



# Parasitoid assemblages of two invading black locust leaf miners, *Phyllonorycter robiniella* and *Parectopa robiniella* in Hungary

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## Abstract

**Background and Purpose:** Two leaf miners, *Parectopa robiniella* and *Phyllonorycter robiniella* (Lepidoptera, Gracillariidae), native to North America, were established in Europe. These two invaders provide an excellent opportunity to study the insertion of new species into an existing host-parasitoid community. The following hypotheses were tested: (i) parasitoids attacking the invaders have a wide rather than a narrow host range; (ii) the invading leaf-miner species on black locust are attacked by fewer species of parasitoids than endemic species; (iii) the parasitoid communities attacking invading species are most similar to those attacking endemic leaf-miners with similar ecology; (iv) how the parasitoid communities affect the population dynamics of invaders; (v) what is the difference between the *Ph. robiniella* and *Pa. robiniella* parasitoid communities.

**Materials and Methods:** Samples were taken at two sites in pure black locust stands: Gödöllő (Pest county) and Visonta (Heves county) and in the western part of Hungary: Csorna, Koronóc, Lövő (Győr-Moson-Sopron County). From each sampling site twenty 60 cm long branches were randomly cut and the first top 15 leaves were checked on each branch: the number of leaflets per leaves and the number of mines per each leaflet were counted. 300 mines of each leaf-miner species were chosen randomly from 10 trees in different canopy levels and were carried to the laboratory for further individual rearing.

**Results and Conclusions:** All the parasitoid species reared from these two leaf-miners are generalists – common and abundant species on different lepidopteran leaf-miners associated with oaks and other woody plants. In both, *Ph. robiniella* and *Pa. robiniella*, the same dominant species of parasitoids were reared. In *Ph. robiniella* the parasitoid species richness was slightly higher than in *Pa. robiniella*. The two invading leaf-miners, *Ph. robiniella* and *Pa. robiniella*, recruited a parasitoid community of nearly the same size as native *Phyllonorycter* species on oaks and this process of shifting onto new hosts was quite rapidly, during 10–20 years. The parasitoid communities of *Parectopa* are simpler than in *Phyllonorycter*, which is probably due to different mine structure and ecology of the two invading hosts.

## INTRODUCTION

Biological invasions are global phenomena, threatening both biodiversity and productivity of cultivated crops including forests. The

number of invasive species and the rate of invasions resulting from anthropogenic activities are permanently increasing (1).

The black locust (*Robinia pseudoacacia* L.) is an important industrial tree species in Hungary. It was introduced to Hungary in the early 18<sup>th</sup> century. Currently black locust forests occupy about 423,000 ha in Hungary, about 23.4% of all forested area. Significant black locust plantations can also be found in Italy, Romania, Moldavia, Southern Ukraine and recently in the Netherlands.

A number of new insect pests of black locust were accidentally introduced to Europe recently and during the last 2–3 decades they became established and widespread throughout most of the continent, particularly in Hungary (2, 3, 4) where black locust is more common and abundant than anywhere else in Europe. Among them two leaf miners, *Parectopa robiniella* (Clemens, 1863) and *Phyllonorycter robiniella* (Clemens, 1859) (Lepidoptera, Gracillariidae), native to North America, were established on the continent.

*Pa. robiniella* was first recorded near Milano, Italy in 1970 (5) and in 1983 it was found in south-western Hungary (6, 7). Currently it is distributed in Italy, Slovenia, Croatia, Austria, Slovakia, Hungary, Romania, Ukraine, Poland and Lithuania (2, 8, 9, 10). It is a bivoltine species, with one larva per mine, pupates in the litter; its development and growth in mines starts at least 2–3 weeks later than in *Ph. robiniella* (2, 3, 7).

*Ph. robiniella* was first recorded in Switzerland, near Basel in 1983 (11) and in 1996 it was found in western and south-western Hungary (12). The species rapidly spread all over Hungary where its host is more abundant than anywhere else in Europe (12, 13, 14). It is currently distributed in Switzerland, Italy, Austria, Slovakia, Hungary, Slovenia, Croatia, Bosnia and Herzegovina, Romania, Ukraine and Lithuania (2, 8, 9, 10, 11, 15). It has three generations per year, 1 to 5–8 larvae per mine; pupation takes place in the mine (2, 3, 8, 16, 17, 18, 19).

Parasitoids have larger impact on community interactions during animal invasions than was previously acknowledged (1, 20). Invaders can often escape from their parasitoids in the process of their invasion. This leads to higher demographic success of invaders, which might give them a competitive advantage over natives (21). However, parasitoids of native counterparts influence the population dynamics and success of invaders, and new host-parasitoid associations can easily be established. Therefore, investigation of the likelihood of such transmission between natives and invaders is crucial to our understanding of invasion success. Such invasions provide natural experiments that can be used to test hypotheses about how parasitoid communities are structured (22). The colonization of novel herbivores by native parasitoids is also of applied interest, because they can play an important role in the population density regulation of a newly introduced pest. An invading phytophagous insect is likely to have less parasitoid species because it generally lacks native specialist parasitoids and

more generalist species may not search the new ecological niche occupied by the invader (23, 24). Studies on parasitoids of the stated two invading black locust leaf miners (particularly *Ph. robiniella*) in Europe were undertaken by several researchers (15, 17, 18, 25, 26, 27, 28); however, they were mainly limited to establishing the species of parasitoids that attack the invaders, and how effective they are in population density regulation. Parasitoid assemblages of *Parectopa* were studied much less than that of *Phyllonorycter*. So, our knowledge of these communities is still very superficial.

We described here the parasitoid community attacking the two invader leaf-miner species in Hungary based on the data collected in 2001–2003, less than 10 years after the introduction of *Ph. robiniella* and ca. 20 years since *Pa. robiniella* was found for the first time in Hungary. These two invaders provide an excellent opportunity to study the insertion of a new species into an existing host-parasitoid community, because leaf miners support rich parasitoid communities (29) and parasitoids of *Phyllonorycter* species, especially on woody plants, have been intensively studied (30, 31, 32, 33, 34, 35). We tested the following hypotheses: (i) parasitoids attacking the invaders have a wide rather than a narrow host range; (ii) the invading leaf-miner species on black locust are attacked by fewer species of parasitoids than endemic species; (iii) the parasitoid communities attacking invading species are most similar to those attacking endemic leaf-miners with similar ecology; (iv) how the parasitoid communities affect the population dynamics of invaders; (v) what is the difference between the *Ph. robiniella* and *Pa. robiniella* parasitoid communities.

## MATERIAL AND METHODS

In the 2001–2002 period, we performed investigation at two sampling sites in pure black locust stands: Gödöllő (Pest county) and Visonta (Heves county) in Hungary. These sampling sites were surrounded by other black locust stands. In 2003, three sampling sites with pure *R. pseudoacacia* stands were chosen in the western part of Hungary: Csorna, Koroncó, Lövő (Győr-Moson-Sopron County).

At all sampling sites, both *Ph. robiniella* and *Pa. robiniella* were present but with different abundance at different places. Both species were abundant enough for sampling at Gödöllő, Visonta (in 2001–2002) and at Koroncó (in 2003). *Parectopa* was dominant and *Phyllonorycter* was rare at Csorna. Contrary to this, *Phyllonorycter* was dominant and *Parectopa* very rare at Lövő. These 3 sampling sites were located in the neighborhood of oak forest where, due to parasitoid transmission from the native, leaf-miner hosts were expected. Samples were taken biweekly.

From each sampling site, twenty 60 cm long branches were randomly cut and the first top 15 leaves were examined on each branch: the number of leaflets per leaves and the number of mines per each leaflet were counted. For *Ph. robiniella* the number of larvae per mine was also taken into account.

300 mines of each leaf-miner species were chosen randomly from 10 trees in different canopy levels and were carried to laboratory for further individual rearing. In the laboratory, under controlled 18–20 °C, and 70–80% relative humidity, each collected leaf with mine(s) was put into a separate vial. Check on parasitoid and moth emergence was done once per week. The collected mines were kept in laboratory for four weeks and then dissected, and mortality factors and parasitization level were determined.

Diversity comparisons were carried out using the Veg-an package (36) of the R Software (37).

## RESULTS

The ranges of parasitization for the two hosts and different sites are given in Table 1.

### *Phyllonorycter robiniella* parasitoids

From all sampling sites, 19 parasitoid species were reared: *Pholetesor nanus* Reinhard (Braconidae), *Holcothorax testaceipes* Ratzeburg (Encyrtidae), *Necremnus hungaricus* Erdős, *Pnigalio pectinicornis* L., *P. soemius* Walker, *Sympiesis acalle* Walker, *S. gordius* Walker, *S. sericeicornis* Nees, *Cirrospilus lyncus* Walker, *C. talitzkii* Bouček, *C. viticola* Rondani, *Pediobius saulius* Walker, *Closterocerus trifasciatus* Westwood, *Chrysocharis pentheus* Walker, *Neochrysocharis formosa* Westwood, *Achyssocharoides cilla* Walker, *Minotetrastichus frontalis* (Nees), *Baryscapus nigroviolaceus* (Nees) (Eulophidae), *Eupelmus urozonus* Dalman (Eupelmidae).

Parasitization rate in Koroncó showed a considerable variation during the sampling season (Figure 1), with a decreasing trend. The maximum rate (41.9%) was detected in the sample taken on 3<sup>rd</sup> July.

Similar variation in parasitization rate during the season could be observed in Lövő, with slightly more definite decreasing trend.

At both sampling sites, *A. cilla* was the dominant parasitoid species, *H. testaceipes* and *M. frontalis* were present in a smaller number, while *P. nanus*, *C. lyncus*, *B. nigroviolaceus* and some others were relatively rare (Figures 3, 4). *P. saulius* was observed mainly as a secondary parasitoid in *P. nanus* and *A. cilla*.

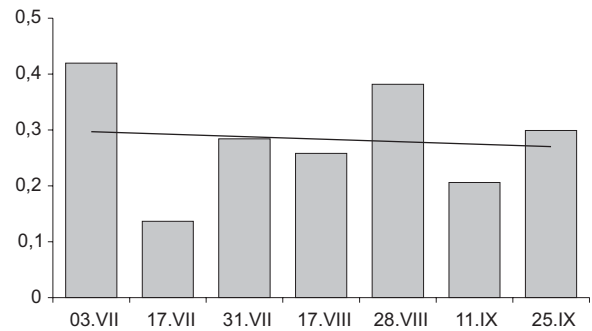


Figure 1. *Phyllonorycter robiniella* parasitization rate during the season (Koroncó, 2003).

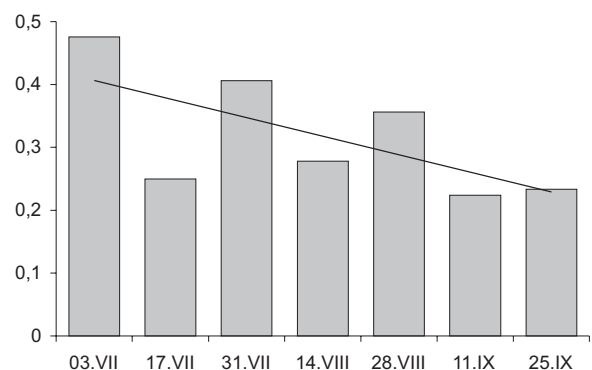


Figure 2. *Phyllonorycter robiniella* parasitization rate during the season (Lövő, 2003).

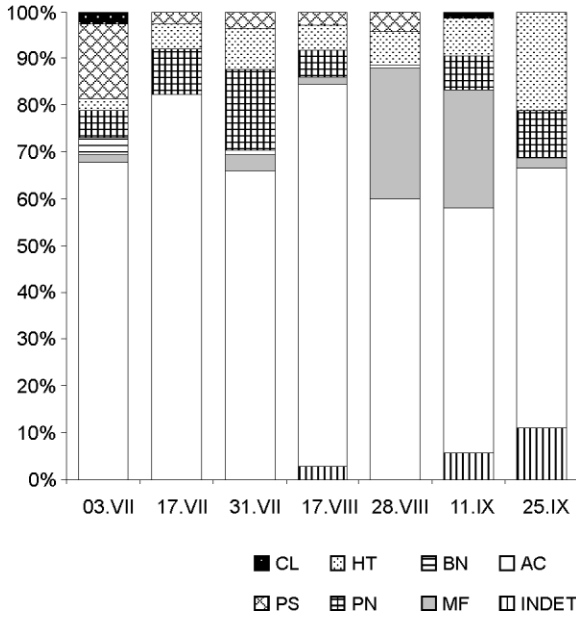
At both sampling sites, Koroncó and Lövő, several main parasitoid species in the *Ph. robiniella* parasitoid complexes were more abundant than all other species (Figure 5). In sampling sites where *Ph. robiniella* was present in large numbers, species diversity was found to be very similar (Figure 6).

In two other sampling sites, Gödöllő and Visonta, the dominant parasitoid species were *P. nanus*, *P. saulius* and *S. sericeicornis* (Table 2). The role of *P. nanus* in parasitoid communities at both sites was more significant than that of all other parasitoid species together (8.9 and 12.6% in Gödöllő and Visonta, respectively). During season, the parasitization rate of *Ph. robiniella* at both sites also

TABLE 1

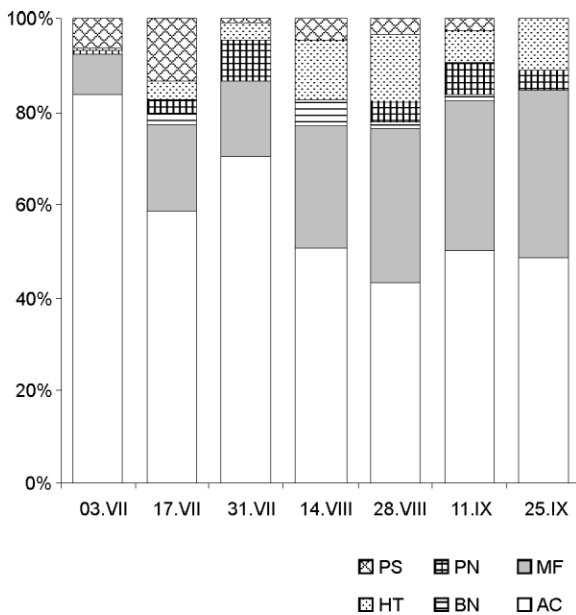
Ranges of parasitization rate (%) for the 2 hosts and 5 sites (N= not sampled at all, L= not sampled due to low density).

Host/Year	<i>Phyllonorycter robiniella</i>			<i>Parectopa robiniella</i>		
	2001	2002	2003	2001	2002	2003
Csorna	N	N	L	N	N	0.6–15.3
Gödöllő	0.3–14.0	1.7–20.5	N	0.3	0–0.6	N
Koroncó	N	N	13.6–41.9	N	N	0.7–8.0
Lövő	N	N	22.4–47.6	N	N	L
Visonta	0.7–16.5	0.7–34.3	N	0–1.0	0	N

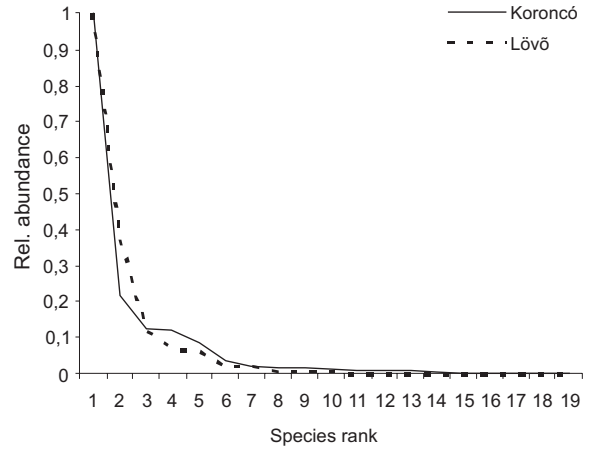


**Figure 3.** Parasitoid species diversity of *Phyllonorycter robinella* (%) (Koronc6, 2003) (AC=Achrysocharoides cilla, BN=Baryscapus nigroviolaceus, PS=Pediobius saulius, HT=Holcothorax testaceipes, PN=Pholetesor nanus, MF=Minotetrastichus frontalis, CL=Cirrospilus lynceus).

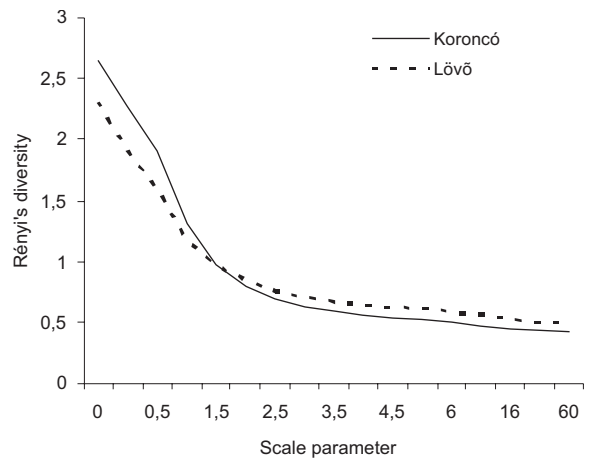
changed – it was much higher in the 1<sup>st</sup> generation and then was essentially decreasing till the 3<sup>rd</sup> generation. Decreasing parasitization rate was similar, but less definite in Koronc6 and L6v6 during 2003.



**Figure 4.** Parasitoid species diversity of *Phyllonorycter robinella* (%) (L6v6, 2003) (AC=Achrysocharoides cilla, BN=Baryscapus nigroviolaceus, PS=Pediobius saulius, HT=Holcothorax testaceipes, PN=Pholetesor nanus, MF=Minotetrastichus frontalis).



**Figure 5.** Dominance-diversity curves of the *Phyllonorycter robinella* parasitoids



**Figure 6.** Comparison of the *Phyllonorycter robinella* parasitoid diversity between sites (Rényi's diversity ordering).

It is also interesting that *P. saulius* appeared at these sampling sites (G6d6ll6 and Visonta), mainly as a primary parasitoid in larvae and pupae of *Ph. robinella* and not as a secondary parasitoid like at the other three sampling sites in western Hungary.

**Parectopa robinella parasitoids**

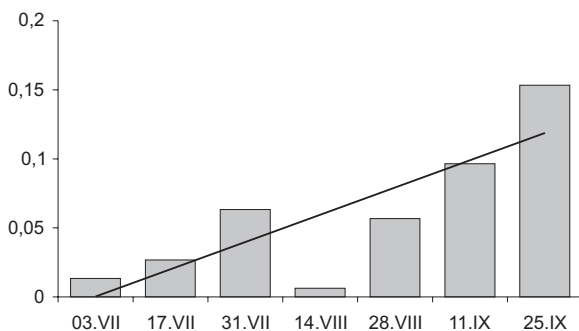
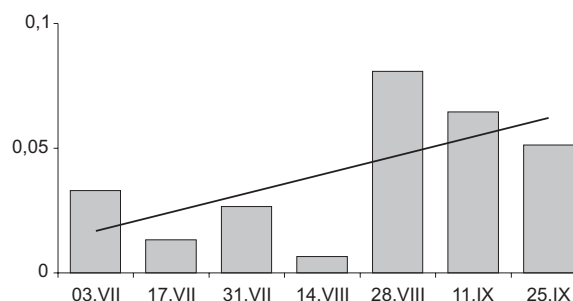
From all sampling sites in western Hungary, 12 parasitoid species were reared: *P. nanus*, *H. testaceipes*, *P. soemius*, *S. acalle*, *S. sericeicornis*, *C. viticola*, *P. saulius*, *C. trifasciatus*, *N. formosa*, *A. cilla*, *M. frontalis*, *E. urozonus*. The rate of parasitization was much lower than in case of *Ph. robinella* (Table 1). Parasitization rate during sampling season showed an increasing trend at both sites, so the parasitization of the 2<sup>nd</sup> generation of this species was higher than in the 1<sup>st</sup> generation (Figures 7–8).

During season, *A. cilla* was the dominant parasitoid species at both sampling sites, while *M. frontalis*, *P. soemius*, *S. acalle*, *S. sericeicornis*, *C. viticola*, *C. trifasciatus* and *N. formosa* were present in a smaller number. *P. saulius* ap-

TABLE 2

Parasitization rate of *Phyllonorycter robiniella* (%) (Gödöllő & Visonta, 2001–2002).

Year/Generation	Gödöllő						Visonta					
	2001			2002			2001			2002		
	1	2	3	1	2	3	1	2	3	1	2	3
<i>Pholetesor nanus</i>	8.9	7.5	0.4	7.5	4.5	0.4	12.6	8.5	0.4	12.6	21.4	0.2
<i>Pediobius saulius</i>	2.0	2.2	0.4	7.3	0.1	0.8	0.7	2.2	0.2	11.5	4.1	0
<i>Sympiesis sericeicornis</i>	0.2	1.1	0	2.5	0	0.1	1.2	1.7	0	3.6	0.5	0
Other species	2.9	3.1	0.1	3.3	0.1	0.4	2.0	2.1	0.1	6.6	2.3	0.5
<b>Total</b>	<b>14.0</b>	<b>13.9</b>	<b>0.9</b>	<b>20.6</b>	<b>4.7</b>	<b>1.7</b>	<b>16.5</b>	<b>14.5</b>	<b>0.7</b>	<b>34.3</b>	<b>28.3</b>	<b>0.7</b>

Figure 7. *Parectopa robiniella* parasitization rate during season (Csorna, 2003)Figure 8. *Parectopa robiniella* parasitization rate during season (Koronc, 2003)

peared to be mainly secondary parasitoid in *A. cilla* and other primary parasitoids. After dissection of mines, we found many parasitoid larvae which we were unable to identify.

In Gödöllő and Visonta, *Pa. robiniella* was also present in a large number and samples for laboratory rearings were taken. However, the level of parasitization was very low, below 1.0% and thus we omitted this data here. However, this low parasitization rate is probably not adequate to the real situation. The laboratory rearing of *Pa. robiniella* was carried out in a wrong way, under insufficient relative humidity, and as a result, the majority of *Parectopa* larvae dried out before the parasitoids were able to develop and emerge, which was confirmed by our dissections.

## DISCUSSION

Our preliminary data showed that polyphagous parasitoid species can easily shift onto newly introduced invader hosts, in our case – *Ph. robiniella* and *Pa. robiniella*. All the parasitoid species we reared from these two leaf-miners are generalists – common and abundant species on different lepidopteran leaf-miners associated with oaks and other woody plants (31, 32). Three of our sampling sites were located in the close neighborhood of oak stands where *Phyllonorycter* and *Tischeria* leaf-miner species were very common and abundant during sampling periods.

Parasitoid species composition of two black locust leaf-miners is identical to those we reared simultaneously from oak leaf-mines. The only difference is that the species richness of parasitoids on oak leaf-miners appeared to be slightly higher compared to the one obtained for the two black locust leaf-miners. The likely explanation for this is that some parasitoids are more specialized oak leaf-miners and thus a shift on a new host had not yet occurred.

## Parasitoid species diversity in *Phyllonorycter* and *Parectopa*

In both, *Ph. robiniella* and *Pa. robiniella*, the same dominant species of parasitoids were reared. In *Ph. robiniella*, the parasitoid species richness was slightly higher than in *Pa. robiniella* (19 and 12 species, respectively) although this difference might be overestimated due to dif-

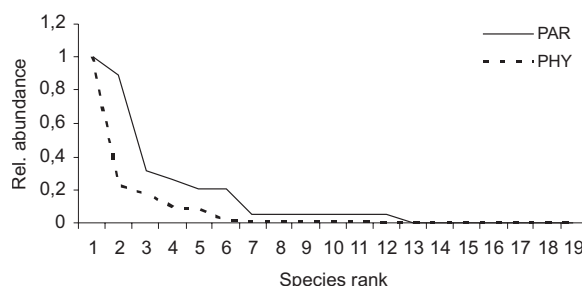


Figure 9. Dominance-diversity curves in different hosts.



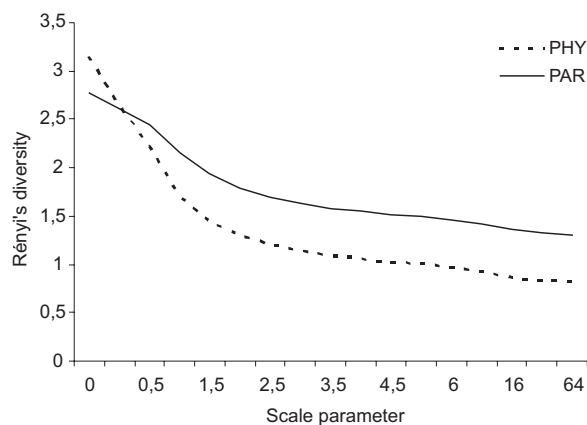


Figure 10. Rényi's diversity ordering in different hosts.

ferent sample sizes. It is too early to make any conclusions about it yet some possible interpretation is to follow below.

The dominance of a parasitoid species seems to be more pronounced in *Phyllonorycter* (Figure 9); however, species richness and species composition are very similar. The diversity of the two communities is not comparable (Figure 10). Nevertheless, it suggests the same tendencies. *Parectopa* has higher parasitoid diversity in case of rare species.

### Life cycle and mine structure versus parasitoid diversity

*Ph. robiniella* has 3 generations per year and the larvae pupate in the mine, while *Pa. robiniella* has only 2 generations and the larva, when it stops finished the feeding, leaves the mine and pupates in the litter. Furthermore, *Parectopa* starts to develop 2–3 weeks later than *Phyllonorycter*. The majority of parasitoids are probably more adapted to *Phyllonorycter* species than to those of *Parectopa* and thus the emergence of subsequent generations of parasitoids is better synchronized with the life-cycles of the *Phyllonorycter* species rather than those of *Parectopa*. The mine structure and its location on the leaf are quite different in *Phyllonorycter* and *Parectopa* (3, 19). In the Hungarian fauna, more than 60 native species of *Phyllonorycter* have been recorded on different hosts, and only 2 *Parectopa* species (including *Pa. robiniella*) are known (38). Parasitoids trophically associated with *Parectopa* species are more specific than those on *Phyllonorycter* so that the formation of parasitoid complexes and host-shifting onto invasive *Parectopa* species will supposedly be a much slower process than in the case of *Ph. robiniella*. Moreover, the pupation in the litter in *Parectopa* also increases the survival of this species in comparison to *Phyllonorycter* whose pupae are exposed to parasitoids which probably never or very rarely leave the tree canopy and thus less affect the *Parectopa* pupae. These biological peculiarities somehow explain much lower parasitization rate and less diverse parasitoid communities of *Parectopa* in comparison to *Phyllonorycter*.

### The rate of parasitism and species composition during sampling season

The rate of parasitism of *Ph. robiniella* showed a considerable difference during sampling season, and rather decreased at all sites. Further sampling is necessary for appropriate understanding of this phenomenon.

In different sampling sites and years, the dominant parasitoid species were different in the same leaf-miner species and across the two species. In *Ph. robiniella* mines, *A. cilla* appeared to be the dominant parasitoid species in Koroncó and Lövő (2003), while in Gödöllő and Visonta (2001–2002) *P. nanus* was pre dominant. Later, the role of some other parasitoid species also increased (Figures 3–4). In *Parectopa* (Koroncó, 2003), *P. saulius* and *P. soemius* were dominant at the beginning of sampling season, later *Achrysocharoides cilla* became the most abundant, while in Csorna *A. cilla* was the most abundant species during the entire sampling season, in both generations.

### Parasitism rate and the population density of leaf-miners

In *Ph. robiniella* no correlation between population density and parasitism rate was found, while in *Pa. robiniella* parasitization level slightly increased with leaf-miner density. However, these are only preliminary results and must be confirmed by further samplings.

In summary, it is clear that the two invading leaf-miners, *Ph. robiniella* and *Pa. robiniella*, recruited a parasitoid community similar to native *Phyllonorycter* species on oaks and that this process of shifting onto new hosts has quite rapidly occurred, during 10–20 years.

Parasitoid communities of *Ph. robiniella* and *Pa. robiniella* also show considerable similarity to that of the invading horse chestnut leaf miner, *Cameraria ohridella* (39–42).

The parasitoid communities of *Parectopa* are simpler than in *Phyllonorycter*, which is probably due to the different mine structure and ecology of two invading hosts.

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