Distinct parasitoid communities associated with host races of the leaf-mining moth *Acrocercops transecta* on distantly related host plants (Juglandaceae and Ericaceae)

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Differences in host-plant species may affect the structure of parasitoid communities of phytophagous insects. Phytophagous insect species often consist of host races, and comparisons between host races enable us to infer the effects of host-plant differences on parasitoid communities. Here, we compare the parasitoid fauna of two host races of the leaf-mining moth *Acrocercops transecta*, which are associated with either Juglandaceae or *Lyonia ovalifolia* (Ericaceae). Field collection and subsequent laboratory rearing revealed a significantly higher parasitization ratio in the Juglandaceae race than in the *Lyonia* race. In the Juglandaceae race the majority of parasitoids were Braconidae, whereas in the *Lyonia* race the majority were Eulophidae. Furthermore, even within the same host race the most abundant parasitoid species differed between populations with different host associations. Hence, the present results strongly indicate that different host-plant taxa affect the structure of parasitoid communities even within a single phytophagous insect species.

**Keywords:** Braconidae; differential parasitism; Eulophidae; host–parasite interaction; Pteromalidae

Introduction

The structure of the parasitoid community of phytophagous insects is influenced by the host plants that the latter consume (Price et al. 1980; Gross and Price 1988; Rott and Godfray 2000; Sugiura 2011; Leppänen et al. 2013). Several studies have demonstrated clear differences in parasitism rates and parasitoid faunas between herbivore communities on different host-plant species (Le Corff et al. 2000; Barbosa et al. 2001; Sugiura 2011). Lill et al. (2002) has further shown that, even within the same species, caterpillars on different host plants experience differential parasitism rates. These results suggest the possibility of top–down effects on host-plant use in phytophagous insects and that shifts to a novel host plant may provide opportunity in the form of enemy-free spaces.

Phytophagous insect species often consist of sub-populations that are specialized to different plant species, e.g. host races. Host races are ecologically (e.g. host preference, larval performance, or phenology) and genetically differentiated but are distributed without geographic isolation (Berlocher and Feder 2002; Dres and Mallet 2002; Matsubayashi et al. 2010). Hence, host races are suitable systems for
elucidating the consequences of host shifts in phytophagous insects on the structure of their parasitoid community. However, few studies have assessed differences in parasitoid faunas between host races, and previous studies have only compared parasitoid communities between host races associated with closely related host plants (e.g. within the same genus or family) (Forbes et al. 2009; Dickey and Medina 2011).

The leaf-mining moth *Acrocercops transecta* (Gracillariidae) is associated with distantly related host plants, Juglandaceae and Ericaceae, in northeast Asia (Kumata et al. 1988). This species consists of two host races that use either several Juglandaceae plants or *Lyonia ovalifolia* (Ericaceae) as a larval food, respectively (Ohshima 2008). Although both host races make mines of similar form on the host plants (Figure 1), the two host races differ clearly in the host preferences of ovipositing females and larval resistance to secondary chemical compounds of host plants (Ohshima 2008, 2012). A mitochondrial DNA-based phylogeny has revealed that the *Lyonia* race evolved once from the Juglandaceae race, and hence that a host shift occurred from Juglandaceae plants to *Lyonia* in *A. transecta* (Ohshima 2008; Ohshima and Yoshizawa 2010). At many sites, the Juglandaceae and *Lyonia* races are sympatric in the sense that the two populations are within the range of the normal dispersal ability of the adult moth. Hence, the comparison of parasitoid communities between the Juglandaceae race and the *Lyonia* race would provide insights into the effect of host shifts on the parasitoid structure of phytophagous insects.

In this study, we compare the structure and composition of parasitoid communities between the Juglandaceae-associated race and the *Lyonia*-associated race of *A. transecta*, using sympatric and allopatic populations of the two host races. We also compare the parasitoids of the Juglandaceae race on two different juglandaceous plants, *Juglans mandshurica* and *Platycarya strobilacea*. We describe the modes of parasitism of respective parasitoid wasps and discuss the possible factors that may be responsible for differences in the parasitoid communities on *A. transecta* on these different host plants.

**Material and methods**

We collected larvae of *A. transecta* from three sympatric (both Juglandaceae and *Lyonia* host races present) and two allopatic (only one host race present) localities in

![Figure 1. Leaf mines of *Acrocercops transecta*. (A) Three mines of the Juglandaceae race on a leaflet of *Juglans mandshurica*; (B) a mine of the *Lyonia* race on *Lyonia ovalifolia*.](image)
Japan (Figure 2). The three sympatric localities were Sendai (38°15' N, 140°49' E; Miyagi Prefecture, northern Honshu), Kaida (35°94' N, 137°60' E; Kiso, Nagano Prefecture, central Honshu) and Niimi (34°59' N, 133°25' E; Okayama Prefecture, western Honshu). *J. mandshurica* (Juglandaceae) and *L. ovalifolia* are both found at Sendai and Kaida, and *P. strobilacea* (Juglandaceae) is found at Niimi in addition to the former two host plants (Table 1). The two allopatric localities were Yamadera (38°31' N, 140°44' E; Yamagata Prefecture, northern Honshu) and Kirishima (31°51' N, 130°46' E; Kagoshima Prefecture, Kyushu) (Figure 2), where either only the Juglandaceae race or the *Lyonia* race is distributed, respectively (Table 1). Collections were conducted from July to August in 2009 and 2012 at Sendai, in August in 2008 at Kaida, from May to July in 2008 and 2012 at Niimi, from July to August in 2008 at Yamadera and from May to July in 2008 and 2009 at Kirishima.

Figure 2. Map of Japan showing the sampling localities of *Acrocercops transecta* mines.
Mined leaves containing live larvae of *A. transecta* were collected from the host plants in the field. Moth larvae were reared in the laboratory from the leaves in which they were collected following the method described by Ohshima (2005) and examined for the emergence of moths or wasps.

**Results**

**Association with host plants of host insects**

Total parasitism rates were significantly higher in the Juglandaceae race (28.1%, *n* = 1292) than in the *Lyonia* race (16.1%, *n* = 931; Fisher’s exact test, *p* = 2.69e−11). Although parasitoid wasp communities in both host races consisted of Braconidae, Eulophidae, and Pteromalidae, rates of parasitism by the three families differed significantly between the two moth host races (Fisher’s exact test, *p* = 2.2e−16, Table 2): in the Juglandaceae race > 90% of emerged parasitoid wasps were Braconidae, whereas in the *Lyonia* race < 70% were Braconidae (Table 2). Nearly 80% of the Braconidae that emerged from the *Lyonia* race were collected at a single locality (Kirishima, see “Geographic variation” below); consequently at other localities the majority of parasitoids of the *Lyonia* race were Eulophidae (Table 2). These patterns did not differ between sympatric and allopatric populations of the two host races.

In the Juglandaceae race, there were no significant differences in the parasitism rate between moths collected from *J. mandshurica* (30.2%, *n* = 792) and from *P. strobilacea* (25.4%, *n* = 500; Fisher’s exact test, *p* = 0.0662). However, the most abundant parasitoid wasps differed between the two host plants. Almost all emerged parasitoids from the Juglandaceae race on *J. mandshurica* were *Aneurobracon philippinensis* (Braconidae: Agathidinae, Figure 3), whereas the most abundant parasitoids from the Juglandaceae race fed on *P. strobilacea* were *Choeras* sp. (Braconidae: Microgastrinae) (Table 2). Five and three morphospecies of Eulophidae and of Pteromalidae, respectively, emerged from the Juglandaceae race.

In the *Lyonia* race, the parasitism rate by Braconidae was 1.9% except for the Kirishima population. Only six *An. philippinensis* and 15 *Choeras* sp., which were the dominant parasitoids in the Juglandaceae race, emerged from the *Lyonia* race (Table 2). In contrast, parasitism by Eulophidae was significantly higher in the *Lyonia* race (5.5%) than in the Juglandaceae race (1.5%; Fisher’s exact test, *p* = 1.40e−06). In total, 10 morphospecies of Eulophidae emerged, of which two were shared with the Juglandaceae race (Table 2, Supplemental Material).
Table 2. Associations between parasitoids and host plants of *Acrocercops transecta*; numbers of emerged adult wasps and of moth larvae collected are shown for each host-plant species.

| Parasitoid wasp | Braconidae | Eulophidae | Pteromalidae | Total |
|-----------------|------------|------------|--------------|-------|
| *Aeneurobracon philippinensis* | 226 | 36 | 262 | 6 | 268 |
| Choeras sp. | 3 | 61 | 64 | 15 | 79 |
| Pholetesor sp. | 1 | 0 | 1 | 78 | 79 |
| Total | 230 | 97 | 327 | 99 | 426 |
| Lyonia ovalifolia | 2 | 11 | 13 | 17 | 30 |
| Total | 8 | 14 | 22 | 51 | 70 |
| *Eulophidae* | | | | | |
| sp. 1 | 2 | 11 | 13 | 17 | 30 |
| sp. 2 | 0 | 0 | 0 | 1 | 1 |
| sp. 3 | 0 | 0 | 0 | 9 | 9 |
| sp. 4 | 2 | 0 | 2 | 12 | 14 |
| sp. 5 | 0 | 0 | 0 | 1 | 1 |
| sp. 6 | 0 | 0 | 0 | 1 | 1 |
| sp. 7 | 0 | 0 | 0 | 4 | 4 |
| sp. 8 | 0 | 0 | 0 | 1 | 1 |
| sp. 9 | 0 | 0 | 0 | 3 | 3 |
| sp. 10 | 0 | 0 | 0 | 2 | 2 |
| sp. 11 | 2 | 2 | 4 | 0 | 4 |
| sp. 12 | 0 | 1 | 1 | 0 | 1 |
| sp. 13 | 2 | 0 | 2 | 0 | 2 |
| Total | 8 | 14 | 22 | 51 | 70 |
| *Pteromalidae* | | | | | |
| sp. 1 | 1 | 0 | 1 | 0 | 1 |
| sp. 2 | 0 | 15 | 15 | 0 | 15 |
| sp. 3 | 0 | 1 | 1 | 0 | 1 |
| Total | 1 | 16 | 17 | 0 | 17 |
| Total | 239 | 127 | 366 | 150 | 516 |
| Number of moth larvae collected | 792 | 500 | 1292 | 931 | 2223 |
Geographic variation

Aneurobracon philippinensis was collected from all the sampling localities (Table 3). However, the other two Braconidae species, Choeras sp. and Pholetesor sp., were more restricted in distribution (Table 3). Choeras sp. was obtained only from Niimi. Pholetesor sp. was collected from two localities; one individual from Sendai and 78 from Kirishima (Table 3). Of the 13 Eulophidae species seven species were only collected from Niimi, but this bias is partially a result of the large sampling size of mined A. transecta larvae from that locality.

Modes of parasitism

All Braconidae parasitoids obtained in the present study were koinobionts and solitary endoparasitoids of A. transecta larvae. In An. philippinensis, female wasps laid their eggs in the first or second instars of A. transecta, which make linear mines on the upper surface of leaves (Figure 4A). Fully grown final instars of A. transecta leave the mines and start making cocoons for pupation (Figure 4B). After A. transecta larvae finish making their cocoons, parasitoid larvae rapidly finish their larval development, feeding on prepupae of A. transecta inside the cocoons (Figure 4C). Hence, An. philippinensis larvae do not make their cocoons by themselves but instead pupate in the cocoons made by A. transecta larvae (Figure 4D).

Choeras sp. shows parasitism patterns similar to An. philippinensis; like the latter, their larvae finish their larval development rapidly after A. transecta finish making cocoons. Larvae of Choeras sp. pupate inside A. transecta cocoons (Figure 4E). We were able to observe oviposition in the laboratory by Choeras sp. females in second stadium larvae of A. transecta, indicating that Choeras sp. also attacks early instars of A. transecta.

Larvae of Pholetesor sp. make their cocoons by themselves. When larvae of A. transecta reach the fifth stadium, Pholetesor sp. larvae open a hole in the host’s lateral abdominal segment (Figure 4F) and exit the host body. Larvae of Pholetesor sp. make white cocoons inside or outside the mines made by their host (Figure 4G). We induced
Table 3. Geographic variation in the parasitoid fauna of *Acrocercops transecta*; numbers of emerged adult wasps and of moth larvae collected are shown for each collecting locality.

| Sampling locality | Yamadera | Sendai | Kaida | Niimi | Kirishima | Total |
|-------------------|----------|--------|-------|-------|-----------|-------|
| Parasitoid wasp    |          |        |       |       |           |       |
| Braconidae         |          |        |       |       |           |       |
| *Aneurobracon philippinensis* | 14 | 41 | 31 | 178 | 4 | 268 |
| *Choeras* sp.      | 0        | 0      | 0     | 79   | 0          | 79   |
| *Pholetesor* sp.   | 0        | 1      | 0     | 0    | 78         | 79   |
| Total              | 14       | 42     | 31    | 257  | 82         | 426  |
| Eulophidae         |          |        |       |       |           |       |
| sp. 1              | 0        | 4      | 2     | 23   | 1          | 30   |
| sp. 2              | 0        | 0      | 0     | 0    | 1          | 1    |
| sp. 3              | 0        | 0      | 0     | 8    | 1          | 9    |
| sp. 4              | 0        | 0      | 0     | 10   | 4          | 14   |
| sp. 5              | 0        | 0      | 0     | 0    | 1          | 1    |
| sp. 6              | 0        | 0      | 0     | 1    | 0          | 1    |
| sp. 7              | 0        | 0      | 0     | 4    | 0          | 4    |
| sp. 8              | 0        | 0      | 0     | 1    | 0          | 1    |
| sp. 9              | 0        | 0      | 0     | 3    | 0          | 3    |
| sp. 10             | 0        | 2      | 0     | 0    | 0          | 2    |
| sp. 11             | 0        | 0      | 0     | 4    | 0          | 4    |
| sp. 12             | 0        | 0      | 0     | 1    | 0          | 1    |
| sp. 13             | 0        | 0      | 0     | 2    | 0          | 2    |
| Total              | 0        | 6      | 2     | 57   | 8          | 70   |
| Pteromalidae       |          |        |       |       |           |       |
| sp. 1              | 0        | 1      | 0     | 0    | 0          | 1    |
| sp. 2              | 0        | 0      | 0     | 15   | 0          | 15   |
| sp. 3              | 0        | 0      | 0     | 1    | 0          | 1    |
| Total              | 0        | 1      | 0     | 16   | 0          | 17   |
| Total              | 14       | 49     | 33    | 330  | 90         | 516  |
| Number of moth larvae collected | 22 | 541 | 112 | 1246 | 302 | 2223 |

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females of *Pholetesor* sp. to oviposit by supplying them with leaves containing second instar mines, indicating that *Pholetesor* sp. also attacks early instars of *A. transecta*.

All Eulophidae and Pteromalidae larvae killed their host larvae between the host’s first and third stadia (*Figure 4H*), and field-collected host larvae that had already been parasitized did not finish development in the laboratory. Hence, Eulophidae and Pteromalidae wasps are thought to be idiobionts that oviposit in the eggs or early instars of *A. transecta* (e.g. first to third instars). Fully grown parasitoid larvae exit the body of the host larvae and pupate inside the mines made by their host (*Figure 4I*).

**Discussion**

Parasitoid communities that emerged from *A. transecta* varied between the two host races irrespective of their distributional regimens (i.e. sympatry or allopatry),
indicating that differences in host plants affect the structure of parasitoid communities. These results are consistent with previous studies (Price et al. 1980; Le Corff et al. 2000; Rott and Godfray 2000; Barbosa et al. 2001; Lill et al. 2002; Sugiura 2011; Leppänen et al. 2013), and the present results further demonstrate that such host-plant-dependent parasitoid community differences appear even within the same host species (i.e. between host races). Thus, the present results strengthen the importance of host-plant differences in determining the structure of parasitoid communities of phytophagous insects.

**Factors differentiating parasitoid communities between host races**

Several host-plant-associated factors have been proposed as mechanisms responsible for differences in parasitoid communities: differences in host-plant morphological traits (Sugiura 2011), differences in female parasitoid oviposition preferences for different host-plant volatiles (Elzen et al. 1983; De Moraes et al. 1998; Stelinski and Liburd 2005; Forbes et al. 2009), and/or nutritional differences between host plants resulting in different parasitoid wasp growth rates (Greenblatt and Barbosa 1981; Mueller 1983). However, responsible mechanisms are likely to vary among systems (Lill et al. 2002).

Sugiura (2011) reported different parasitism rates by *Acrysocharoides* sp. (Eulophidae) on two different leaf-roller moths, *Caloptilia azaleella* and *Caloptilia leucothoes* (Gracillariidae), associated with *Rhododendron macrosepalum* and *R. reticulatum* (Ericaceae), respectively. He found that the significantly reduced parasitism on *C. azaleella* was due to glandular hairs on *R. macrosepalum*, which trap and kill many tiny insects including *A*. sp. (Sugiura and Yamazaki 2006). In the *A. transecta* system, *J. mandshurica* has glandular hairs and sticky leaves compared with *Platycarya strobilacea* and *L. ovalifolia*. The present results show significantly lower parasitism rates by Eulophidae and Pteromalidae on the Juglandaceae race on *J. mandshurica* (1.1%) compared with on *P. strobilacea* (6%) and on the *Lyonia* race (5.5%; Fisher’s exact test, compared with *P. strobilacea p* = 1.03e–06; compared with *Lyonia p* = 4.53e–16). Hence, the glandular hairs and sticky leaves of *J. mandshurica* have the potential to prevent Eulophidae and Pteromalidae, which are tiny wasps, from oviposition, and this possibility should be tested by behavioural observations in future studies.

The two host-plant families of *A. transecta* (Juglandaceae and Ericaceae) belong to different plant orders (Fagales and Ericales, respectively) which are not phylogenetically closely related (APG III 2009). This suggests that there are substantial differences in the plant volatiles attracting ovipositing females and in the secondary chemical compounds affecting larval growth. Many parasitoid wasps use host-plant-related odours (Turlings et al. 1990; Whitman and Eller 1990; Dicke and Minkenberg 1991; De Moraes et al. 1998) as infochemicals (Dicke and Sabelis 1988). Forbes et al. (2009) reported that parasitoid wasps from different host races of the maggot fly *Rhagoletis pomonella* showed different preferences for host-plant volatiles: parasitoid wasps could discriminate the odour of host plants on which their host fly larvae fed from the odours of other host plants. Hence, given the differences in plant volatiles between juglandaceous plants and *L. ovalifolia*, host-plant discrimination by ovipositing females is a likely mechanism leading to different parasitoid communities on the two host races of *A. transecta*. 
Another possible mechanism differentiating the structure of parasitoid communities is the nutrimental quality of each host plant. Greenblatt and Barbosa (1981) showed that pupal weight of parasitoid wasps was correlated with the weight of the pupae of their host (the gypsy moth *Lymantria dispar*), which in turn varied among host plants. In addition, Mueller (1983) showed that the survivorship of parasitoid wasps differed significantly among nine host plants of *Heliothis* species and that the wasps laid more eggs in host larvae on the host plant that had the highest parasitoid survivorship. Although there are no body size differences in adult moths, and consequently no size differences in larvae and pupae, between Juglandaceae and *Lyonia* races (Kumata et al. 1988), *L. ovalifolia* has a unique and toxic secondary chemical compound, lyonioxin (Ohta and Hikino 1981). This implies that parasitoid wasps feeding on larvae of the *Lyonia* race encounter this *Lyonia*-specific toxin and would need to overcome this toxin to develop in larvae of the *Lyonia* race. If this lyonioxin-associated performance reduction exists for the parasitoid wasps, natural selection should favour the evolution of biased oviposition preferences for the Juglandaceae race. Hence, laboratory experiments on oviposition preferences and larval performances of parasitoid wasps will provide insights into the mechanisms underlying differential parasitism rates between Juglandaceae and *Lyonia* races.

The present results demonstrate distinct parasitoid community structures not only between Juglandaceae and *Lyonia* races but also within the Juglandaceae race. The fact that very few *An. philippinensis* emerged from the Juglandaceae race on *P. strobilacea* suggests the presence of interspecific competition among parasitoid wasps because all the parasitoid wasps obtained in the present study are solitary endoparasitoids. Previous studies on leaf-miner parasitoids have revealed the presence of competitively dominant parasitoid species and have proposed that asymmetric interspecific competition is an important factor shaping the structure of parasitoid communities on each host plant (Kato 1994; Sugiura 2011). However, we know little about the extent to which parasitoids compete and about the detailed mechanisms of competition among parasitoid wasps (Harvey et al. 2013). Fortunately, *A. transecta* has a multivoltine life cycle with a short generation time (ca. 3 weeks per generation) and is easy to rear successively in the laboratory (Ohshima 2005). We have also established methods for laboratory rearing of *An. philippinensis* and *Choeras* sp. (Kawamura et al. unpublished data). Hence, the future competition experiments using the *A. transecta* parasitoid system will contribute to uncovering the mechanisms of interspecies competition as well as the factors differentiating the parasitoid communities between *J. mandshurica* and *P. strobilacea*.

**Modes of parasitism**

*Aneurobracon philippinensis* and *Choeras* sp. show a unique mode of parasitism in that fully grown parasitoid larvae take over the cocoons made by their host gracillariid larvae. As the two genera belong to different subfamilies (Agathidinae and Microgastrinae), this highly specialized behaviour has probably evolved independently, as a consequence of adaptation to gracillariid larvae. Although the genus *Aneurobracon* and its allied genera in the subtribe Mesocoelina are specialists on gracillariid moths (Achterberg 1990), host ranges of the genus *Choeras* spread across several microlepidopteran families (e.g. Tineidae, Plutellidae, Psychidae, Tortricidae, Pterophoridae; Achterberg 2002). Hence inferring that the species phylogeny of
*Choeras* will contribute to the elucidation of the evolutionary pathways of the unique mode of parasitism in *Choeras*. sp.

Behavioural observations in the laboratory and field revealed that all parasitoid wasps collected in the present study lay their eggs in early instars of *A. transecta*. Fourth and fifth instars of *A. transecta* make deeper tent-like mines than do first to third instars, and third instars make blotch-like mines; these complex mine structures probably hinder oviposition by parasitoid wasps. In contrast, oviposition in first and second instars, which make linear mines, is probably easier for parasitoid females.

**Consequences of differential parasitism on the formation and maintenance of the two host races in *A. transecta***

Although the Juglandaceae and *Lyonia* races of *A. transecta* show distinct female preferences and larval performances, mating between the two host races is successful regardless of the directions of crossing in the laboratory (Ohshima 2008). Behavioural experiments of mating-site selection have shown that males of the *Lyonia* race and both sexes of the *Juglans* race show no preference for either plant, although *Lyonia*-race females significantly prefer *Lyonia* (Ohshima 2010). This weak correlation between habitat and mating-site selection, coupled with high mating compatibility in the laboratory, indicates that there are no pre-mating isolation barriers between the two host races. Experiments on the viability of *F*₁, *F*₂ and backcross generations also revealed that there are no genomic incompatibilities between the two host races (Ohshima 2008, 2012).

However, the *F*₁ hybrid larvae cannot survive when they feed on *Lyonia* as a host plant. But on *Juglans*, hybrids as well as the Juglandaceae race grow to adulthood (Ohshima 2008). These results indicate that in the wild, gene flow from the *Lyonia* race to the Juglandaceae race could occur, while introgression in the opposite direction should be completely hindered. Hence, the abundance and survival rate of the *F*₁ hybrid larvae on juglandaceous plants are of fundamental importance for the maintenance of the two host races. The present results demonstrate a significantly higher parasitism rate on juglandaceous plants than on *L. ovalifolia*, which implies that this higher parasitoid pressure on one suitable host for the *F*₁ hybrid larvae could contribute to the isolation between the two host races. In contrast, lower parasitoid pressure on the derived host plant, *L. ovalifolia*, probably facilitated the host shift from juglandaceous plants as enemy-free space, even though *L. ovalifolia* contains toxic secondary chemical compounds. The key requirement for enemy-driven top-down host divergence is that enemies are specialists on herbivore niches rather than on herbivore lineages (Leppänen et al. 2013), and this is consistent in the present case. Therefore, the differences in parasitoid pressure among host-plant species may have the potential to strengthen the isolating barriers between populations with different host associations, and specialized parasitoid preference for herbivores on specific plants could influence the direction of host-plant shifts in herbivores.

**Conclusion**

The structure of parasitoid communities clearly differs between host races of *A. transecta*, and the lower parasitism rate, especially by Braconidae, in the derived *Lyonia* race suggests that a shifting to a novel host plant provides an enemy-free
space. However, mechanisms differentiating the structure of parasitoid communities are still unclear and both host-plant-dependent and -independent effects should be tested by laboratory experiments. The present results were based only on successfully emerged parasitoid wasps. Hence, developing species-specific primers or sequencing a DNA barcoding region (Hebert, Cywinska, et al. 2003; Hebert, Ratnasingham, et al. 2003) for each parasitoid wasp, coupled with molecular analysis of parasitoid linkages (MAPL), which is a recently established approach by Rougerie et al. (2011) and Wirta et al. (2014), will provide more accurate determination of parasitoid communities, and these methods could contribute to the identification of taxonomically difficult parasitoid taxa. Also, more extensive sampling through the occurrence season of A. transecta mines and using samples from additional localities will shed light on the seasonal dynamics and geographic variation of the structure of parasitoid communities.

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Supplemental material
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