Biological Control 126 (2018) 90–96

Contents lists available at ScienceDirect

Biological Control

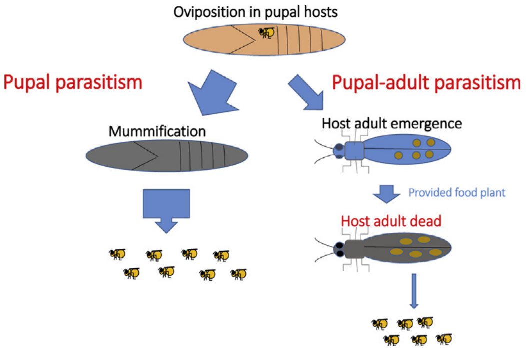
journal homepage: www.elsevier.com/locate/ybcon

Pupal-adult parasitism of the coconut hispine beetle by the koinobiont pupal parasitoid *Tetrastichus brontispae*

Keiji Takasu[a,*], Jemimah N. Ndabarua[b,1], Hieu Thi Pham[b,2], Shun-ichiro Takano[a]

[a] *Faculty of Agriculture, Kyushu University, Fukuoka 819-0395, Japan*
[b] *Graduate School of Bioresources and Bioenvironmental Sciences, Kyushu University, Fukuoka 819-0395, Japan*

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Host age selection
Host adult mortality
Parasitic wasp
Pupal-adult parasitism

ABSTRACT

Pupal parasitoids are considered to be idiobionts. However, some pupal parasitoids do not seem to kill or paralyze hosts at oviposition, including *Tetrastichus brontispae*, an endo-pupal parasitoid of *Brontispa longissima.* We conducted laboratory experiments to determine if *T. brontispae* parasitizes pupal hosts with a koinobiont developmental strategy. For this to be the case, its immatures would have to develop during the host transition from pupa to adult and in adulthood. With 0-d-old to 5-d-old pupal hosts, the *T. brontispae* females stung hosts of all ages with over 80% frequency. When the pupal hosts were parasitized, there were two modes of parasitism: pupal parasitism and pupal-adult parasitism. For pupal hosts that were parasitized, the majority showed a typical pupal parasitism, involving first the mummification of parasitized pupae and then the development of the parasitoid immatures within and emergence from these mummified pupae. However, the parasitized pupae that did not become mummified developed to host adults in which the parasitoid immatures developed, indicating pupal-adult parasitism. When parasitized pupal hosts were mummified, the 0-d-old to 3-d-old pupae were more likely than the older pupae to be mummified and had a greater number of parasitoid adults produced per host. The pupal-adult parasitism was not as successful. The parasitoid adults only successfully emerged from two host adults, which were parasitized as 2-d-old pupae, and one host adult parasitized as a 3-d-old pupa. The parasitoid eggs laid in 4-d-old and 5-d-old pupae continued to develop during and after the host transition from the pupa to adult. However, when the host adults died within 10 days after emergence, all the parasitoids died without completing their development. These results suggest that *T. brontispae* is a koinobiont parasitoid that successfully parasitizes young pupal hosts mainly through host mummification and rarely through pupal-adult parasitism.

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* Corresponding author.
E-mail address: takasu@brs.kyushu-u.ac.jp (K. Takasu).
[1] Present address: Technical and Advisory Services Department, Horticultural Crops Directorate, P.O. Box 42601-00100, Nairobi, Kenya.
[2] Present address: School of biosciences, University of Melbourne, Victoria 3010, Australia.

https://doi.org/10.1016/j.biocontrol.2018.07.013
Received 21 March 2018; Received in revised form 23 July 2018; Accepted 26 July 2018

The fact that many parasitized 4-d-old or 5-d-old pupae died at the pupal or adult stage without producing any parasitoids, may also suggest that, under natural conditions, the pupal and adult mortality of *B. longissima* resulting from parasitism by *T. brontispae* is significant.

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1. Introduction

Based on their developmental strategy, parasitoids can be dichotomously divided into idiobionts and koinobionts. Idiobiont parasitoids kill or paralyze their hosts at oviposition, while koinobionts allow their hosts to continue to move, feed, and develop (Godfray, 1994; Quicke, 1997; Mills, 2009). Endo-parasitoids that only attack the host's pupal stage are generally considered to be idiobionts (Mills, 2009). However, for parasitoids that attack and complete their development in an inactive stage of the life cycle, such as the pupa, the distinction between idiobionts and koinobionts is not always as apparent (Mills, 2009). For example, the endo-pupal parasitoids *Tetrastichus brontispae* Ferrière and *Diadromus subtilicornis* (Gravenhorst) do not appear to kill or paralyze their hosts at oviposition, and the parasitized pupal hosts continue to survive for a few days (Tran and Takasu, 2000; Nguyen et al., 2012). They may be regarded as koinobiont parasitoids.

In the case of a koinobiont pupal parasitoid, the immature parasitoid may continue to develop in hosts while the host pupae transition to adult stage, and eventually the parasitoid adult might emerge from the host adult. Unlike idiobiont pupal parasitoids that use nongrowing or paralyzing hosts, the successful koinobiont pupal parasitoid that has prolonged interaction with physiologically active developing hosts must regulate host development and overcome the host immune responses (Harvey and Vet, 1997). With increasing age, because the pupae have undergone differentiation and are developing body structures of the adult insect that would significantly reduce nutritional accessibility to parasitoids, the suitability of the pupal hosts for parasitism generally decreases (Harvey and Malcicka, 2016). Pupae and adults also have immune responses against parasitoids (Pennacchio and Strand, 2006). Parasitoid eggs that invade the host hemocoel provoke a series of immune responses mediated mainly by circulating hemocytes that form multilayer capsules around the eggs (Carton et al., 2008). Koinobionts are known to have various strategies to conform their development with that of their hosts or to regulate host development, as well as to evade or suppress the host's defensive immune responses (Vinson, 1990; Pennacchio and Strand, 2006). Pupal-adult parasitism by parasitoids that mainly attack hosts' pupal stages has not been reported, although, when it parasitizes larvae of *Coccinella septempunctata* L. (Coleopera: Coccinellidae), the adult parasitoid *Dinocampus* (=*Perilitus*) *coccinellae* (Schrank) (Hymenoptera: Braconidae) shows larval-pupal-adult parasitism (Kadono-Okuda et al., 1995).

The gregarious endo-parasitoid *Tetrastichus brontispae* Ferrière (Hymenoptera: Eulophidae) successfully parasitizes the final-instar larvae, prepupae and pupae of the coconut hispine beetle *Brontispa longissima* (Gestro) (Coleoptera: Chrysomelidae) (Chen et al., 2010; Nguyen et al., 2012*)*. When the *T. brontispae* parasitizes fourth-instar larvae or prepupae of *B. longissima,* the parasitized larvae or prepupae first successfully pupate and are then mummified. Subsequently, the parasitoid adults emerge from the mummified hosts (H.P.T., unpublished observation), suggesting that *T. brontispae* is a koinobiont since the host apparently continues developing after parasitism. Therefore, in the present study, we conducted laboratory experiments to determine if *T. brontispae* parasitizes pupal hosts with a koinobiont development strategy. If so, the parasitoid eggs laid in the host pupae may continue to develop during and after the host transition from pupa to adult. We first examined the effects of host pupal age at the time of parasitism on the immature development and survival of the host and parasitoid. When the host adults emerged from the parasitized host pupae, we continued to rear them, and examined the immature *T. brontispae* development and adult emergence. We also examined the effect of such parasitoid development on host adult mortality.

2. Materials and methods

2.1. Insects

The *Brontispa longissima* used in this study originated from larvae, pupae and adults collected by hand-picking in Dili, East Timor (08°33′S, 125°33′E to 08°34′S, 125°36′E) on 19th July 2014. The coconut hispine beetle colony was reared in plastic containers (13 × 10 × 6 cm) with a ventilated lid. The larvae and adults were maintained separately. Canary Island date palms *Phoenix canariensis* hort. ex Chabaud (Arecales: Arecaceae) were used in the experiments based on their suitability (Wu et al., 2006; Yamashita and Takasu, 2010; Takano et al., 2017). Six pieces of leaflets (5–7 cm in length) of *P. canariensis* were tightly bundled together with rubber bands on each edge as described by Yamashita and Takasu (2010). The beetle colony was reared in an incubation chamber at 25 °C with a photoperiod of 12L:12D.

The *Tetrastichus brontispae* used in this study originated from a total of ca. 20 adults that had emerged from a *B. longissima* mummy that had been collected in the same location as mentioned above in Dili, East Timor on 19 July 2014. This parasitoid colony was maintained in the laboratory using 0-d-old *B. longissima* pupae. In the present study, day 0 was defined as the day of molting from the prepupa to the pupal stage (Nguyen et al., 2012). The adult wasps were provided a drop of undiluted honey as a food source in Petri dishes (90 mm in diameter, 30 mm in height). The parasitoids were reared at 25 °C with 12L:12D for 2 days before experiments.

2.2. Parasitoid attack of hosts of different ages

Host pupae that were 0, 1, 2, 3, 4 and 5-d-old were each exposed to a single 2-d-old *T. brontispae* female without any ovipositional experience in a glass vial (13 mm in diameter, 75 mm in length). When a *T. brontispae* female encountered a *B. longissima* pupa, she either drummed the host surface for 10–70 s and then stung the host for 20–30 min, or she left the host soon after contacting it. We observed whether a *T. brontispae* female stung a host when she made first contact with the host. When a female did not contact the host within 30 min, the observation was terminated. Soon after the *T. brontispae* females left the host after a sting, the stung host was removed from the vial and reared for the following experiments. In this experiment, we did not examine whether the *T. brontispae* actually laid eggs in the hosts. A total of 100 parasitoid females were used for each host age.

2.3. Host's fate and parasitoid development

Soon after each host had been stung by a single parasitoid in the parasitoid attack experiment described above, the host was transferred to a Petri dish (60 mm in diameter, 25 mm in height) and reared in an incubator at 25 °C with 12L:12D. The hosts were checked daily for mummification (Supplementary figure S1A), parasitoid emergence or adult beetle emergence. Mummification is defined as the condition whereby larval development of the parasitoid inside the host causes the host pupae to harden and darken (Quicke, 1997; Nguyen et al., 2012). The emerging host adults from parasitized pupae were each provided with a fresh leaf bundle of *P. canariensis* every 2 days and checked for survival every day until 10 days after emergence. After the dead host adults dried completely, we dissected their abdomens to determine if they contained immature parasitoids and their stages at death such as larva, pupa or adult) (Supplementary figure S1B). When immature parasitoid movement was observed in the abdomens of dead host adults (Supplementary figure S1C), we continued to keep them in leaf bundles as mentioned above until the parasitoid adults emerged. When the parasitoid adults emerged, we recorded the date of emergence. After parasitoid emergence, we examined the number and sex of the parasitoid adults that had emerged from each host. As a control, a total of 100 unparasitized pupae were reared in the same way as described above, and their pupal period, emergence rate and survival after 10 days of emergence were examined.

2.4. Statistical analysis

The parasitoid attack rate was compared between hosts based on age with the chi-square test, followed by post hoc multiple comparisons in proportion to the sample for tests of homogeneity for all possible pairwise comparisons (Malascuilo and McSweney,1977). The proportions of mummification, adult emergence and host death and the proportions of host adults alive, host adults with dead parasitoids, and host adults producing parasitoid adults were analyzed with the chi-square test, followed by the post hoc multiple comparisons in sample proportions. The proportions of developmental stages of dead parasitoids in host adults were compared among hosts parasitized at 3, 4 and 5-d-old pupae with chi-square test. Mean number of parasitoid adults emerged per host, mean developmental time and the arcsine-transformed proportion of females in the emerged adults were compared with a one-way ANOVA, followed by the Tukey-Kramer HSD test. Host adult survival censored at 10 days after emergence was estimated using the Kaplan-Meier method. Using the log-rank test, the adult survival was then compared between the adults that had emerged from the parasitized 0, 1, 2, 3, 4 or 5-d-old pupae, and the control adults that had emerged from the unparasitized pupae. JMP12.2 (SAS Institute Inc.) was used for the statistical analysis.

3. Results

3.1. Development of unparasitized pupae

All the unparasitized *B. longissima* pupae emerged. The pupal period was 6.6 ± 0.6 (Mean ± SD) days. Subsequently, all the adults reared on the plants survived for 10 days after emergence.

3.2. Parasitoid attack of hosts of different ages

When *T. brontispae* females encountered 0 to 5-d-old pupae of *B. longissima*, they stung the pupae of all the ages tested with greater than 80% frequency ($\chi^2 = 15.5$, df = 5, P = 0.009) (Fig. 1). The attack rate of the 4-d-old pupae was significantly higher than that of the 1-d-old pupae (post hoc multiple comparisons for sample proportions for tests of homogeneity, $P < 0.05$) (Fig. 1).

3.3. Fate of parasitized pupae

After the pupal hosts were each parasitized by a *T. brontispae* female, they either underwent mummification, yielded host adults, or died (Fig. 2). The percentage of mummified pupal hosts decreased significantly with increasing host pupal age at the time of parasitism ($\chi^2 = 286.5$, df = 5, $P < 0.0001$) (Fig. 2). Although more than 85% of the 3-d-old or younger host pupae stung by *T. brontispae* were mummified, only 48% of the stung 4-day-old pupae and only 2% of 5-d-old stung pupae were mummified (Fig. 2). There were significant differences among 3-d-old or younger hosts, 4-d-old hosts, and 5-d-old hosts (post hoc multiple comparisons in sample proportion for tests of homogeneity, $P < 0.05$). Conversely, as host age at the time of parasitism increased, the percentage of host adult emergence from the parasitized host pupae increased significantly from 2% for the 0-d-old to 82% for the 5-d-old pupae ($\chi^2 = 209.8$, df = 5, $P < 0.0001$) (Fig. 2). The pupae parasitized at 4 or 5 d of age emerged significantly more frequently than those parasitized at 2 d or younger (post hoc multiple comparisons for sample proportions for tests of homogeneity, $P < 0.05$). The percentage of the dead pupal hosts was also higher in the hosts parasitized as 4-d-old and 5-d-old pupae, than those parasitized as younger hosts ($\chi^2 = 61.7$, df = 5, P = 0.0001; post hoc multiple comparisons for sample proportions for tests of homogeneity, $P < 0.05$) (Fig. 2).

3.4. Parasitoid development in mummified pupae

Most of the mummified pupae yielded parasitoid adults regardless of the pupal age attacked ($\chi^2 = 10.4$, df = 5, $P > 0.05$) (Table 1). The mean developmental time of the parasitoids from oviposition to adult emergence was 22–23 days (F = 2.91, df = 5,339, $P > 0.05$) (Table 1). The mean number of the parasitoid adults emerging from the parasitized host pupae decreased significantly with increasing host age at the time of parasitism, ranging from 8.5 from 5-d-old pupae to 13.2 from 0-d-old pupae (F = 7.27, df = 5, 339, $P < 0.001$) (Table 1). The proportion of female parasitoids among the emerged adults was not significantly different (F = 0.5, df = 3,336, $P > 0.05$) (Table 1).

3.5. Parasitoid development in adult hosts

After host adults emerged from pupae that had been parasitized by *T. brontispae*, the adults were reared on fresh plants. More than 40% of the adults emerged from pupae parasitized at 1-d-old or at older ages died within 10 days after emergence, while all the control adults that emerged from the unparasitized pupae survived 10 days after emergence (Fig. 3). There were significant differences in the survival between adults that had emerged from pupae parasitized at 2 d, 3 d, 4 d, or 5 d and the control adults that had emerged from the unparasitized pupae (2 d vs control, $\chi^2 = 99.3$, $p < 0.0001$; 3 d vs control, $\chi^2 = 91.6$, $p < 0.0001$; 4 d vs control, $\chi^2 = 85.9$, $p < 0.0001$; 5 d vs control, $\chi^2 = 57.6$, $P < 0.0001$) (Fig. 3).

The host adults that died after emergence either yielded adult parasitoids (Supplementary Figure S1D), contained dead parasitoids in their abdomens (Supplementary Figure S1B), or had no symptoms of parasitism (Fig. 4). The overall percentage of dead host adults containing dead parasitoids was 59% (45/76), and the percentage significantly varied from 0% for adult beetles parasitized as 1-d-old pupae to 81% of those parasitized as 4-d-old pupae ($\chi^2 = 18.1$, df = 4, P = 0.01) (Fig. 4). The parasitoids were found dead at larval, pupal and adult stages in abdomens of the dead host adults (Table 2). There was no significant difference in the proportion of larval, pupal and adult

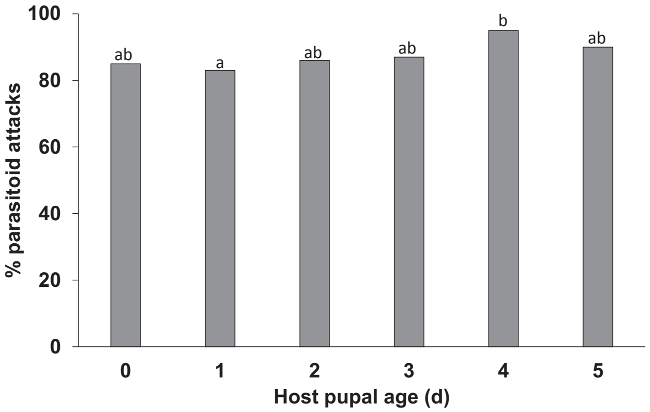

Fig. 1. Attack of *B. longissima* pupae of different ages by *T. Brontispae.* Bars with the same letter were not significantly different by post hoc multiple comparisons in sample proportion for tests of homogeneity (P = 0.05).

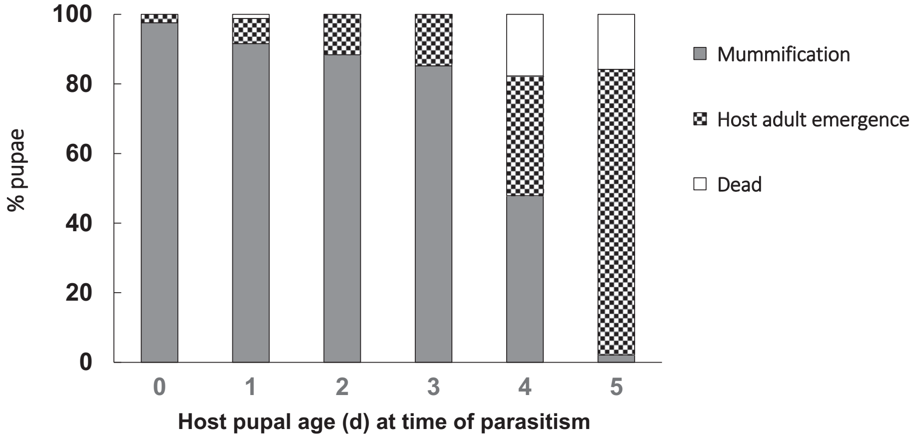

Fig. 2. Fate of *B. longissima* pupae parasitized by *T. brontispae.*

Table 1
Development of *T. brontispae* in mummified *B. longissima* pupae.

Host pupal age (day) at time of parasitization[1]	No. of hosts mummified[1]	% mummies yielding parasitoid adults[1]	Development time Mean ± SE[1]	No. parasitoid adults emerged mean ± SE[1]	% females of emerged adults[2]
0	82	98.8a	22.1 ± 0.01a	13.2 ± 0.03a	88.2a
1	76	98.7a	22.2 ± 0.01a	12.8 ± 0.03ab	85.3a
2	76	97.3a	22.1 ± 0.01a	12.9 ± 0.02ab	86.6a
3	75	96.0a	22.3 ± 0.01a	12.0 ± 0.03bc	88.1a
4	46	89.1a	22.4 ± 0.01a	11.1 ± 0.06c	87.5a
5	2	100a	23.0 ± 0a	8.5 ± 0.35bc	88.2a

[1] Percentages and means followed by the same letters in the same columns were not significantly different by chi-square test and the Tukey-Kramer HSD test ($P = 0.05$), respectively.

[2] The arcsine-transformed percentages of females in emerged adults were not significantly different by the Tukey-Krammer HSD test ($P = 0.05$).

stages among the host adults emerged from pupae parasitized at 3, 4 and 5 d ($\chi^2 = 5.8$, df = 4, $P > 0.05$) (Table 2). Only two host adults emerged from pupae parasitized at 2 d, and one adult from pupae parasitized at 3 d yielded *T. brontispae* adults. In the cases when the host adults successfully yielded parasitoid adults, the host adults, which had emerged from the pupae parasitized at 2 d and 3 d, died on the day of emergence or one day later, respectively. That is, they died within 6–7 days after parasitism. The parasitoid adults emerged from the abdomens of the host adults that had died 17–18 days earlier (Supplementary Figure S1D). In the cases when the parasitoids adults successfully emerged from the host adults, the developmental time from oviposition to adult emergence was 23 to 25 days, 1–3 days longer than those which had emerged from the mummified pupae (Table 3). The number of parasitoid adults that had emerged from each host adult was between 5 and 7, a smaller number than those that had emerged from the mummified pupae (Table 3). Statistical comparisons of the development time and the number of parasitoid adults that had emerged per host could not be conducted because of the small sample sizes (n = 2 for 2-d, n = 1 for 3-d).

4. Discussion

We showed that *T. brontispae* can parasitize 0 to 5-d-old pupae of *B. longissima.* Our results, showing that most of the pupae parasitized at 3-d-old or younger became mummified and later yielded parasitoid adults, are consistent with previous studies (Chen et al., 2010; Nguyen

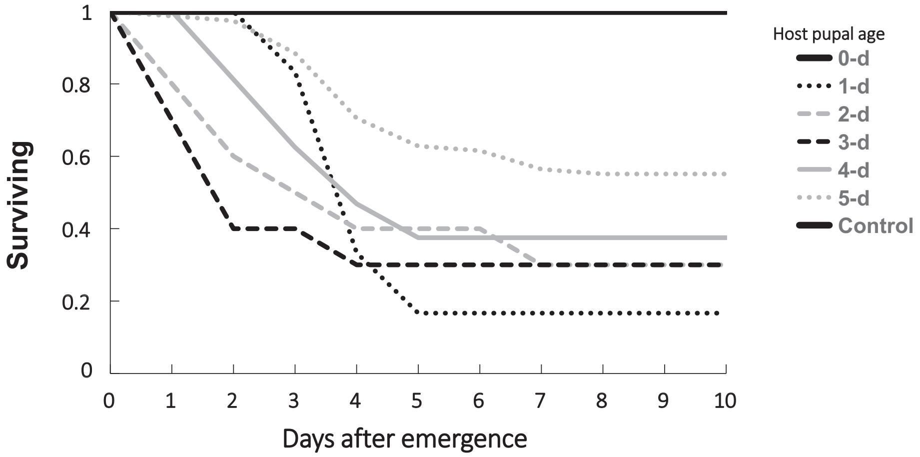

Fig. 3. Survival of adults emerged from unparasitized pupae or from pupae parasitized at 0-d,1-d,2-d,3d-,4-d, or 5-d. The data was censored at 10 days after emergence.

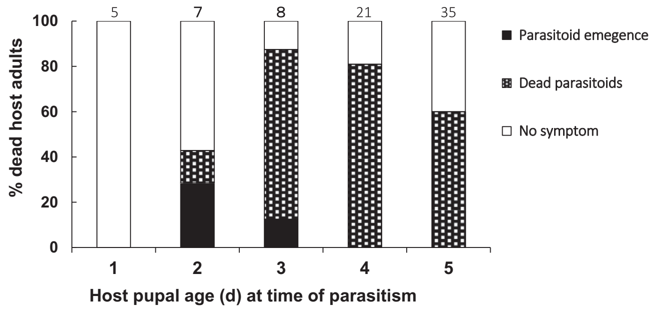

Fig. 4. Development of *T. brontispae* in the abdomen of *B. longissima* adults that had emerged from the parasitized pupae and died. The number at the top of each bar indicates the number of adults examined.

Table 2
Unsuccessful development of *T. brontispae* in *B. longissima* adults.

Host pupal age (day) at oviposition	No. hosts containing dead parasitoids	% hosts containing dead parasitoids at stage of		
		Larva	Pupa	Adult
2	1	0	0	100
3	6	33.3	66.7	0
4	17	52.9	41.1	5.9
5	21	76.2	23.8	0

There was no significant difference in the proportion of parasitoid stages among 3-d, 4-d and 5-d-old pupae at the time of oviposition by chi-square test (P = 0.05). For the analysis, the data for 2-d-old pupa was not included because there was only one sample.

Table 3
Successful development of *T. brontispae* in *B. longissima* adults.

Host pupal age (day) at oviposition	No. of host adults yielded parasitoid adults	Development time Mean ± SE	No. parasitoid adults emerged mean ± SE	% females of emerged adults
2	2	23.5 ± 0.35	5.5 ± 1.1	72.7
3	1	25.0	6.0	100

et al., 2012). In addition, we found that after the parasitized pupal hosts emerged as adult beetles, the parasitoids inside the pupal hosts continued to develop during the transition from the host's pupal to adult stage and did so in the abdomen of the host adults. Most of the parasitoids in the host adult abdomens were dead after the host adults died. Parasitoid adults successfully emerged from only three host adults. This is the first study to report pupal-adult parasitism by an endo-parasitoid that mainly attacks pupal hosts, although larval-pupal-adult or pupal-adult parasitism by the parasitoid *D. coccinellae* has been observed, which mainly attacks adult hosts but occasionally also larval or pupal hosts (Obrycki et al., 1985; Kadono-Okuda et al., 1995).

When a *T. brontispae* female contacted a pupal host, she stung the host more than 80% of the time regardless of host age. The results are consistent with Nguyen et al. (2012), which showed that *T. brontispae* attacks host pupae of all ages. Based on external host examination by their antennae and internal host examination by their ovipositor, parasitoid females decide to oviposit in hosts, and, using internal examination after drilling, they sometimes reject unsuitable hosts (Godfray, 1994). In the present study, we did not know whether the parasitoid attacks resulted in actual oviposition unless the stung hosts produced parasitoid adults or contained dead parasitoids. In fact, some of the host pupae stung by *T. brontispae* developed to adults, and the adults survived 10 days without any symptom of parasitism. The percentage of stung host adults alive for 10 days increased with increasing host age from 2 to 6% for 0 d to 3 d to 13% for 4 d to 45% for 5 d. The results may suggest that, after internal host examination by its ovipositor, *T. brontispae* more often reject older hosts.

Pupal age is known to strongly affect host suitability (Tran and Takasu, 2000; Ueno, 2014). The young pupal stage is generally considered to be more suitable for parasitism by idiobiont species, because, as the development of the pupa proceeds, the host tissue is converted into the morphological structures of the adult. The immature parasitoids can more easily feed from the relatively undifferentiated young pupae (Chabora and Pimentel, 1977; Ueno, 2014). In the present study, when the parasitized hosts were mummified, the suitability of the host pupae was highest at 0 d and decreased with increasing host age. The mummification rate and the number of parasitoid adults produced per host were highest at 0 d and decreased with host age. The results are consistent with previous studies (Chen et al., 2010; Nguyen et al., 2012). The fact that the pupal period of *B. longissima* is approximately 7 days, but that its pupae parasitized at 3-d-old or older became mummified in 4–6 days, may suggest that *T. brontispae* regulates the development of the *B. longissima* pupae for host mummification. Further study is needed to determine the physiological mechanism of host regulation and mummification of the host pupae by this parasitoid.

Although pupal-adult parasitism by *T. brontispae* was observed, the host adults were not as suitable for parasitism as the host pupae. Successful parasitoid emergence from host adults was only observed in host adults that emerged from pupae parasitized at 2 d (n = 2) and 3 d (n = 1). All the parasitoids that initiated parasitizing in 4-d-old and 5-d-old pupae died in the dead host adults. The dead parasitoids were found in the abdominal cavities of the *B. longissima* adults. The host adults were apparently less suitable for parasitism than the host pupae, because in the host adults only the abdomen is available for the immature parasitoids. The number of the parasitoid adults that successfully emerged from the host adults was 5–7 and smaller than those from the mummified pupae (8–13 wasps emerged from each host pupa). We also speculate that the mummified *B. longissima* pupal casing may protect the immature parasitoids from desiccation for a longer period after the host's death due to parasitism than the thinner exoskeleton of the adult. In addition, the successful emergence of *T. brontispae* from host adults that had been parasitized as 2-d-old and 3-d-old pupae may be because, when the host adults died a few days after emergence, the parasitoids in them would have likely developed to the final instar larvae that had completed feeding. As such, they were better positioned to develop to adulthood in the relatively dry conditions of the dead adult host. The parasitoids that were initiated in 4-d-old or 5-d-old pupa may have been younger larvae when the host adults died and were perhaps incapable of survival without the host hemolymph. Further, the immune response of *B. longissima* may cause the death of the immature parasitoids in host adults. A major immune response of the hosts provoked by the endoparasitoid eggs is the melanotic encapsulation by the immunocytes and plasma proteins (Vinson, 1990; Pennacchio and Strand, 2006). Liu et al (2008) showed that parasitism by *T. brontispae* causes changes in the immunoreaction of the *B. longissima* pupae. In *Octodonta nipae* (Coleoptera: Chrysomelidae), a relative species of *B. longissima*, its immune response is suspected to lead to the lower successful parasitism rate of older pupae by *T. brontispae*. This is because, as the pupal age advances, new hemocytes are synthesized and there is a higher resistance ability (Meng et al., 2016). In the present study, we could not determine if the death of the immature parasitoids in host adults was caused by encapsulation.

Optimal foraging theory predicts that whether parasitoid females oviposit in a host or not depends on the host's profitability and availability (Godfray, 1994). Previous studies of host age selection by pupal parasitoids showed a positive correlation between acceptance rate and host suitability (Chabora and Pimentel, 1977; Tran and Takasu, 2000; Stacconi et al., 2015; Mehmood et al., 2018). In the present study, however, there was imprecise correspondence between host acceptance and host suitability. Although *T. brontispae* stung all the pupal ages of hosts with more than 80% frequency, the host suitability significantly decreased with increasing host age. The high attack rate of suboptimal hosts such as 4- or 5-d-old pupae in the present study may be explained by the following reasons. First, as we explained earlier, *T. brontispae* might more often reject the older pupae after ovipositor insertion. Second, experimental conditions in the present study may have affected the parasitoid attack rate. We provided hosts to a 2-d-old female that did not have any ovipositional experience in the nonchoice condition. It has been shown that a suboptimal host is used either if a parasitoid female is inexperienced with any host or, in the absence of optimal hosts, when only suboptimal hosts are available (Godfray, 1994; Mackauer, et al., 1996; Acebes and Messing, 2013). Chen et al. (2010) showed that *T. brontispae* preferred to parasitize 0-d-old pupae over other ages in a choice test where 4th and 5th instar larvae and 0-d-old to 5-d-old pupae were presented to parasitoids together.

The pupal parasitoids that mummify hosts after parasitism have been considered idiobionts (Mills, 2009). Although *T. brontispae* also mummified most young pupal hosts after parasitism, its females did not seem to kill or paralyze hosts at oviposition, and its eggs and larvae were capable of surviving and developing in the developing pupal and adult hosts. Many of the pupal hosts parasitized at 4 and 5 d of age continued to develop to adults, while the immature parasitoids were partially developing. When the parasitized hosts became mummified, mummification did not occur in the 4–6 days after parasitism. In parasitized 2-d-old and 3-d-old pupae, where most were mummified, some were able to develop to host adults and thereafter produced parasitoid adults. Since it takes 2–3 days for the *T. brontispae* eggs to hatch (Chen et al., 2010), the feeding and development of the parasitoid larvae may have caused host death and mummification. Like other koinobiont endoparasitoids, *T. brontispae* also has physiological mechanisms to escape encapsulation in hosts (Meng et al., 2016), which allows the eggs and larvae to survive and develop in physiologically active host pupae and adults. Therefore, *T. brontispae* is considered as a koinobiont pupal parasitoid that uses relatively young host pupae mainly through host mummification and rarely through pupal-adult parasitism. We do not know the adaptive significance of pupal-adult parasitism by *T. brontispae* because only a few cases of pupal-adult parasitism were successful, and, even when successful, only a small number of wasps emerged from the adults.

Although the unsuccessful parasitism of hosts could possibly result in host death, this aspect has often been ignored or underestimated as a component of parasitoid effectiveness in biological control (Desneux et al., 2009; Abram et al., 2016). In the present study, more than 10% of 4-d-old and 5-d-old pupae died without producing any parasitoids. More than 50% of the host adults that emerged from pupae that had been parasitized at 4 or 5 d died within 10 days and prior to reproduction. Additionally, all the control adults that emerged from the unparasitized pupae were alive in 10 days. The average longevity of the unparasitized female beetles is 143 days and 100% of the emerged females have been reported to lay eggs in laboratory conditions (Takano et al., 2012, 2013). All ages of pupae and emerging adults of *B. longissima* are normally found in brown, dry withered leaves (Supplementary figure S2), and this parasitoid preferentially searches for hosts in these dry, brown, and folded leaf parts (Takasu, unpublished). Therefore, it is quite possible that, under natural conditions, *T. brontispae* encounters and parasitizes 4-d or 5-d-old pupae as well as younger pupae. Parasitism at the pupal stage of *B. longissima* by *T. brontispae,* even if not obviously successful as evidenced by adult host emergence, can significantly reduce *B. longissima* populations by causing the mortality of both the pupae and teneral adults. These parasitoid attributes may be partly responsible for the previously reported successful biological control of *B. longissima* (Voegele, 1989; Chang, 1991; Nakamura et al. 2006).

5. CRediT authorship contribution statement

Keiji Takasu: Conceptualization, Writing-Reviewing and Editing, Supervision. **Jemimah N. Ndabarua**: Investigation, Writing-Original draft preparation. **Hieu Thi Pham**: Methodology, Investigation. **Shunichiro Takano**: Validation, Investigation.

Acknowledgements

We thank Olson, D. and Ruberson, J. R. for reading the draft and their valuable comments. This research was partly supported by JSPS KAKENHI [grant Numbers JP 2125008 and JP26850031] and Science and Technology Research Partnership for Sustainable Development (SATREPS), Japan Science and Technology Agency (JST) / Japan International Cooperation Agency (JICA).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.biocontrol.2018.07.013.

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