**Propagule Pressure, Genetic Structure, and Geographic Origins of *Chondrilla juncea* (Asteraceae): An Apomictic Invader on Three Continents**


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- **Premise of the study:** Assessing propagule pressure and geographic origins of invasive species provides insight into the invasion process. Rush skeletonweed (*Chondrilla juncea; Asteraceae*) is an apomictic, perennial plant that is invasive in Australia, South America (Argentina, and North America (Canada and the United States). This study comprehensively compares propagule pressure and geographic structure of genotypes to improve our understanding of a clonal invasion and enhance management strategies.

- **Methods:** We analyzed 1056 native range plants from Eurasia and 1156 plants from three invaded continents using amplified fragment length polymorphism (AFLP) techniques. We used measures of diversity (Simpson’s *D*) and evenness (*E*), analysis of molecular variance, and Mantel tests to compare invasions, and genotype similarity to determine origins of invasive genotypes.

- **Key results:** We found 682 unique genotypes in the native range, but only 13 in the invaded regions. Each invaded region contained distinct AFLP genotypes, suggesting independent introduction events, probably with different geographic origins. Relatively low propagule pressure was associated with each introduction around the globe, but levels of among-population variation differed. We found exact AFLP genotype matches between the native and invaded ranges for five of the 13 invasive genotypes.

- **Conclusions:** Invasion dynamics can vary across invaded ranges within a species. Intensive sampling for molecular analyses can provide insight for understanding intraspecific invasion dynamics, which can hold significance for the management of plant species, especially by finding origins and distributions of invasive genotypes for classical biological control efforts.

**Key words:** AFLPs; Asteraceae; biological control; *Chondrilla juncea*; invasive; origins; propagule pressure; rush skeletonweed; weed.

The invasion process can be viewed as a series of steps in which propagules of a species (e.g., seeds, eggs, larvae, rhizome and stem fragments, mature individuals) are taken up from the native or already invaded range and transported to a new area (Kolar and Lodge, 2001; Sakai et al., 2001). Across the range of a widespread invasive species there may be dissimilarities in propagule pressure (founder population size and/or number of introduction events), genetic diversity, allocation of resources, levels of competitive ability, or resistance or tolerance to control methods including natural enemies, all of which have implications for ongoing invasion dynamics (e.g., Hänfling et al., 2002; Urban et al., 2008; Farrer et al., 2011; Robert et al., 2012).

Propagule pressure is now recognized as a predictor of establishment success and the likelihood of invasion (Kolar and Lodge, 2001; Colautti and MacIsaac, 2004; Lockwood et al., 2009; Simberloff, 2009) and is determined by the propagule size, or propagule number, or both (sensu Simberloff, 2009). Propagule size is defined as the number of individuals in a propagule (founder population size), and propagule number is defined as the number of discrete introduction events (Simberloff, 2009). Whether the propagules come from one source or from different sources in a metapopulation (“propagule pool” vs. “migrant pool” sensu Slatkin (1977)) can have bearing on the genetic diversity of the invasion. Recent literature has focused on the importance of multiple introduction events during biological invasions, and a growing body of evidence suggests that multiple introductions may be the rule rather than the exception (Novak and Mack, 2001, 2005; Wares et al., 2005; Lavergne and Molofsky, 2007; Dlugosch and Parker, 2008; Kolbe et al., 2008; Wilson et al., 2009; Keller and Taylor, 2010; Estoup and Guillemaud, 2010).

With the number of biological invasions and their negative consequences expected to increase in the future, determining how, when, and from where invaders are introduced becomes crucial to gaining a better understanding of the role of propagule pressure in the invasion process (Novak, 2007; Wilson et al., 2009; Estoup and Guillemaud, 2010). Determining the propagule...
pressure of a biological invasion can be done using historical information (e.g., herbarium or museum specimens, or written accounts) that provides insights into the number of founders and/or the number of introduction events in the new range (Simberloff, 2009; Estoup and Guillemaud, 2010; Huttanus et al., 2011), but this information is often lacking for accidentally introduced plants. The manner in which a species is introduced into its new range (i.e., its propague pressure) holds genetic consequences for invasive populations. Thus, molecular markers and population genetic parameters can be used to estimate the genetic signature of propague pressure and identify the geographic origins of invasive populations (Estoup and Guillemaud, 2010; Novak, 2011). The use of molecular markers can also provide insight into the genetic variation of invasive populations that may be driven by founder effects, postintroduction hybridization, and/or natural selection (Ellstrand and Schierenbeck, 2000; Sakai et al., 2001; Lee, 2002; Petit, 2004; Goolsby et al., 2006a; Keller and Taylor, 2008; Le Roux and Wieczorek, 2009; Prentis et al., 2009). In addition, understanding the amount and distribution of genetic diversity within and among invasive populations can enhance the efficacy of management programs (Roderick and Navajas, 2003; Müller-Schirer et al., 2004; Strong, 2004; Gaskin et al., 2011). For example, determining the geographic origins of invasive genotypes can aid in the search for effective classical biological control agents, as natural enemies can be host-specific at the population or genotype level (e.g., Garcia-Rossi et al., 2003; Evans et al., 2005; Goolsby et al., 2006b).

Rush skeletonweed (Chondrilla juncea L., Asteraceae) is an invasive perennial herb native to Eurasia and North Africa (McVean, 1966; Panetta and Dodd, 1987) that primarily reproduces clonally via autonomous gametophytic apomixis, though there may be some residual sexuality in the native range (Chaboudez, 1994). This species has been accidently introduced to and has invaded Australia, Argentina, Canada, and the United States (Schirman and Robocker, 1967; Cullen and Groves, 1977; Tortosa and Medan, 1977). In Australia, C. juncea is recognized as the most problematic weed of wheat-growing regions (Cuthbertson, 1967; Panetta and Dodd, 1987). Negative economic consequences include reductions of up to 80% in wheat yield (Panetta and Dodd, 1987) and damage to wheat harvest machinery (Cuthbertson, 1967). In Argentina, the species is considered a National Plague of Agriculture by the Fiscalización Fitosanitaria. In the northwestern United States, C. juncea now occupies approximately 2.5 million ha of croplands, grazing lands, and natural areas (Cuthbertson, 1967; Sheley and Hudak, 1995). In Idaho alone, the infested area has increased from 20 ha in the 1960s to 1.4 million ha in the mid-1980s, most notably in national forests (Piper and Andres, 1995).

Previous studies using morphological, phenological, and allozyme data indicated that C. juncea invasions consist of multiple introduced biotypes (Chaboudez, 1994; Hasan et al., 1995; McCaffrey et al., 1996) that have different susceptibility to certain biological control agents and herbicides (Burdon et al., 1984; Black et al., 1998; Campanella et al., 2009). For example, due to extreme host-specificity, imported strains of the rust biological control agent Puccinia chondrillina Bubak & Syd. are not effective against two of the three C. juncea biotypes in Australia, one of the three biotypes in the United States, and have had little effect on plants in Argentina. In Australia, the two rust-resistant biotypes of C. juncea have extended their distribution to replace the susceptible biotype (Burdon et al., 1981). For these and other reasons, biological control of C. juncea has not been considered successful on any invaded continent (Burdon et al., 1981; Prather, 1993; Vigna et al., 1993; Milan et al., 2006).

Chondrilla juncea possesses a wide geographic distribution in its native range (McVean, 1966; Panetta and Dodd, 1987), but many native range regions have not been sampled or had plants analyzed with genetic markers. In addition, C. juncea biotypes in Canada, western Australia, and eastern North America invasions have not been identified, and there has been no comprehensive comparison of C. juncea genotypes among the three invaded continents. Morphological and phenological biotypes of C. juncea are difficult to identify unless plants are grown under common garden conditions (Hill and Groves, 1973). Allozyme techniques were used to identify three biotypes in Australia, three in the United States, and three in Argentina, and plants from Australia and the United States have been compared with plants from a portion of the native range: the former Yugoslavia, Italy, Greece, and Turkey (Chaboudez et al., 1992; Hasan et al., 1995). However, the use of allozymes generally underestimates genetic variability compared with more recently developed marker systems such as AFLPs, simple sequence repeats (SSRs) and inter-simple sequence repeats (ISSRs) (Cabrera et al., 2001; Ipek et al., 2003; Le Roux and Wieczorek, 2009).

The goal of this study was to improve our understanding of the C. juncea invasion. We hypothesized that low propagule pressure (i.e., few genotypes and few introduction events) of this invasion led to strong geographic structuring on a regional basis. We also hypothesized that the use of highly polymorphic molecular markers such as AFLPs, along with more comprehensive sampling, will provide a more complete inventory of native and invasive genotypes, improving our ability to find invasion origins. We will discuss the implications of these results on future biological control efforts directed toward C. juncea and other clonally propagating invasive plant species.

**Materials and Methods**

**Plant Collections**—Ligules (petal-like corollas of ray florets found in composite flowers) were collected because earlier attempts at DNA amplification from C. juncea leaf tissue were unsuccessful, possibly due to secondary compounds associated with the latex in vegetative portions of these plants. To avoid foreign pollen contamination, collectors endeavored to collect flowering heads just prior to their opening. Each flower head remains open for approximately 1 d (Panetta and Dodd, 1987), though on any given day, multiple flower heads can be open on a plant. Collectors did not obtain samples from plants closer than 5 m apart to avoid collecting ramets connected by underground rhizomes.

We sampled a total of 2206 plants on five continents. In the native range, we sampled 1050 plants from 149 populations in 21 countries ranging from Spain to Uzbekistan (Fig. 1; Appendix S1, see Supplemental Data with the online version of this article). The final number of plants sampled from each native population varied from 1 to 20, with an average ±SD of 7.0 ± 4.3 plants per population. In the invaded range, we sampled 1156 plants from 154 populations (North America: 721 plants, 96 populations; Australia: 377 plants, 52 populations; Argentina: 58 plants, six populations; Figs. 2, 3A–C, Appendix S2, see online Supplemental Data). The final number of plants sampled from each invasive population varied from 1 to 20, with an average of 7.5 ± 3.9 plants per population. The number of populations sampled was higher in putative areas of original introduction such as the vicinity of Wagga Wagga, New South Wales, Australia and Spokane, Washington, United States, and in densely invaded areas such as southern Idaho, and the central California foothills, United States. We sampled relatively few populations in Argentina as the invasion is limited to an area approximately 200 x 300 km, and also in western Australia and the eastern United States, where C. juncea is subject to a rigorous eradication program or is less common, respectively. We also genotyped Australia’s Commonwealth
Diversity and geographic structure of genotypes — To determine how different genotypes are geographically distributed in the invasive and native ranges, we used the Dice similarity coefficient: 2|G|/|A|, where |G| is the number of invasive genotypes and |A| is the number of native genotypes. This coefficient considers that when both samples are missing the same genotype, the Dice similarity is zero. When one sample is completely different from the other, the Dice coefficient is also zero. Intermediate similarity values range from 0 to 1, with higher values indicating that the number of plants representing each genotype is more evenly distributed.

To assess how genetic variation is distributed within and among populations and regions, we used analysis of molecular variance (AMOVA), as implemented in the program Arlequin version 3.5.1.2 (Excoffier et al., 1992). For some populations, we collected only a few individuals; thus, we assessed the bias of omitting under-collected populations by performing AMOVA both with and without populations containing fewer than five individuals.

For both the native and invaded ranges, we determined the correlation between genetic and geographic distances using a Mantel test. To obtain genetic distances between populations, we used the statistical software R v 2.13.0 (R Development Core Team, 2011) with the Hickory function from AFLPdat (Ehrich, 2006) to convert AFLP data into the correct format for Hickory v1.1 (Holsinger et al., 2002). Using default parameters in Hickory for burn-in, sampling, and thinning, we used the option that does not assume Hardy–Weinberg equilibrium (the f-free model) to estimate FST. The FST estimates (Eurasia, 0.497; North America and Argentina, 0.499; Australia, 0.498) were incorporated into the program AFLP-surv (Vekemans, 2002) to determine Nei’s genetic distance (Nei, 1972), with Lynch and Milligan’s (1994) correction, between populations. Estimates of allelic frequencies were compared with the fourth option in AFLP-surv; “Bayesian method with non-uniform prior distribution of allele frequencies”. Nei’s genetic distances between populations were compared to geographic distances between populations (calculated from decimal-degree coordinates using the AFLPdat function Geodist in R) in a Mantel test using the program IBD v 1.53 (Jensen et al., 2005) with 1000 permutations. Mantel tests were performed on what we considered contiguous ranges of C. juncea (Eurasia, western North America, eastern Australia, western Australia, and Argentina).

AFLP error rate and number of genotypes — From the two pairs of AFLP primers, we selected 121 variable loci for the
2206 *C. juncea* plants analyzed in this study. AFLP data are shown in Appendix S3 (see Supplemental Data with the online version of this article). There were 129/5808 mismatched AFLP calls for the 48 repeated plants, equaling a 2.2% (2.8/121 loci per plant) error rate.

In the native range, assuming that plants are actually identical genotypes if their alleles differed at a maximum of three AFLP loci, we found 682 unique genotypes (*G*) among the 1050 plants analyzed, with 576 singletons and 474 plants that were genetically identical to one or more other plants. One of the 149 native range populations was monotypic (data not shown). The number of clones (plants) for each genotype was \(n = 45, 39, 33, 15, 14, 13\); all other genotypes consisted of fewer than 10 clones. We were able to distinguish 13 AFLP genotypes among the three invaded regions, with seven in North America (genotypes 1, 1a, 2, 3, 3a, 8, 9) and three each in Australia (genotypes 5, 6, 7) and Argentina (genotypes 4, 4a, 4b) (Figs. 2, 3A–C; Table 1). An “a” or “b” after the genotype number indicates that the genotype was very similar (≥0.95 Dice similarity) to a genotype designated by an integer only; e.g., genotypes 3

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**Fig. 2.** Map of sampled *Chondrilla juncea* L. populations in western North America. Population identification number is inside, or next to, each circle. Circles of same color are the same genotype. Our estimates of current contiguous distributions of populations are shown by solid lines based on USDA (2005), Rice (2005), Calflora (2008), WeedMapper (2009), herbarium collections, and our personal field observations.
Fig. 3. Maps of sampled *Chondrilla juncea* L. populations in (A) midwestern and eastern North America, (B) Australia, and (C) Argentina. Population identification number is inside, or next to, each circle. Circles of same color are the same genotype, and no genotypes are shared among invasions on different continents. Our estimates of current contiguous distributions of populations are shown by solid lines, or dashed lines for historical (and probably extinct) distributions, and are based on Hill and Groves (1973), Vigna and Lopez (1989), USDA (2005), Rice (2005), Calflora (2008), A.V.H. (2009), WeedMapper (2009), herbarium collections, and our personal field observations.
which was monotypic. We also performed AMOVAs on sets of populations (<i>P</i> < 0.001; online Appendix S5). The AMOVA of Argentina samples showed little differentiation (7.4%, <i>P</i> = 0.08) among populations, whereas all other invaded regions and contiguous invasions contained 87–96% of variation among populations (<i>P</i> < 0.001), except for the central United States, which was monotypic. We also performed AMOVAs on sets of data with populations containing fewer than five individuals included (data not shown). Percentages of variation among or within populations did not vary by more than 1% from the results shown in Appendix S5.

The presence of only one native population that was monotypic was surprising in comparison with the many monotypic populations in North America and Australia. Chaboudez (1994) found high diversity among populations in Turkey (91 genotypes across 123 populations). Along with our finding of only a single monotypic population, that suggests that sexual reproduction in the native range may be more common than previously suspected. The low to moderate correlation between genetic distance and geographic distance suggests gene flow decreases with distance across the native range. This also appears to be true for North America and Australia, whereas gene flow does not appear to be limited in the relatively small geographic area invaded by <i>C. juncea</i> in Argentina.

The majority of genetic variation in North America is distributed among populations as indicated by the AMOVA results.
and high percentage of monotypic populations, suggesting that, barring the extirpation of genotypes through stochastic events or natural selection, the few population founding events usually contained single propagules or multiple genotypically identical propagules from the same source population. In addition, our data suggest low propagule pressure from genetically distinct established populations during subsequent range expansion. The same pattern appears to hold for Australian populations, where genetic variation is mostly partitioned among populations. Thus, Australian populations also appear to have been
The new genotypes (8 and 9) found in the eastern United States have no previous biotype designations.

Three biotypes and three allozyme genotypes had previously been reported for Australia (Burdon et al., 1980), and we did not find any additional genotypes. Our AFLP genotypes correlate with Australian biotypes as follows: genotype 5 = Form C-broad rosette leaves; 6 = Form A-narrow rosette leaves; 7 = Form B-intermediate width rosette leaves. Hill and Groves (1973) suggested that Form A once had the most widespread distribution in eastern Australia, from southeastern Queensland to the New South Wales/Victoria border, and west into South Australia. Form B and Form C were found to be limited to east-central New South Wales. Burdon et al. (1981) found that Forms B and C had increased their range since the 1973 study, probably because biological control efforts created a decline in the frequency of Form A in central New South Wales (Fig. 3B). In contrast, we find that all three AFLP genotypes are relatively common and evenly represented in eastern Australia. These findings may be explained by two scenarios: (1) Form A was not reduced by biological control methods as suggested by Burdon et al. (1981), or (2) Form A may have increased in abundance since the 1981 study despite biocontrol efforts. Additional research will be required to assess which of these scenarios explains our result. In a previous study in Argentina (Sacco, 1988), it was unclear how the common and rare allozyme genotypes were distributed within and among populations of this invasion, so we can make no comparisons to our present data.

**Comparison to earlier studies**—In western North America AFLP genotypes 1 and 1a correlate with the “Banks” biotype, genotype 2 with the “Washington early-flowering” biotype, and genotypes 3 and 3a, with the “Washington late-flowering” biotype (Schirman and Robocker, 1967; Rosenthal et al., 1968; Lee, 1986; Hasan et al., 1995; McCaffrey et al., 1996). Our wider sampling increased the known number of invasive genotypes described in North America from three to seven, with two of these (8 and 9) being genetically distinct from previously identified genotypes.

**Geographic origins**—In the native populations, we found approximately double the number of genotypes found in an earlier study (682 genotypes from 1050 plants [65%] compared to 326 genotypes from 983 plants [33%]; Chaboudez et al., 1992), suggesting that more intensive plant sampling in the native range and the use of AFLP markers improved our fine-scale knowledge of established following low propagule pressure (i.e., from single or multiple genetically homogenous propagules). In Argentina, the opposite pattern is observed, where genetic variation is found mostly within populations, but still propagule pressure appears low, with few genotypes introduced, and these are likely from a single introduction event and origin since they are genetically very similar.

<table>
<thead>
<tr>
<th>Region</th>
<th>No. of plants sampled</th>
<th>G</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eurasia</td>
<td>1050</td>
<td>682</td>
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<td>0.96</td>
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<tr>
<td>Argentina</td>
<td>58</td>
<td>3</td>
<td>0.20</td>
<td>0.38</td>
</tr>
<tr>
<td>Australia</td>
<td>377</td>
<td>3</td>
<td>0.66</td>
<td>0.98</td>
</tr>
<tr>
<td>Western Australia</td>
<td>110</td>
<td>3</td>
<td>0.57</td>
<td>0.88</td>
</tr>
<tr>
<td>Eastern Australia</td>
<td>267</td>
<td>3</td>
<td>0.63</td>
<td>0.94</td>
</tr>
<tr>
<td>North America (NA)</td>
<td>721</td>
<td>7</td>
<td>0.73</td>
<td>0.84</td>
</tr>
<tr>
<td>Western NA</td>
<td>632</td>
<td>5</td>
<td>0.67</td>
<td>0.84</td>
</tr>
<tr>
<td>Midwestern NA</td>
<td>32</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Eastern NA</td>
<td>57</td>
<td>3</td>
<td>0.25</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Notes: G, number of genotypes found in region; D, Simpson’s diversity index corrected for finite sampling; E, Evenness.

**Table 3. Highest Dice pairwise similarity coefficients between native and invasive Chondrilla juncea. Highest match of an invasive genotype to a native genotype is followed by highest match to a native genotype from an alternate population.**

<table>
<thead>
<tr>
<th>Invasive genotype</th>
<th>Invasion</th>
<th>Dice similarity</th>
<th>Country</th>
<th>Pop. no.</th>
<th>Individual no.</th>
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<td>Bulgaria</td>
<td>7</td>
<td>236</td>
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<td></td>
<td></td>
<td>0.97</td>
<td>Macedonia</td>
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<td>860</td>
</tr>
<tr>
<td>1a</td>
<td>North America</td>
<td>1.00</td>
<td>Bulgaria</td>
<td>7</td>
<td>235</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.97</td>
<td>Macedonia</td>
<td>86</td>
<td>860</td>
</tr>
<tr>
<td>2</td>
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<td>Germany</td>
<td>43</td>
<td>425</td>
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<tr>
<td></td>
<td></td>
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<td>Spain</td>
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<td>401</td>
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<td>North America</td>
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<td>France</td>
<td>27</td>
<td>230</td>
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<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>Spain</td>
<td>112</td>
<td>173</td>
</tr>
<tr>
<td>3a</td>
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<td></td>
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<td>Russia</td>
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Notes: For population location and GPS coordinates, see Appendix S1.
invaded range origins. Our data indicate that populations of *C. juncea* in North America, Australia, and Argentina do not share genotypes, suggesting that the introductions into these three regions were independent events, probably with different geographic origins, as has been found in other invasions (e.g., Besnard et al., 2007; Reusch et al., 2010).

To determine geographic origins of invasive genotypes, it is helpful, but not required, for native populations to possess significant genetic structure (Keller and Taylor, 2008; Novak, 2011). If genetic diversity is highly structured, genotype comparisons may be used to pinpoint origins to a specific population (e.g., the invasion came from population A), and most individuals in an invasive population are genetically similar to individuals in population A (e.g., Novak and Mack, 2001; Goolsby et al., 2006a). If genetic diversity is less structured, origins may not be linked to a specific population but may be associated with a certain region in the native range (e.g., Milne and Abbott, 2004). In our study, we were able to describe exact matches between native and invasive AFLP genotypes for five of the 13 invasive genotypes. In addition, we found high genetic similarity (>0.95) for three other genotypes. Identifying the geographic origins of an invasive is not only facilitated by high genetic structure, but increases in accuracy with more intensive sampling of native populations (Muirhead et al., 2008). In addition, the probability of identifying the geographic origins of an invasion is much higher for species with relatively low genetic diversity, such as self-pollinating plants and species that reproduce through asexual (clonal) means (Novak and Mack, 2001; Facon et al., 2003; Novak, 2011).

We found Dice similarity matches between invasive genotypes and individual native plants that differ from assessments of geographic origins previously described. In earlier work, plants from the former Yugoslavia, Italy, Greece, and Turkey (Chaboudez et al., 1992; Chaboudez, 1994; Hasan et al., 1995) were analyzed, and Hasan et al. (1995) found allozyme genotypes in Yugoslavia and Greece that matched two of the three patterns found in the USA (our genotypes 1 and 3, and perhaps 1a and 3a). Hasan et al. (1995) also suggested that the genetic relationships among native and invasive populations might be further explored by sampling plants more broadly across Eurasia and using a more polymorphic genetic marker. Just such an approach was taken in our study. In general, we found the best genotype matches in different regions of the native range compared with the regions reported in Hasan et al. (1995). For genotypes 1 and 1a, our best match occurred in Bulgaria, which is geographically close to their matches from Yugoslavia and Greece, but for genotypes 3 and 3a, our best matches occurred in populations from western Europe (France and Spain) instead of their match in Yugoslavia. We also found a 100% match in Germany for genotype 2, for which there was no previous native range allozyme match.

Chaboudez et al. (1992) focused on comparisons of Australian genotypes with plants from former Yugoslavia, Italy, Greece, and Turkey. Later, Chaboudez (1994) focused solely on collections from Turkey. Neither study found an exact match between native and invasive populations using allozymes. We also failed to find an exact match for Australian genotypes 5, 6, and 7, but did find a 0.95 match in Greece for genotype 6 and a 0.99 match in Macedonia for genotype 7. Although we sampled heavily in Turkey (Fig. 1), we did not find any Dice similarities high enough to imply that this country is the origin of Australia’s *C. juncea*.

**Implications for biological control**—Some present *C. juncea* biological control agents are host-specific at the genotypic level (Emge et al., 1981; Cullen and Moore, 1983) and thus not effective on all genotypes associated with an invasion. The discovery of identical or highly similar AFLP genotypes between the invasive and native ranges will allow future testing to determine whether the most highly coevolved agents (those found on plants with the same genotype) will be the most effective biological control agents (but see Hokkanen and Pimentel [1989]) for a discussion of “new associations”).

In western North America, we found that certain genotypes dominate certain areas of the invasion. Though the outcomes of biological control efforts are dependent on many factors, including life history, genetics of the control agent and environmental conditions (Chaboudez and Sheppard, 1995), if the invasion in a certain region is genetically monotypic (e.g., California) or nearly monotypic (e.g., southern Idaho), the regional invasion may be easier to control if an effective agent is found (Müller-Schärer et al., 2004).

While we did not find any additional genotypes in Australia, our study provides a better description of the geographic distribution of genotypes, allowing for more precise application of biological control agents. In western Australia, genotypes had not previously been identified, and based on our results genotype 6 is the most common (Fig. 3B; Table 1). This is the only Australian genotype susceptible to the rust *Puccinia chondrillina* released in eastern Australia, suggesting that the use of that agent in western Australia may be effective in the future. Our inability to find closer matches for genotype 5 and 6 suggests that the origins for these genotypes remain unsampled and may possibly occur in North Africa or further east in Asia.

In Australia and North America, the large amount of genetic variation partitioned among populations and the lack of genetic similarity between many genotypes poses the challenge of finding highly host-specific biological control agents for each genotype or finding an agent that is effective against all invasive genotypes in these regions. The control of only a subset of genotypes present in a population or region may lead to expansion of the more tolerant/resistant genotypes (Burdon et al., 1981), i.e., “self-defeating biological control” (Garcia-Rossi et al., 2003).

Currently, Argentina has used biological control agents developed for Australia and/or the USA, and foreign exploration for rust strains with high specificity to any of Sacco’s (1988) three allozyme genotypes has not occurred. We failed to find high similarity matches (>0.95) for Argentine genotypes, indicating that the geographic origins of this invasion are undetermined. However, our results do point to France or Spain as regions for more intensive population sampling. Because the Argentina invasion encompasses a relatively small area compared to the invasions in North America or Australia, and most populations are mixtures of genetically similar AFLP genotypes (0.97–0.99 similarity), it is possible that all three genotypes will respond similarly to biological control agents (Sacco, 1988), but we suggest that all three genotypes should be included in any potential agent testing.

**Conclusions**—Highly variable AFLP markers are useful for distinguishing between lineages of clonal invasive species such as *C. juncea*. Only a small number of invasive genotypes (*n* = 13) were detected across the three invaded continents, compared with 682 genotypes across all native populations. This result alone indicates relatively low propagule pressure for invasion. Through the consistent use of the same molecular marker system across the entire range of *C. juncea*, we were able
to find the geographic origins for many genotypes. In addition, we have detected differences in levels of AFLP diversity, evenness, and genetic structure of invaded regions and contiguous invasions. In some instances the use of more comprehensive population sampling and AFLP analysis produced results that mirror those previously obtained using allozymes (genotypes or biotypes introduced into Australia), and in other instances our study provided additional information (seven rather than three genotypes in North America).

Results of this study should enhance coordinated, multinational efforts to control this important invasive species and should be considered a useful addition to a classical biological control program. Identification of all genotypes in an invasion, which is likely possible in many clonally reproducing species, allows more complete testing of the host-specificity of potential biological control agents, thus lowering the risk that there will be unexpected resistance or tolerance to introduced biological control agents.

LITERATURE CITED


