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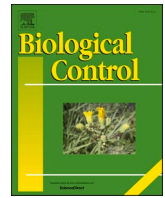
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Development and oviposition strategies in two congeneric gregarious larval-pupal endoparasitoids of the seven-spot ladybird, *Coccinella septempunctata*

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HIGHLIGHTS

- Oviposition time was longer for *Oomyzus spiraculus* than for *O. scaposus*.
- Offspring sex ratios in both parasitoids were heavily female-biased.
- Offspring development time decreased with host instar.
- *Oomyzus scaposus* offspring adults were generally larger than *O. spiraculus* adults.
- There is some variability in life-history traits in two parasitoid species.

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ABSTRACT

Aphids are serious pests of many crops in agroecosystems and their biological control is focused on enhancing the performance of specialized natural enemies of aphids such as parasitoid wasps and predators like ladybirds. However, ladybirds are often attacked by their own parasitoids in the fourth trophic level that can negatively affect ladybird performance and, hence, their effectiveness as control agents. The biology and ecology of these parasitoids has been less well explored. This study compared various life-history traits in two closely related parasitoids of the seven-spot ladybird, *Coccinella septempunctata* (Coleoptera: Coccinellidae): *Oomyzus scaposus* and *O. spiraculus* (Hymenoptera: Eulophidae), which naturally co-occur in eastern China and are facultatively gregarious koinobiont larval-pupal endoparasitoids of ladybird beetles. They can oviposit and develop in all four larval instars of their hosts but kill and emerge from the pupae. Both parasitoids did not have a clear oviposition preference for any host instar, but oviposition duration tended to increase in first to third instar hosts. Moreover, oviposition time was significantly longer for *O. spiraculus* than for *O. scaposus*. Adult eclosion from parasitized hosts (ranging between 35 and 45%) and sex ratios (85–90% female) were similar in both species and did not differ among host instars. Brood sizes were similar in both species but tended to increase in first to third instar hosts. Egg-to-adult development time was shorter and the eclosing adults of *O. scaposus* were heavier than those of *O. spiraculus*. In both species, development time decreased with host instar at parasitism, but instar-specific effects on biomass differed between the two species: heavier *O. scaposus* adults developed from second and third instar hosts, whereas biomass of for *O. spiraculus* tended to decrease with instar at parasitism. In both species, females were significantly larger than males. Our results show that expression of some of the life-history traits vary depending on which instar is parasitized, but do not point at a specific instar optimal for each of the parasitoids. Moreover, despite being closely related, there is some variability in the expression of life-history traits in both parasitoids.

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1. Introduction

Ladybird beetles (Coleoptera: Coccinellidae) are highly effective predators of aphids because both larval and adult stages voraciously consume large numbers of aphid nymphs and adults (Hodek et al., 2012). Therefore, they are used as biological control agents of aphids of which many species are serious pests of important agricultural crops worldwide, not only as herbivores, but also as viral vectors (van Emden & Harrington, 2017). One of the major impediments in the success of biological control programs against herbivores is that their natural enemies in the third trophic level harbour their own natural enemies in the fourth trophic level (Schooler et al., 2011; Tougeron & Tena, 2019; Cusumano et al., 2020). For instance, most parasitoid wasps are attacked by primary (larval) and/or secondary (pupal or mummy) hyperparasitoids, and these can sometimes decimate the abundance of their primary parasitoid hosts, thus greatly reducing their effectiveness as biological control agents (Cusumano et al., 2020).

Most ladybird beetles are attacked by primary parasitoids that, considering their position in the food chain, occupy the fourth trophic level (Orr & Boethel, 1986). Despite their potential importance in disrupting biological control programs, the biology and ecology of most ladybird parasitoids have been little explored. What studies have been performed investigated parasitism rates of introduced ladybird species like *Harmoni axyridis* (Pallas) (Berkvens et al., 2010; Roy et al., 2011; Ceryngier et al., 2018), the effect of parasitism on the foraging efficiency of ladybird larvae (Bayoumy, 2011; Bayoumy & Michaud, 2012), or the adaptive manipulation of the behaviour of adult beetles by the parasitoids, turning them into surrogate ‘bodyguards’ (Maure et al., 2011, 2014; Dheilly et al., 2015). Much less is known about life-history and developmental strategies in ladybird parasitoids, in particular larval or larval-pupal endoparasitoids.

Several species of unrelated solitary and gregarious parasitoids are known to attack the larval and/or pupal stages of ladybirds (Ceryngier et al. 2012). Most are koinobionts whose hosts continue feeding and growing throughout the course of parasitism (Askew & Shaw, 1986; Harvey, 2005). For koinobionts, the host represents a dynamic resource that may vary quite dramatically in size (and quality) between parasitism and death (Godfray, 1994; Harvey, 2005). Perhaps the best studied parasitoid of ladybirds is *Dinocampus coccinellae* (Schrank), a solitary koinobiont that is able to parasitize and develop in larvae, pupae and adult ladybirds (Kadono-Okuda et al., 1995; Obrycki et al., 1985). However, several gregarious larval-pupal parasitoids in the genus *Oomyzus* (Hymenoptera: Eulophidae) have also been reported from ladybirds in Eurasia (Triltsch, 1996; Ali et al., 2013; Song et al., 2020; Ullah et al., 2020). Although preference and performance studies have been conducted with many koinobiont parasitoids (Harvey, 2005), few, if any, have been done with gregarious larval-pupal parasitoids of ladybirds.

This study examines and compares oviposition behavior, parasitism success, development and offspring sex ratios in two closely related parasitoids, *Oomyzus scaposus* Thomson and *O. spiraculus* Song, Fei & Cao in the four different larval instars of their host, the seven-spot ladybird *Coccinella septempunctata* L. This species is the most abundant ladybird across much of Eurasia and North America, and is an economically important predator of aphids and whiteflies (Hodek et al., 2012). In China, it is considered to be the most important natural enemy of the cotton-melon aphid, *Aphis gossypii* Glover (Xia et al., 2018). Several parasitoids of *C. septempunctata* have been previously described in the literature (Schaefer & Semyanov, 1992; Triltsch, 1996) but life-history or developmental parameters in *O. scaposus* or *O. spiraculus* are thus far less studied. Both species are obligate larval-pupal endoparasitoids: they oviposit in ladybird larvae but only emerge from the host pupae.

Given that they are sister species and exhibit significantly overlapping traits, we hypothesize that there is strong congruity between these two species in their oviposition behavior and development. We

argue that more attention needs to be paid to the potentially disruptive effects of parasitoids in the fourth trophic level, hyperparasitoids of herbivores and parasitoids of predatory arthropods, in pest control programs. We also make a plea for more studies examining the biology and ecology of ladybird parasitoids in natural communities.

2. Materials and methods

2.1. Insect cultures

The seven-spot ladybird, *C. septempunctata*, together with its two endoparasitoids *O. scaposus* and *O. spiraculus*, were originally collected from vetch fields near Nanjing, east China. The prey of the seven-spot ladybird, vetch aphids, *Megoura viciae* Mordvilko, originated from a long-standing laboratory colony maintained at the Jiangsu Academy of Agricultural Science and was maintained on bean *Vicia faba* L. The ladybird colony was established from 30 seven-spot ladybirds randomly collected from the field and was maintained in insect rearing cages (40 × 40 × 40 cm) containing bean *Vicia faba* L. infested with vetch aphids as food. Cultures of *O. scaposus* and *O. spiraculus* were reared on seven-spot ladybird larvae. Ladybird larvae were parasitized by presenting them individually at the end of a small brush to 2-to-3 day-old female wasps of each species in a plastic vial. A larva was considered to be parasitized when the female wasp had inserted her ovipositor into the host for at least 30 s. The parasitized larvae were then transferred to a Petri dish (diameter of 5 cm) with vetch aphids that were replenished daily. All insects were maintained in a climate room at temperature of 22 ± 2 °C, relative humidity of 50–70% and photoperiod of 16L: 8D. The female wasps used in the experiment were 2–3 days old, mated, and naive.

2.2. Experimental protocol

2.2.1. Instar-specific parasitoid oviposition preference

Dual-choice tests were performed in 5-cm Petri dishes to determine whether female *O. scaposus* and *O. spiraculus* preferred to oviposit in specific host instars. Pairs of two different larval instars were offered to each parasitoid species and all instar combinations were tested. Two different instar larvae of the seven-spot ladybird were gently transferred to either side of a Petri dish with a soft brush. A female wasp (3 days old) was placed with the head facing away from the prey in the center of the Petri dish using a soft brush. A bioassay was valid when a female wasp had inserted her ovipositor in one of the host larvae for >30 s. When a wasp did not land on one of the host larvae within 30 min, it was recorded as “non-responding” and this data point was excluded from the statistical analysis. Female wasps were used only once. Each pair of host instars was used once and the position of the instars in the Petri dish was randomly switched between replicates. The bioassay was replicated at least 60 times for each parasitoid species.

2.2.2. Host-instar-specific parasitoid performance

To determine instar-specific performance of the two parasitic wasps, each of the four instars were separately exposed to either parasitoid species in 5-cm Petri dishes. A female wasp (3 days old) was allowed to parasitize the host once until leaving the host voluntarily, and then the oviposition time was recorded. Each female wasp was used once. Each parasitized larva was transferred into a glass tube and provided with vetch aphids until the adult wasps emerged. The development time from oviposition to adult eclosion, the number of emerging wasps (brood size), and the sex ratio (proportion male adult parasitoids) were recorded. At eclosion, five female and all male (normally 1 or 2) wasps from each brood were dried in an oven at 60 °C for 72 h and weighed on a microbalance. The parasitism success rate (proportion of hosts giving rise to wasps) was also measured. The bioassay was replicated at least 40 times for each parasitoid species.

2.3. Statistical analyses

Instar-specific parasitoid oviposition preference was analyzed using binomial tests for each instar combination with H_0 (no preference): $p = 0.5$. The response variable was the number of trials in which the female preferred to parasitize host instar A over B and the number of trials was the total number of trials in which a successful parasitism occurred.

A generalized linear model (GLM) with a binomial distribution and logit link function was employed to analyze parasitism success rate. Parasitoid species and host instar were entered as explanatory variables and the response variable was individual trial success (i.e. a host producing adult parasitoids or not). Similarly, sex ratio data were analyzed with the proportion of males per brood as response variable. A GLM with a Poisson error distribution and a log-link function was used to analyze oviposition time, brood size and egg-to-adult development time. Overdispersion of data was taken into account via empirical estimation of scaling parameters. Parasitoid species and host instar were the explanatory variables and the response variable was the time of a female wasp to parasitize a host once till leaving the host voluntarily (oviposition time), and the number of emerging wasps per host (brood size), respectively. All wasps from each host eclosed on the same day resulting in a single data entry per host for development time. In the latter model brood size was entered additionally as a covariate. A simple linear regression model (LM) was used to analyze adult biomass, with parasitoid species, host instar and brood size (covariate) as explanatory variables. The response variable was the mean biomass of the parasitoids eclosing from the same host individual. Biomass was log-transformed to fit model assumptions and it was analyzed separately for males and females.

If any of the main factors was significant in the models described above, Tukey multiple comparison tests among the means were conducted. All statistical analyses were undertaken with R statistical software (R Core Team, 2018) using the R basic package “stats” and package of “multcomp” (Hothorn et al, 2008).

3. Results

3.1. Instar-specific parasitoid oviposition preference.

O. scaposus preferred fourth over third instar ladybird larvae (Binomial test: $P = 0.013$; Fig. 1a), but did not show instar preference when offered any of the other instar combinations. *O. spiraculus*, did not exhibit oviposition preference for any host development stage (Binomial tests: $P > 0.05$, Fig. 1b).

3.2. Instar-specific parasitoid performance

Oviposition duration. Oviposition duration differed between parasitoid species (GLM: $\chi^2 = 157$, $df = 1$, $P < 0.001$) and among host instars (GLM: $\chi^2 = 34.1$, $df = 3$, $P < 0.001$), whereas the interaction between

these two factors was not significant (GLM: $\chi^2 = 3.21$ $df = 3$, $P = 0.36$). The effect of host instar on oviposition time was less pronounced for *O. scaposus* than for *O. spiraculus* (Fig. 3a). Oviposition duration tended to increase with host instar till the third instar for both parasitoid species. Oviposition duration of *O. spiraculus* was two to three times longer than that of *O. scaposus* (Fig. 3a).

Parasitism success. Successful parasitism was neither affected by parasitoid species (GLM: $\chi^2 = 1.53$, $df = 1$, $P = 0.22$), host instar (GLM: $\chi^2 = 7.49$, $df = 3$, $P = 0.060$), nor their interaction (GLM: $\chi^2 = 0.72$, $df = 3$, $P = 0.87$, Fig. 2). In general, parasitism success (successful development into adults) ranged between 30 and 50% (Fig. 2).

Sex ratio. Sex ratio of parasitoid offspring was not affected by host instar (GLM: $\chi^2 = 6.51$, $df = 3$, $P = 0.09$), parasitoid species (GLM: $\chi^2 = 0.58$, $df = 1$, $P = 0.45$) or their interaction (GLM: $\chi^2 = 1.57$, $df = 3$, $P = 0.67$). The sex ratio was highly female biased: the average male proportion was 0.13 for both species (Fig. 3b).

Brood size. Host instar had a significant effect on parasitoid brood size (GLM: $\chi^2 = 37.2$, $df = 3$, $P < 0.001$) and this effect was similar for the two parasitoid species (GLM: species: $\chi^2 = 0.32$, $df = 1$, $P = 0.57$; species-host instar interaction: $\chi^2 = 4.59$, $df = 3$, $P = 0.20$). Parasitoid species tended to lay more eggs in later instar hosts (Fig. 3c).

Development time. Egg-to-adult development time varied depending on host instar at the time of parasitism, parasitoid species, and brood size (GLM: host instar: $\chi^2 = 159$, $df = 3$, $P < 0.001$; species: $\chi^2 = 52.3$, $df = 1$, $P < 0.001$; brood size: $\chi^2 = 167$, $df = 1$, $P < 0.001$), but was not affected by the interaction between host instar and parasitoid species (GLM: $\chi^2 = 6.52$, $df = 3$, $P = 0.90$). In both species, development time tended to decrease with host instar (Fig. 3d). Development time of

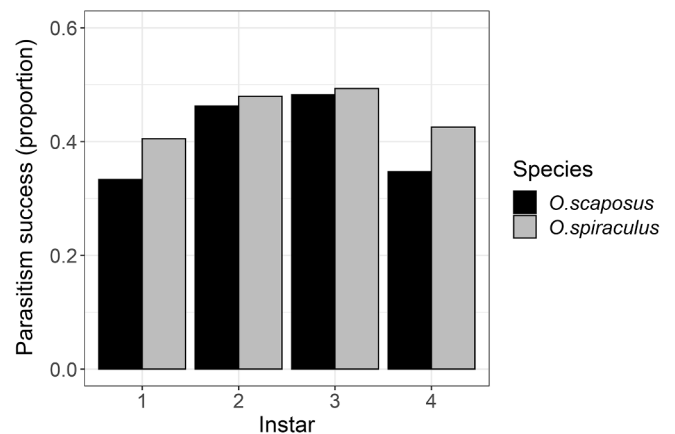


Fig. 2. Parasitism success (proportion of parasitized host producing adults) of *Oomyzus scaposus* (black bars) and *O. spiraculus* (grey bars) parasitizing first to fourth instar *Coccinella septempunctata* hosts. Bars represent the proportion of parasitized hosts producing adult wasps in each instar. At least 40 hosts of each instar were parasitized.

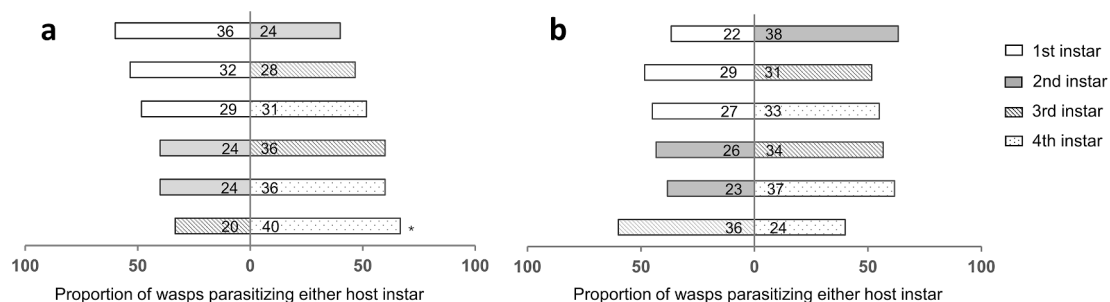


Fig. 1. Oviposition preference of female *Oomyzus spiraculus* (a) and *O. scaposus* (b) in pair-wise choice assays with first (white bar), second (grey bar), third (dashed bar), and fourth (dotted bar) instar *Coccinella septempunctata* hosts. Bars represent preference proportions for either host instar out of total choices with the numbers of females depicted in the bars. The asterisk denotes a significant preference in this host pair combination (binomial test).

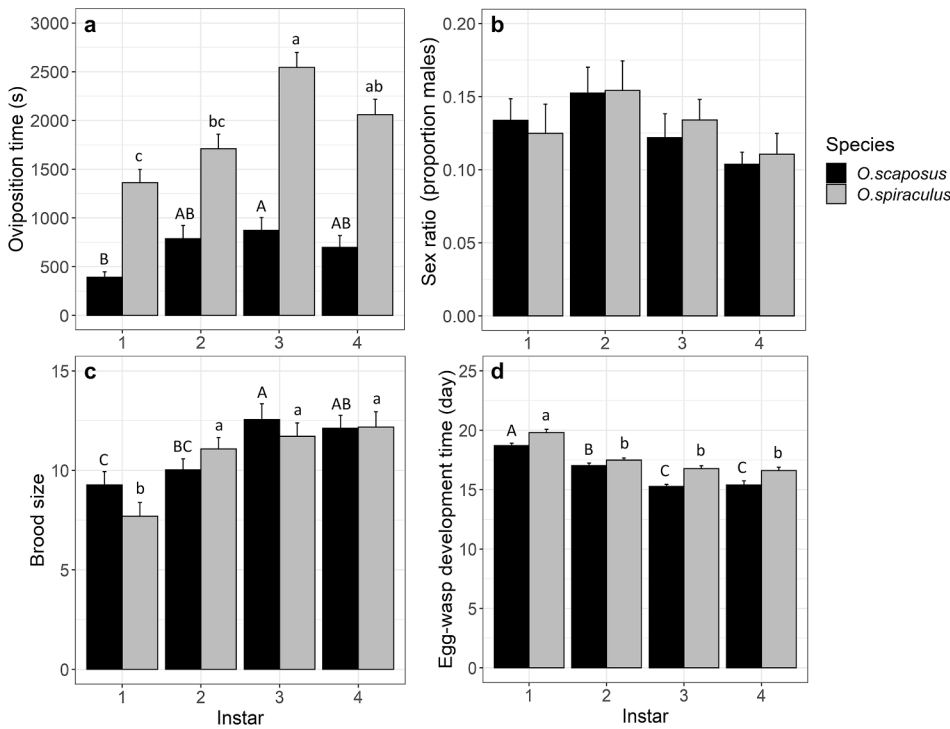


Fig. 3. Oviposition duration (a), offspring sex ratio (proportion of males) (b), brood size (c), and egg-to-adult development time of *Oomyzus scaposus* (black bars) and *O. spiraculus* (grey bars) parasitizing first to fourth instar *Coccinella septempunctata* hosts. Bars represent means + SE. Comparisons among means were conducted for each of the two species in separate analyses (Tukey). Upper (*O. scaposus*) and lower (*O. spiraculus*) case letters denote significant differences among means ($P < 0.05$). Only when the effect of instar was significant multiple comparison tests were conducted.

O. spiraculus was longer than that of *O. scaposus* (Fig. 3d). In both species, development times of male wasps were a few hours shorter than those of females.

Biomass. Adult biomass of males was affected by host instar (LM: $F_{3, 198} = 4.39, P < 0.01$), parasitoid species (LM: $F_{1, 198} = 35.46, P < 0.001$), brood size (LM: $F_{20, 198} = 2.01, P < 0.01$), and the interaction between parasitoid species and host instar (LM: $F_{3, 198} = 6.75, P < 0.001$). Similarly, adult biomass of females was affected by host instar (LM: $F_{3,232} = 8.00, P < 0.001$), parasitoid species (LM: $F_{1, 232} = 38.53, P < 0.001$), but was neither affected by brood size (LM: $F_{21,232} = 1.38, P = 0.13$) nor the interaction between parasitoid species and host instar (LM: $F_{3,232} = 1.65, P = 0.18$). Both male and female wasps of *O. scaposus* were heavier than those of *O. spiraculus* (all Tukey- test within instar comparisons $P < 0.05$, except for males in first instar hosts, and females in first and fourth instar hosts Fig. 4). In *O. spiraculus* adult biomass of both sexes tended to decrease with host instar at parasitism, whereas for *O. scaposus* biomass of male and female wasps was lower in the first and fourth instar host compared to the two middle host instars (Fig. 4).

4. Discussion

The results of this study show that there was both divergence and convergence in ovipositional and developmental traits in *O. scaposus* and *O. spiraculus*. Both parasitoids laid broods of 8–13 eggs in larvae of *C. septempunctata*, but generally more in later than in earlier instars (Fig. 3c). Offspring sex ratios in both parasitoids were also heavily female-biased across all instars of *C. septempunctata* (Fig. 3b). The duration of oviposition of both *Oomyzus* species tended to increase from first to third instar host (Fig. 3a). Parasitism success was similar in both species and did not differ across instars (Fig. 2). Egg to adult development time tended to decrease with host instar. In *O. spiraculus* faster development in later instars coincided with reduced adult biomass, whereas in *O. scaposus* biomass was highest from second and third instar hosts (Fig. 3d, 4). Adult females were also significantly larger than males in both species. Despite the similarity in brood size in both species (Fig. 3c), adult *O. scaposus* wasps handled hosts much more rapidly than *O. spiraculus* (Fig. 3a). This reveals that *O. scaposus* is able to lay more

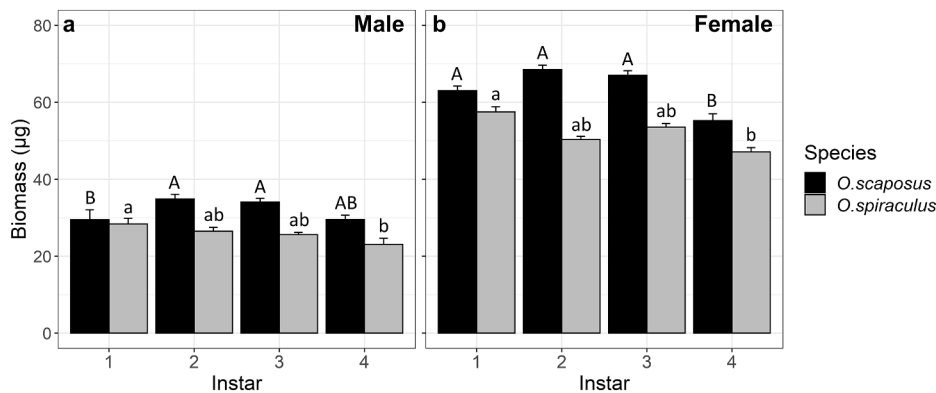


Fig. 4. Adult dry mass of *Oomyzus scaposus* (black bars) and *O. spiraculus* (grey bars) of males (a) and females (b) that parasitized first to fourth instar *Coccinella septempunctata* hosts. Bars represent means + SE. Comparisons among means were conducted for each of the two species in separate analyses (Tukey). Upper (*O. scaposus*) and lower (*O. spiraculus*) case letters denote significant differences among means ($P < 0.05$).

eggs per unit of time than *O. spiraculus*. Moreover, adult *O. scaposus* were also larger than *O. spiraculus* and emerged as adults earlier (Fig. 3d, 4). These results suggest that *O. scaposus* is somewhat better adapted or more efficient with their ladybird host.

Several (but not all) developmental parameters in both *Oomyzus* species were remarkably similar with those of a closely related species, *Oomyzus sokolowskii* (Kurdjumov), developing in different instars and pupae of the diamondback moth, *Plutella xylostella* (L.) (Wang et al., 1999; Nakamura and Noda, 2002; Li et al., 2017). For example, offspring sex ratios were heavily female-biased, and brood sizes among the species were also very similar (Li et al., 2017, Fig. 3b). The overlap of some traits among different *Oomyzus* species suggests that host-related constraints like size (hence resource availability) are similar among them, and second, that the expression of ontogenetic traits in this genus are phylogenetically conserved, even when attacking unrelated hosts in the second (diamondback moth larvae) and third (ladybird beetle larvae) trophic levels. This is not surprising, because phylogeny constrains the ability of closely related species (e.g. congeners) to evolve traits in response to novel ecological challenges (McKittrick, 1993; Price, 2003).

Patterns of host utilization and development in the two species studied here were similar to those displayed by other gregarious, koinobiont parasitoids of herbivorous insects (Godfray, 1994; Harvey, 2005; Jervis et al., 2008; Song et al. 2017). For example, sexual-size dimorphism (i.e. larger females) is very commonly reported in the parasitic Hymenoptera, even across many unrelated taxa (Godfray, 1994; Mackauer, 1996; Quicke, 1997, 2015) although the inverse pattern (i.e. larger males) occasionally occurs (Harvey & Strand, 2003). Furthermore, in koinobiont parasitoids, brood sizes may or may not increase with host size, with this parameter depending on when the parasitoid terminates its relationship with the host (Harvey, 2005). Furthermore, the heavily female-biased sex ratios in both species is indicative of 'local mate competition (LMC)', whereby males emerge just before females siblings and mate with them upon female eclosion (Shuker et al., 2005). LMC is commonly observed in many other gregarious parasitoids. Our results thus show that primary parasitoids of both herbivores and predators exhibit similar functional traits that reflect both phylogeny and overlap in biological and ecological characteristics of their hosts, regardless of their trophic status.

An important question is how our lab-based results translate to field conditions in predicting the possible impact of the two *Oomyzus* species *C. septempunctata*. Preliminary data at several locations in the east and south of China reveal that parasitism rates of *C. septempunctata* by *O. scaposus*, especially later in the season, can be significant (>50%), whereas parasitism by *O. spiraculus* is less variable over the season but rarely higher than 20% (Fei et al. unpublished data). The shorter oviposition times of *O. scaposus* may clearly give it a competitive edge in the field over *O. spiraculus*. Few studies have measured the effects of body size in parasitoids under field conditions, but Visser (1994) and Ellers et al. (1998) showed that demographic traits like reproduction and survival in parasitoids are enhanced in larger females in nature. For parasitoids in the fourth trophic level, where hosts are presumably more scarce than the herbivore hosts of parasitoids, large size may be even more important for fitness (Harvey, 2005).

The combined high parasitism rates of *C. septempunctata* by the two *Oomyzus* species observed in the field at multiple locations in China suggest that these parasitoids can significantly reduce ladybird populations and this in turn can clearly hamper biological control efforts of pests such as *A. gossypii*. Hyperparasitoids of primary parasitoids are known to inflict serious losses on important biological control agents (Schooler et al., 2011; Tougeron & Tena, 2019). Given that ladybirds are among the most important predators of aphids, mealybugs, scale insects, psyllids and other pests in many important commercial crops (Dixon, 2000; Pugh et al., 2015), a more thorough knowledge of the biology, behavior and ecology of their parasitoids in both natural and managed ecosystems, such as greenhouses, may therefore help agronomists to develop strategies to mitigate their impacts. Cusumano et al. (2020)

suggest exploiting the chemical ecology of plants to lure hyperparasitoids away from their primary parasitoids. Perhaps it is possible to use a similar strategy to reduce the impacts of *Oomyzus* species on their ladybird hosts. Moreover, practices that have been used to attract primary parasitoids of herbivore pests, such as flower strips bordering agricultural crops, will also attract hyperparasitoids and parasitoids of predators (Araj et al., 2008). The costs and benefits of these practices on pest control need to be investigated further.

In summary, this study compared and contrasted detailed aspects of the development and life-history in two closely related larval-pupal parasitoids of ladybirds. We show that *O. scaposus* and *O. spiraculus* are both well adapted to *C. septempunctata*, but with the former species exhibiting somewhat higher success on this host. Translating the results of lab-based studies to the field where conditions are unpredictable is a major challenge. The ecology and biology of both the parasitoids and their hosts are also influenced by an array of biotic and abiotic factors in nature - competitors, predators, pathogens, wind, rain, heat - that will affect their ontogeny and behavior. Moreover, how the sensory environment as well as structural differences in ladybird habitat (e.g. in managed agroecosystems) affect the expression and importance of life-history traits in parasitoids has thus far been little studied. Although difficult, incorporating more complexity into life-history studies might yield very useful information on management strategies to minimize the harmful impacts of parasitoids attacking important predators like *C. septempunctata*.

Further experiments examining parasitism rates of *C. septempunctata* in different crop cultivars as well as extrinsic and intrinsic competition between the two parasitoids are underway and will help to shed light on trait-based effects on parasitoid success and of possible indirect effects (e.g. via mutual interference) on pest abundance. It will also be interesting to test if parasitism leads to compensatory feeding by ladybird larvae as has been shown with several other species of gregarious koinobiont parasitoids attacking herbivorous hosts (Harvey & Malcicka, 2016). If parasitized ladybirds actually consume more aphids than healthy ladybird larvae, then the short term impacts on aphid abundance may be to enhance aphid predation. Moreover, given that several parasitoid species are also major natural enemies of aphids, it is important to determine how ladybird parasitoids and aphid parasitoid hyperparasitoids interact to affect the relative abundance of aphids via intraguild effects.

CRediT authorship contribution statement

Minghui Fei: Conceptualization, Methodology, Formal analysis, Writing - original draft, Writing - review & editing, Funding acquisition. **Haowu Hu:** Methodology, Investigation. **Rieta Gols:** Methodology, Formal analysis, Writing - review & editing. **Shengnan Liu:** Investigation. **Xiaolin Wan:** Investigation. **Baoping Li:** Conceptualization, Resources, Writing - review & editing. **Jeffrey A. Harvey:** Methodology, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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