Landscape genetics and climatic associations of flea beetle lineages and implications for biological control of tansy ragwort

M. Szücs¹, C.L. Anderson² and M. Schwarzländer³

Summary

While *Longitarsus jacobaeae* Waterhouse (Coleoptera: Chrysomelidae) has shown the best results for biocontrol of tansy ragwort, *Senecio jacobaea* L. (Asteraceae), the precise effects of this flea beetle on tansy ragwort distribution and abundance are confused by the introduction in North America of two distinct strains, of Italian and Swiss origin. Beetles of the two biotypes differ in their phenology, while hybrids of the two strains show life-cycle characteristics different from either parental strain. However, it is not known which biotype(s) currently provides control of tansy ragwort infestations or whether both biotypes or hybrids of the two have been established in the USA. Moreover, mixed populations may exhibit lower efficiency in controlling the target weed relative to pure strains, and specificity for the invasive host may be compromised. In this study, molecular markers were employed to distinguish between biotypes of *L. jacobaeae*. Analysis of mitochondrial sequence of specimens from Switzerland, Oregon and California revealed higher than expected sequence variation, within and between strains. Despite the high levels of polymorphism, cladistic analysis did not show distinct separation of strains into well-defined clades. These results indicate that Swiss *L. jacobaeae* beetles may have established in North America in contrast with the general assumptions. As the revealed mitochondrial DNA (mtDNA) polymorphisms themselves will not identify populations, we are now employing nuclear markers to characterize North American populations, their ancestry and their association with certain climatic regions. The goal is to match the most effective genotypes of *L. jacobaeae* with new tansy ragwort infestations east of the Cascade mountain range.

Keywords: COI, sequence variation, hybrid strain, insect biotypes.

Introduction

Tansy ragwort is one of the fastest spreading invasive plants in the western USA since its introduction in 1922 (McEvoy, 1984). It is particularly prevalent in Washington, California and Oregon, has spread recently into Montana and Idaho and is listed as a noxious weed in eight states. In the west of the Cascade Mountain range, tansy ragwort infestations have been most effectively controlled with biological control agents (McEvoy et al., 1991). Tansy ragwort biomass was reduced by 93% in western Oregon (Coombs et al., 1996) and by 99% in sites in northern California (Hawkes and Johnson, 1978) after the introduction of three biological control agents, but the success is primarily attributed to *Longitarsus jacobaeae* Waterhouse (McEvoy et al., 1991). Although *L. jacobaeae* is the most effective biocontrol agent, quantifying the effects of this flea beetle on tansy ragwort distribution and abundance is difficult due to the introduction of two distinct biotypes, or strains, of Italian and Swiss origin in North America (Frick, 1971; Frick and Johnson, 1973).

Beetles of the two biotypes differ in their phenology and environmental requirements, and hybrids of the two biotypes show phenologies different from that of either
parental strain (Frick, 1971; Frick and Johnson, 1973). While the establishment of the Italian biotype of *L. jacobaeae* is widely assumed in western coastal areas, the fate of the originally released Swiss beetles in Del Norte County, California (Frick, 1970) is not known. Thus, it is not clear which biotype(s) are currently providing effective control of coastal infestations of tansy ragwort or whether hybrids of these biotypes have been established in the USA. Beetles derived from these North American coastal populations have failed to establish in areas east of the Cascade Mountain range, which are characterized by a continental climate with cold winters (Turner and McEvoy, 1995). Mixed populations might be less effective in controlling tansy ragwort infestations, and hybrids may differ in their host specificity, placing native coniferal plants at risk (Hoffman et al., 2002). Consequently, it is a matter of great importance to determine the origins and composition of extant populations in North America.

Comparison of the genetic composition of extant *L. jacobaeae* populations in North America with European source populations will not only indicate the origins of North American populations but also reveal patterns of hybridization and climatic associations of *L. jacobaeae* lineages. In this study, we investigated two introduced North American populations and one Swiss population of *L. jacobaeae* to assess whether mtDNA sequences can reveal sufficient genetic variation and differentiation between strains that may allow development of molecular markers that can distinguish biotypes of the flea beetle.

## Methods and materials

### DNA extraction

DNA was extracted from 50 individuals using a DNeasy Tissue Kit (Qiagen Inc., Valencia, CA), following the manufacturer’s protocol. Twenty-two individuals were collected from Mettembert, Switzerland (47°24′N, 7°20′E), 14 specimens were collected both from Salem, OR (44°93′N, 122°99′W) and from Crescent City, CA (41°44′N, 124°08′W).

### DNA amplification

Polymerase chain reaction (PCR) was performed with general-purpose insect-derived primers that are known to amplify a fragment of the coleopteran mtDNA genome (Szalanski and Owens, 2003):

C1-J-2797: 5′-CCTCAGCAGTTATCTAGATTAC-3′
C2-N-3400: 5′-TCAATATCATTGATGACCAAT-3′

These primers amplify the 3′ end of the cytochrome oxidase I gene, the transfer RNA for leucine, and the 5′ end of cytochrome oxidase II gene. Each reaction was carried out in 20 μl reaction volume, containing 1× Colorless GoTaq buffer pH 8.5, 1.5 mM MgCl₂, 1 U of GoTaq DNA polymerase (Promega), 10 pmol of each primer, 0.2 mM deoxyribonucleotide triphosphate and approximately 25 ng DNA. Thermal cycling conditions were 3 min at 94°C, followed by 40 cycles of 30 s at 94°C, 20 s at 51°C, 50 s at 72°C, with a final step of 3 min at 72°C.

### DNA sequencing and analysis

PCR products were prepared for sequencing using ExoSAPit (GE Healthcare Corp., Piscataway, NJ), following the manufacturer’s protocol. Cleaned samples were sequenced with Big Dye version 3.1 following the manufacturer’s protocol and the reactions run on an Applied Biosystems 3130xl automated sequencer. Sequences were edited and aligned with Sequecher 4.1 (Gene Codes Corp., Ann Arbor, MI). Parsimony analysis was carried out using PAUP 4.0b10 for Macintosh (Swofford, 2002). For the tree shown in Fig. 1, *Aphthona cyparisiae* Koch was used as an outgroup to root the tree (accession number gi88810025), incorporating 541 bp of sequence data. Thirty-two sites were parsimony-informative, including 50 haplotypes from North American and Swiss populations. Search was full heuristic, with branch swapping [tree bisection-reconnection (TBR)] and gaps treated as missing data. To gauge the reliability of the observed topology, we did a further round of bootstrap analysis using a different outgroup, *Diabrotica barberi* Smith and Lawrence (accession number gi11275649), and a reduced number of taxa. For the bootstrap analysis, we used 27 haplotype samples representing all the major branches of the tree shown in Fig. 1, incorporating 588 bp of sequence data, of which 13 sites were parsimony-informative. Search was full heuristic with branch swapping, gaps were treated as missing data and 100 replicates were performed.

### Results

The *L. jacobaeae* sequences revealed an unexpectedly high level of polymorphism. Ninety-eight polymorphic sites were detected within the analysed 541 bp region, of which 32 were parsimony-informative. The sequences were obtained from 50 individuals from three populations (two North American and one Swiss). By way of comparison, sequencing the same region from 22 individuals of the southern corn rootworm, *Diabrotica undecimpunctata*, revealed two haplotypes, differentiated by a single nucleotide polymorphism (Szalanski and Owens, 2003). High levels of polymorphism, notwithstanding, parsimony analysis of the unique haplotypes did not show clustering of strains into well-defined clades. Results of this analysis are shown in Fig. 1. To test the reliability of the topology we obtained in our parsimony analysis, we carried out bootstrap analysis (Hillis and Bull, 1993). Bootstrap analysis indicated a 50% majority-rule consensus tree in which
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all North American and Swiss samples cluster in a simple polytomy (Fig. 2).

**Discussion**

Our results show that there is substantial sequence variation in the mitochondrial genome of *L. jacobaeae*, both within and between strains, suggesting that further investigation of other regions will yield genetic markers indicative of strain type. Both Italian- and Swiss-strain beetles were originally released in California, but only the establishment of the Italian biotype was confirmed, and it is now widely accepted that all beetles distributed along the west coast are of Italian origin (Turner and McEvoy, 1995). According to this assumption, the CA and OR populations sampled for this study...
were derived from the Italian strain. Our results are surprising in light of this general assumption. Because of the strikingly high level of observed polymorphism, a trend would be expected for the clustering of haplotypes if the sampled beetles represent two distinct biotypes, such as the Swiss and Italian. One possible explanation for the lack of segregation of strains may be that Swiss beetles from the original releases have established in California, and either significant hybridization or introgression occurred between the Italian and Swiss beetles. This seems a plausible scenario, as L. jacobaeae used for this analysis was collected in Del Norte County in California where Swiss beetles were initially released and Californian populations later provided the source populations for the redistribution of flea beetles along the west coast.

However, definitive conclusions cannot be drawn from this sampling because it lacks representative beetle strains of known Italian origin. Therefore, we are employing nuclear markers to assess the ancestry of North American populations and their association with certain climatic regions. The goal is to match the most effective genotypes of L. jacobaeae with new tansy ragwort infestations east of the Cascade Mountain range.

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References


Frick, K. E. (1971) Longitarsus jacobaeae (Coleoptera: Chrysomelidae), a flea beetle for the biological control of tansy ragwort. II. Life history of a Swiss biotype. Annals of the Entomological Society of America 64, 834–840.


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