The hyperparasitoid *Marietta picta* (Hymenoptera: Aphelinidae) mediates competitive interactions between two parasitoids of *Paratrioza sinica* (Hemiptera: Psyllidae): *Tamarixia lyciumi* (Hymenoptera: Eulophidae) and *Psyllaephagus arenarius* (Hymenoptera: Encyrtidae)

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**GRAPHICAL ABSTRACT**

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**ABSTRACT**

Both the ectoparasitoid *Tamarixia lyciumi* Yang (Hymenoptera: Eulophidae) and the endoparasitoid *Psyllaephagus arenarius* (Hymenoptera: Encyrtidae) are solitary nymphal parasitoids of *Paratrioza sinica* (Hemiptera: Psyllidae), a pest of goji berry, *Lycium barbarum*. Moreover, *T. lyciumi* is frequently attacked by a hyperparasitoid *Marietta picta* Andre (Hymenoptera: Aphelinidae) in the field. To analyze competitive interactions between *T. lyciumi* and *P. arenarius* mediated by *M. picta*, we evaluated interspecific host discrimination and larval competition between *T. lyciumi* and *P. arenarius*, and the effect of *M. picta* on their competitive interactions, under laboratory conditions. The results showed that *T. lyciumi* could discriminate hosts pre-parasitized by *P. arenarius*, but not vice versa. However, *P. arenarius* was a superior intrinsic competitor in multiparasitized hosts regardless the order of parasitism. Although multiparasitized hosts produced more *P. arenarius* and fewer *T. lyciumi* after exposure to *M. picta*, the total number of emerging *T. lyciumi* and *P. arenarius* did not decline significantly, and sex ratios did not change. Thus, larval superiority would appear to favor the establishment of newly introduced *P. arenarius* in the Ningxia region, while the inferior competitor *T. lyciumi* is able to utilize interspecific host discrimination to avoid...
1. Introduction

As biological control agents, parasitoids play an important role in community structures in terms of ecological and economic values (Hawkins et al., 1999; LaSalle and Gould, 1993). Many larval/nymphal parasitoids (primary parasitoids) emerge from herbivores, and a part of hyperparasitoids (secondary parasitoids) successfully develop on other parasitoids (Jervis et al., 1993). Interspecific competition containing extrinsic and intrinsic competition is valuable in shaping ecological structures as competing species hardly share resources in the identical ecosystem (De Moraes et al., 1999; Godfray, 1994). Some views are presented that newly introduced species may disrupt existing stable communities (Denoth et al., 2002; Didham et al., 2005; Mooney and Cleland, 2001), whereas others indicate that various species can effectively reduce pest populations via synergistic effects (DeBach, 1966; Stiling and Cornelissen, 2005). Interspecific competition should be considered by biological control programs as competitive interactions between/among parasitoid species are likely to influence the fitness of their progeny and thus impact pest suppression (Follett et al., 2000). So far the effect of extrinsic competition between species remains unclear. Thus, studies on intrinsic competition are extremely valuable, which not only provide theoretical foundations, but also improve biological control strategies. When a new parasitoid species is introduced in an ecosystem, the type (parasitoid or hyperparasitoid) of species and the extent of impact should be considered to define its role (neutral, positive or negative) in the ecosystem. Potential interactions with native species in identical food webs may affect the practical results of intentional or accidental new-species introduction, so the purpose of studying newly introduced species is to synergistically facilitate pest suppression and maintain ecosystem stability.

*Parastriona sinica* Yang et Li (Homoptera: Psyllidae) is a leaf-sucking pest widely distributed across goji berry (*Lycium barbarum*) orchards in northern China (Liu et al., 2013). Currently, chemical pesticides are potentially harmful to human health and ecological environment (Garrett and Kennedy, 2006; Youn et al., 2003). Thus, the utilization of biological control agents deserves to draw more attention (Leskey et al., 2012; Lee et al., 2013). Two solitary nymphal parasitoids *Tamarixia lyciuni* Yang and *Psyllaphagus arenarius* with high parasitism rates are explored as common biological control agents of *P. sinica* in Ningxia province, China. *Tamarixia lyciuni* is a ectoparasitoid originally described in 1997 (Tang, 1997), whereas the endoparasitoid *P. arenarius* is first recorded only in 2016 from China (Zhang et al., 2017). The parasitism rates of *T. lyciuni* and *P. arenarius* in the field reach occasionally up to 86.0% and 89.7%, respectively (Duan et al., 2002; Wu et al., 2017), and both of them showed positive preferences for 4th-instar nymphs of *P. sinica* compared to the nymphs at other development stages (Wang et al., 2010; Wu et al., 2017). When 4th-instar nymphs are provided, the resource competition between *T. lyciuni* and *P. arenarius* is more intensive, the extent and interaction of the biological control of *P. sinica* can be shown more clearly and directly, so the 4th-instar nymphs are more appropriate as treated hosts. Only one parasitoid emerges from a *P. sinica* nymph when multiparasitism occurs due to interspecific intrinsic interactions. Therefore, competitive interactions between the classical biological control agent *T. lyciuni* and the newly introduced natural enemy *P. arenarius* should be examined to generate a coexistence in the biological control of *P. sinica*.

Hyperparasitoid, a parasitoid attacking larvae/pupae of primary parasitoids (Poelman et al., 2012), is considered as a critical element in the fourth trophic level of insect communities (Sullivan and Völk, 1999). Some studies indicate hyperparasitoids may disrupt the coexistence mediated by primary parasitoids and then influence their biocontrol efficiency (Yang et al., 2017; Gomez-Marco et al., 2015; Schooler et al., 2011; Rand et al., 2012). However, others suggest that hyperparasitoids can facilitate biological control by stabilizing host-parasitoid and parasitoid–parasitoid dynamics (Sullivan, 1987; Hassell and Waage, 1984). From a biological control perspective, hyperparasitoids play an important role in mediating interactions between primary parasitoids (Ye et al., 2017). *Marietta picta* Andre (Hymenoptera: Aphelinidae), a hyperparasitoid of *T. lyciuni*, was originally discovered in 2016 from Ningxia. *Marietta picta* shows its significant preference for mature larvae of *T. lyciuni*, and the parasitism rate described in the field sometimes reaches up to 24.59% (Ma, unpublished data). In order to survey the role of *M. picta* in the establishment of successful biological control programs in Ningxia, the study on its effect on competitive interactions between *T. lyciuni* and *P. arenarius* is meaningful.

Studies on the coexistence of parasitoids in natural ecosystems are valuable in IPM programme as host–parasitoid–hyperparasitoid interactions may either disrupt the existing ecological balance or establish a collective coexistence in terms of biological control. In this study, we addressed 2 main questions: (1) Whether there is an interspecific host discrimination between *T. lyciuni* and *P. arenarius*, and which species is superior in the larval competition. (2) What is the effect of *M. picta* on the intrinsic competition between larval *T. lyciuni* and *P. arenarius*. Consequently, from a coexistence viewpoint, competitive interactions between *T. lyciuni* and *P. arenarius* mediated by *M. picta* in the biological control of *P. sinica* are assessed.

2. Materials and methods

2.1. Insect

Since both *T. lyciuni* and *P. arenarius* showed high parasitism rates and remarkable preferences for 4th-instar nymphs of *P. sinica*, 4th-instar nymphs of *P. sinica* on the leaves were collected from the large-scale goji berry orchard of Dadi, Zhongning County, Ningxia Province, China, and kept in plastic containers (length 45 cm × width 108 cm × height 151 cm). The colonies of *T. lyciuni*, *P. arenarius* and *M. picta* were also established from the goji berry orchard of Dadi. As host feeding of *T. lyciuni*, *P. arenarius* and *M. picta* failed to occur, their adults needed to be supplied additional honey, Thus, both fresh hosts and drops of 10% honey were provided to the parasitoids twice a week. All 4th-instar nymphs of *P. sinica* used in experiments were < 12 h old, all female parasitoids were 4-d-old and mated. The experiments were performed in conditions at LD 16:8, 25 °C and 40–60% RH.

2.2. Interspecific host discrimination and larval competition between *T. lyciuni* and *P. arenarius*

To evaluate the interspecific host discrimination and the larval competition between the two species, 30 fresh 4th-instar nymphs of *P. sinica* on the leaves were firstly exposed to 10 *T. lyciuni* females in a Petri dish (9 cm diameter). Oviposition by parasitoids could be identified by the presence of a distinctive scar on the nymph, the oviposition attempt of *T. lyciuni* was about 4.6 min per nymph. Zero-, 1-, 2-, 3-, 4-, 5-, 6- or 7 days after being attacked by *T. lyciuni*, the 30 paralyzed hosts were displayed to a *P. arenarius* female in the Petri dish. Similarly, 10 *P. arenarius* females were introduced to attack fresh 4th-instar nymphs of *P. sinica* until all the 30 nymphs were parasitized, the handling time of *P. arenarius* was about 2.3 min per nymph. Then a
female *T. lyciumi* was allowed into the Petri dish containing the 30 parasitized nymphs with different intervals (0-, 1-, 2-, 3-, 4-, 5-, 6- or 7 days) between ovipositions. The petri dish was observed until the female parasitoid (subsequently introduced) stopped interacting with the nymphs for at least 30 min, and the number of multiparasitized nymphs was counted. After 3 weeks, species sex and the emerging parasitoids were identified via morphological identification. The proportions of *T. lyciumi* and *P. arenarius* emerging from multiparasitized hosts ( emergence rate), and the proportion of multiparasitized nymphs producing no parasitoids (host mortality rate) were calculated. Moreover, the sex of emerging parasitoids was recorded. Each treatment was replicated 10 times simultaneously.

In order to assess overall parasitism rates and emergence rates of parasitoids in non-competitive situations, 30 fresh 4th-instar nymphs of *P. sinica* were presented to a female *T. lyciumi* and *P. arenarius*, respectively, as controls. The number of parasitized nymphs was examined after the female stopped interacting with the nymphs for more than 30 min, and then the number and sex of emerging parasitoids were recorded after 3 weeks. The experiment was replicated 10 times simultaneously.

2.3. Effect of *M. picta* on the larval competition between *T. lyciumi* and *P. arenarius*

To analyze whether interspecific intrinsic interactions between *T. lyciumi* and *P. arenarius* could be influenced by the hyperparasitoid *M. picta*, 30 multiparasitized 4th-instar nymphs of *P. sinica* on the leaves were selected. The hosts were previously parasitized by *T. lyciumi*, and subsequently attacked by *P. arenarius* 0-, 1-, 2-, 3-, 4-, 5-, 6- or 7 days later. A similar treatment was followed, only the oviposition sequence of the two species was reversed. As *M. picta* showed a significant preference for mature larvae of *T. lyciumi* (3.5-day old) with high parasitism rates (Ma, unpublished data), the multiparasitized hosts were presented to 10 *M. picta* females in a Petri dish 3.5 days after the parasitization by *T. lyciumi*. Emergence rates of *T. lyciumi* and *P. arenarius* from multiparasitized nymphs were examined after three weeks to test the population change (decrease or increase) of *T. lyciumi* or *P. arenarius* when the multiparasitized hosts were exposed to *M. picta*. The sex of emerging primary parasitoids was also recorded. Moreover, in order to evaluate the long-term possibility of mediation by *M. picta* in the local area, its parasitism rate, emergence rate and sex ratio of progeny under multiparasitism situations were recorded as well. And thirty 3.5-day old larvae of *T. lyciumi* on 4th-instar nymphs were presented to 10 *M. picta* females in a Petri dish as controls. Each treatment was replicated 10 times simultaneously.

Decreased or increased rate of the primary parasitoid was calculated according to $\% = [(\text{emergence rate with the presence of } M.\ pica) - \text{emergence rate without the presence of } M.\ pica]/(\text{emergence rate without the presence of } M.\ pica) \times 100$.

2.4. Data analysis

Descriptive statistics were given as the mean values and standard errors of the mean. Proportions of multiparasitized nymphs, developmental outcomes containing emergence rates of parasitoids from multiparasitized nymphs and host mortality rates, and sex ratios of emerging parasitoids were analyzed with $X^2$ tests. The values for each combination of factors were calculated according to the resulting standardized residual via crosstab analysis, and compared to Bonferroni-corrected $P$ values testing whether each multiparasitism or emergence rate was significantly different from the average rate across various time intervals. Differences among average developmental outcomes (host mortality rate and emergence rates of *T. lyciumi* and *P. arenarius*) were analyzed using one-way ANOVA with the Tukey HSD test. Other data were examined using independent t-tests. In one-way ANOVA and t-tests, $P$ values < 0.05 were considered significant. All statistical analyses were performed using SPSS 20.0 software (IBM, Armonk, NY).

3. Results

3.1. Interspecific host discrimination and larval competition between *T. lyciumi* and *P. arenarius*

*Psyllaephagus arenarius* could not discriminate among *P. sinica* nymphs of various ages pre-parasitized by *T. lyciumi* ($\chi^2_{(5, N=2400)} = 29.395$, $P < 0.0001$; 16 comparison tests, adjusted $\alpha = 0.003125$, $P$ of each test $> \alpha$; Fig. 1A). No significant difference was observed between the average multiparasitism rate (50.8% ± 2.1%) and that of control (parasitism rate of *P. arenarius* only; 60.7% ± 5.9%) ($t = 1.567$, df = 11.165, $P = 0.145$). Nevertheless, *T. lyciumi* showed significantly positive preference for hosts recently (0–2 days) parasitized by *P. arenarius*, but reluctantly accepted older (6–7 days) ones ($\chi^2_{(5, N=2400)} = 202.998$, $P < 0.0001$; 16 comparison tests, adjusted $\alpha = 0.003125$; Fig. 1B). The average multiparasitism rate (27.3% ± 4.9%) was notably lower than that of control
(parasitism rate of *T. lyciumi* only; 58.7% ± 6.2%) \( (t = 3.808, df = 16, P = 0.002) \).

Only one parasitoid had a possibility of emerging from a *P. sinica* nymph under multiparasitism situations, while there were still some of multiparasitized nymphs yielding no parasitoids. Developmental outcomes depended on the order of oviposition and the interval between ovipositions. When *P. sinica* nymphs were previously exposed to *T. lyciumi* and subsequently offered to *P. arenarius*, *T. lyciumi* developed extraordinarily well when multiparasitism occurred without time intervals (0-day) between parasitizations, but its emergence rates from multiparasitized nymphs gradually decreased as intervals were increased. However, proportions of *P. arenarius* produced from multiparasitized hosts increased with increased intervals. *Psyllaephagus arenarius* was a superior larval competitor in hosts 3–5 days following exposure to *T. lyciumi*, but rarely emerged from multiparasitized nymphs from the remaining time intervals (6–7 days) between ovipositions \( (\chi^2_{(14,N=1200)} = 149.369, P < 0.0001; 24\) comparison tests, adjusted \( \alpha = 0.002083; \) Fig. 2A). In control experiments, emergence rates of *T. lyciumi* and *P. arenarius* from hosts were 85.0% ± 4.6% and 74.9% ± 4.7%, respectively. Thus, under multiparasitism situations, both *T. lyciumi* (average emergence rate, 26.1% ± 2.1%; \( t = 9.766, df = 88, P < 0.001 \)) and *P. arenarius* \( (30.7\% ± 2.1\%; \ t = 7.146, df = 88, P < 0.001) \) suffered huge losses compared to control groups due to the lethal larval competition. Overall, although *P. arenarius* slightly outcompeted *T. lyciumi* in the larval competition, a significantly higher proportion (average host mortality, 43.2% ± 2.8%) of host nymphs producing no parasitoids was detected \( (F_{2,237} = 14.214, P < 0.001) \). In terms of sex, the time interval between the parasitizations failed to affect sex ratios of emerging *T. lyciumi* \( (\chi^2_{(7,N=310)} = 10.546, P = 0.16) \) and *P. arenarius* \( (\chi^2_{(7,N=374)} = 13.082, P = 0.07) \). When *T. lyciumi* was the first species to parasitize hosts, the average proportion of *T. lyciumi* females \( (56.1 ± 4.2%; \ t = 0.24, df = 16, P = 0.813) \), whereas the average proportion of *P. arenarius* females \( (50.5 ± 5.0\%); \ t = 2.833, df = 16, P = 0.012 \) was clearly superior in the larval competition, and the greatly competitive inferiority of *P. arenarius* \( (30.7\% ± 2.1\%; \ t = 7.146, df = 88, P < 0.001) \) was examined compared to control groups \( (68.8 ± 4.1\%); \ t = 2.833, df = 16, P = 0.012 \).

When the oviposition by *P. arenarius* was followed by that by *T. lyciumi*, more *T. lyciumi* rather than *P. arenarius* emerged when multiparasitized nymphs were generated within 24 h, and then emergence rates of *T. lyciumi* and *P. arenarius* gradually decreased and increased with increased time intervals, respectively. *Tamarixia lyciumi* hardly emerged from multiparasitized nymphs 5–7 days following exposure to *P. arenarius*, whereas *P. arenarius* successfully developed in the hosts from those intervals \( (\chi^2_{(14,N=650)} = 96.094, P < 0.0001; 24\) comparison tests, adjusted \( \alpha = 0.002083; \) Fig. 2B). The average emergence rates of both *T. lyciumi* \( (22.8\% ± 2.6\%; \ t = 8.274, df = 88, P < 0.001) \) and *P. arenarius* \( (38.4\% ± 3.4\%; \ t = 6.367, df = 20.081, P < 0.001) \) from multiparasitized hosts were observably lower than those of control, respectively. Overall, *P. arenarius* was clearly superior to *T. lyciumi* in the larval competition, and there was still a significant proportion \( (38.8\% ± 2.9\%); \) of multiparasitized hosts yielding neither parasitoid species \( (F_{2,237} = 9.409, P < 0.001) \). In addition, sex ratios of emerging *P. arenarius* \( (\chi^2_{(7,N=189)} = 7.863, P = 0.345) \) and *T. lyciumi* \( (\chi^2_{(7,N=374)} = 5.858, P = 0.556) \) could not be impacted by the time interval. The female proportion of *P. arenarius* \( (62.0 ± 3.8\%); \ t = 2.208, df = 16, P = 0.042) \) was consistent with that of control \( (68.8 ± 4.1\%) \) when it acted as the initial species to parasitize hosts \( (t = 1.195, df = 16, P = 0.25) \), but the significantly decreased proportion of *T. lyciumi* females \( (34.8 ± 8.1\%); \) was examined compared to control groups \( (54.6 ± 4.8\%); \ t = 2.208, df = 16, P = 0.042) \).

**3.2. Effect of *M. picta* on the larval competition between *T. lyciumi* and *P. arenarius***

With the presence of *M. picta*, more *P. arenarius* emerged from multiparasitized nymphs irrespective of the interval between the initial oviposition by *T. lyciumi* and subsequent parasitization by *P. arenarius*, especially the 0–4 days intervals, but signally more hosts produced no parasitoids from the remaining intervals \( (\chi^2_{(14,N=2400)} = 256.118, P < 0.0001; 24\) comparison tests, adjusted \( \alpha = 0.002083; \) Fig. 3A). Overall, *P. arenarius* \( (39.0\% ± 2.4\%); \) had a dominant position in the larval competition, and the greatly competitive inferiority of *T. lyciumi* \( (16.4\% ± 1.1\%); \) was mainly due to the hyperparasitism of *M. picta*, but there were still many multiparalyzed nymphs \( (44.6\% ± 2.8\%); \) producing no parasitoids \( (F_{2,237} = 45.767, P < 0.001) \). Fewer *T. lyciumi* successfully developed from multiparasitized hosts after exposure to *M. picta* with an average decreased rate of 37.2%, whereas the number of emerging *P. arenarius* increased with an average rate of 26.9%, and the overall number of *T. lyciumi* and *P. arenarius* produced from multiparalyzed nymphs went down 2.5%. The interval between ovipositions could not affect the sex ratios of emerging *T. lyciumi* \( (\chi^2_{(7,N=1305)} = 13.035, P = 0.071) \) and *P. arenarius* \( (\chi^2_{(7,N=935)} = 12.266, P = 0.092) \), and the female proportions of *T. lyciumi* \( (22.8\% ± 2.6\%; \ t = 0.813, df = 14, P = 0.494) \) and *P. arenarius* \( (50.5 ± 5.0\%); \ t = 0.293, df = 14, P = 0.774) \) were still a signification from the mean expected sex ratios of emerging parasitoids \( (*\) indicate proportions of each developmental outcome (Fig. 2). (A) Hosts were firstly parasitized by *T. lyciumi*; (B) hosts were initially parasitized by *P. arenarius*. Asterisks (*) indicate proportions of each developmental outcome (*T. lyciumi*, *P. arenarius* or nothing) differ significantly from the mean expected proportion across various time intervals, according to \( \chi^2 \) tests with Bonferroni corrections.

![Fig. 2. Proportions of multiparalyzed nymphs producing *T. lyciumi*, *P. arenarius* or nothing at various intervals between ovipositions. (A) Hosts were firstly parasitized by *T. lyciumi*; (B) hosts were initially parasitized by *P. arenarius*. Asterisks (*) indicate proportions of each developmental outcome (*T. lyciumi*, *P. arenarius* or nothing) differ significantly from the mean expected proportion across various time intervals, according to \( \chi^2 \) tests with Bonferroni corrections.](image-url)
subsequently exposed to $M.\ picta$ in $< 24$ h intervals when hosts were

$P = 0.482$) were not impacted by the time interval, and the presence of

Proportions of multiparasitized nymphs producing $T.\ lyciumi$ significantly increased by 32.3% and 22.5%, respectively, when multiparasitized hosts emerged from a $P.\ sinica$ nymph, suggesting the lack of interspecific discrimination could trigger a lethal competition between larval parasitoids ($Okuda\ and\ Yeargan, 1988$). In order to avoid massive losses, inferior competitors tend to evolve interspecific host discrimination ($Pijls\ et\ al.,\ 1995;\ van\ Alphen\ and\ Visser,\ 1990$). Thus, the inferior competitor $T.\ lyciumi$ potentially benefited by discriminating hosts prepared by $P.\ arenarius$ after evaluating the cost of multiparasitism. Even though parasitoids pay increasing attention to interspecific discrimination ($van\ Baaren\ et\ al.,\ 1994;\ Weber\ et\ al.,\ 1996$), multiparasitism cannot be avoided once various species of parasitoids exist in an ecosystem.

4.2. $Psyllaephagus\ arenarius$ is superior in the larval competition

Interspecific intrinsic interactions between two parasitoid species are characterized by studies in which hosts are exposed to parasitoids in different sequences and intervals ($Irvin\ et\ al.,\ 2006;\ Alim\ and\ Lim,\ 2011$). In general, larvae of the first species are benefited as they exploit and obtain limited host resources before competitors, which restricts the development of opponents within the identical host ($Casumano\ et\ al.,\ 2012$). However, $P.\ arenarius$ was successful under multi-parasitism situations when it was served as both initial and subsequent species, implying the asymmetrical result that one species outcompeted another regardless the order of ovipositions ($Kfir\ and\ van\ Hamburg, 1988$). The result was consistent with the previous study that $Oenocyrus\ telemonoides$ dominated the intrinsic competition irrespective of the parasitization sequence and won even when hosts were offered to $Trissolcus\ basalis$ 7 days before ($Casumano\ et\ al.,\ 2011$). Although $P.\ arenarius$ usually won the intrinsic competition, its larval superiority was partly affected by the sequence of parasitization and the interval between parasitizations in terms of developmental
outcomes. In sequential exploitative experiments, P. arenarius could dominate the larval competition as the first species, but failed to show an overwhelming superiority when competing as the subsequent species. When P. arenarius was served as the first species, its larval superiority was unaffected by the time interval between ovipositions (Fig. 2B), presumably because of age differences between P. arenarius and T. lycium. In the case of interspecific intrinsic interactions between solitary parasitoids, the older larva outcompete the younger one in general (McBrien and Mackauer, 1990). Our study from dissected P. sinica nymphs showed that P. arenarius eggs began to hatch 0.77 ± 0.06 day from the oviposition and the larvae subsequently develop within 4.34 ± 0.19 days (Wu, unpublished data). The egg incubation period and the larval period of T. lycium are 1.35 ± 0.07 and 5.00 ± 0.87 days, respectively (Liu et al., 2010).

The outcome that P. arenarius spent less time on the egg incubation and the larval development than T. lycium, also explained the competitive superiority of P. arenarius in hosts where the oviposition by T. lycium was followed by that by P. arenarius when eggs of two parasitoid species were laid within 5 days (Fig. 2A). Our study was analogous to past studies that many species of Telenomus sp. with shorter egg incubation periods or larval periods were superior in interspecific intrinsic interactions (Gerling, 1972; Navasero and Oatman, 1989). However, P. arenarius failed to completely dominate the larval competition as the subsequent species, and host mortality rates increased with increased time intervals between ovipositions, especially 6-day and 7-day intervals. This indicates age differences between P. arenarius and T. lycium trigger the lethal competition for finite resources when P. arenarius was served as the subsequent species. Similarly, when the parasitization by Trissolcus nigripes follows that by Telenomus gi-fuensis, survival rates of both species decrease with increased intervals due to age differences (Mahmoud and Lim, 2008).

Interestingly, we found the inferior competitor T. lycium survived relatively better than P. arenarius when multiparasitism occurred within 24 h regardless the order of parasitizations. These data imply that the short-term venom adverse to P. arenarius may be injected by T. lycium females to arrest the development of host nymphs. Arrestment factors injected by female parasitoids during ovipositions are used to restrict their opponents through a series of physiological changes in hosts (Konopka et al., 2017). For instance, eupelmid parasitoids profit from the multiparasitism by injecting the paralyzing venom into hosts (Doury et al., 1997; Nakamatsu and Tanaka, 2003).

In terms of sex ratios, the host containing a parasitoid species is thought of as a low-quality resource, causing fewer female offspring produced by subsequent species (Godfray, 1994). And this is the reason why both T. lycium and P. arenarius significantly decreased their proportions of female eggs when they were subsequent species to parasitize hosts.

4.3. Marietta picta mediates competitive interactions between P. arenarius and T. lycium

Even if the parasitoid species under consideration is superior in the interspecific larval competition, it is still negatively affected by the multiparasitism from a biological control viewpoint (Mackauer, 1990). It is a fact that superior competitors still suffer from low-quality resources as the hosts are exploited jointly by opponents (Boivin and Brodeur, 2006), although at a minor extent, which may trigger a sequence of developmental deficiencies of the emerging superior parasitoids (Harvey et al., 2009). Thus, it is necessary for biological control programme to further strengthen the intrinsic advantages of superior species by restricting competitive abilities of opponents. Hyperparasitoids can influence the populations of both hosts and primary parasitoids (Sunderland et al., 1997; Rosenheim, 1998). When the multiparasitized nymphs were exposed to M. picta, larval P. arenarius was almost dominant in the intrinsic competition regardless the order and interval of parasitizations by the primary parasitoids. More P. arenarius and fewer T. lycium emerged from hosts, and the overall number of the primary parasitoids did not decline significantly. Besides, the female proportion of emerging P. arenarius failed to increase even with the help of M. picta, indicating the sex ratio of emerging P. arenarius depended mainly on the change in host quality, even if at a minor extent. Thus, although the exploitation of T. lycium was restrict by M. picta, P. arenarius still did not produce more female eggs when it encountered pre-parasitized hosts. From the hyperparasitoid perspective, the parasitization by M. picta was not affected by multiparasitism, M. picta could still successfully develop from T. lycium larvae under multiparasitism situations. Its parasitism rate, emergence rate and sex ratio of emerging adults were unchanged although larval T. lycium was in competition with the opponent, indicating M. picta could survive in this ecosystem and mediate the interactions between T. lycium and P. arenarius in the long term.

Our study suggests M. picta may play an important role in mediating and balancing the competitive interactions between native T. lycium and introduced P. arenarius in the field by increasing the fitness and density of the latter without destroying the overall population of the primary parasitoids. Both T. lycium and P. arenarius have excellent parasitic capabilities, but P. arenarius was a superior competitor in multiparasitism situations thanks to its shorter developmental periods. Therefore, the larval superiority of P. arenarius and the mediation by M. picta are some of the most significant factors contributing to the establishment of newly introduced P. arenarius in Ningxia.

4.4. The potential coexistence between T. lycium and P. arenarius in the biological control of P. sinica

Our previous surveys in the field revealed that there was almost no overlap between the population peaks of P. arenarius and T. lycium, which occurred in early July and late July, respectively (Wu et al., 2017). Tamarixia lycium is widespread in Ningxia as early as 1997 (Tang, 1997), whereas P. arenarius (Zhang et al., 2017) and M. picta (Ma, unpublished data) are not recorded in this region until 2016. In many cases, newly introduced species are likely to cause adverse effects on native species through competition and trophic interactions (Rodriguez, 2006; Berthon, 2015). Ideally, introduced biological control agents can coexist with native species to improve pest management (Schultheiss et al., 2001). Our laboratory research indicates that, although native T. lycium has a large population in Ningxia, as an introduced biological control agent, P. arenarius has a great chance to establish a population in the local area due to its larval superiority in the intrinsic competition and the mediation by M. picta. Besides, the interspecific host discrimination of T. lycium and no overlap between the population peaks of P. arenarius and T. lycium in the field also benefit the establishment of the newcomer P. arenarius and its coexistence with T. lycium. In fact, the host–parasitoid–hyperparasitoid interactions have the potential to establish a stable ecosystem and generate a collective coexistence in the biological control of P. sinica. However, the availability of such interactions need to be confirmed with further field investigations. The laboratory results cannot be directly extrapolated to field populations as laboratory experiments are simplified systems (Bogran et al., 2002). From a coexistence perspective, understanding competitive interactions between T. lycium and P. arenarius mediated by M. picta contributes greatly to practical applications of the parasitoids in the biological control of P. sinica.

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