absent in *N. formosa*. Both parasitoids underwent oosorption when starved, while feeding on host larvae promoted oogenesis and egg maturation. In both, oogenesis and vitellogenesis commenced on the first day of the pupal stage rather than after eclosion. Formation of ovarioles in *D. isaea* commenced 1 day earlier than in *N. formosa*. Mature eggs were rarely observed in ovaries of newly emerged *D. isaea*, but usually a few were present in *N. formosa*. When hosts (second–third instar *Liriomyza sativae* larvae) were provided, the number of mature eggs in *D. isaea* ovaries initially increased and then stabilized, while in *N. formosa*, the number first increased and then decreased. *Diglyphus isaea* had fewer but larger eggs than *N. formosa* did. Thus, synovigenic divergence begins at the pupal stage and may result in different life-history traits of adults.

Keywords Synovigenic parasitoid · Oosorption · Anhydropic egg · Body size · Life history traits

Introduction

Adult parasitoid nutrition is derived from two sources: ‘capital nutrients’ are obtained during growth and development in the host, while ‘income nutrients’ are acquired during adult feeding (Casas et al. 2005; Jervis and Ferns 2004). Capital nutrients of the adult parasitoid are formed in the immature stages (before adult eclosion) and then do not increase after eclosion, as most parasitoid species do not replenish lipid levels after emergence from their host (Visser and Ellers 2008; Visser et al. 2010). Therefore, income nutrients significantly influence life history traits of adult wasps, particularly adult feeding (Heimpel and Collier 1996; Jervis et al. 2008). Heimpel and Collier (1996) divided parasitoid food sources into host food (host hemolymph and tissues) and non-host food (e.g., nectar, fermented fruits, insect honeydew). Female parasitoids that need to feed on the host to meet the nutrient requirements of egg maturation, continuous oviposition and survival are called host-feeding parasitoids. More than 140 species in 17 families of Hymenoptera feed on their host after adult eclosion (Jervis and Kidd 1986). However, the total number of host-feeding parasitoid species is nearly 100,000, about one-third of all parasitoids species (Kidd and Jervis 1991).

Because host-feeding parasitoids often adversely affect their hosts, and may even kill them through oviposition and feeding, they have greater potential as biocontrol agents

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than non-host-feeding wasps (Heimpel and Collier 1996; Kidd and Jervis 1989, 1991). When host-feeding parasitoids encounter hosts, the females face a trade-off between current and future reproduction (i.e., decreased survival and future reproduction as a function of present reproduction). As both may be determined by a combination of environmental and physiological factors (Bernstein and Jervis 2008; Heimpel and Collier 1996), it is vital that host-feeding parasitoids adjust oogenesis to host availability (Bodin et al. 2009).

In recent years, the relationships between the dynamics of ovarian development (the balance between oogenesis and oosorption) and the life history of female parasitoids have received considerable attentions (Bodin et al. 2007, 2009; Ellers and Jervis 2004; Harvey 2008; Kapranas and Luck 2008; Papaj 2000; Ralec 1995; Richard and Casas 2009). Flanders (1950) divided parasitoids into proovigenic parasitoids and synovigenic parasitoids, based on the number of mature eggs in the ovaries of female adults at emergence. The degree of synovigeny is characterized by an ovigeny index (OI), which is the ratio of the mean egg complement at adult emergence to the mean maximum lifetime egg complement. For a strictly proovigenic species, OI = 1, and for a strictly synovigenic species, OI = 0 (Jervis et al. 2001). Recent evidence supports a continuum of ovigeny indices (the ovigeny index hypothesis) rather than a simple dichotomy (Jervis and Ferns 2011).

The OI of female parasitoids has a negative relationship with longevity or body size (Jervis and Ferns 2011; Jervis et al. 2001). Jervis et al. (2001) and Jervis and Ferns (2011) speculated that parasitoid synovigeny is associated with resource allocation strategies that balance the acquisition of capital versus income nutrients. Strongly synovigenic wasps have relatively low capital nutrients; body maintenance and reproduction of adult female wasps mainly depend on adult feeding. Hence, in female wasps, oogenesis is affected by life-history traits, particularly the way in which parasitoids obtain nutrients. However, relatively few studies have been conducted on the relationship between oogenesis and adult life history traits.

All host-feeding parasitoids are considered to be synovigenic (Heimpel and Collier 1996; Jervis and Ferns 2011; Jervis and Kidd 1986). Most host-feeding parasitoids feed on host tissues and hemolymph, enhancing their reproduction and increasing their longevity (Giron et al. 2004; Heimpel and Collier 1996; Jervis and Ferns 2011). While different host-feeding parasitoids have different levels of synovigeny, corresponding differences in their oogenesis patterns have not been well studied.

As important natural enemies of *Liriomyza* leafminers, the idiobiont parasitoids *Diglyphus isaea* (Walker) and *Neochrysocharis formosa* (Westwood) (Hymenoptera: Eulophidae) have wide distributions and ecological adaptability and strong pest-control potential (Liu et al. 2013; Wang et al. 2012). These two parasitoids are sympatric in some areas of China, e.g., in celery fields in Kunming, Yunnan (Wan-Xue et al., personal observation). Both *D. isaea* and *N. formosa* are host-feeding parasitoids. Both of them feed on the host larvae and may kill them. However, their synovigeny indices (OI) differ, being 0.002 and 0.12 for *D. isaea* and *N. formosa*, respectively. The two species also have different developmental duration, body size, longevity, reproductive capability, and host-feeding capacity (Chien et al. 2005; Liu et al. 2013; Wang et al. 2012; Zhang et al. 2011).

The objective of this study was to determine the oogenesis and maturation patterns of *D. isaea* and *N. formosa*, and to examine the effects of host feeding on oogenesis based on the structure of their reproductive systems. This should lead to a better understanding of the resource allocation strategies of these two parasitoids where they coexist.

Materials and methods

Both *D. isaea* and *N. formosa* were obtained by collecting *Liriomyza huidobrensis* (Blanchard) hosts in celery fields in Gongxian, Yunnan, China, in April 2011. Subsequently, parasitoids were reared on second or third instars of *L. sativae* Blanchard in a greenhouse. Host larvae for this rearing colony were collected from a bean field at the Langfang Experimental Station of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. Parasitoid colonies were maintained on *L. sativae* reared on dwarf oil-bean plants kept at 25–30 °C, 70–80 % relative humidity, and natural light conditions.

Freshly emerged *D. isaea* and *N. formosa* adults were provided with excess second or third instar *L. sativae* for host feeding and oviposition. Twenty 2- to 3-day-old *D. isaea* and *N. formosa* females were randomly selected, held at −20 °C for 20 min, and then dissected on a slide with PBS buffer (pH 7.2), where the structure of the reproductive system was observed and photographed under a stereoscopic microscope (LX73, Olympus, Tokyo, Japan). Twenty adult females of *D. isaea* and *N. formosa* were provided with late second to early third instar *L. sativae* larvae for 2 h to stimulate oviposition in order to obtain pupae and, later, adults at the same developmental stage. Parasitized host larvae were transferred to an artificial climate chamber and kept at 27 ± 1 °C, 70 ± 5 % RH, and a 14:10 L:D photoperiod. Based on the results of preliminary experiments, mature parasitoid larvae at the same developmental stage could be collected on the fifth morning after the hosts were parasitized. These immature parasitoids were then transferred for further development under the above conditions.

Ovarian development and oogenesis were observed at the pupal and adult stages. To study ovarian development during the pupal stage, cohorts of pupae of equal size were selected under a stereomicroscope (SZ61, Olympus) and dissected every 24 h after pupation. Male pupae were discarded, and up to ten female pupae were dissected at each sampling time. To study oogenesis in the adult stage, individual newly emerged adult parasitoids were exposed daily to one bean leaf with about 20 late second to early third instar *L. sativae* larvae in a glass petri dish (9 cm diameter, 2.2 cm height). Each day, the parasitoids were transferred to a new dish with a fresh supply of host larvae. The dishes were kept at 25 ± 1 °C, 70 ± 5 % RH, and 14:10 L:D photoperiod. Within 48 h, oviposition and host feeding of each parasitoid were recorded under a binocular stereomicroscope. Ten females were randomly selected at times 0, 6, 12, 24, 48, 96, 120, 144, 168, 192, 216, and 240 h after provision of the host larvae. Control adult female parasitoids were provided with water over the same time period, and ten females were randomly selected at each of the 6, 12, 24, 48, and 96 h time points. The wasps were frozen for 5 min at −20 °C and dissected immediately.

Ovarian development and oogenesis were observed during the dissection; stages of oocyte development were categorized and their numbers recorded using the scheme of Bodin et al. (2009). Oocytes were classified into three stages of development: group I, when the area of yolk deposition occupied less than half of the whole volume of the egg chamber; group II, when the area of yolk deposition occupied half or more of the egg chamber but did not completely fill it; group III, when oocytes had their full complement of yolk. We measured the length (*L*) and width (*W*) of two mature eggs (group III) on each side of the ovary with a micrometer. When there were fewer than four group III eggs, the lengths and widths of all group III eggs were measured. The volume of eggs (*V*) was calculated as $V = (\pi \times L \times W^2)/6$ (Avelar 1993). Measurements were compared using SAS (Version 12.0), while mean oogenesis as well as ovarian development was subjected to one-way analysis of variance. Mean values in the text are given ± SEM.

Results

Reproductive morphology

The reproductive systems of *D. isaea* and *N. formosa* each consisted of one pair of ovaries (Fig. 1). Of the 520 adult females of *D. isaea* tested, 99.2 % had six ovarioles (three in each ovary). The remaining 0.8 % had five ovarioles. Of 520 *N. formosa*, 97.7 % had six ovarioles, while a few had four, five, or seven ovarioles, accounting for 0.2, 1.9, and 0.2 % of the total, respectively.

The left and right ovaries of both species extended from the genitalia to the anterior wall of abdomen (Fig. 1). A yellow spermatheca (Fig. 1E) was located on the dorsal side of the junction between the two lateral oviducts, and, at their junction with the common oviduct, three protruding accessory glands and two spherical sacs containing transparent fluid were observed (Fig. 1). The larger sac was identified as a mucous gland, and the smaller one, in the form of along curved tube, as Dufour's gland (Fig. 1G). In *D. isaea*, an accessory gland also protruded from both sides of the common oviduct (Fig. 1a).

We observed no obvious difference in the number of mature eggs on the two sides of the oviduct in either of the two wasps. While mature unlaid eggs were present in the ovaries, the wasps continued to host-feed for a short time (1–2 h) and new eggs continued to develop. The maximum number of mature eggs held in one ovariole by *D. isaea* was six and by *N. formosa* was five. At the onset of oosorption, mature eggs in the midsections of the ovarioles on both sides were affected first, followed by those further along the ovarioles (toward the common oviduct). The

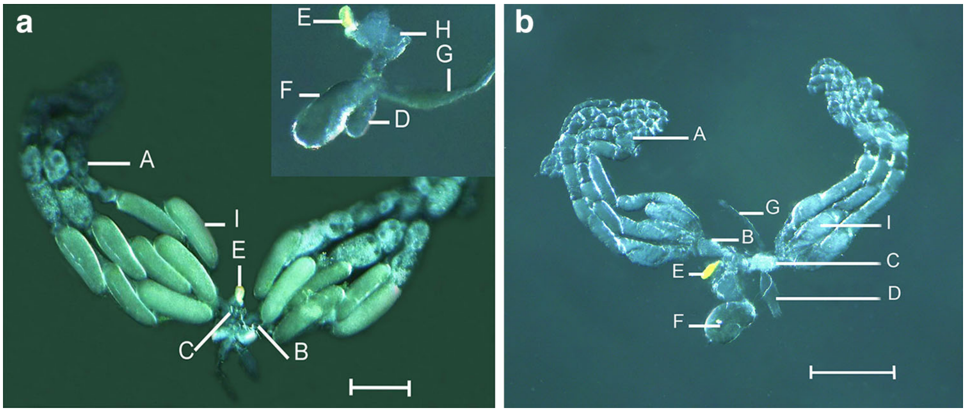


Fig. 1 Anatomy of the reproductive system of female *Diglyphus isaea* (**a**) and *Neochrysocharis formosa* (**b**) on the third day after eclosion. *A* ovariole, *B* lateral oviduct, *C* common oviduct, *D* poison gland, *E* spermatheca, *F* colleterial gland, *G* Dufour's gland, *H* oviduct gland, *I* mature oocyte. *Scale bar* 0.1 mm

most mature eggs in the ovaries of both species were found in the lateral oviducts.

Ovarian development and oogenesis

The durations of the pupal stages of *D. isaea* and *N. formosa* were 5.2 ± 0.03 days ($n = 100$) and 5.5 ± 0.05 days ($n = 100$), respectively, with a significant difference between the two ($F_{1,199} = 48.31$, $p < 0.0001$). Ovary formation commenced 24 h after pupation in both species. Initially, the ovaries appeared as thin threads, with no differentiation of the ovarioles or egg chamber. Ovarioles and egg chambers started to form in *D. isaea* at 96 h after pupation and yolk deposition started at 120 h (Fig. 2a). In *N. formosa*, ovarioles started to form at 48 h and were clearly visible 72 h after pupation. Formation of the egg chambers and yolk deposition began at 96 h.

The ovarioles of both wasps progressively increased in length, starting 24 h after pupation (Fig. 3). However, the ovarioles of *D. isaea* increased at a faster rate and were larger overall than those of *N. formosa*. Ovariole length of in *N. formosa* increased rapidly from 96–120 h after pupation.

Numerous group I and a few group II eggs were present in the ovaries of *D. isaea* between 96 and 120 h after pupation (Fig. 4). Many group I eggs had formed in the ovaries of *N. formosa* 72 h after pupation, but their numbers decreased after 96 h, and many group II eggs were present at 120 h after pupation (Fig. 4). At emergence, few group III eggs were found in *D. isaea* (mean 0.1 ± 0.07, $n = 20$, Fig. 5a), while in *N. formosa*, 5.6 ± 0.5 ($n = 20$) group III eggs were found (Fig. 5b).

Diglyphus isaea adults fed on host larvae soon after emergence and the number of group III eggs in their ovaries increased within 48 h, peaking at 17.0 ± 0.8 eggs/female ($n = 10$) at 96 h, after which the number remained relatively stable (Fig. 6a). The number of group II eggs stabilized within 48 h, and the number of group I eggs increased steadily until 120 h. The total number of eggs exhibited a time course similar to that of the group III eggs.

When newly emerged *N. formosa* adults were provided with host larvae, the number of group III eggs in their ovaries increased steadily for the first 120 h, decreased somewhat after that, increased again to a maximum of 27.5 ± 2.1 eggs/female at 168 h (significantly higher than the maximum egg load of *D. isaea*, $F_{1,19} = 175.5$, $p < 0.0001$), after which the number again decreased (Fig. 6b). The number of group II eggs in the ovaries increased sharply for the first 12 h, after which the number remained relatively stable. The number of group I eggs

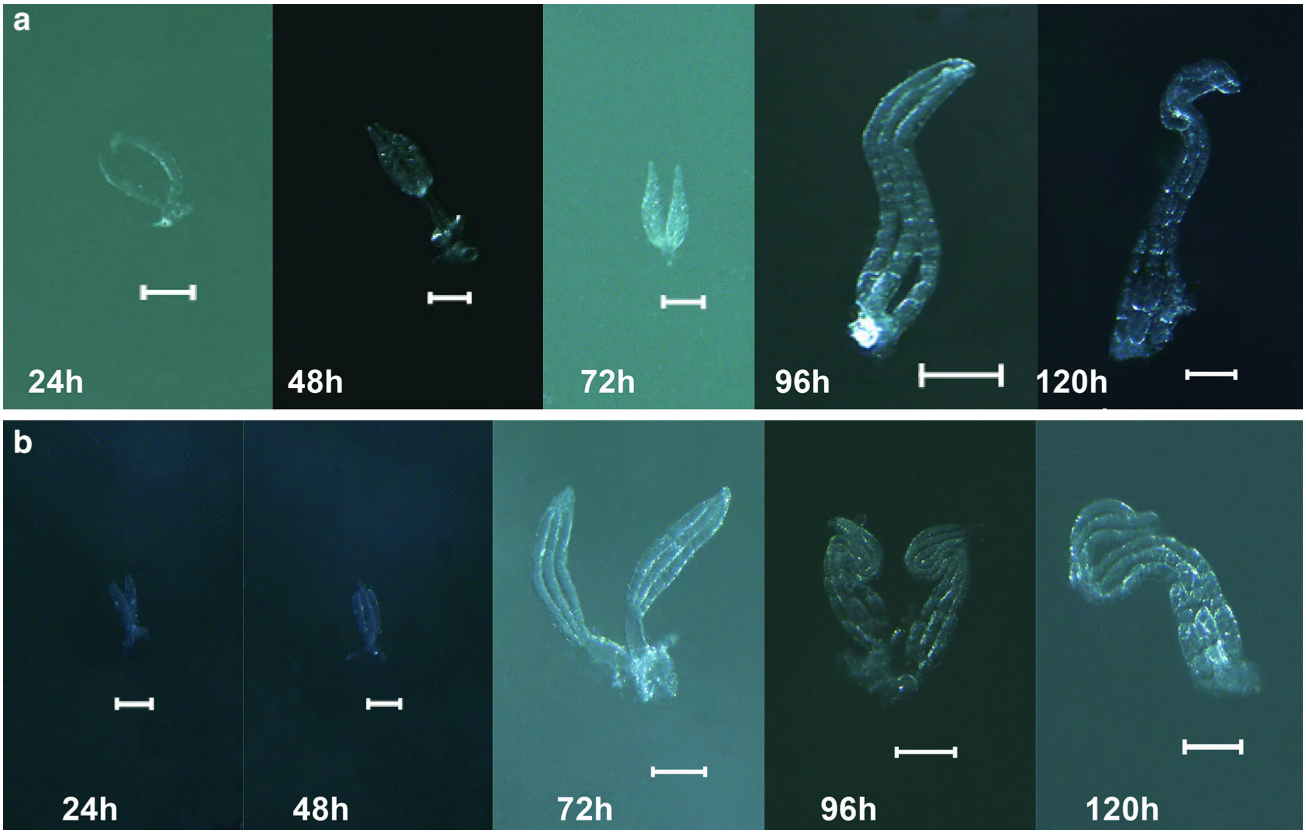


Fig. 2 Ovarian development of female *Diglyphus isaea* (**a**) and *Neochrysocharis formosa* (**b**) at the pupal stage. *Scale bar* 0.05 mm

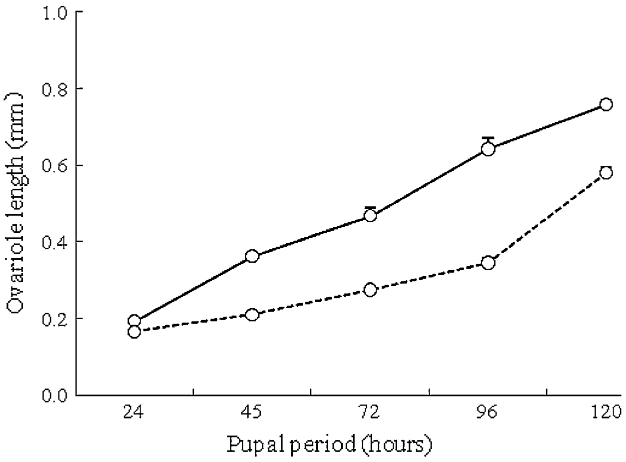


Fig. 3 Change in ovariole length of *Diglyphus isaea* (*continuous line*) and *Neochrysocharis formosa* (*dotted line*) at the pupal stage. Data are means; *vertical lines* indicate ± SE

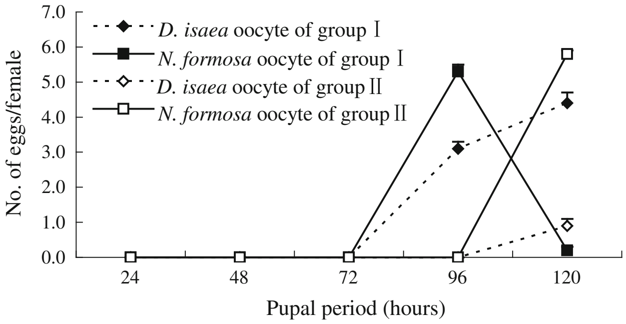


Fig. 4 Oogenesis of female *Diglyphus isaea* and *Neochrysocharis formosa* at the pupal stage

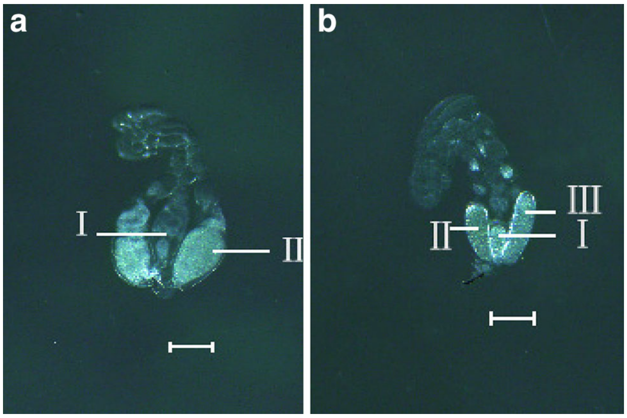


Fig. 5 Ovaries of female adults of *Diglyphus isaea* (**a**) and *Neochrysocharis formosa* (**b**) at the time of emergence. *Scale bar* 0.1 mm

increased sharply in the first 6 h and then continued to increase at a relatively constant rate. In general, the total number of eggs in the ovaries of *N. formosa* adults exhibited a similar time course to that of group III eggs. The total number of eggs in *N. formosa* was clearly greater than in *D. isaea*.

Newly emerged *D. isaea* females provided only with water had a maximum longevity of 4 days and mean longevity of 3.3 ± 0.9 days ($n = 20$) (Fig. 7a). The number of group III eggs in the ovaries increased gradually for the first 12 h and peaked at 2.8 ± 0.9 eggs/female ($n = 10$), after which oosorption commenced. The number of group II eggs decreased steadily, and all groups II and III eggs were reabsorbed. The number of group I eggs increased during the first 48 h and decreased after that.

Newly emerged *N. formosa* females provided only with water had a maximum longevity of 4 days and a mean longevity of 2.6 ± 0.2 days ($n = 20$) (Fig. 7b). The number of group III eggs increased somewhat during the first 12 h and then decreased sharply. The number of group II eggs increased during the first 24 h and then decreased. All group II and III eggs had been reabsorbed 72 h after emergence. The number of group I eggs decreased steadily from 6 h after emergence.

Change in volume of group III eggs in the ovaries of adults

The volume of group III eggs in *D. isaea* ovaries feeding on host larvae was greatest within 12 h of emergence; it then decreased and remained stable from 24 h (Fig. 8). The volume of group III eggs in the ovaries of *D. isaea* provided with only water increased throughout the trial, but the eggs were smaller than those in ovaries of *D. isaea* feeding on host larvae. The volume of group III eggs in the ovaries of *N. formosa* increased for the first 6 h after emergence and then essentially stabilized, peaking at 48 h. The volume of group III eggs in the ovaries of *N. formosa* provided with only water decreased after 6 h, and the eggs were smaller than those in the ovaries of *N. formosa* feeding on host larvae.

Discussion

While the ovaries of most parasitoid wasps share a similar anatomical structure, the number of ovarioles varies greatly among families and genera. For example, although most parasitoids possess 2–20 pairs of ovarioles (Iwata 1959), *Aphidius* spp. possess a single pair, and *Euceros* spp. possess more than 200 pairs. In Ichneumonidea, the number of ovarioles is associated with life-history traits. Generally, koinobiont parasitoids have more ovarioles than idiobionts (Iwata 1960). The number of ovarioles among individuals varies little within species with few ovarioles, but in species with many ovarioles it may vary greatly and

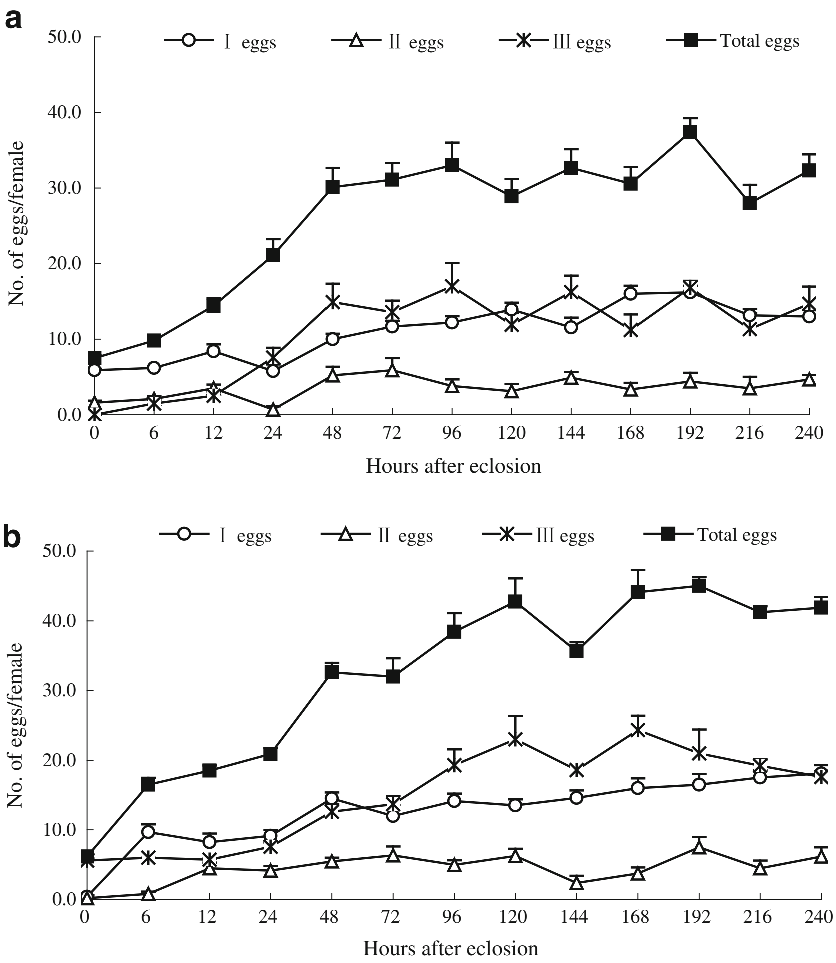


Fig. 6 Time course of oogenesis of female *Diglyphus isaea* (**a**) and *Neochrysocharis formosa* (**b**) provided with host larvae. Data are means; *vertical lines* indicate ± SE

can even differ between the left and right ovaries of the same individual (Iwata 1959, 1960).

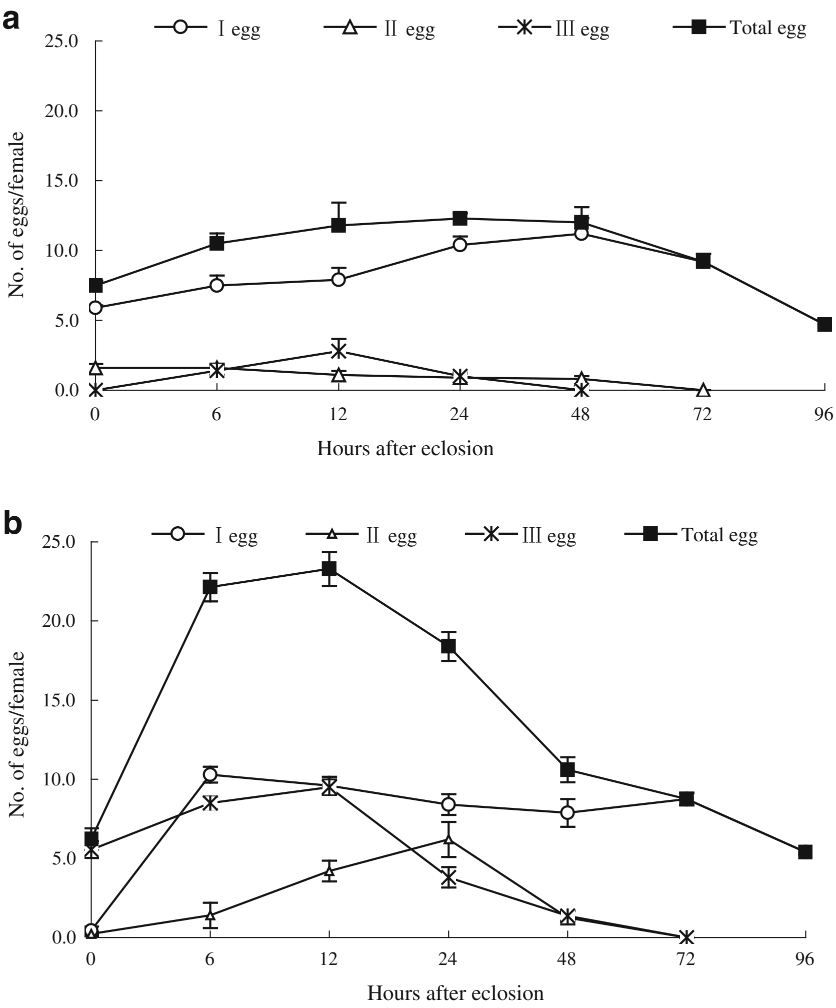
Although *D. isaea* and *N. formosa* are sympatric parasitoids of different subfamilies (Eulophinae and Entedoninae, respectively) and both are idiobionts, the former is ectoparasitic, while the latter is endoparasitic. The ovarian structures of the two parasitoids were quite similar, with three pairs of meristic polytrophic ovarioles in most of the ovaries dissected. This agrees with the pattern described by Iwata (Iwata 1959, 1960). For example, *Brachymeria intermedia* (Nees) (Hymenoptera: Chalcididae) has two ovaries, each with three polytrophic ovarioles. Each ovariole contains eight to ten follicles and one or two ripe eggs (Dindo et al. 1996).

We also found that the number of ovarioles per female did not differ among females of different sizes, but larger females carried more mature and immature eggs, which is also the case in the ectoparasitoid *Agrothereutes lanceolatus* Walker (Hymenoptera: Ichneumonidae) (Ueno 1999). Not all synovigenic endoparasitoids display the same pattern. In *Venturia canescens* (Gravenhorst), there is a positive and significant positive correlation between wasp size and egg load as well as ovariole number; large wasps possess significantly more ovarioles and mature eggs than do small ones (Eliopoulos et al. 2003).

No eggs were found in the oviducts of either *D. isaea* or *N. formosa*, suggesting that both species laid eggs rapidly. However, a pair of oviduct accessory glands was clearly visible in female *D. isaea* but absent in *N. formosa*. The eggs of *D. isaea* were nearly twice as large as those of *N. formosa*, and it is possible that the accessory glands of *D. isaea* may secrete mucus to lubricate the oviducts or facilitate further absorption of nutrients by the eggs in the oviducts (Flanders 1942). These accessory glands may also secrete host-marking pheromones concerned with host recognition and parasitization by female wasps (Nacro and Nenon 2008; Rosi et al. 2001).

Since the work of Flanders (1942, 1950), host feeding in female parasitic Hymenoptera has been associated with the production of large, yolk-rich eggs that have been termed ‘anhydropic’ because they do not swell during embryogenesis. Such host feeding has also been linked to synovigeny. Yolkless eggs, in contrast, are termed ‘hydropic,’ and these are generally laid by proovigenic parasitoids. Proovigenic parasitoids generally lay relatively fewer hydropic eggs than anhydropic eggs compared with

Fig. 7 Time course of oogenesis of female *Diglyphus isaea* (**a**) and *Neochrysocharis formosa* (**b**) provided with water only



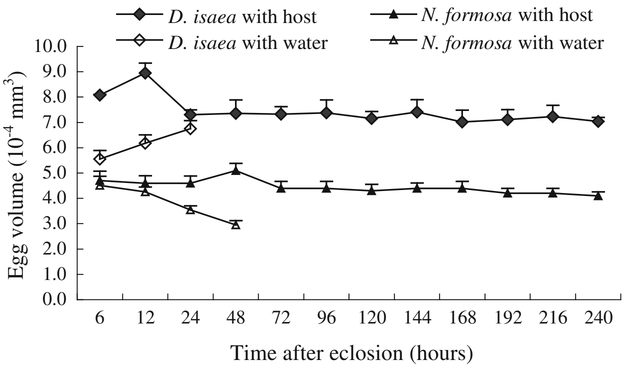


Fig. 8 Volume changes in group III eggs of female *Diglyphus isaea* and *Neochrysocharis formosa* provided with either host larvae or water. Data are means; vertical lines indicate ± SE

synovigenic parasitoids on the same diet. However, proovigenic parasitoids are able to mature their anhydropic eggs in a shorter time after encountering hosts, decreasing the chance that adult parasitoids will be preyed upon by natural enemies while searching for food. The eggs of both parasitoids studied here were of the anhydropic type. Eggs laid by *D. isaea* were large and yellow and probably contained more yolk than eggs of *N. formosa*, which were smaller and white. Mature eggs swell slightly when held in water for at least 5 min, but the size of mature eggs held in PBS (pH 7.2) for a long time showed no obvious change (Wan-Xue et al., personal observation).

Most host-feeding parasitoids (especially destructive host-feeding parasitoids) produce anhydropic eggs, e.g., *Tetrastichus incertus* (Ratz.) (Eulophidae) (Dowell 1978), *Aphelinus asychis* (Walker) (Aphelinidae) (Bai and Mackauer 1990), *Epidinocarsis lopezi* (De Santis) (Encyrtidae), *Leptomastix dactylopii* Howard (Encyrtidae), *Asaphes vulgaris* Walker (Pteromalidae) (Ralec 1995), *Pimpla nipponica* Uchida (Ichneumonidae) (Ueno 1999), *Aphelinus abdominalis* (Dalman) (Aphelinidae) (Couty and Poppy 2001; Ralec 1995), *Encarsia formosa* Gahan (Aphelinidae) (Burger et al. 2004), and *Eretmocerus eremicus* Rose and Zolnerowich (Aphelinidae) (Asplen and Byrne 2006).

Eggs laid by host feeding, but non-destructive, koinobiont parasitoids such as *Opius caricivorae* Fischer (Hymenoptera: Braconidae), an endoparasitoid of dipteran leafminers, tend to be hydropic (Chien and Chang 2012; Xu et al. 2007). Some non-host-feeding synovigenic parasitoids, including the synovigenic, secondary ectoparasitoid *Dendrocerus carpenteri* (Curtis) (Megaspilidae) (Ralec 1995), *Coccophagus atratus* Compere (Aphelinidae), and several heteronomous hyperparasitoids (Donaldson and Walter 1988), also produce anhydropic eggs. However, some non-host-feeding synovigenic parasitoids, such as *V. canescens* (Hymenoptera: Ichneumonidae), produce micro-type hydropic eggs (Harvey et al. 2001). It therefore seems possible that destructive host feeding has evolved to enable females to sustain the production of high-quality anhydropic eggs. Oosorption is a common process in insects where anhydropic (high yolk content) eggs are broken down into their constituents, which are returned to the somatic nutrient pool for future reproduction (Bell and Bohm 1975; Papaj 2000). This is usually an active female response to resource deprivation and may be triggered by dietary stress, lack of available oviposition sites, or the absence of males (Bell and Bohm 1975).

Generally, oosorption occurs in anhydropic eggs of synovigenic parasitoids. However, there are exceptions, including the synovigenic, secondary ectoparasitoid *Dendrocerus carpenteri* (Megaspilidae) (Ralec 1995) and *Eulophus pennicornis* (Nees) (Eulophidae), a gregarious ectoparasitoid of the tomato moth *Lacanobia oleracea* (Linnaeus) (Wakefield et al. 2010), in which anhydropic eggs are stored. Similarly, egg resorption does not occur in the synovigenic endoparasitoid, *V. canescens*, in which egg production stops in the absence of food (Eliopoulos et al. 2003).

Oosorption occurs in all host-feeding parasitoids reported to date (Asplen and Byrne 2006; Bodin et al. 2009; Liu and Ueno 2012; Takasu et al. 2012; Tschudi-Rein and Dorn 2001). Our study revealed that oosorption occurred in both of our study species and that their oosorption pattern was consistent with that in other insects, i.e., a tendency for absorption of mature or nearly mature eggs (Bell and Bohm 1975). Under a starvation diet (only water provided), female parasitoids are able of utilizing capital nutrients to continue egg maturation for the first 12 h after emergence. After this length of time, oosorption occurs. On a starvation diet, within 48 h after emergence, group I eggs of *D. isaea* continued to develop to group II eggs, while group III eggs continued to be produced, and the total number of eggs increased. After 48 h, the number of eggs laid decreased. On a starvation diet, although group I eggs of *N. formosa* continued to develop to group II eggs, group III eggs were no longer laid. Therefore, the total number of eggs produced decreased from 12 h after emergence.

Oosorption differed between the two parasitoids. Both wasps retained a number of immature (group I) eggs, allowing the rapid resumption of oviposition and reproduction upon obtaining host food. When host larvae were provided, the ovaries of *D. isaea* and *N. formosa* contained at most 34 and 30 mature oocytes, respectively, and each ovariole contained a maximum of six and five mature oocytes, respectively. In another host-feeding parasitoid, *Eupelmus vuilleti*, in the presence of a host, each ovariole generally contained three oocytes: one fully mature egg, one nearly mature, and one immature. Host-deprived females reabsorbed their most mature and their smallest oocytes, keeping just one almost mature oocyte per ovariole (Bodin et al. 2009). Host-feeding parasitoids are synovigenic, and host feeding contributes to their longevity and reproductive capability (Heimpel and Collier 1996; Jervis and Ferns 2011; Jervis and Kidd 1986).

Our previous studies (Zhang et al. 2011) and this research have shown that *D. isaea* and *N. formosa* are strongly synovigenic parasitoids in which host feeding facilitates oogenesis and egg maturation and increases their longevity. Of the two, *D. isaea* had greater synovigeny (OI = 0.002) than *N. formosa* (OI = 0.12) (Zhang et al. 2011; Yi-Bo et al., unpublished data); mature eggs were rarely found in newly emerged *D. isaea* females, but a few mature eggs were found in newly emerged *N. formosa*.

Yolk deposition occurred at the pupal stage in both wasps, suggesting that oogenesis in females was initiated at this stage. However, the two wasps showed obvious differences in the dynamics of ovarian development and oogenesis. The formation of the ovarian chamber, oogenesis, and egg maturation was slower in *D. isaea* than in *N. formosa*. At the adult stage, the number of mature eggs peaked in *D. isaea* and then remained stable for a long period before decreasing. In contrast, mature egg numbers of *N. formosa* first increased and then immediately decreased.

Under the same conditions, the total number of eggs produced by the two wasps was similar but, in *D. isaea*, there were fewer larger eggs than in *N. formosa*, possibly reflecting different nutrient restraints. When the parasitoids were provided with the same nutrients, larger but fewer eggs were produced. Therefore, it appears that *D. isaea* needs more nutrients to complete egg development than does *N. formosa*. This could explain why *D. isaea* has a stronger synovigenic level than *N. formosa*. In addition, the larger body size of *D. isaea* may provide a greater range of host-feeding and non-host-feeding opportunities. Although these two idiobiont host-feeding parasitoids are sympatric, belong to the same family, and parasitize the same hosts, differences in their oogenesis could be related to differences in their life history characteristics, e.g., *D. isaea* is larger and lives longer than *N. formosa* (Wan-Xue et al., unpublished results). This is consistent with the "oogenesis hypothesis" suggested by Jervis and Ferns (2011).

Because both wasps are idiobionts, they cannot gain host resources by altering host metabolism. However, such constraints may be reduced as they obtain resources for oogenesis and somatic maintenance from host feeding. Because these parasitoids feed on and kill the host by stinging without oviposition, their potential use as control agents is enhanced, and they are now used around the world as important natural enemies of leafminer pests (Liu et al. 2013; Wang et al. 2012; Zhang et al. 2011).

The sympatricity of these species is especially important for their combined use as biological control agents. For example, they co-occur in celery fields damaged by *L. huidobrensis* in Kunming, Yunnan, China (Wan-Xue et al., unpublished observations). This coexistence results from niche partitioning and allows increased resource exploitation because of differences in their life histories and oogenesis. Because of these differences in the strategies for resource allocation and exploitation, *D. isaea* and *N. formosa* could be released concurrently to control leafminer pests. *D. isaea* plays a steady long-term role in control because of its greater longevity and low mature egg number in early adult life after emergence. In contrast, *N. formosa* could be used for more immediate control because of its shorter longevity in adult life and more rapid egg maturation in the early days after emergence. A further advantage of the combined release of these two parasitoids may be that their host-killing behaviors are selective for different sizes of host larvae.

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