Unexpected Use of Pupal Stage of a Lepidopteran Host by the Ectoparasitoid Wasp Agrothereutes lanceolatus (Hymenoptera: Ichneumonidae)

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Abstract

In general, the majority of ectoparasitoids of Lepidoptera parasitize larval and/or prepupal stages of the host and do not use the pupal stage, suggesting the presence of ecological and physiological constraints in the evolution of host range. Here, the evidence is given that Agrothereutes lanceolatus, a solitary ectoparasitoid wasp, can exceptionally parasitize host pupae, succeeding to extend its host range to pupal hosts that have a hardened cuticle. Laboratory experiments confirmed that A. lanceolatus developed successfully to adult both on host prepupae and on young pupae in equal proportions. Development time and size of the resulting wasps did not differ between the host types. However, the percentages of hosts producing wasp offspring differed among age classes within the pupal stage. The offspring survival was high except the oldest class of host pupae. With increasing host pupal ages, the offspring size decreased whereas the development time increased. Older host pupae hence were less suitable for the development of offspring. The offspring mortality mostly occurred during the 1st larval stadium without signs of growth, suggesting a difficulty in feeding on host pupae from an external position. Thus, A. lanceolatus can use a wider range of hosts than expected from its ectoparasitic development. The fact may enable A. lanceolatus to be a common and widespread polyphagous species among ectoparasitic Cryptinae.

Keywords: Offspring fitness; Pupal parasitoid; Homona magnanima; Host quality

Introduction

An ovipositing female parasitoid in the field encounters hosts of different stages and ages, and select to oviposit on some of them because a variety of stage and age of a given host species are likely to be present simultaneously in time and space. Parasitoids in general prefer some host stages or ages to the others [1-3] though host preference depends on host availability and parasitoids’ physiological state, i.e., egg load, life expectancy, etc. [4-6].

Host stage and age usually affect parasitoid offspring survival, development rate, and sex ratio [4,7-11]. Fitness (size, fecundity, etc.) of the resulting offspring is also determined by the host stage and age that are parasitized [9-13]. Host stage and age are thus important determinants of ‘host suitability’ for many parasitoids. Investigations into the relationships between host-stage or -age and host suitability have been undertaken for a wide range of parasitoids [7,8,13,14].

The host range of ectoparasitoids of lepidopterans is mostly restricted to host prepupae and larvae in protected situations such as those in the cocoon, plant stem and leaf roll [15-17]. One major reason for this may be that lepidopteran pupae are covered with a hardened cuticle, which makes it difficult for ectoparasitic larvae to feed on the tissue within from an external position [18]. In contrast, many endoparasitoids parasitize lepidopteran pupae and prepupae [15,17]. Curiously, ectoparasitoids attacking Hymenoptera and Coleoptera can use the pupal stage of hosts, which have a soft cuticle. Also, ectoparasitoids such as Nasonia spp. parasitize coarctate dipteran pupae; however, they lay eggs on a soft pupa inside a hardened larval skin and the parasitoid larvae consume the soft host. These facts suggest the presence of ecological and physiological constraints in the evolution of host range [19,20].

The present study focuses on the ectoparasitoid wasp Agrothereutes lanceolatus Walker (Hymenoptera: Ichneumonidae: Cryptinae), which can be an exceptional example, extending its host range through inclusion of the host pupal stage. Agrothereutes lanceolatus is a common ectoparasitic wasp that is distributed throughout East Asia including Japan [19]. Members of the Cryptinae, including A. lanceolatus, principally use Lepidoptera hosts [16,19]. Agrothereutes lanceolatus is widely seen in a variety of environments and attacks several tortricids and pyralids, including important pests such as Chilo suppressalis Walker, Glyphodes pyloi Walker, and Homona magnanima Diakonoff [19-23].

Host records evidently indicate that A. lanceolatus is polyphagous whereas most Cryptinae are rather monophagous or oligophagous [20]. Thus, A. lanceolatus may have life history traits that enable it being polyphagous [22,23]. Furthermore, careful examination of specimens preserved with the host mummy suggests that it may also parasitize host pupae (Ueno, personal observations). If this holds true, A. lanceolatus is an exceptional ectoparasitic species and is an excellent test parasitoid to examine host range evolution in ecto- versus endoparasitoids. However, it is also likely that the parasitoid develops as a hyperparasitoid of endoparasitoids inside lepidopteran pupae. No previous studies have given evidence that ectoparasitoids can use the pupal stage of lepidopterans. Here I hypothesize that A. lanceolatus can

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parasitize the pupal stage of hosts, which makes it to use a wide range of host species.

Accordingly, in the present study, offspring development and survival of *A. lanceolatus* on host pupae versus pupae were examined to test the hypothesis. The impact of host pupal age on the offspring survival, body size, and development was also examined. Based on the results, life history and host selection behavior of *A. lanceolatus* is discussed.

Materials and Methods

General biology of *A. lanceolatus*

Although many aspects of the biology of *A. lanceolatus* are poorly understood, current information on its biology is summarized as follows. *Agrothereutes lanceolatus* is multivoltine, appearing from early in spring to late autumn, after which overwinterers as a prepupa inside the cocoon. Adult female longevity is about one month at a constant 20°C in the laboratory. Females are synovigenic and produce yolk-rich, anhydropic eggs continuously during the lifetime [22,23]. In common with many other synovigenic species, destructive host feeding is essential for egg production [23]. *A. lanceolatus* is an idioibiont, and the female paralyzes the host permanently with venom upon oviposition. It can also be a facultative hyperparasitoid [22]. *A. lanceolatus* is easily mass-reared on a factitious host, *Galleria mellonella* (Linnaeus) [22].

Parasitoid and host preparation

A laboratory colony of *A. lanceolatus* was established using adult parasitoids collected from the campus of Nagoya University, Nagoya City, Japan. Female parasitoids were placed individually in plastic containers (10 cm in diameter, 4.5 cm in height), together with tissue paper saturated with honey solution. The tissue paper was replaced twice a week thereafter. The containers were kept at 20 ± 1°C, 60-70% RH with a 16L: 8D photoperiod. The colony was maintained on prepupae of a laboratory host, *Galleria mellonella*. Host cocoons containing prepupae were presented to female *A. lanceolatus* in the plastic containers. Parasitized hosts were removed and held at 25 ± 1°C with a 16L: 8D photoperiod, until parasitoid emergence. Newly emerging females were placed individually in the plastic containers, were paired with a male and were maintained as described above.

Host stage effect: Host prepupa versus pupa

This experiment was aimed to examine the effect of host developmental stage on the performance of the offspring *A. lanceolatus*. Offspring survival rate, size, and developmental time were used as measures for performance. Host cocoons of 16-18 mm in length were selected to use. Host cocoons containing prepupae and pupae (2–4 days old after pupation) were used as test hosts.

Each test female wasp was allowed access to both prepupal and pupal hosts. One host prepupa and one pupa were presented for each female for two hours every two days, allowing the female wasp to experience with both types of hosts. This treatment was repeated 3–4 times before the experiment was initiated and, as a result, female wasps six to eight days old were used for testing.

A pair of host cocoons (one prepupa and one pupa) was presented to each test female wasps in the containers. After a single oviposition had been confirmed, hosts were removed from the containers to avoid them being superparasitized. Oviposition was confirmed by observing the base of the wasp ovipositor where it was possible to see an egg passing through [24,25]. In all, 38 females were used and thus 76 hosts were parasitized in this experiment.

Parasitized hosts were individually placed in plastic containers and were kept at 20 ± 1°C under a 16L: 8D photoperiod, until adult parasitoid emergence. The containers were checked daily for parasitoid emergence. The sex of emerging parasitoids was recorded, and, subsequently, the forewing length was measured under a stereoscopic microscope. Forewing length was used as an index of offspring size. Hosts that had not produced parasitoid offspring one month post oviposition were dissected under a binocular microscope to examine the stage at which parasitoids had died.

Effect of host pupal age

The effects of host pupal age on parasitoid development were examined. Host pupae were classified into four age classes according to their age: Young pupal class (1–3 days old since pupation); eye pigmentation class (4–5 days old); wing formation class (6–8 days old); and pharate class (9–10 days old). Although the cocoon morphology (size, color, etc) did not change with age, the internal physiology of host pupae would dramatically be different among the age classes.

Female wasps used for testing were provided with different aged host pupae enclosed in the cocoon of different ages before experimentation. During the test, each female was provided with 2–4 hosts of different ages. The number of hosts presented was not same among test females because some females did not respond to hosts after attacking 2–3 hosts. After a single oviposition was confirmed, hosts were removed from the container. When wasp offspring emerged, the day of emergence and the forewing length of the wasps were recorded. The day from oviposition to emergence was calculated as a measure of development time. The other procedures were the same with the above experiment.

Data were analyzed with the aid of JMP [26]. The percentages of successful parasitism, i.e., production of the offspring, were analyzed with chi-squared tests. Two-way ANOVAs were used to analyze the effect of host age and wasp sex on offspring size and developmental time. Factors affecting developmental rate were analyzed by an ANCOVA.

Results

Host stage effect: Host prepupa versus pupa

A total of 61 adult *A. lanceolatus* emerged from 76 hosts parasitized. Success of parasitism, i.e., emergence of wasp offspring, was high in both host prepupae and pupae. The percentages of successful parasitism were 78.9% and 81.6%, respectively, and no significant difference was found between the host stage groups (Chi-squared test; N=76, χ²=0.08, P=0.77). Development time in days from egg (=oviposition) to wasp emergence was not affected by the host stages (Tables 1 and 2), though it was about 1 day longer in female than male offspring (Table 1). The mean size of emerging wasp offspring did not differ between host types or between wasp sexes (Table 2) (two-way ANOVA; F=0.71, P=0.41 for host stage; F=0.77, P=0.39 for wasp sex). Offspring sex ratio (% females) also did not differ between the host types (54.6% versus 46.4%; chi squared test, N=61, χ²=0.40, P=0.53).

Effect of host pupal age

In all, 173 hosts were parasitized. Survival from egg to wasp emergence was strongly affected by host pupal age. The percentages of hosts producing wasp offspring differed significantly between the host
The interaction term was not included in the analysis because interaction between the two factors was not significant.

**Table 1:** Two-way ANOVAs for estimating effects of host prepupae versus pupae on development time of *A. lanceolatus*.

| Factors          | df | F-value | P-value |
|------------------|----|---------|---------|
| Host stage       | 1  | 1.07    | 0.30    |
| Wasp sex         | 1  | 45.17   | <0.0001 |

Host stage had no significant effect on parasitoid offspring size and developmental time (ANOVA, *P*=0.05, see the Results for details).

**Table 2:** Mean offspring size (forewing length in mm) and development time (days) of *A. lanceolatus* on host prepupae versus young pupae.

| Factors          | df | F-value | P-value |
|------------------|----|---------|---------|
| Host stage       | 1  | 1.07    | 0.307   |
| Wasp sex         | 1  | 1.07    | 0.307   |

**Table 3:** Two-way ANOVA for estimating the effect of host pupal age and wasp sex on developmental time of *A. lanceolatus*.

| Factors          | df | F-value | P-value |
|------------------|----|---------|---------|
| Host age         | 1  | 89.8    | <0.0001 |
| Wasp sex         | 1  | 17.7    | <0.0001 |

**Figure 1:** Offspring survival of *A. lanceolatus* in relation to host age classes within the pupal stadium. The survival differed significantly among the groups (see the Results for statistical details). Host age class: 1 = fresh pupa; 2 = pupa with eye pigmentation; 3 = pupa with wing formation; and 4 = pharate adults.

**Figure 2:** Developmental time in days from egg to adult wasp emergence in *A. lanceolatus* in relation to host age classes within the pupal stadium (above: male, below: female). Developmental time differed significantly among the groups (see Table 3 for statistical details). Data were shown as mean ± SE.
the size of emerging wasp offspring does not differ between the host stages (Table 2). For *A. lanceolatus*, host quality is equal between host prepupae and pupae. It is not known how ectoparasitic larvae of *A. lanceolatus* gain and maintain access to the host resources and, also, how they mobilize resources present within the host pupae. It is valuable to examine feeding behavior of *A. lanceolatus* larvae in the future study.

**Host pupal age and parasitoid performance**

For a number of pupal endoparasitoid wasps of Lepidoptera, the age at which the host pupa has been parasitized influences offspring survival and performance [14,27-29]. Likewise, the present study has provided evidence that host pupal age affects the offspring survival and performance of ectoparasitic *A. lanceolatus*. On younger host pupae, *A. lanceolatus* offspring survives better, grows larger and faster (Figures 1-3). These results suggest that host suitability is higher in younger host pupae. The majority of available literatures show similar results [14,29-32]; a decrease of host suitability with pupal age appears to be the general rule.

Factors df F-value P-value
Host age 3 11.3 <0.0001
Wasp sex 1 3.75 0.057

The interaction term was not included in the analysis because interaction between the two factors was not significant.

**Table 4:** Two-way ANOVA for estimating the effect of host pupal age and wasp sex on offspring size of *A. lanceolatus*.

| Factors          | df | F-value | P-value |
|------------------|----|---------|---------|
| Host age         | 3  | 11.3    | <0.0001 |
| Wasp sex         | 1  | 3.75    | 0.057   |

Data were shown as mean ± SE.

**Table 5:** ANCOVA for estimating the effect of host pupal age, wasp sex and size on offspring developmental rate per size.

**Figure 3:** Offspring size (forewing length) of *A. lanceolatus* when host age classes within the pupal stadium varied (above: male, below: female). Offspring size differed significantly among the groups (see Table 4 for statistical details).

**Figure 4:** Relationships between developmental rate per size and offspring size in *A. lanceolatus*. Regressions obtained were significant (P<0.05).

**Figure 5:** Offspring survival at the early stage of development, i.e., 1st stadium in relation to host pupal age (A) and comparison of developmental stages at which parasitoid offspring died (B). For simplicity, data from youngest (dotted line) and oldest (undotted line) classes of hosts are shown to illustrate survival curves (B).
regardless of the age of host pupae parasitized. The presence of toxins or toxin-like substances is not known substances in G. mellonella pupa and adult moth, suggesting an accumulation of toxins in older pupae cannot be the reason.

The nutritional inadequacy in old host pupae is a potential reason. During the pupal stage of a host, larval tissue is reconstructed dramatically to form adult tissue as the pupa ages. Newly constructed adult tissue is sclerotized in particular around the pupal body surface. Developing parasitoid larvae may have a difficulty to digest such sclerotized tissues. Further, sclerotized adult tissues around pupal surface may prevent the developing larvae from feeding on pupal contents from an external position of the host pupa. Indirect evidence that may support this idea is that many developing A. lanceolatus die during the earliest larval stage (Figure 5). Mortality often takes place before the offspring starts to grow. It is likely that the first stadium of A. lanceolatus has difficulty to feed on host pupa and starves to death due to a failure to access the tissues within the host.

The present study also shows that offspring size and development time differ among host age classes within the pupal stage. The difference may result from changes in the nutritional composition of the host. Alternatively, host age effects may be caused by changes in the amounts of nutrient available to the developing parasitoid. Host resources converted to adult sclerotized tissues should not be usable for the developing parasitoid. Consequently, the amounts of edible host resources may be reduced with increasing pupal age, leading to an increase in development time and a decrease in offspring size. For a number of pupal parasitoids, adults that emerge from older host pupae are smaller than those from younger ones [29,31].

An intriguing result in the present study is that larger parasitoids show higher developmental rate per size (=offspring size/developmental day) (Figure 4). This means that larger offspring develops faster than smaller one. Intuitively, smaller parasitoids should consume smaller quantities of host resource and, hence, could pupate earlier [4,37]. However, this is not the case for A. lanceolatus. Slower developmental rate in less suitable, old hosts (Figure 4) is reasonable, but higher developmental rate for larger parasitoid offspring is difficult to explain. The development strategies of parasitoids have been received less attention than reproductive strategies, such as host selection and sex allocation [11,37]. The positive relationship between development and size in A. lanceolatus poses a question regarding the developmental strategies in parasitoids.

The ability of parasitoids to cope with hosts of variable quality would have the overall effect determining host range. The present study gives evidence that A. lanceolatus has a potential to use a relatively wide range of host pupal age classes in the laboratory situation. This suggests that host range of A. lanceolatus is wider than expected based on its ectoparasitic nature. It is unclear what life history traits enable the ectoparasitic A. lanceolatus to use the host pupal stage, and this remains to be investigated. Again, the feeding strategy of developing larvae should be addressed to answer the question in future research.

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