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Phylogeny: can it help us to understand host choice by biological weed control agents?

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Abstract. The host-specificity of potential control agents is a critical element in the practice of biological weed control. Ecological processes affecting host selection of an organism are often constrained by its phylogeny. Thus, an understanding of the evolution of host-choice will provide greater confidence in experimental determinations of host ranges and should help to underpin the scientific basis to host-specificity testing. This is especially true where different host races exist within a particular species. One approach to studying how host-associations have evolved has been to compare the phylogenies of specialized insect groups with that of their host-plants. This paper discusses the usefulness of phylogenetic studies to biological weed control, illustrated by examples of the evolution of host-choice within the weevil genera *Larinus* and *Rhinocyllus*, both of which are currently being used as agents.

Introduction

To be used for biological control, the safety of a potential agent with respect to non-target plant species has to be clearly demonstrated. Therefore, from a purely applied sense, the subject of host-choice and specialization has been a key concern of biological control workers. However, most published work has concentrated on practical aspects of host-specificity testing such as the testing protocol e.g. centrifugal-phylogenetic testing (Wapshere 1974), maximizing genetic diversity of test plants (Weidemann and TeBeest 1990), and methodology, open or cage testing (Clements and Cristofaro 1996), culturing the insects (Shepherd 1989), life-stages to be tested and choice or non-choice tests (Cullen 1989). At the same time, an increasing interest in the more fundamental aspects of host use and selection has developed amongst evolutionary ecologists (see Jermy 1984; Jaenike 1990; Courtney and Kibota 1990; Bernays and Chapman 1994) particularly with regard to how the evolution of biological diversity may be driven by changes in patterns of resource utilization.

These fundamental issues fall into two categories; investigation of proximate causes for host-selection, such as phytochemicals, plant morphology, behaviour, prior experience and the interaction of physiology and sensory cues (see Szentesi and Jermy 1990; Bernays and Chapman 1994; Chew and Renwick 1995) and the study of the ultimate causes for host-selection, namely genetic control of the mechanisms used to choose particular hosts or incorporate novel hosts into an insect's diet (see Futuyma and Peterson 1985) and the ecological and evolutionary processes that underpin them (see Thompson 1988; Via 1990; Mitter and Farrell 1991). Weed biological control workers have drawn from studies on the mechanisms of host-selection and ecology of the agent to help interpret host-choice tests (Cullen 1989), but have given little consideration to the value of evolutionary studies in the interpretation of such data and the selection of agents. The well-known centrifugal phylogenetic testing strategy (Wapshere 1974) goes part of the way, but considers neither the phylogeny of the agent itself nor the dynamic interaction between the evolution of agent and host-plant.

In recent years there has been a renewal of interest in the use of phylogenetic studies, particularly with the development of new types of genetic markers (see Harvey *et al.* 1995). A phylogenetic approach has been used to obtain new insights into the evolution of host-choice in specialist insects. For example, Farrell and Mitter (1990) used phylogenetic comparisons to provide strong evidence for parallel diversification between the monophagous leaf beetles in the genus *Phyllobrotica* and their Lamiales host plants. This would suggest that in this group the evolution of host-choice is constrained by plant speciation events. Using a similar methodology, Futuyma and co-workers (Futuyma and McCafferty 1990; Futuyma *et al.* 1995) also found strong phylogenetic constraints in host shifts by the oligophagous beetles in the genus *Ophraella*, although these did not parallel the evolution of these plant taxa. The lack of genetic variability with regard to host-plant affiliation and presence of phylogenetic constraints in this group was confirmed through comparative studies of female behaviour and larval fitness on potential host-plants (Futuyma 1992; Futuyma *et al.* 1995).

If phylogenetic studies can help reconstruct the evolution of host-specialization within insect taxa and indicate the limits of host-shifts, they are an invaluable aid to our understanding of host choice *vis a vis* biological weed control. This paper uses two case studies to illustrate the latter point.

Case studies

The two examples used here to illustrate the role of phylogeny in understanding the evolution of host-choice both concern biological control agents of thistles (Asteraceae: Cardueae). The Cardueae are a good group to study from this point of view for, not only have they provided a large number of important weeds outside of their Eurasian range, but they contain numerous examples of host-biotype formation (Eber *et al.* 1991; Zwölfer and Römstöck-Völkl 1991; Briese and Sheppard 1992; Sobhian 1993; Müller-Schärer *et al.* 1991).

The first case involves oligophagous species of the genus *Larinus*, known to attack the capitula of thistles of the genera *Onopordum* and *Cynara* (Briese and Sheppard 1992). One species, *L. latus*, has been recently released in Australia against weedy, introduced *Onopordum* species (D. Briese, A. Walker and W. Pettit unpublished data). Before *L. latus* could be approved for release, it was necessary to verify that it was a distinct species to *L. cynarae*, which contains populations that attack *Onopordum* species and others that attack *Cynara* species (Briese and Sheppard 1992; Sheppard *et al.* 1995). The two taxa had previously been described as subspecies (Hoffmann 1954). This was done using allozyme electrophoresis (Michalakis *et al.* 1992) and, in order to clarify the observed geographic distribution of host biotypes of *L. cynarae*, Briese *et al.* (1996) extended the study to consider micro-evolution within Mediterranean populations of these taxa.

The second case involves another oligophagous weevil, *Rhinocyllus conicus*, which attacks the capitula of thistles of the genera *Carduus*, *Cirsium*, *Silybum* and *Notobasis* and is well known for having host 'ecotypes', several of which have been identified for use as biocontrol agents against *Carduus nutans*, *C. pycnocephalus*, *Silybum marianum*, *Cirsium vulgare* and *C. arvense* (Zwölfer and Preiss 1983). In a classic study, Goeden *et al.* (1985) showed that two of the forms introduced into America were genetically distinct and this explained their relative success and failure on different host thistles. This work was later extended to identify which of the two introduced biotypes was colonizing native-American *Cirsium* species (Unruh and Goeden 1987). More recently, though, Klein (1991) and Klein and Seitz (1994) carried out a major study of 44 populations of the weevil from western Europe to Israel and obtained data on their host utilization and genetic structure using allozyme electrophoresis.

Methodology

Raw data on the allozyme frequencies of 14 regional populations of *R. conicus* from Klein and Seitz (1994) and 18 populations of *Larinus* species (from Briese *et al.* 1996) have been reanalyzed to produce phylogenetic trees for these taxa. Details of the methodologies used to obtain the original allozyme frequencies are found in Klein and Seitz (1994) and Briese *et al.* (1996), respectively.

To be consistent, the phylogenies of both *Larinus* and *Rhinocyllus* species were produced by the distance-Wagner method of Farris (1972) using the BIOSYS-1 software package (Swofford and Selander 1989). The trees were based on the Cavalli-Sforza chord distance (Cavalli-Sforza and Edwards 1969), which satisfies important assumptions about rates of evolution and the role of genetic drift (Swofford and Olsen 1990). It is desirable to include an outgroup population to provide evolutionary direction in a phylogeny, though the method of mid-point rooting (Farris 1972) can be used in the absence of a fixed root. As Klein and Seitz (1994) did not include an outgroup for *R. conicus*, the outgroup used to root the original phylogenetic tree of *Larinus* species (Briese *et al.* 1996) was omitted in the current analysis, for consistency. This did not change the branching pattern and the unrooted tree produced for the 18 populations of *Larinus* species was essentially the same as that originally rooted to *Larinus scolymi* to provide evolutionary direction (see Briese *et al.* 1996).

Phylogeny of the *Larinus latus / cynarae* group

Surveys undertaken by Briese and co-workers (Briese and Sheppard 1992; Sheppard *et al.* 1995) in the northern Mediterranean basin showed that the eastern Mediterranean species, *L. latus*, was affiliated only with *Onopordum* species, while the western Mediterranean species, *L. cynarae*, formed apparent host biotypes on *Onopordum* species in France and northern Spain, on *Cynara humilis* in southern Spain and on *Cynara cardunculus* in Italy and Greece (Fig. 1). The phylogenetic tree for *Larinus* species (Fig. 2) shows that the genetic relationships between these populations reflects the geographic separation and pattern in host-utilization. There is a clear separation between the eastern Mediterranean species, *L. latus*, and the western Mediterranean species, *L. cynarae*, while the populations sampled from each host-biotype of *L. cynarae* form separate clusters. The French populations of *L. cynarae*, affiliated with *Onopordum* species, were found to be genetically closer to *L. latus* than the Italian and Greek populations, which were affiliated more with *Cynara* species, despite the much closer proximity of the latter groups to the range of *L. latus* (Briese *et al.* 1996).

When the evolutionary history of their host plant is considered, the host-affiliation patterns become clearer. The genus *Onopordum* has its centre of origin in west central Asia and moved westward into Europe. Vicariance events in the Mediterranean area led to a separation of *Onopordum* species and secondary centres of speciation of the genus in both the Iberian/north African area and Greece/Turkey regions.

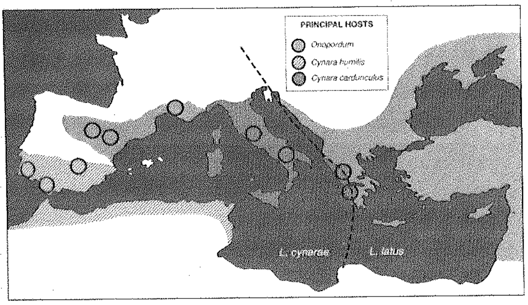

Fig. 1. Distribution of *Larinus latus* and the host biotypes of *L. cynarae* in the Mediterranean basin. Sites sampled for allozyme electrophoresis are indicated by circles.

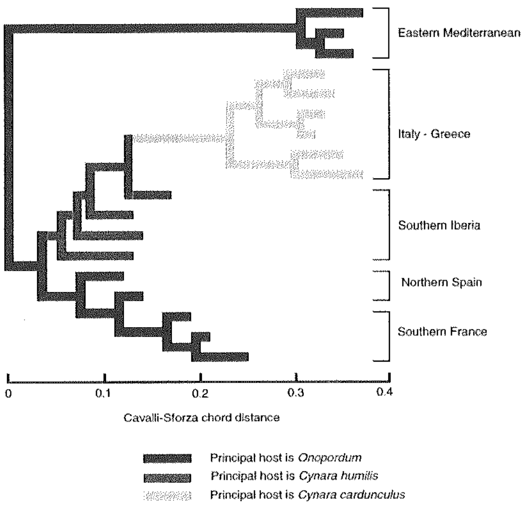

Fig. 2. Phylogenetic tree of three populations of *Larinus latus* and 15 populations of *L. cynarae* in Mediterranean Europe and their host preferences. The tree is based on the Cavalli-Sforza chord distance and has a cophenetic correlation of 0.964. Original gene frequencies used to produce tree from Briese *et al.* (1996).

The closely-related genus *Cynara* (Fig. 3) originated in the area of Iberia/north Africa and developed two main branches; one (once considered a separate genus *Bourgeia*), leading to *C. humilis*, evolved primarily in the area of origin while the other, leading to *C. cardunculus*, spread into the central and eastern Mediterranean region (Wiklund 1992). Briese *et al.* (1996) have interpreted these patterns to indicate that the ancestral form of the genus *Larinus* was affiliated with the genus *Onopordum* and spread westward from the centre of origin with this genus. Allopatric separation led to speciation between *L. latus* and *L. cynarae*, while within *L. cynarae* further dispersal into new regions has restricted gene flow. This, coupled with different patterns in the availability of the original host genus *Onopordum* and a suitable novel host, *Cynara* species, has led to shifts in host-plant affiliation. Allopatry appears essential as there was no evidence for genetic differentiation between neighbouring population-pairs in southern Spain or Italy that were collected from different hosts (Briese *et al.* 1996; see also Michalakis and Olivieri 1992). The fact that *L. cynarae* did not become associated with other available thistle genera in the absence of *Onopordum*, supports the idea that such host-shifts are restricted by the evolutionary association of this lineage of the genus *Larinus* with a particular lineage of Cardueae thistles that includes only *Onopordum* species and *Cynara* species (Fig. 3). Host-shifts also appear to have occurred subsequent to host-plant speciation rather than in parallel. Strict co-evolution does not occur and weevils of the genus *Larinus* appear to have responded to speciation within the genus *Onopordum* by incorporating new species into their host-spectrum rather than co-speciating, as was the case with the beetles in the genus *Phyllobrotica* mentioned earlier (Farrell and Mitter 1990).

Phylogeny of *Rhinocyllus conicus*

Klein (1991) sampled populations of *R. conicus* from all regions of western and Mediterranean Europe where biotypes had been recorded, including Israel (Fig. 4). The data suggest a separation into host-biotypes based on regional climate types; a Mediterranean-climate group using principally *Silybum* species, but also having an affiliation with the genera *Carduus* and *Notobasis*, a continental-climate group restricted to the genera *Carduus* and *Cirsium*, and an oceanic-climate group in western Europe, specializing on *Cirsium* species in the field (see also Zwölfer and Preiss 1983). As for *Larinus* species, the phylogenetic tree reflects this separation (Fig. 5).

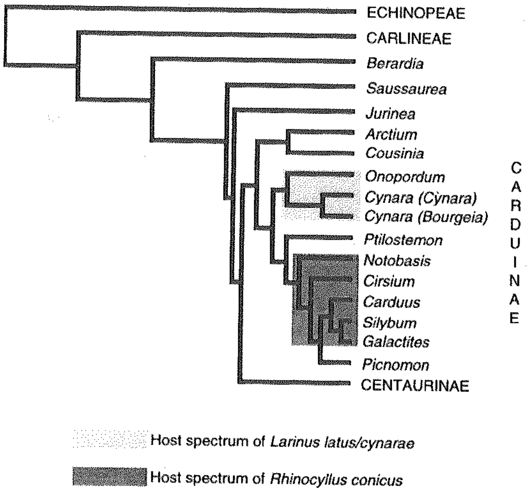

Fig. 3. Phylogenetic tree of genera of the sub-tribe Carduinae and other sub-tribes of Cardueae (Asteraceae) based on Petit (1990). The genus *Cynara* has been split into two to show the major divisions described by Wiklund (1992). Genera included in the host spectra of *Larinus latus/cynarae* and *Rhinocyllus conicus* are shaded.

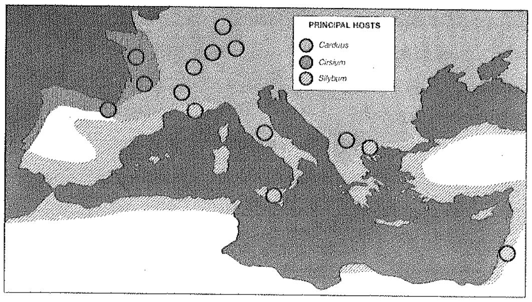

Fig. 4. Distribution of host biotypes of *Rhinocyllus conicus* in western Europe and the Mediterranean basin. Sites sampled for allozyme electrophoresis are indicated by circles (derived from data of Klein and Seitz 1994).

Following Petit (1990), the host-genera belong to a single clade of Cardueae thistles, and thus, there again appears to be a phylogenetic constraint on host-affiliation. In fact, Hoffmann (1954) also gives the genus *Galactites*, another member of this clade (see Fig. 5), as a host of *R. conicus*.

Klein (1991) showed that continental *R. conicus* are capable of breeding on *Silybum* species, but do not do so in northern Europe because the activity period of the weevil is out of synchrony with the flowering period of the thistle. The Mediterranean biotype of *R. conicus* is active four weeks earlier with a shorter oviposition period and thus is adapted to *Silybum* species as hosts. In southern France, *Carduus pycnocephalus* is attacked once *S. marianum* has finished flowering (personal observation), while in

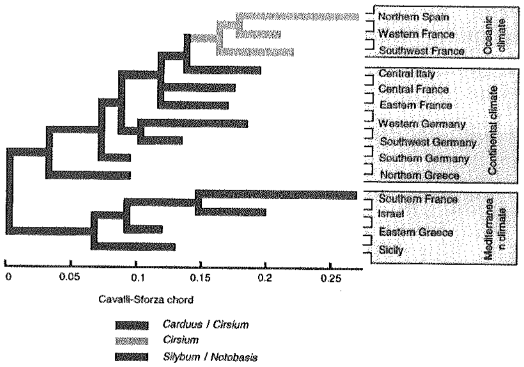

Fig. 5. Phylogenetic tree of 14 regional populations of *Rhinocyllus conicus* in west central and Mediterranean Europe and their host preferences. The tree is based on the Cavalli-Sforza chord distance and has a cophenetic correlation of 0.816. Original gene frequencies used to produce tree from Klein and Seitz (1994).

Israel, there is sequential attack on *Carduus argentatus*, *Notobasis syriaca* and *S. marianum*, following their flowering periods (Klein 1991). In addition, Klein and Seitz (1994) noted a strong concordance of morphological and genetic differences between the continental and Mediterranean groups and suggested that differences were sufficient to invoke subspecific status for the two forms. Thus, allopatric separation and the resultant disruption to gene flow, coupled with differences in relative host-plant abundance, have lead to ecological and phenological shifts in the insect populations which reinforce the speciation process. In fact, on the basis of description and geographic range, Klein and Seitz (1994) suspect that specimens described in the past as *Rhinocyllus oblongus* are probably identical to the Mediterranean form of *R. conicus*.

Klein and Seitz (1994) did not recognize the oceanic-climate group (Fig. 4) as different from the continental-climate group, as genetic differences are relatively small and are correlated with geographic distance. However, the oceanic-climate group does show a difference in activity pattern, with a longer pre-oviposition period after hibernation (Zwölfer and Römstöck-Völkl 1989) and is no longer in synchrony with the flowering period of *Carduus* species. *Cirsium* is the dominant genus of thistle in this climatic region and the activity pattern of *R. conicus* in this area favours an association with species in this genus. This suggests an adaptive cline, particularly as climate factors alone would favour an earlier onset of activity in this warmer region and maintain a synchrony with *Carduus* species. While adults from the oceanic-climate group showed no clear preference between *Cirsium* and *Carduus* species when exposed to suitable heads of each genus at the same time (Zwölfer and Preiss 1983), Klein (1991) noted a gradient in the relative field-use of *Carduus* species compared with *Cirsium* species along an east-west axis. The phylogenetic tree (Fig. 5) supports a degree of differentiation, although genetic separation is obviously not as complete as in the case of the Mediterranean-continental split. The lack of allopatry on this east-west axis means that gene flow would not be as restricted and would tend to delay the formation of biotypes exhibiting preference-differences. In continental-Europe, host-utilization patterns depend on the synchrony of flowering periods with *R. conicus* activity, with *Carduus* species being earlier than *Cirsium* species. Within regions, there was no pattern of genetic differentiation between populations from the two host-genera, and within-region genetic variation was generally less than that found between regions (Klein 1991). This implies that, for these weevils, host-race formation cannot occur in sympatry.

An evolutionary interpretation of the data would suggest that this particular *R. conicus* lineage arose in south-eastern Europe and was historically associated with a particular clade of Cardueae that included *Cirsium*, *Silybum* and *Carduus* species (the latter genus provides a link between the biotypes). Initial separation of the continental and Mediterranean groups was probably due to glaciation events, which Zwölfer (1986) considers are responsible for most biotype formation in insects associated with Cardueae thistles. Under this hypothesis, with the retreat of the ice, the continental form has spread northward through Germany and into western Europe, maintaining a strong association with the genus *Carduus*. At the western margin of their range, further differentiation is leading to specialization on *Cirsium* species, although geographic barriers are lacking and gene flow appears too high to allow the formation of biotypes with genuine host-preference-differences. The Mediterranean form has spread along the northern and southern Mediterranean shores and has become adapted principally to *Silybum* species in the north. In other areas it has also incorporated regionally-abundant members of this clade of Cardueae such as *Notobasis* species whose flowering periods overlap with weevil activity periods.

Discussion

Apart from some insect taxa that appear to track particular phytochemicals (Mitter and Farrell 1991), there is no unifying factor in the evolution of host specialization (Bernays and Chapman 1994). However, a pre-requisite for host-shifts in herbivorous insects is the existence of genetic variability for oviposition and feeding preference and larval fitness (see Carrière and Roitberg 1995). Such variability has been identified in many species, particularly polyphages (see Jaenike 1990; Via 1990; Bernays and Chapman 1994), but recent studies involving more host-specialized insects have indicated that genetic variability for host-affiliation in such groups is limited (Futuyma *et al.* 1995). In addition, comparisons of the phylogenies of specialized groups with those of their host-taxa support the notion of a strong phylogenetic conservatism in host associations (Mitter *et al.* 1991; Futuyma 1992). The two case studies here, stemming more from an interest in host-choice as it pertains to biological weed control, support the idea that host-choice can be constrained by phylogeny. In both cases, host-shifts at levels that have lead to either species, subspecies or biotypes have been confined to a few plant taxa that share a common, relatively recent, origin. Even though conservatism of host-affiliation is prevalent, phylogenetic studies tell us that it does not usually manifest itself as a strict parallel co-evolution between plant and herbivore, but rather as shifts within a limiting set of taxa (Mitter and Farrell 1991).

Given that there are strong phylogenetic constraints to the formation of new host-plant associations, the problem remains: under what conditions are such shifts likely to occur? The two studies reported here provide insights into this, for they examine situations where speciation is in the process of occurring. In both cases, a necessary condition for shifts in host-affiliation in the field has been allopatry, through either vicariance events such as glaciation, or dispersal into new regions. Such events may involve exposure to a different array of plants, and, while the range of potential hosts may be constrained by phylogeny (i.e. by the past association of ancestral forms of the herbivore with a particular lineage of host-plant), utilization patterns will depend on the relative abundance and availability of plants within these constraints. Ecological differences lead to different adaptive peaks of populations in different areas, which, reinforced by the reduced gene flow due to geographic separation, lead to behavioural shifts and host-biotype formation (e.g. biotypes of *L. cynarae* and *R. conicus*), morphological as well as ecological shifts leading to subspecific distinction (e.g. the continental and Mediterranean forms of *R. conicus*) and eventual speciation (*L. latus* and *L. cynarae*). Within the phylogenetic constraints imposed by long-term historical events, host-choice at the regional level is determined by local ecological processes. If this is coupled with restricted gene flow, new biotypes and species having new host-plant associations will arise eventually (Fig. 6).

The phylogenetic conservatism demonstrated by these studies should be noted by officials charged with regulation of agent introductions and allay their fears of sudden and unexpected host-switches. Indeed, a thorough understanding of the evolutionary history of host-association of a particular agent would enable us to ascribe host limitations and predict what species, if any, might be incorporated into the host-spectrum of an agent once released in the new region. For example, the attack of the '*Carduus*' strain of *R. conicus* on native American species of *Cirsium* was predicted and would have been entirely foreseeable from Klein's (1991) phylogenetic study.

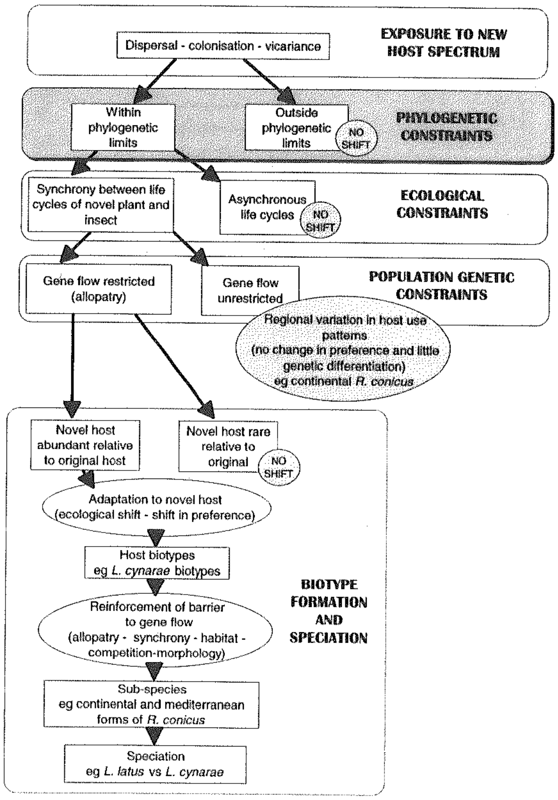

Fig. 6. Constraints to shifts in host affiliation and a proposed pathway for the formation of host biotypes and speciation in the two weevil groups studied.

It is also clear from these studies that the presence of an insect on a particular plant species does not necessarily mean that it is an appropriate agent for that plant. For example, the Mediterranean biotype of *R. conicus* will attack *C. pycnocephalus* in southern Europe, but is not in strict synchrony with it and is better adapted to *S. marianum*. The more suitable agent for *C. pycnocephalus* was the continental form of the weevil (see Goeden *et al.* 1985). Furthermore, within both the continental and Mediterranean groups, the association of *R. conicus* with different Cardueae species within its host-limits depends on the degree of synchrony between flowering and weevil activity. However, weevil phenology does not appear to be as closely associated with climate as does plant phenology. Different climatic conditions prevailing in regions where the host-plant has been introduced may therefore lead to changes in the relative phenologies of host and agent in the newly-colonized areas. Such shifts in synchrony may explain the lack of success of *R. conicus* on *C. nutans* in Australia (see Woodburn and Cullen 1993) compared to North America (see Kok and Surles 1975) although both originated from the same region in continental Europe. The relative plasticity of weevil phenology appears linked to their phylogeny and levels of gene flow and this can provide important information for predicting agent impact in areas of introduction. Where gene flow remains high between populations associated with different hosts, this association is more likely to be due to ecological factors rather than a genetically-based difference in preference.

The evolutionary constraints on many specialist insect groups may lead to phylogenetic inertia, with a retention of the ability to develop on an ancestral plant lineage. Given sufficient time and the continuity of selective pressures, such relationships will decline for older associations, but a herbivore could ‘shift’ to such a plant if the association is renewed through dispersal or colonization. Clearly, it is advantageous to know the history of the relationship between herbivore and plant hosts to make accurate decisions on the suitability of agents.

In general, this knowledge will enable weed biological control workers to define host-specificity with more assurance. Of equal importance, we are in an excellent position to undertake studies that help explore further the evolutionary ecology of herbivore-plant associations and its role in the generation of diversity. In this way we can contribute to the wider debate on the evolution of host-specialization as well as profiting from it.

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