**Know Your Enemy: The Use of Molecular Ecology in the Onopordum Biological Control Project**

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**Abstract**

Experience has repeatedly shown that accurate identification of the target weed(s) for a biological control project is critical to the success of a biological control project. This is particularly true where the weed may comprise different biotypes or be part of a species complex, where hybridisation is suspected or where the agent-host plant relationship is very tight. Molecular ecology provides a number of powerful tools for resolving the identity of weeds, and their use in understanding a hybrid swarm of *Onopordum* spp. in Australia is described to illustrate some recently developed techniques. Some implications of hybridisation between *O. acanthium* and *O. illyricum* for the impact of particular biological control agents are discussed. In addition to this applied aspect, molecular markers can be used to better understand the phylogeny of plant groups containing the target weed(s). *Onopordum* is used as an example of how this can help our theoretical understanding of the host-selection process by potential control agents.

**Keywords:** Biological control, molecular ecology, *Onopordum* spp., hybridisation, phylogeny

**Introduction**

The host plant/control agent relationship is central to the science of weed biological control. From a practical viewpoint, it is critical to be certain of the identity of the target weed and of candidate agent. Correct identification can be used to pinpoint where in the native range an introduced weed comes from and can thus focus the search for potential control agents (Chaboudez et al. 1992, Scott et al. 1998). Failure to do so can lead to lack of agent establishment and/or incomplete control, particularly in cases involving biotypes of a single weed species (e.g. Chaboudez 1994), weed species complexes (e.g. leafy spurge (Mahlberg 1990), blackberries (Bruzzese and Hasan 1986)) or agent host-races (e.g. the thistle capitulum weevil, *Rhinocyllus conicus* (Goeden et al. 1985), skeleton weed rust, *Puccinia chondrillina* (Espiau et al. 1998)). From a more fundamental viewpoint, it is important to better understand how such host/agent relationships have evolved in oligophagous insects. Briese (1996) has shown how phylogenetic studies of plant and herbivore taxa give clues to the evolution of host-choice in agents. Such knowledge can help biological control practitioners to design better tests for evaluating host-range of candidate agents and maximise the chances of safely introducing effective agents into a new habitat.
Techniques used in biological control projects where the identity of and relationships between organisms have been studied vary from morphology (Hull and Groves 1973), chromosome counts (Morrison and Scott 1995), plant secondary chemicals (Mahlberg 1990) and protein electrophoresis (Goeden et al. 1985, Morrison and Scott 1996). However, recent developments in molecular techniques give the biological control practitioner a selection of powerful tools for resolving the identity and relationships of both target weed and candidate agent (see reviews by Nissen et al. 1995, O’Hanlon and Peakall, 2000). The increased power of these methods is demonstrated by a recent study on blackberries by Evans et al. (1998), who, using DNA markers, identified 20 genotypes from 13 previously described morphological taxa (e.g. Evans et al. 1998). The importance of this knowledge is obvious in the light of differential susceptibility to a rust fungus control agent by different members of the blackberry species complex (Bruzzese and Hasan 1986).

The present paper describes the use of molecular techniques to clarify doubts concerning the identity of Onopordum spp. thistles in Australia and demonstrate the existence of widespread hybridisation between species. In doing so it discusses the implications of such hybridisation for the Onopordum biocontrol project. The use of molecular techniques to demonstrate phylogenetic relationships both within the genus Onopordum and between Onopordum and other genera of the Carduinae thistles is also described, and the importance of knowing such relationships is discussed in terms of seeking to better understand host-range restrictions of candidate biological control agents.

Identity of Australian Onopordum

Pastures in south-eastern Australia are considered to have been invaded by two species of Onopordum of European origin, Scotch thistle (O. acanthium L.) and Illyrian thistle (O. illyricum L.) (Parsons and Cuthbertson 1992). These weedy thistles are currently the target of a biological control project (Woodburn and Briese 1996). However, there has been some doubt concerning the identity of some populations (see Groves et al. 1990), as morphological analysis revealed that many infestations of Onopordum in Australia did not correspond with any of the known species. Instead, they were morphologically very diverse, but generally intermediate between O. acanthium and O. illyricum. Amplified

![Fig. 1.](attachment:image.png)

Non-metric multidimensional scaling scatterplot of genetic distances between Australian Onopordum and putative European source taxa (shaded). Circles indicate individual Australian populations.
fragment length polymorphism (AFLP) was therefore used to determine the uniqueness of Australian *Onopordum* populations, whether they are descended from hybridisation between Scotch and Illyrian thistles, and how closely related they are to other species of *Onopordum*.

Of more than 100 polymorphic DNA fragments generated by AFLP, those diagnostic for Scotch and Illyrian thistles were demonstrated to segregate in putative hybrids, confirming their hybrid origin (Fig. 1) (O’Hanlon *et al.* 1999). Genetic exchange between these thistles may confer a fitness advantage to weedy *Onopordum* in the new Australian environment (see Vila and d’Antonio 1998), and also contribute to adaptive variation in the group. Indeed, preliminary transplant experiments suggest that hybrid genotypes have higher fitness in certain contexts and a demographic genetic study revealed heterozygote advantage within a hybrid population (O’Hanlon, unpublished data).

The existence of widespread hybridisation amongst *Onopordum* in Australia also has implications for the management of these weedy thistles, as the wide variability exhibited in certain plant characters may effect control methods, including the use of biological control agents, and their impact (Table 1). For example, both parent species of

<table>
<thead>
<tr>
<th>Character</th>
<th><em>O. acanthium</em></th>
<th><em>O. illyricum</em></th>
<th>Possible effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Life-history</td>
<td>adapted to cooler continental climates</td>
<td>adapted to warmer Mediterranean climates</td>
<td>• selection of best-adapted hybrid populations in all infested localities&lt;br&gt;• hybrid vigour</td>
</tr>
<tr>
<td>Life-history</td>
<td>biennial</td>
<td>facultative perennial</td>
<td>• rosette feeding by agents may lead to varying degrees of perenniation with flow on effects for plant life-cycle</td>
</tr>
<tr>
<td>Capitula</td>
<td>more, smaller, shorter time to maturity</td>
<td>fewer, larger, longer time to maturity</td>
<td>• differential oviposition patterns and survival of seed-feeders&lt;br&gt;• differential impact on seed production</td>
</tr>
<tr>
<td>Architecture</td>
<td>bushy, highly branched</td>
<td>more open, fewer branches</td>
<td>• differential survival of stem feeding agents&lt;br&gt;• differential exposure of agents to predation</td>
</tr>
<tr>
<td>Leaf structure</td>
<td>broader with dense woolly tomentosum</td>
<td>indented with less dense tomentosum</td>
<td>• differential protection for eggs laid on leaves&lt;br&gt;• differential palatability to grazing stock&lt;br&gt;• differential uptake of herbicides</td>
</tr>
</tbody>
</table>
Onopordum are monocarpic, but *O. acanthium* is a strict biennial while *O. illyricum* is a facultative perennial. Rees *et al.* (1999) found that in *O. illyricum* bolting is dependent on rosettes reaching a critical size, so that stresses such as rosette herbivory may reduce plant size and thus promote perenniation of rosettes. Three such rosette-feeding agents have been released, the weevil *Trichosirocalus* sp. nov., the moth *Eublemma respersa* and the fly, *Botanophila spinosa*, each with slightly different feeding niches and periods of attack. Perenniation induced by these agents could favour their population build-up by maintaining resource levels, but may also have complex flow on effects, through reduced seed production and input into soil seed reserves, which could affect capitulum attacking (the weevil, *Larinus latus*) or stem-boring agents (the weevil, *Lixus cardui*). Another important difference between the two parent species is that *O. illyricum* has much larger capitula, which favours survival of *L. latus* (Briese 1996). Hybrid forms tend to show variability in capitulum size and number as well as varying degrees of perenniation (Pettit *et al.* 1996), and the presence of wide genetic variability in these and other characters will undoubted compound the plant/herbivore interactions alluded to above. Moreover, it could lead to selection for particular plant traits under continued herbivore pressure from introduced agents. Variability in other characters such as leaf form and texture may also affect palatability and chemical uptake of hybrid thistle populations (see Pierce 1996), which needs to be considered in the integration of biological control with tactical grazing and herbicides, such as described by Huwer *et al.* (1999) for *Onopordum* thistles.

**Phylogeny of European Onopordum**

A previous taxonomic treatment of morphological variation in European *Onopordum* defined two subgenera, *Onopordum* comprising four sections, and *Acaulon*, comprising a single section (Amaral Franco 1975). However, phylogenetic analyses of AFLP data found this to be an unnatural classification, with section *Echinata* being non-monophyletic. In addition, the solitary species from section *Acaulon*, *O. acaulon*, grouped with other species in rather than forming a separate clade, as suggested from morphological studies. The AFLP phylogeny is supported by biogeographic evidence as the different clades correspond to regional distributions of *Onopordum* spp. in Europe (Fig. 2).

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**Fig. 2.** AFLP and morphological phylogenies of nine European species of *Onopordum* (*Cynara* spp. used as outgroups).

A = species restricted to Iberian peninsula  
B = species occurring Aegean region  
C = widespread species
Some *Onopordum* species showing strong morphological and habitat affinities are only distantly related. The discordance between molecular and morphological analyses may result from a pattern of colonisation and evolution (O’Hanlon et al. 1999). Given that the genus is considered to have originated in central Asia, it could be hypothesised that *Onopordum* species invaded continental Europe from the east. During colonisation, different species hybridised, resulting in genetically diverse founder populations. The Iberian Peninsula and Aegean regions became isolated, and speciation within these regions caused sorting of adaptive traits with ecological preferences, resulting in convergent morphological evolution. Some subsequent spread of species such as *O. illyricum* may have occurred along ecocline corridors such as the Mediterranean coastal strip. This proposed evolutionary pathway for European *Onopordum* spp. is concordant with the speciation of a group of capitulum-feeding weevils of the genus *Larinus* into eastern and western European taxa specialising on the genus *Onopordum* (see Briese et al. 1996).

Such “lineage sorting” is supported by the recurrent formation of hybrids in Europe (Gonzales Sierra et al. 1992) and the continual gene flow between species (O’Hanlon et al. 1999). This may help explain why the oligophagous insect fauna found on *Onopordum* spp. remains genus specific (Briese et al. 1994) rather than having developed more specialised relationships with individual species.

**Phylogenetic relationships between thistles**

Knowledge of the phylogenetic relationships of target weeds and related plants is important considering the need for very high degrees of specificity in biological control agents, particularly given current concern for non-target impacts (see Louda et al. 1997). Thistles appear to be a modern group with genera diverging relatively recently. Consequently, relationships between thistles have been difficult to resolve. Different data sets produced phylogenetic trees that, while differing in the levels of resolution obtained, were mostly congruent (Fig. 3). When combined, a “total evidence” tree produced a more...
fully resolved than trees produced from individual data sources, with strong support for
several clades. Arctium-Cousinia formed a monophyletic stem group to the remaining
genera which were divided into two clades; (1) relatives of Carduus and (2) relatives of
Cynara. Within the former clade, Picnomon-Notobasis, Carduus-Tyrimnus, and Silybum-
Galactites were identified as sister genera. Within the latter clade, relationships between
Cynara, Ptilostemon and Onopordum remained unresolved.

Despite uncertainty within the clade, it is clear that Cynara and Ptilostemon are more
closely related to Onopordum than are the other genera. Following Wapshere’s (1974)
centrifugal phylogenetic testing procedure, this justifies the use of Cynara species as key
test plants to determine whether it is worth pursuing a more generalised and expensive
host-testing protocol. The restriction of a group of closely related seed weevils within the
genus Larinus to the Cynara-Onopordum clade also supports this and provides clues to
the evolution of host-choice (Briese et al. 1996).

Conclusions

DNA markers have proved a powerful tool to better understand the system in which
biological control of Onopordum spp. is being attempted. They provided, either alone or
through combination with other character sets, a clearer picture of past and current evo-
lution of this group of thistles, and the importance of hybridisation in this process. More
precise knowledge of the phylogenetic relationships of Onopordum spp. have given a bet-
ter idea of how the natural enemies of this group of thistles evolved with their hosts. The
ability to unravel the existence of hybridisation and the extent of its occurrence in
Australia means that researchers can better anticipate agent impact and modify control
strategy if needed.

Despite their power, the use of molecular techniques for better understanding the biology
of weeds is often inhibited by the lack of expertise, and the time and cost involved in
performing such studies. Furthermore, such costs are rarely included in biocontrol project
budgets and often the benefits are not considered sufficiently important by outcome-orien-
ted funding sources. As in this case study of Onopordum, this problem can be resolved
through collaboration with researchers interested in plant population genetics and evolu-
tion. Weed biological control, through targeting invasive plant species and through the
deliberate transfer of organisms to new habitats, can provide excellent models for study
in these important fields of considerable current research interest. In return, the biological
control practitioner can obtain a much clearer picture of the system he hopes to manipu-
late.

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