Native and introduced parasitoids attacking the invasive chestnut gall wasp *Dryocosmus kuriphilus*

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The globally invasive chestnut gall wasp *Dryocosmus kuriphilus* was recently reported in Italy and threatens European chestnut orchards and native forests. Of Chinese origin, this species has invaded Japan, Korea, the USA, Nepal and Europe and in each region it has been attacked by parasitoids exploiting oak gall wasps. Classical biological control using the parasitoid *Torymus sinensis* (Hymenoptera: Torymidae) successfully reduced infestation in Japan. Subsequent work in Japan and Korea showed this parasitoid to represent a poorly understood and biologically diverse species complex. Following its success in Japan, *T. sinensis* was released in Italy in 2005. A growing appreciation of the taxonomic and ecological complexity of the *T. sinensis* complex in Japan and Korea has stimulated ongoing DNA-based work on relationships and interbreeding of species in this group. Oak cynipid parasitoids provide potential for native augmentative or conservation biological control of *D. kuriphilus* in Europe. Exploitation of this potential requires improved understanding of the taxonomy of these parasitoids, particularly of morphologically highly conserved lineages such as *Eurytoma* and *Eupelmus* in which recognised species may contain many cryptic lineages. There is a growing need for a molecular survey of the communities centered on chestnut and oak gall wasps to: (1) ascertain the identity of *T. sinensis* lineages released in Italy (2) track the dispersal of *T. sinensis* in the native community, either in its original form or as hybrids with native species (3) identify possible biological control agents in the native parasitoid community.

Introduction

Natural communities are under constant threat from invasive organisms. Invasion can result from a natural range expansion where a given species exploits new ecological niches or can be facilitated by human transportation. Global warming and climate change represent an increasingly important anthropogenic factor affecting the natural distribution of numerous organisms and there is a growing need to understand the impact of past or recent invasion events or introductions on native communities (Schönrogge *et al*., 1995; Stone *et al*., 1995; Schönrogge *et al*., 1996a,b, 1998; Schönrogge & Crawley, 2000; Hayward & Stone, 2006). We need tools to predict the outcome of such events (Schönrogge *et al*., 2006) and, where necessary, to minimize their impacts.

The chestnut gall wasp, *Dryocosmus kuriphilus* (Hymenoptera: Cynipidae) is considered to be the most important pest of chestnut (*Castanea*) worldwide (Brussino *et al*., 2002; Moriya *et al*., 1990; Murakami *et al*., 1995; Payne *et al*., 1983). Originating from China, this pest colonized Japan in 1958 (Murakami *et al*., 1980), Korea in 1958 (Cho & Lee, 1963) and the USA in 1974 (Payne *et al*., 1976). Very recently, it was detected for the first time in Europe (in Piedmont, Italy) in 2002 (Brussino *et al*., 2002) and Nepal (Abe *et al*., 2007). Human exchange of infected cultivars and material for grafting among chestnut growers is thought to be the main factor facilitating its dispersal (Aebi *et al*., 2006).

Dryocosmus kuriphilus is a member of the oak gall wasp tribe Cynipini, and is one of only two species in this tribe to induce galls on *Castanea* (Felt, 1940; Stone *et al*., 2002). While oak gall wasps rarely affect their host tree's fitness, attack by *D. kuriphilus* on chestnut commonly reduces wood production (Kato & Hijii, 1997) and fruit yield by 50–75% (Payne *et al*., 1983). Additionally, severe consecutive attacks are thought to result in the death of the tree (Moriya *et al*., 2003), probably in combination with other detrimental factors such as fungal infection, drought or severe attack by other herbivores.

Chestnut cultivation fulfils several important commercial, socio-cultural, and ecological roles in southern Europe. In Piedmont, chestnut orchards are traditionally planted for fruit and wood production, representing a significant additional long-term income for many farmers. From an ecological point of view, chestnut forests are the major woodland type in Northern Italy (Bounous, 2002).

Table 1 Parasitoids recruited by *D. kuriphilus* in Italy with origin (No, native from oak cynipids; I, introduced) and natural distribution (WP, western palaearctic; M, Mediterranean Europe)

Parasitoid species	Family	Origin	Distribution
Sycophila iracemae	Eurytomidae	No	M
Sycophila variegata	Eurytomidae	No	WP
Sycophila biguttata	Eurytomidae	No	WP
Eurytoma pistacina	Eurytomidae	No	WP
Eurytoma brunniventris*	Eurytomidae	No	WP
Eurytoma sp. new species	Eurytomidae	No	?
Mesopolobus mediterraneus	Pteromalidae	No	M
Mesopolobus sericeus	Pteromalidae	No	WP
Mesopolobus tarsatus	Pteromalidae	No	M
Torymus sinensis	Torymidae	I	China, Korea
Torymus auratus	Torymidae	No	WP
Torymus flavipes	Torymidae	No	WP
Torymus scutellaris	Torymidae	No	WP
*Megastigmus dorsalis**	Torymidae	No	WP
Eupelmus urozonus*	Eupelmidae	No	WP
Baryscapus pallidae	Eupelmidae	No	WP
Ormyrus pomaceus	Ormyridae	No	WP

*Indicates taxon with cryptic species identified molecularly.
Species also attacking *D. kuriphilus* in China, Korea and Japan are named in **bold**.

Chestnut trees play an important role in stabilizing steep slopes and their litter contributes to the maintenance of characteristic soils. Significant reduction of managed chestnut orchards and native forests would have major socio-economic impacts on local communities.

Several approaches to reducing populations of *D. kuriphilus* have been attempted. Use of chemical pesticides has proved inefficient as the immature stages are protected within the gall (Murakami, 1981; Torii, 1959). Mechanical removal of infested twigs (pruning) and the protection of immature twigs with nets, although effective, do not represent practical solutions because of their labour intensiveness. Breeding of resistant chestnut varieties was successful for about 20 years in Japan, but these were eventually rapidly overcome by a novel virulent strain of *D. kuriphilus* (Murakami, 1981). While Italian researchers are conducting further work on the use of pesticide and the development of resistant varieties, the only effective method for reducing *D. kuriphilus* infestation to date is the use of hymenopteran parasitoids as biological control agents.

In a recent review, Aebi *et al*. (2006) described the parasitoid communities associated with *D. kuriphilus* in its native and invaded ranges. As it became established in new environments, *D. kuriphilus* rapidly recruited parasitoids attacking oak gall wasps locally. The same is apparent in Italy (Table 1) (Aebi *et al*., 2006; Stone *et al*., 2002).

Successful biological control in Japan

Biological control using parasitoid Hymenoptera was pioneered in Japan, whose scientists studied the natural enemies of *D. kuriphilus* in its Chinese native range in detail (Aebi *et al*., 2006 and references therein). Of a total of 11 chalcid species reared from *D. kuriphilus* in China, *Torymus sinensis* (Hymenoptera: Torymidae) was selected as a suitable biological control agent because its life cycle appeared well synchronized with that of its host. Within 5 years of its release, *T. sinensis* successfully reduced local infestation levels from 43% to 3%, well below the tolerable injury level of 30% (proportion of infected shoots, Gyoutoku & Uemura, 1985; Moriya *et al*., 1990; Ôtake *et al*., 1984). In Japan infestation levels have stayed low since 1982.

Taxonomy of *T. sinensis* and non-target effects

Subsequent work in Korea and Japan has shown that *T. sinensis* is part of a taxonomically complex set of closely related species that are biologically diverse, but difficult to distinguish morphologically (Murakami, 1988; Yara *et al*., 2000; Yara, 2004). Native Korean parasitoids identified morphologically as *T. sinensis* can be divided into two ecotypes on the basis of their adult emergence periods (Murakami *et al*., 1995). The two ecotypes are thought to be derived from native oak cynipid hosts with differing phenology. Neither of these ecotypes was able to provide effective biological control of *D. kuriphilus*, due primarily to phenological mismatches in both strains between adult emergence and the development of galls of the chestnut gall wasp in the field (Murakami *et al*., 1995).

Further taxonomic complexity in this group is derived from the ability of introduced Chinese *T. sinensis* to hybridize with a closely related species native to Japan, *T. beneficus*. Hybridization was suspected (Shiga, 1999) and in 1992, Moriya and colleagues successfully crossed *T. sinensis* and *T. beneficus* in the laboratory to produce fertile hybrid females. Hybrids were also detected in the field (Moriya *et al*., 1992, 2003; Yara *et al*., 2000) and molecular markers proved their hybrid origin (Izawa *et al*., 1992, 1995, 1996; Toda *et al*., 2000; Yara, 2004, 2006; Yara *et al*., 2000). Female morphology has been used in the past to distinguish *T. sinensis* and *T. beneficus*. The ratio of the ovipositor sheath length to the thorax length (O/T ratio) (Ôtake, 1987) in combination with the adult emergence time was used to identify the two species. *T. sinensis* has a larger O/T ratio than *T. beneficus* (Otake, 1987). *T. sinensis* females emerge later than *T. beneficus* females (5th to 23rd of April and 10th of March to 8th of April, respectively, Murakami, 1981). However, analyses by Yara (2004) using Cytochrome oxidase I sequence data (a marker widely used in molecular systematics; Caterino *et al*., 2000) have shown that the O/T ratio is an unreliable character in discrimination of *T. sinensis* and *T. beneficus*.

An additional twist in the tale is the demonstration of three distinct ecotypes in Japanese *T. beneficus*, whose adults differ in their emergence phenology between an early season emerging strain (TbE, second half of March to first half of April), a late-season emerging strain (TbL, late April to early May) and an autumn strain (TbA, October to November) (Otake, 1987; Murakami, 1988; Yara, 2004). All three strains attack *D. kuriphilus* in Japan, but are thought to have originated from different oak cynipid hosts. The original host of the early season emerging strain is thought to be an *Andricus* sp. leaf gall on *Quercus serrata*, while the hosts of the other ecotypes are unknown (Murakami, 1988). Yara (2004) showed that the early season and autumn strains of *T. beneficus* are not strongly differentiated genetically, and that the late-season strain of *T. beneficus* is more closely related to *T. sinensis* than to its early season emerging strain.

Taken together, these results show the limitations of existing morphological taxonomy in this group of Torymidae and highlight the need to develop molecular markers able to reliably distinguish the different species and ecotypes attacking *D. kuriphilus* and oak cynipids in eastern Asia. These results also show that morphologically indistinguishable populations of wasps harvested in the wild in Japan probably constitute a biologically diverse assemblage of species, ecotypes, and their hybrids.

Initiation of biological control in Italy

The rapid development of severe chestnut infestation in Italy and the success of Japanese scientists in biological control of *D. kuriphilus* led to the swift introduction of Japan-sourced *T. sinensis*. After 2 years of trials that were unsuccessful due to early emergence of the imported *T. sinensis* relative to the development of its target in the field, 90 mated Japan-sourced *T. sinensis* females were released for the first time in the field in three localities in 2005 (Aebi *et al*., 2006). Their establishment was assessed by the collection and rearing of more than 9000 *D. kuriphilus* galls in these localities. In 2006, 1058 couples were released in 11 locations. The source material for these rearings was 25 500 galls imported from Japan, producing more than 1660 individuals of a further four unidentified parasitoid species (currently being molecularly identified) in addition to *T. sinensis*: *Torymus* sp., *Eurytoma* sp., *Eupelmus* sp. and *Ormyrus* sp. To facilitate further releases of *T. sinensis*, a mass rearing attempt was initiated in tents containing young chestnut trees infested with *D. kuriphilus*.

The significance of parasitoid taxonomy in biological control

Given the complex biology of *T. sinensis*, the material introduced from Japan and now released could encompass a range of biological species, ecotypes, and hybrids (Murakami *et al*., 1995; Yara, 2004). While this diversity may contribute to selection for a particular phenotype that matches *D. kuriphilus* phenology in Europe, it may complicate the optimization of rearing and release strategies. Precise identification of natural enemies associated with a pest is a key factor in the importation and establishment phases of a biological control programme (Rosen, 1986). Mass production of introduced or indigenous species relies heavily on correct identification as cultures may become contaminated by undesirable and ineffective species (Rosen & DeBach, 1973). For example, mass cultures of the parasitoid *Encarsia perniociosi* (Hymenoptera: Aphelinidae) used in Europe against the San Jose Scale *Quadraspidiotus perniciosus* were invaded and overtaken by a related but ineffective species, *E. fasciata*, which had a greater competitive ability under storage conditions (Rosen & DeBach, 1973). The recognition of different biological races within a species is as important as the identification of different parasitoid species (Rosen, 1986). Because ecotypes have different ecological requirements (Drès & Mallet, 2002), detecting them and exploiting the diversity they represent can enhance a biological control programme (DeBach, 1969). For example, control of the walnut aphid *Chromaphis juglandicola* (Homoptera: Aphididae) along the coast and in central and northern parts of California required the introduction of two ecotypes of the parasitoid *Tryoxys pallidus* (Hymenoptera: Aphidiidae) from France and Iran. The French ecotype was an effective control agent in Mediterranean coastal climates, but was unable to establish itself in the dryer inland environment, while the Iranian ecotype was better adapted to inland conditions (van den Bosh *et al*., 1979). Appreciation of the possible consequences of release of *T. sinensis* thus requires a careful assessment of what has actually been introduced.

Potential for biological control using native parasitoids

Native parasitoids could potentially be exploited in either augmentative biological control (rearing and subsequent release of native parasitoids) or conservation biological control (an approach used in closed spaces such as breeding cages or granaries) to enhance native parasitoid populations. Sixteen parasitoid species have so far recruited naturally to *D. kuriphilus* in Italy (Table 1). However, their attack rates of the invading gall remain very low (between 0.5 and 1.6%). This may well be because *D. kuriphilus* galls develop midway between the spring and summer/autumn generations characteristic of oak cynipids, resulting in a mismatch between emergence of native parasitoid adults and chestnut galls in an appropriate developmental stage for attack (Aebi *et al*., 2006).

The rapid recruitment of oak cynipid parasitoids to *D. kuriphilus* suggests that despite current low attack rates there may be some value in an augmentative or conservation biological control programme using native species. Here again, accurate taxonomic assessment of the component species is essential for the reasons described above. Recent phylogeographic work has shown the existence of cryptic lineages in several taxa previously regarded as single species, including *Eurytoma brunniventris* (Eurytomidae), and *Megastigmus dorsalis* (Torymidae). These cryptic lineages are not host plant specific, but attack overlapping sets of hosts on shared host plants. The extent to which they interbreed and are biologically different (as in the *Torymus sinensis* complex) is unclear. The cryptic lineages can be differentiated using DNA sequence data, allowing the establishment if required of pure lineage rearing programmes. These same molecular approaches would allow the detection of hybridization between released *T. sinensis* and native Torymid parasitoids in Italy. The potential for such uncontrolled interactions is clearly shown for *T. sinensis* and *T. beneficus* in Japan, but their biological impact is hard to predict.

DNA barcodes have recently emerged as a tool to minimize risks of biological harm resulting from biological invasions (Armstrong & Ball, 2005). The Department of Exploitation and Protection of Agricultural and Forestry Resources is actively involved in the application of molecular tools to the monitoring of community level impacts of biocontrol agents' introduction.

We are currently characterizing the parasitoid community centered on *D. kuriphilus* in Japan and Italy using DNA sequence data. This will allow us to (i) assess the diversity present in the introduced *T. sinensis*, and (ii) provide diagnostic DNA sequence 'barcodes' for other potential biocontrol agents. This survey has already found the taxonomic diversity of parasitoids associated with Japanese *D. kuriphilus* populations to be higher than previously thought. For example, one taxon initially identified as the widespread generalist parasitoid *Eupelmus urozonus* (Eupelmidae) has been shown to consist of at least 3 morphologically indistinguishable species. The complex issues raised above for *T. sinensis* are thus probably widespread in parasitoid Hymenoptera. We also have extensive sequence data for native European parasitoids of oak cynipid galls. Monitoring of native parasitoid communities around heavy infestation sites of *D. kuriphilus* and at release sites of *T. sinensis* will allow us to (iii) detect and track the integration of introduced parasitoids into native communities, and (iv) quantify their hybridization with related native parasitoid species. Finally, the same tools will allow us to identify which specific lineages within European native parasitoids make the host shift from oak (or possibly rose) gall wasp hosts to *D. kuriphilus*, and hence which lineages might be selectively reared for augmentative biological control.

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Parasitoïdes indigènes et introduits attaquant l'espèce envahissante *Dryocosmus kuriphilus* (cynips du châtaignier)

Dryocosmus kuriphilus est un cynips du châtaigner envahissant au niveau mondial. Il a été récemment signalé en Italie et menace les châtaigneraies et les forêts indigènes en Europe. D'origine chinoise, cette espèce a envahi le Japon, la Corée, les Etats-Unis, le Népal et l'Europe et dans chaque région il a été attaqué par des parasitoïdes exploitant des cynips du chêne. La lutte biologique classique utilisant un parasitoïde, *Torymus sinensis*, (Hymenoptera: Torymidae) a réussi à réduire l'infestation au Japon. Le travail ultérieur au Japon et en Corée a montré que ce parasitoïde représentait un complexe d'espèces biologiquement diverses et mal comprises. A la suite de son succès au Japon, *T. sinensis* a été relâché en Italie en 2005. Une meilleure appréciation de la complexité taxonomique et écologique du complexe *T. sinensis* au Japon et en Corée a stimulé la poursuite de recherches moléculaires sur les relations et les croisements d'espèces dans ce groupe.

Les parasitoïdes des cynips du chêne fournissent un potentiel pour une lutte biologique augmentative ou par conservation contre *D. kuriphilus* en Europe. L'exploitation de ce potentiel exige une meilleure compréhension de la taxonomie de ces parasitoïdes, en particulier des lignées morphologiquement bien conservées comme *Eurytoma* et *Eupelmus* dans lesquelles des espèces reconnues peuvent comprendre de nombreuses lignées cryptiques. Il existe un besoin croissant pour une étude moléculaire des communautés centrée sur le châtaignier et les cynips du chêne pour: (1) déterminer l'identité des lignées de *T. sinensis* relâchées en Italie (2) retracer la dispersion de *T. sinensis* dans la communauté indigène, soit sous sa forme originale soit comme hybrides avec les espèces indigènes (3) identifier de possibles agents de lutte biologique dans la communauté des parasitoïdes indigènes.

Аборигенные и интродуцированные паразитоиды в борьбе с инвазивной каштановой орехотворкой *Dryocosmus kuriphilus*.

Об инвазии каштановой орехотворки *Dryocosmus kuriphilus* в мировом масштабе недавно сообщалось в Италии; она угрожает европейским каштановым садам и аборигенным лесам. Будучи китайского происхождения, этот вид распространился в Японии, Корее, США, Непале и Европе, и в каждом регионе он подвергался нападению со стороны паразитоидов дубовых орехотворок. Классическая биологическая борьба с использованием паразитоида *Torymus sinensis* (Hymenoptera: Torymidae) успешно сократила заражение в Японии. Последующая работа в Японии и Корее показала, что этот паразитоид представляет собой плохо изученный и биологически разнообразный комплекс различных видов. После его успеха в Японии *T. sinensis* выпускался в Италии в 2005 г. Растущее понимание таксономической и экологической сложности комплекса *T. sinensis* в Японии и Корее стимулировало ведущуюся сейчас на базе ДНК работу по изучению взаимоотношений и интербридинга видов в этой группе. Паразитоиды дубовых орехотворок обеспечивают потенциал для увеличения естественной биологической борьбы с *D. kuriphilus* в Европе. Эксплуатация этого потенциала требует более продвинутого понимания таксономии паразитоидов, главным образом морфологически хорошо сохранившихся линий, таких как *Eurytoma* и *Eupelmus*, в которых признанные виды могут содержать множество скрытых линий. Увеличивается потребность в молекулярном исследовании сообществ, связанных с орехотворками каштана и дуба, с тем чтобы: (1) установить идентичность линий *T. sinensis*, выпущенных в Италии, (2) проследить распространение *T. sinensis* в природном сообществе, либо в его

первоначальной форме, либо в качестве гибридов с аборигенными видами, (3) определить возможных агентов биологической борьбы в аборигенном сообществе паразитоидов.

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