

Finding the Area of Origin of the Horse-Chestnut Leaf Miner: a Challenge

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Abstract - The horse-chestnut leaf miner, *Cameraria ohridella*, is a moth of unknown origin that was first discovered in Macedonia in 1984. Twenty years later, it is found in most European cities, defoliating nearly all horse-chestnut trees. A multidisciplinary program has been set up to find its area of origin. It includes studies of the parasitoid complex of *C. ohridella* in the Balkans and of other *Cameraria* spp. in other parts of the world; host tree screening tests; surveys in the Balkans, Asia and North America; and molecular studies on *C. ohridella* and congeneric species.

I. Introduction

When an invasive alien pest invades a new region, determining the area of origin of the pest is a key step in the development of control strategies, particularly with biological control. In most cases, the species is already known in other parts of the world. However, sometimes the pest is new to science. Then, finding its area of origin may become a real detective story. Among insects, one of the most famous cases is the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero, a pest that suddenly appeared in 1972 in Central Africa. This species posed a serious threat for the subsistence of millions of people for whom cassava is the major food crop. After an intensive collaborative research project involving several national and international organisations, the area of origin of the cassava mealy bug was finally found in 1981, in Paraguay. A parasitoid was selected and introduced to Central Africa, which led to a successful biological control program [13].

A more recent example is the horse-chestnut leafminer, *Cameraria ohridella* Deschka and Dimic (Lep.: Gracillariidae). This insect was first found in Macedonia in

the early 1980's [2]. Since then, it has spread over most of Europe, and its distribution now covers an area from England to Ukraine and from Sweden to Spain, Italy and Greece [11]. Its main host, the common horse-chestnut (*Aesculus hippocastanum*) is a major urban tree in most of Europe. In most regions where the pest occurs, horse-chestnut trees are severely defoliated, year after year. The trees are not killed, but the aesthetic damage is so severe that municipalities are already replacing this highly valuable tree by other species. The horse-chestnut is endemic to the Balkans where the few remaining natural stands are also attacked, causing concern for the survival of this rare tree species. Furthermore, the moth can also attack and develop on maple species (*Acer pseudoplatanus* and *A. pseudoplatanoides*) [5, 12] and, considering the pressure on the moth to find new suitable host plants after having totally defoliated horse-chestnut trees, it is likely that the damage sustained by maples will increase in the near future.

For the moment, control methods are limited to the aerial spraying of diflubenzuron, the injection of systemic insecticides, and the removal of dead leaves, in which the moth overwinters [11]. However, these methods are expensive and practically difficult to conduct over large areas. The moth is attacked by a range of native natural enemies, particularly parasitoids, but their impact is very limited, and there is no sign that parasitism rates are going to improve in the near future [4, 7]. The only method that may provide a long-term and sustainable solution to the problem is classical biological control, through the release of natural enemies from the region of origin of the moth. Unfortunately, this region of origin is not yet known. It was first thought that the moth came from the Balkans [2], the region where the moth was first found and from where the European

horse-chestnut originates. Although the Balkans option cannot be ruled out [8, 12], there are several arguments against it [15, 17]. Firstly, if the moth originated in Europe, why does it only spread now, and so rapidly? Secondly, the lepidopteran fauna in the Balkans is quite well known, and the moth had never been observed before causing outbreaks in the early 1980's. Furthermore, *Cameraria* is not a European genus. Most species occur in North America, and a few are known from East, South and Central Asia. Additionally, only very polyphagous parasitoids have been reared from *C. ohridella* in Europe, whereas, in their native range, most insects are attacked by some specific or oligophagous parasitoids. However, the best argument in favour of an extra-European origin is that, in the Balkans where *C. ohridella* was first observed twenty years ago outbreaks have continued unabated (however note that there are indications that outbreaks actually started 30 years ago in Macedonia -See [22]). Such long-lasting outbreaks characterize exotic rather than native species.

Other possible areas of origin include East and Central Asia, and North America, where other *Cameraria* species occur. These are also the regions where other trees of the genus *Aesculus* occur but, since *C. ohridella* can also attack and develop on maple species (*Acer* spp.) in Europe, it cannot be excluded that the host tree in the area of origin belongs to another genus, e.g. *Acer* or other trees not occurring in Europe [12].

A combination of various methods are presently used to locate the area of origin of *C. ohridella*. These include: (1) studies of the parasitoid complex of *C. ohridella* in the Balkans and of other *Cameraria* spp. in other parts of the world; (2) host tree screening tests; (3) surveys in the Balkans, Asia and North America; (4) molecular studies on *C. ohridella* and congeneric species. This paper briefly presents these methods. Investigations are still on-going, and most results have not yet been published. Therefore, only methods and preliminary results are presented herein.

II. Parasitoids of *C. Ohridella* in the Balkans and *Cameraria* spp. in North America and Asia

We are working on two hypotheses. Firstly, we assume that if *C. ohridella* originates from natural horse-chestnut stands in the Balkans, it must have a larger parasitoid complex in this region, including specific parasitoids, than in the regions where it has been recently introduced. Our second assumption is that, if *C. ohridella* in the Balkans has a lower parasitism rate and a more restricted parasitoid complex than other *Cameraria* spp. in their native range, we can suspect that the Balkans are not the area of origin of the moth.

Until now, only very polyphagous parasitoids have been found on *C. ohridella*, and parasitism rates in Europe are unusually low for a leaf miner (e.g. [7, 12]). This has already been used as an argument against the European origin of *C. ohridella* (e.g. [17,]Kenis, 1997, Pschorn-Walcher, 1997). However, until recently, observations on parasitism had been

made exclusively in newly invaded regions in Central Europe. Therefore, it was important to survey the Balkans and the regions where the horse chestnut is endemic. Extensive collections were made in Greece, Macedonia and Bulgaria. The first results show that the same polyphagous parasitoids occur in the Balkans and in Central Europe. No specific or oligophagous parasitoid has been found in natural stands in the Balkans (G. Grabenweger, R. Tomov, N. Avtzis, unpublished). The main difference between the Balkans and the rest of Europe is the higher prevalence of *Pediobius saulius*, a eulophid pupal parasitoid known from many other gracillariid moths. Thanks to *P. saulius*, parasitism rates are usually higher in the Balkans (typically 5-30%, [4]; G. Grabenweger, R. Tomov, N. Avtzis, unpublished) than in Central Europe, but they remain very low for a leaf miner [10], suggesting that Europe is probably not the area of origin of the moth.

The literature on parasitism of other *Cameraria* spp. is limited to a few studies on American species, in particular the pecan leaf miner *Cameraria caryaefoliella* (Clemens) [14] and the oak leaf miners *Cameraria hamadryadella* (Clemens) and another *Cameraria* species [1, 3]. These studies suggest higher parasitism rates than in Europe [1, 3] and parasitoid species that appear less polyphagous than those attacking *C. ohridella* [14]. Collections are presently made of other *Cameraria* spp. in China and Japan. Preliminary results suggest parasitism rates up to 80% (M. Kenis, unpublished).

III. Host Tree Screening Tests

C. ohridella is presently screened on possible host trees. Both no-choice tests in field cages and open-field tests are carried out. Oviposition rates and development successes are observed. Tests naturally started with species of the genus *Aesculus* [5, 6]. Eleven of the 15-18 world *Aesculus* species have been screened, in addition to many hybrids and varieties. Although, in no-choice tests, oviposition occurred on all species, *C. ohridella* could develop on a few species only. The European *A. hippocastanum* and the Japanese *A. turbinata* were the most suitable trees. Some successful development was observed on some North American species, *A. octandra*, *A. pavia*, *A. glabra* and *A. sylvatica*, although, on these trees, few larvae successfully developed to the adult stage. On other North American species (*A. californica*, *A. parviflora*) and Asian species (*A. indica*, *A. chinensis*, *A. assamica*), the moth could not develop [5, 6]. Since *C. ohridella* is also found occasionally on maple (*Acer pseudoplatanus* and *Acer platanoides*), screening tests are being conducted on *Acer* species from various origins. Results are not yet available.

IV. Surveys

Surveys are carried out in potential regions of origin using two methods. Firstly, trees are inspected visually for mines

of *Cameraria* spp. Larvae and adults are compared to *C. ohridella*. Secondly, since an efficient and highly specific pheromone of *C. ohridella* is available [16], pheromone traps are placed in various regions of the world to locate low populations of the moth. Surveys have already been made in the Balkans, Asia, and North America.

In Greece and Bulgaria, all stands of *A. hippocastanum* are infested, at various levels. Several species of *Acer* have been inspected at the vicinity of horse chestnut stands, but only a few mines were found on *A. pseudoplatanus*, as it is occasionally observed in Central Europe. No mines were found on *A. obtusatum*, *A. monosperulatum*, *A. campestre* and *A. platanoides*. Pheromone traps were placed in various regions of Greece in *Acer* spp. stands far from *A. hippocastanum* stands, but no moth was caught. Surveys are continuing on more native *Acer* species and other tree genera.

In Asia, surveys were made horse-chestnut stands in China (*Aesculus chinensis* and *A. wilsonii*), Japan (*A. turbinata*) and Pakistan (*A. indica*). An undescribed *Cameraria* sp., different from *C. ohridella* has been found in Japan on *A. turbinata*. Other potential host trees were surveyed, in particular *Acer* spp., on which other *Cameraria* spp. were observed (M. Kenis, unpublished). These species were used for molecular studies (see below).

In North America, surveys were carried out less intensively, because the leaf miner fauna is supposed to be better known than in Asia. Indeed, another species, *Cameraria aesculisella*, occurs on *Aesculus* spp. in eastern North America [12]. Nevertheless, casual surveys have been made and pheromone traps have been placed in *Aesculus* spp. stand in various US States, without success.

V. Molecular Studies

Molecular studies on *Cameraria* spp. have two different objectives. Firstly, a phylogeographic study is carried out with *C. ohridella* in Europe, to assess the genetic variability of European populations and to evaluate if European populations come from a single introduction. RAPD-PCR and mtDNA sequencing are used. Preliminary results were presented in Kovács et al. [18], who showed very little genetic variability in European populations, suggesting a single introduction. However, these results were based on populations collected in plantations in Central Europe only. Similar tests are presently being carried out with populations from natural horse-chestnut stands in the Balkans.

Secondly, we are carrying out a phylogenetic study of the genus *Cameraria*. Both the nuclear gene 28S rDNA (D1-D3 expansion regions) and the mitochondrial COI are used to reconstruct a combined molecular phylogeny at species level. The aim is to rebuild the ancestral host use and patterns of host shifts of *Cameraria* to see how these have changed through evolutionary time. In a similar study, Lopez-Vaamonde et al. [19] studied the evolution of host plant use of *Phyllonorycter*, the genus most closely-related to *Cameraria*. The authors showed high levels of

phylogenetic conservatism that is, closely related *Phyllonorycter* species tend to feed on closely related host plant species. If *Cameraria*'s host plant use is also phylogenetically conserved, we would expect a molecular phylogeny where *Cameraria* species would cluster according to their host plant group. Thus, if *A. hippocastanum* is the original host plant of the latter moth species and not the result of a recent colonization event, we would expect to obtain a clade of *Aesculus* -feeding *Cameraria* spp. including *Cameraria ohridella*.

VI. Conclusion

Finding the area of origin of *C. ohridella* is a real challenge and is proving more difficult than expected. We cannot totally rule out that *C. ohridella* comes from the Balkans. However, if this is the case, the dramatic increase in population densities of a previously undetected species still needs to be explained. The persistent outbreaks on *A. hippocastanum* in the Balkans and the lack of specific natural enemies strongly suggest that *A. hippocastanum* is not its original host. A sudden host shift has been proposed, e.g. by Hellrigl [12] who suggests an *Acer* species as a possible host of origin but, until now, this host of origin still has to be found. The adoption of new host plants by herbivores is more common when either the herbivore is introduced in a new region, or native herbivores are in contact with new plant species (e.g. introduced crop species). Among examples in the microlepidoptera; the gracillariid moth *Phyllonorycter messaniella* (Zeller), attacking Fagaceae, Betulaceae and, occasionally, Rosaceae in Europe; increased its host range to many other families when it was introduced into New Zealand [23]. The Mexican tortricid *Platynota sultana* (Walsingham) adopted many new hosts when it expanded its range to California [20]. Also in California, the native gracillariid *Marmara gulosa* Guillén and Davis apparently expanded its host range from native willow to various introduced plants such as citrus, avocado, cotton and oleander [9].

There is an increasing amount of evidences that *C. ohridella* originates from another continent, from an *Aesculus* species or another host tree. Most of the worlds *Aesculus* species have been surveyed for *C. ohridella*, without success. Furthermore, the majority of them seem to be unsuitable for the development of *C. ohridella*, and *C. ohridella* is morphologically much closer to the Japanese *Acer*-feeding *Cameraria nipponica* Kumata than to any other species, including the *Aesculus*-feeding *C. aesculisella* [12]. Thus, it becomes more and more likely that the moth originates not only from another continent, but also from another host tree, e.g. *Acer* or another tree genus from the Sapindales that does not occur in Europe. In this case, looking for its area of origin may look like searching for a needle in a haystack. This challenge definitely warrants a multidisciplinary approach involving a combination of bio-ecological and molecular studies, as presented here, ideally with more sampling efforts and funding.

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