Multiple introductions from the Iberian peninsula are responsible for invasion of *Crupina vulgaris* in western North America

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Received: *3 September 2001*
Accepted: *6 December 2001*

Summary

- *Crupina vulgaris* is a relatively recent invader to grasslands and other open habitats in western North America. Like related *Centaurea* species, it was introduced from the Mediterranean region, where it does not exhibit ruderal behavior. Determining the number and sources of invasion founders allows fuller interpretation of colonization dynamics and recognition of potential intercontinental carriers, both critical factors for curbing the spread of invasive species.
- We chose the molecular technique of random amplified polymorphic DNA (RAPD) to identify the number and sources of invasion founders from the eastern hemisphere, by comparing indigenous and invasive populations.
- Our results indicated that the five North American populations derived from three or more successful invasion events whose founders originated in the Iberian peninsula.
- Also inferred by the similarity clustering among eastern hemisphere populations is a more ancient origin of the genus to the east of the Mediterranean, a concept supported by the scarcity of suitable nonanthropic habitat in Spain. Its epizoochoric association with migratory movements of domestic herds suggests probable routes of migration first to southern Europe, then later to North America.

Key words: *Asteraceae*, colonization, *Crupina vulgaris*, invasion founders, molecular markers, RAPDs.

© *New Phytologist* (2002) **154**: 419–428

Introduction

Crupina vulgaris (Asteraceae: Cynareae) is a diminutive winter annual forb from the Mediterranean region, whose original name, *Centaurea crupina* L., indicates its close relationship with the genus *Centaurea* (Bremer, 1994; Garcia-Jacas *et al.*, 2001). Upon discovery of a naturalized population of *Crupina vulgaris* in Idaho in 1968 (Stickney, 1972), scientists were alarmed by its invasive potential, because *Centaurea* comprises some of the most aggressive invaders of western North American arid and semiarid wildlands (e.g. *C. diffusa*, *C. solstitialis*, *C. stoebe*, *C. virgata*). As a new discovery, *Crupina vulgaris* was listed as a Federal noxious weed, and was subsequently found in three other states: California, Washington and Oregon. Current estimates of the infested area exceed 26 000 ha. On overgrazed rangelands, it forms dense, nearly pure stands (Miller & Thill, 1983), a concern because of its relatively low palatability to cattle, the primary resource use (Zamora *et al.*, 1989). Because *Crupina vulgaris* is a tap-rooted annual that primarily infests steep south-facing slopes, the potential for soil erosion is aggravated when it replaces fibrous-rooted grass species, which have more persistent residue. Also, dense populations of this exotic invader reduce biodiversity in native ecosystems (Prather *et al.*, 1991).

History of *C. vulgaris* as invader in North America and Australia

Contrary to previous reports that the first North American record of *C. vulgaris* was in Idaho in 1968 (Stickney, 1972),

the earliest collections of this species date from Massachusetts (Sorrie & Somers, 1999). Specimens at the Harvard University Herbaria, collected by C. E. Perkins in 1877 and 1879 from Boston, and South Boston Flats, Suffolk County, Massachusetts, USA, indicate that *C. vulgaris* was among the numerous species introduced in ship's ballast (NEBC, W. Kittridge, pers. comm.). *Crupina vulgaris* was not collected again and it does not appear in current floras, indicating that it failed to establish in the northeastern United States. Thus, the discovery in Idaho was the first successful introduction. Over the next two decades three additional populations were reported: in 1975 at Santa Rosa, Sonoma County, California (Miller & Thill, 1983), in 1984 at Lake Chelan, Chelan County, Washington (Alverson & Arnett, 1986), and in 1987 at Dry Creek, Umatilla County, Oregon (Couderc-LeVaillant & Roché, 1993). The Sonoma County population was declared eradicated by 1982 (Miller & Thill, 1983), but it was rediscovered 7 y later about 1 km from the original infestation (Davis & Sherman, 1991). In 1990 another new population was reported in Modoc County, California (Calif. Department Food & Agric. records). These five infestations, detected between 1968 and 1990 in four western States, remain widely disjunct from each other (Fig. 1), unconnected by any known satellite or transitional populations.

A report of the introduction and eradication of *C. vulgaris* in British Columbia during the 1980s (Zamora *et al.*, 1989) appears to have been in error (R. Cranston, pers. comm.). In South Australia, where *C. vulgaris* was discovered in 1936 around a reservoir in Hope Valley near Adelaide, scientists believe it was introduced in packing material for the heavy construction equipment, imported from Europe, which was used to build the reservoir (Ising, 1937). Although it has persisted in this location for 65 y, it has not dispersed (Jessop & Toelken, 1986), and is not considered a weed in Australia (Lazarides *et al.*, 1997).

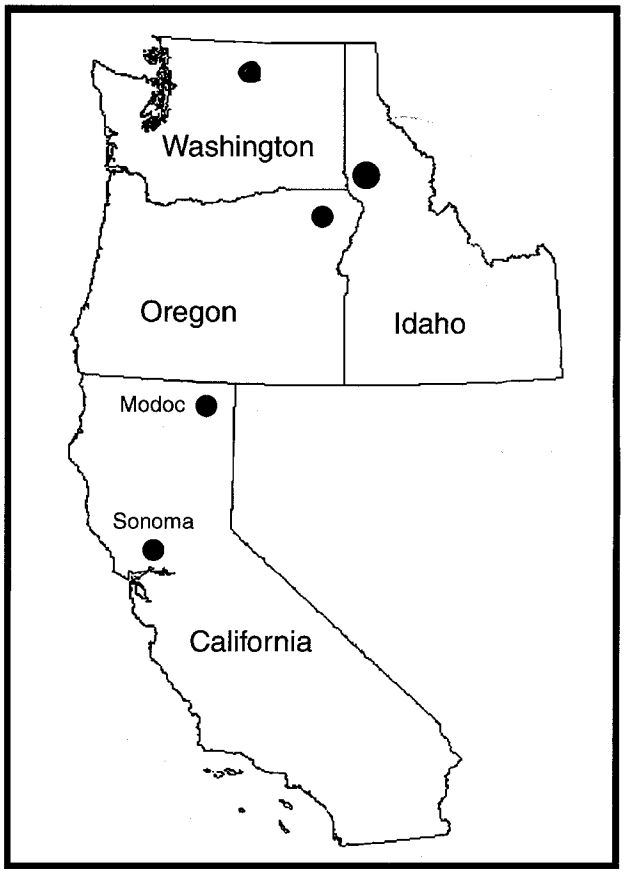

Fig. 1 Location of *Crupina vulgaris* colonies in western North America.

Multiple introductions suspected

Although the means of intercontinental migration was not known, multiple introductions have been suspected in North America, due to morphological and phenological differences between populations (Couderc-LeVaillant & Roché, 1993; Roché, 1996). Morphological differences include cypsela size and pappus length: populations from Modoc and Chelan counties have uniformly smaller cypselas (19 mg) with shorter pappus (4–5 mm) and the remaining three populations (Idaho, Oregon, and Sonoma County, California) are characterized by a larger cypsela (36 mg) with a longer pappus (7–9 mm). The same differentiation among populations was seen in rosette characteristics when populations were grown in a uniform garden study. In this common garden, the shiny, dark green rosette leaves of Chelan plants grew close to the soil surface at all times, in contrast to the lighter green rosette leaves of plants from the Oregon and Idaho populations, which grew upward at an angle away from the soil, except during cold winter conditions (Roché, 1996). In the same study, plants from the Oregon and Idaho populations flowered up to 10 d earlier than the population from Chelan. The Chelan population also required longer vernalization at cold temperatures and was more sensitive to damage from high temperatures than the Oregon and Idaho populations (Roché, 1996).

A pattern of isolated foci is consistent with relatively recent introductions and the species' propagule dispersal system, which, relative to common ruderal species, is highly inefficient (Roché & Thill, 2001). As a self-compatible annual, still relatively early in the invasion process, *C. vulgaris* was a good candidate for use of a molecular marker technique to investigate the invasion genetics among North American populations and compare them with native populations. In the eastern hemisphere, *C. vulgaris* is distributed around the Mediterranean basin, with its western limits in the Iberian peninsula and Morocco, and extending across southern Europe, Crimea, northern Greece and Turkey, northern Iran and Iraq, to the Caucasus region and Uzbekistan, Turkmenistan and northeastern Afghanistan (Kupicha, 1975; Dittrich *et al.*, 1980; Couderc-LeVaillant, 1984; Czerepanov, 1995).

Objectives and RAPD analysis

Isozymes have been used successfully to investigate genetic variation among and within populations of a variety of invading species, for example *Bromus tectorum* (Novak *et al.*, 1993; Novak & Mack, 1993), *Capsella bursa-pastoris* (Neuffer & Hurka, 1999), and *Lythrum salicaria* (Strefeler *et al.*, 1996). However, a limitation of isozyme analysis is that variation may be too low for detection of genetic diversity (Nissen *et al.*, 1995), and preliminary studies showed that this was the case for *C. vulgaris* (T. Garnatje, unpublished data). Randomly amplified polymorphic DNA (RAPD) analysis has effectively identified genotypes and relationships among populations of a variety of organisms (Hadrys *et al.*, 1992). RAPDs often represent the appropriate scale of genetic analysis for studies of colonizing plants, tracking migration pathways, identifying the number of introductions and linking them to ancestral populations (Stiller & Denton, 1995; Neuffer, 1996). Although RAPD markers are dominant, making them a less powerful tool than codominant isozyme markers, this limitation is offset by the greater number of loci that can be compared. Chloroplast DNA restriction fragment length polymorphism (RFLP) has been used to investigate more detailed within-population variability and maternal inheritance (Rowe *et al.*, 1997). We selected RAPDs as the suitable scale of genetic detail to determine the number of introductions and source locations for an outcrossing, but self-compatible annual species.

The objectives of this study were first to examine the appropriateness of RAPD-based DNA fingerprinting to characterize the genetic structure of the invasive *C. vulgaris* populations, second to determine the number of introductions responsible for the North American invasion, and third to identify the most probable source locations in the eastern hemisphere of the invasion founders. This information would be useful for recognizing potential intercontinental carriers and understanding colonization dynamics, as well as a base for various weed management strategies such as biological control, competitive vegetation and prevention tactics.

Materials and Methods

Plant material

We sampled natural populations in Eurasia (Armenia, France, Greece, Iran, Italy, Spain, Switzerland and Uzbekistan) and North America, collecting a single cypsela per plant. Some populations were not used in the analysis because an insufficient number of seeds germinated. A few others were eliminated because clear, reproducible band patterns could not be obtained from their DNA. Collection sites of the 48 populations included in the study are shown in Table 1, with voucher specimens deposited at the Botanic Institute of Barcelona, Spain (BC). Seeds were germinated and DNA was extracted from a fully developed cotyledon of five plants from each population. A duplicate DNA extraction from one plant per population served as a control.

We had two choices when planning the RAPDs amplifications. We could carry out an in-depth analysis with 10–15 primers, if we sacrificed breadth in sampling. Alternatively, we could sample extensively, including more populations, and use fewer primers. After a preliminary survey with 20 primers, we chose the second option. *Crupina vulgaris* is self-compatible, and we found very low levels of intrapopulational variation, as compared with interpopulational differences. Thus we were able to obtain useful inferences with fewer primers than normally used for outcrossing plants.

DNA extraction and RAPD amplifications

Total genomic DNA was isolated from fresh leaf material following the protocol of Doyle & Doyle (1987), as modified by Cullings (1992). Twenty primers (A4, B8, B20, D10, E5, F6, G1-G3, G19, J1-J3, and J4-J10, Operon Technologies, Alameda, CA, USA) were screened to obtain five primers (B8, E5, G2, G19, and J10) that produced reproducible, polymorphic bands. Primers were tested with different DNA concentrations to determine the concentration for optimal RAPD patterns while minimizing artefactual bands. DNA amplification was carried out in 10 µl reaction volumes containing 1.0 µl of template DNA; 5.6 µl sterile distilled water; 1 µl of 10 mM $MgCl_2$; 1.0 µl 10× loading buffer (Promega Corp, Madison, WI, USA); 0.8 µl 1.25 mM dNTP (Promega) and 0.4 µl of 5 pmol primer, and 1 unit (0.2 µl) of Taq polymerase (Promega). Reaction volumes were placed in 0.2 ml microfuge tubes, covered with mineral oil (Sigma-Aldrich, St. Louis, MO, USA) and placed in a thermal cycler (PTC 100, MJ Research Inc., San Francisco, CA, USA). Amplification was carried out with the following thermal profile: (i) initial denaturation at 95°C for 1 min (ii) melting at 95°C for 30 s, annealing at 37°C for 1 min and extension at 72°C for 2 min for 44 cycles (iii) final extension at 72°C for 5 min, after which the samples were maintained at 4°C until removal. Absence of contamination in each run was verified by including a negative control in which DNA was omitted. Reproducibility was confirmed by duplicating randomly selected reactions for DNA samples of each population for each primer.

Amplification products were resolved electrophoretically in 2% agarose gels in 1 × TBE buffer run at 10 V cm^{-1} for about 5 h in 0.5× TBE, and visualized by staining with ethidium bromide. PGEM DNA marker (Promega) was used as a molecular size marker. The gels were photographed under UV light with a Kodak digital camera (DC220) (Eastman-Kodak, Rochester, NY, USA).

Data analysis

Analysis of the RAPD products (loci) included only reliably scored and repeated bands, and a representative of the

Table 1 Plant collections of *Crupina vulgaris* used as sources of DNA. The depository for voucher specimens is the Botanic Institute of Barcelona, Spain (BC)

Country and population	Province or state	Collector and number
France		
Briançonnet	Alpes Maritimes	Roché & Susanna 2020
Briançon	Hautes Alpes	Korfhage & Roché 110
Gap	Hautes Alpes	Korfhage & Roché 103
Puy St. Richard	Hautes Alpes	Korfhage & Roché 119
Les Matelles	Hérault	Sobhian s.n.
St. Jean de Cuculles	Hérault	Sobhian s.n.
Draguignan	Var	Roché & Susanna 2021
St. Maximim la Ste. Baume	Var	Roché & Susanna 2025
Apt	Vaucluse	Roché & Susanna 2028
Saignon	Vaucluse	Sobhian s.n.
Greece		
Kalpáki (Ioánina)	Epiros	Roché & Susanna 1979
Kónitsa (Ioánina)	Epiros	Roché & Susanna 1978
Elefteropouli (Kavala)	Macedonia	Roché & Susanna 1999
Siátista (Kozáni)	Macedonia	Roché & Susanna 1973
Skepasto (Seres)	Macedonia	Roché & Susanna 2007
Skopos (Flórina)	Macedonia	B. Roché s.n.
Thermi (Thessaloníki)	Macedonia	Roché & Susanna 1953
Panagia (Tríkala)	Thessalía	Roché & Susanna 1983
Didimotiho (Evros)	Thraki	Roché & Susanna 1996
Italy		
Aosta	Aosta	Korfhage & Roché 116
Susa	Torino	Korfhage & Roché 118
Spain		
Maeztu-Corres	Álava	Vilatersana 102
Covarrubias	Burgos	Roché & Susanna 1932
Rebolledo de la Torre	Burgos	Garcia-Jacas & Susanna 2079
Santibáñez del Val	Burgos	Roché & Susanna 1933
Huete (Cañaveras)	Cuenca	Susanna 1599
Solán de Cabras	Cuenca	Susanna 1813
Linares de la Sierra (Aracena)	Huelva	Garnatje 56 & Vilatersana
Candasnos	Huesca	Roché & Susanna 1949
Cazorla, Fuente del Oso	Jaén	Garnatje 77 & Vilatersana
Vegacervera	León	Garcia-Jacas & Susanna 2074
Torrelaguna	Madrid	Garcia-Jacas & Susanna 1455
Reinoso de Cerrato	Palencia	Roché & Susanna 1928
El Burgo de Osma	Soria	Roché & Susanna 1936
Pont del Diable	Tarragona	Garcia-Jacas, Martín, Susanna 1472 & Vallès
Huerta de Valdecarábanos	Toledo	Susanna 1884
Pollos	Valladolid	Roché & Susanna 1924
Cervera de la Cañada	Zaragoza	Roché & Susanna 1940
Frasno-Saviñán	Zaragoza	Roché & Susanna 1943
Malanquía	Zaragoza	Roché & Susanna 1938
Salvatierra de Esca	Zaragoza	Vilatersana 87
Villarroya de la Sierra	Zaragoza	Roché & Susanna 1939
United States		
Kelly Springs, Modoc Co.	California	Raymond s.n.
Santa Rosa, Sonoma Co.	California	Lane & Pitcairn 2027
Lawyer Cyn., Clearwater Co.	Idaho	Roché 2028
Dry Creek, Umatilla Co.	Oregon	Roché
Lake Chelan, Chelan Co.	Washington	Roché
Uzbekistan		
30 Km E of Bukhara	Bukhara	Khassanov s.n.

dominant pattern was selected from each population to use for comparison among populations. Each band was assigned a molecular weight using Lane Manager 2.1a (T.D.I.S.A., Madrid, Spain), which performed a semilogarithmic regression with the DNA molecular weight standard on each gel. Bands were then manually scored for each population, 1 for presence and 0 for absence, to generate a binary matrix. This binary matrix of polymorphic bands from all five primers was analyzed using the program NTSYS-pc (Rohlf, 1997), employing SIMQUAL (similarity for qualitative data) and the similarity coefficient, Dice. This coefficient was used in order to generate an unweighted pair-group (UPGMA) dendrogram, through the routine SAHN within NTSYS. The coefficient Dice is equivalent to that of Nei & Li (1979), which was recommended by Lamboy (1994) for analyzing RAPD data. However, dendrograms generated using the Jaccard index were identical except for the length of the branches.

To evaluate the fit of the cluster analysis, a Mantel test was carried out as described by Rohlf (1997). To analyze the possible relationship between genetic and geographic distances in the sampled populations, we generated a matrix of geographic distances by calculating the distance in kilometers between a subset of 33 populations, using the geographic co-ordinates (Bartish *et al.*, 2000). American populations were excluded from this analysis. This matrix was compared with the genetic distance matrix used for the cluster analysis. A Mantel test was carried out with MXCOMP in NTSYS-pc, with 9000 permutations to compute the significance of the correlation. To further assess the correlation between geographic and genetic distances among Iberian and French populations, we carried out a second Mantel test, but this time with only 16 populations from Spain and France.

Results

Clustering of the genetic distances

The five primers employed generated a total of 75 RAPD bands, ranging in size between 200 bp and 1.9 kb. The number of bands scored per primer varied between 11 (J-10) and 21 (E-5). The total number of polymorphic bands was 70, representing 93.3% of all bands. Details of the number of bands and percent polymorphism for each primer are shown in Table 2.

The relationships among populations is presented as a dendrogram generated by UPGMA clustering (Fig. 2). The Mantel test result was r = 0.74556, which indicates a poor fit for the cluster (Rohlf, 1997). The similarity coefficient between the 48 populations varied between 0.53 and 0.94. The clustering is not clean geographically, as populations from different countries did not separate into distinct clusters. Nevertheless, despite dispersal of individual populations from different countries throughout the dendrogram, several general tendencies are apparent (Fig. 2). The Uzbekistan population does not cluster, reflecting the large geographic and genetic distance. The majority of the Greek populations, instead, form a cluster. The rest of the clusters are an intermingling of populations from northern Spain (four) and the Alps (two Italian and three Alp and Maritime Alp populations from France), with two northern Greek populations from western Macedonia (Siátista, Skepasto). The upper branch of the dendrogram comprises 27 populations, including all five American populations. The remainder are populations from northern and central Spain and southern France, except for one Greek population and two southern Spanish populations.

Table 2 Base sequences of the primers used for DNA amplification and the number of polymorphic bands produced by each primer

Primer	Sequence, 5′ to 3′	No. total bands	Polymorphic bands	Polymorphic bands (%)
B-08	GTCCACACGG	12	10	83.3
E-05	TCAGGGAGGT	21	21	100
G-02	GGCACTCAGG	13	11	84.6
G-19	GTCAGGGCAA	18	18	100
J-10	AAGCCCGAGG	11	10	90.9

The five American populations (Fig. 2) segregate into three branches of the dendrogram. Modoc population (California) clustered at 0.90 similarity with the Chelan (Washington) population. These two populations are found associated at 0.87 similarity with two populations from the northern part of the Iberian peninsula, Reinoso de Cerrato and El Burgo de Osma (Fig. 3). In turn these four populations cluster with five more populations from northern Spain and one from Kavala (eastern Macedonia), Greece (Fig. 2). The population from Oregon clustered at 90% similarity with a Spanish population from Huerta de Valdecarábanos. Similarity between the Sonoma (California) and Idaho populations was 0.88 and these were joined at 0.82 similarity to a larger cluster, which included the Oregon population and six Spanish populations: Solán de Cabras, Rebolledo de la Torre, Salvatierra de Esca, Linares de la Sierra, Huerta de Valdecarábanos, and Torrelaguna (Figs 2 and 3).

Correlation between geographic and genetic distances

We found a significant correlation between geographic and genetic distances with our first analysis (normalized Mantel statistic Z r = 0.47851, p[random Z > = observed Z] = 0.0002), including populations from Greece, France, Italy, Spain and Uzbekistan. However, when comparing geographic and genetic distances among Iberian and French populations only, the correlation was significantly lower (normalized Mantel statistic Z r = 0.14127, p[random Z > = observed Z] = 0. 1792).

Fig. 2 Map of Spain, showing locations of populations.

Discussion

Colonization pattern in North America

Clustering of the North American populations by the molecular marker data yields a pattern lacking geographical proximity: that is Idaho with Sonoma County, California; Chelan, Washington, with Modoc County, California; and Oregon separate (Fig. 2). This may indicate one exotic population founding another, but does not preclude separate introductions by the same vectors from similar origins. Unraveling the invasion dynamics would be simpler if one could assume that discovery closely followed initial introduction. But the inconspicuous, delicate stature of *Crupina vulgaris* makes it almost invisible when populations are sparse, so that a considerable amount of time (i.e. decades) could easily elapse between introduction and detection. All six original discoveries of *C. vulgaris* in the western United States were made by professional botanists or weed scientists, and none were reported by casual observers or landowners. In spite of this limitation, a probable invasion order may be inferred from corroborating evidence, such as size and shape of populations and land use. Because the Idaho and Sonoma County discoveries (1968 and 1975) significantly predate the others (1984, 1987, 1990), they likely represent the first introduction(s). This is consistent with the size and distribution pattern of the Idaho population, where more than 20 000 ha are infested, with roadside stringers and satellite populations up to 60 km distant from the perimeter of the core infestation. Which of the other two clusters, Oregon or Chelan/Modoc, represents the second colonization event is not clear because potential dispersal vectors and suitable habitat differ so much between sites. The population at Lake Chelan, Washington, discovered in 1984, also comprises several smaller infestations, scattered along slopes above the northeast shore of the lake for about 10 km. Its related population in Modoc County, California, discovered 6 y later, is contained within a 300-ha polygon. When the Oregon population was detected in 1987, its boundaries enclosed about 200 ha, but by 2000 a satellite population had established several km away from the core infestation.

Founder populations in the eastern hemisphere

The positive correlation between genetic distances and geographic distances allows us to make some general inferences on the geographic origin of the American populations. The study indicates that all five populations of

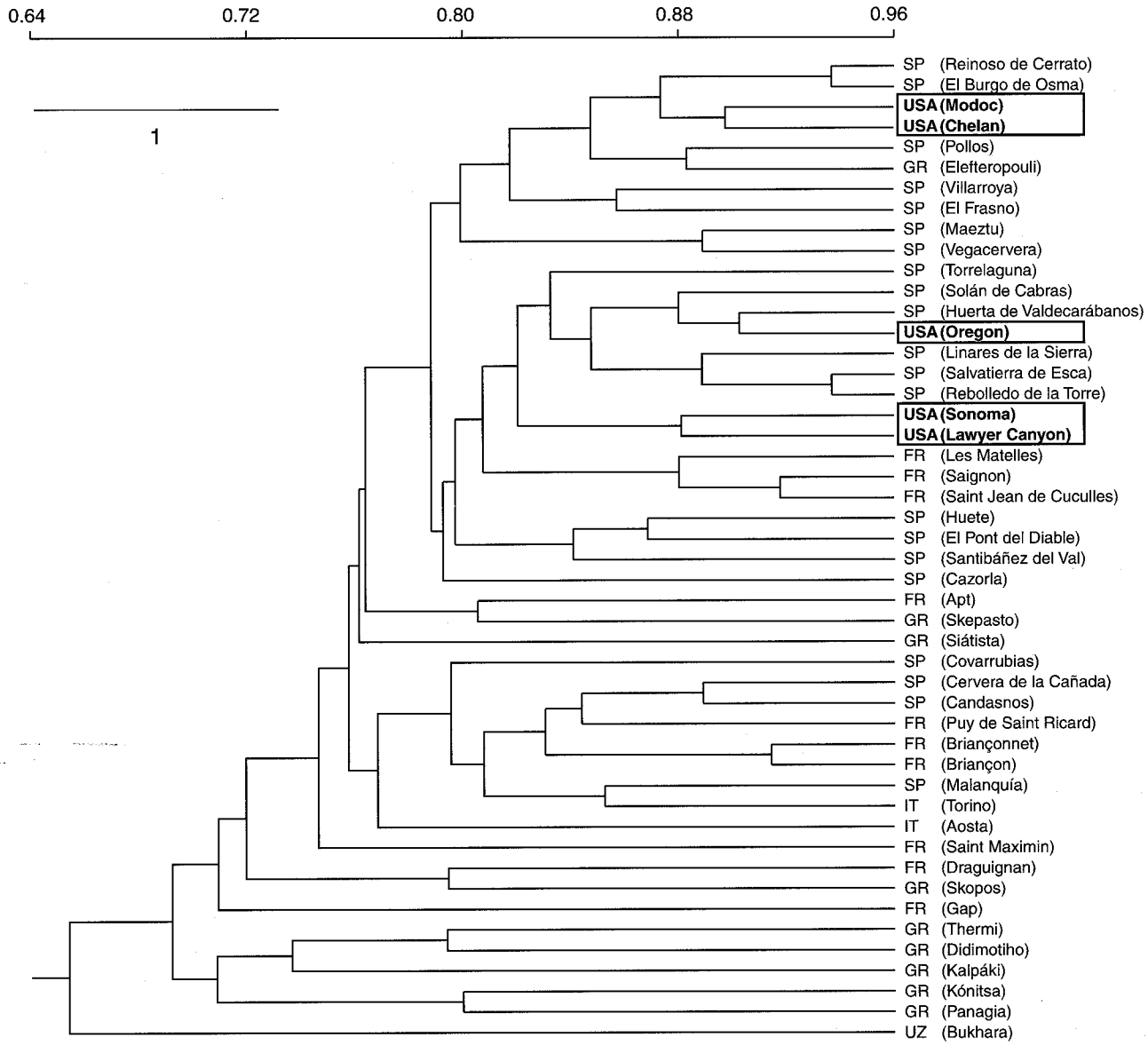

Fig. 3 Dendrogram constructed from UPGMA showing the relationships among populations of *Crupina vulgaris* collected from North America (USA) and France (FR), Greece (GR), Italy (IT), Spain (SP), and Uzbekistan (UZ). Site names are listed in Table 1.

C. vulgaris in North America originated in Spain. That there were at least three successful colonization events suggests that there was a vector for long distance dispersal that facilitated repeated introductions.

Although the weak correlation between geographic distance and genetic distance within the Iberian Peninsula prevents us from precisely pinpointing the origins of the American populations, as was the intent of the paper, it may reveal a previously unsuspected level of migration within the Iberian Peninsula. The Lawyer Canyon and Sonoma populations are linked to a broad area from the central and the north part of the Iberian Peninsula (populations from Torrelaguna, Solán de Cabras and Huerta de Valdecarábanos and from Salvatierra de Esca and Rebolledo de la Torre, respectively), with the inclusion of extreme south population from Linares de la Sierra (Figs 2 and 3). The Chelan and Modoc populations derive from northern Spain, based on similarity with populations near Reinoso de Cerrato and El Burgo de Osma (Figs 2 and 3). The Oregon population most closely resembles a population from the central meseta or plateau of Spain, near Huerta de Valdecarábanos (Fig. 2). That the invasive populations are genetically similar to populations scattered over relatively wide areas of Spain and the grouping of Spanish populations that are not geographically proximate suggests an explanation that *C. vulgaris* has a relatively efficient distribution mechanism (considering the size of the propagule) and may also be a colonizer in the Iberian Peninsula, albeit with a much older chronology.

Origin of the genus and migration in the eastern hemisphere

Several factors support this explanation that *C. vulgaris* may be a colonizer in the Iberian Peninsula: first the location of the center of origin of Cardueae genera, second sparcity of appropriate native habitat, and third position of populations in RAPD dendrogram (i.e. lower correlation between geography and the cluster pattern as an indicator of recent and continuous migrations).

The traditional view is that the center of origin for the subtribe *Centaureinae* to which *Crupina vulgaris* belongs, is east of the Mediterranean region, from whence it spread to occupy semidesert habitats during the Miocene (Small, 1919; Bremer, 1994). *Crupina* may have followed a pattern of westward migration in the Mediterranean region similar to that of numerous other steppe species which expanded their range to occupy anthropic (human modified) habitats that approximated their native environment (Gamarra & Montouto, 1999).

Crupina vulgaris is restricted to open habitats with a Mediterranean climate and well-drained soils, including grasslands, open wood or shrublands, or remnants of these types of vegetation adjacent to cropland (e.g. vineyards, cereal grains, olives). In the Iberian Peninsula, these habitats are almost entirely anthropic. Iberian climax vegetation is largely temperate forest (evergreen or deciduous *Quercus*, or *Fagus*, *Pinus*, or mixed stands), with natural grasslands occurring only above treeline in the mountains and internally drained basins (Montserrat & Fillat, 1990). Natural communities in which *C. vulgaris* grows, *Thero-Brachypodietea*, are limited to nonzonal sites (e.g. shallow soils, rock outcroppings, steep slopes with intense insolation). Extensive expansion of this vegetation type coincided with widespread human destruction of woody vegetation (Bolòs, 1967). Thus, if *Crupina vulgaris* existed in the Iberian Peninsula before anthropic conversion of the landscape, it was confined to small openings on shallow soils or steep, south-facing slopes, isolated by dense shade of surrounding evergreen oak forest.

In the dendrogram, the clustering of Uzbekistan and Greek populations as more distantly related is consistent with an origin of the genus in the region east of the Mediterranean. (It is logical here to speak of the genus because it comprises only three species, one of which is a hybrid between the other two, and all originally known as a single species, *Centaurea crupina*). A pattern of general geographic groupings studded with an intermingling of other populations could easily develop as *Crupina* gradually moved from east to west across the entire width of the Mediterranean region over a period of thousands of years. This migration probably coincided with the Neolithic revolution (Holocene period of 12,000–4000 year B.P.), during which the human population in the Mediterranean region increased more than tenfold (Le Houérou, 1981). The impacts of domestic herds and cultivation reached Spain near the end of that era, and by the 1400s essentially all of the evergreen oak forest of central Spain had been destroyed (Montserrat & Fillat, 1990).

Overlaying this broad pattern of east to west movement in the Mediterranean region is the potential for smaller scale dispersal of *C. vulgaris* within the Iberian Peninsula along the pathways of *trashumancia*. Nomadic movements of flocks, begun in Neolithic times to exploit seasonally available forage, were refined over the centuries to become well-established trails (*cañadas*) between summer and winter pastures (*trashumancia*), which are still in use (Montserrat & Fillat, 1990). Shorter transhumanance routes connect summer grazing in mountains with spring and autumn pastures near villages in adjacent valleys, while longer passages extend nearly the entire north–south length of Spain (Mangas, 1992). Research in Mediterranean chaparral in Israel reported epizoochoric transport of *Crupina* by sheep (Shmida & Ellner, 1983). Thus, transhumanant bands of sheep form a reasonable connection between populations as distant as Cazorla and Covarrubias (Fig. 3).

Transcontinental migration

As noted previously, the large, heavy propagules of *C. vulgaris* are not readily dispersed long distances. Without the pappus, they are about the size and shape of a kernel of *Triticum aestivum*. Although this similarity implicates cereal grains or large seeded grasses as the vehicle for transcontinental migration, several aspects of the case history cast doubt on this argument. First, most of the weedy species that came to North America as contaminants of crop seeds did so before 1912 when the United States began implementing legal protection against weed seeds. The pappus is not readily deciduous, making the nearly black cypsela not only highly conspicuous, but easily removed by screening. Second, weeds introduced as seed contaminants were a common component of the cropping system, or at mimimum were competitive with the crop, in both the Old and New World fields. *Crupina* may have been more competitive under primitive agricultural practices, but it cannot compete in cereal crops grown with mechanized practices in either Spain or the United States. Third, cereal crops were not historically cultivated in the vicinity of all the introductions. This factor would be insignificant if *C. vulgaris* were widespread in western North America, because it could easily be redistributed in straw or animal feedstuffs. The earliest successful colonization was detected in 1968. If it had been growing in cereal fields, it is likely that it would have been noticed by a farmer or farm advisor before several decades had elapsed (representing colonization time for thousands of ha). Interception records maintained by USDA APHIS PPQ for Federal Noxious Weeds indicate that *C. vulgaris* has not been intercepted crossing the US borders since it was listed and records kept on weed interventions, 1983-present (Polly Lehtonin, pers. comm.).

'Known' vectors for transcontinental migration of *C. vulgaris* include ballast soil (Sorrie & Somers, 1999) and packing

material for construction equipment (Ising, 1937); and for short distance migration, sheep (Schmida & Ellner, 1983). By contrast to relatively strict regulations on crop seeds, packing materials and ballast entering the United States in the first half of the 20th century, little attention was paid to wool, and regulations for animals were primarily related to diseases (Randy Westbrooks, pers. comm.). When air transportation became affordable, sheep herders coming from Spain brought only a few personal items; but earlier immigrants (e.g. during the period after World War II) brought their herding dogs with them (Antonio Oros, pers. comm.). Unlike common ruderal species, *C. vulgaris* relies on 'quality, not quantity', producing a few large propagules and sacrificing widespread dispersal for high individual survival rate (Roché & Thill, 2001). That *C. vulgaris* did not colonize every sheep camp tended by Basque herders in the American West is consistent with the very low probability of dispersal. This is counterbalanced by the Spanish origin of all of the successful *C. vulgaris* colonies and that one of the few legal channels for Spanish immigrants in the 20th century was contracts for sheepherding (Douglass & Bilbao, 1975).

Acknowledgements

The authors thank M. Pitcairn, P. Lane and B. Raymond for collection of the California populations. The study was supported by a CSREES NRI CGP strengthening grant to the University of Idaho, in collaboration with the Botanic Institute of Barcelona. We thank the Department of Genetics of IRTA (Cabrils, Spain) for their generous sharing of their laboratories for carrying out the first RAPDs tests. Larry Lass and Brett Bingham prepared the map of North American populations. Two anonymous reviewers made useful commentaries for improving the manuscript.

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