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BIODIVERSITY RESEARCH

Invasive species of *Heracleum* in Europe: an insight into genetic relationships and invasion history

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ABSTRACT

Several species of the genus *Heracleum* (Umbelliferae) were introduced into Europe from south-west Asia in the 19th century and are now widespread in many countries. At least three invasive taxa with unresolved relationships to one another are thought to occur in Europe: *Heracleum mantegazzianum* Sommier & Levier, *H. sosnowskyi* Manden, and *H. persicum* Desf. ex Fischer. They are tall plants forming extensive stands with a high cover. To elucidate genetic relationships between the species, and gain insight into their invasion history, samples were collected from native ranges in Asia and invaded ranges of the three species in Europe and analysed using amplified fragment length polymorphism. Five other *Heracleum* species were also studied and in total, 189 samples from 72 populations were analysed. The results confirmed that there are three distinct tall *Heracleum* species invading in Europe. Within each of the three species, plants collected in the invaded range are genetically close to those from their native ranges. A close genetic relationship between the three invasive *Heracleum* species in Europe was also found. A high overall genetic variability detected in the invaded range suggests that the majority of invading populations were not affected by a genetic bottleneck and that rapid evolution, drift, or hybridization played a role in genetic structuring of invading populations. For *H. mantegazzianum*, genetic distance of populations in the native range significantly decreased with geographical distance, but not in the invaded range. It is likely that the current pattern of genetic diversity in Europe resulted from multiple introductions of all three species.

Keywords

AFLP, alien plant, biogeographical approach, biological invasion, genetic relationships, *Heracleum*.

INTRODUCTION

Invasive alien species are a significant threat to biodiversity worldwide and as such attract increasing attention (Cox, 2004; Rejmánek *et al*., 2005; Sax *et al*., 2005; Pyšek *et al*., 2006; Richardson, 2006). Studies range from the development of scientific concepts aimed at understanding the biological basis of invasion (Hulme, 2003; Richardson & Pyšek, 2006; Stohlgren *et al*., 2006) to practical implications, including control, management and the economic costs imposed by invasive species (Zavaleta, 2000; Pimentel, 2002). Species that have been proven to be successful invaders over a large geographical area have received most of the attention (Weber, 2003). In Europe, *Heracleum mantegazzianum* (giant hogweed) has long been recognized as a prominent example of an invasive alien with a wide distribution, significant impact and remarkable dynamics of spread at both national (Pyšek, 1991; Collingham *et al*., 2000; Wadsworth *et al*., 2000; Müllerová *et al*., 2005) and European scales (Nielsen *et al*., 2005). In 2002, *H. mantegazzianum* was identified as a suitable model species for the development of a sustainable strategy for the management of invasive alien species in Europe and became the subject of a European project 'Giant Alien' (Nielsen *et al*., 2005; Pyšek *et al*., 2007a). However, no sooner had this project begun that it became clear that the taxonomy of *H. mantegazzianum* in its invaded range in Europe was far from clear cut and that what has been traditionally considered as a single species in fact represents a complex of several taxa with unresolved relationships to one another. A study was thus initiated, within the Giant Alien project, to shed light on the genetic relatedness of the *Heracleum* taxa that are known to be invasive in Europe.

Heracleum is a widespread, taxonomically complex genus. Generic delimitation is problematic as recent molecular studies showed that the genus is not monophyletic (Downie *et al*., 2000; J. Paik & M. F. Watson, unpublished data; M. Zych, unpublished

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data). Classification problems are compounded by the general paucity of good herbarium specimens (Pyšek *et al.*, 2007b), inadequacy of historical-type material, and extremely variable nature of morphological characters. Many species are robust and tall; the plants have large inflorescences and basal leaves, therefore collectors tend to select the smaller lateral branches and upper leaves, which are less informative. Good fruiting material is also often lacking, and some taxa are recorded only from a very few collections. Revised classifications have been proposed for parts of the range of the genus, but present knowledge is incomplete (Davis, 1972; Fading & Watson, 2005). Moreover, the different concept of species used by taxonomists in Russia and Western Europe led to further confusion (Jahodová *et al.*, 2007).

It has been suggested that alien species in the invaded region may experience rapid genetic changes under selection pressures in the novel environment (Ellstrand & Schierenbeck, 2000; Bossdorf *et al.*, 2005; Novak & Mack, 2005), and this is considered to be one of the reasons why they attain dominance in introduced ranges (Maron *et al.*, 2004; Hierro *et al.*, 2005). Patterns of genetic similarity can be used to identify closely related populations and molecular markers are useful tools in determining the origin of invasive species. The theory behind the use of genetic markers is that populations with a shared ancestry are genetically more similar (Bond *et al.*, 2002). Based on this theory, the source population from the native range is expected to be more similar to those from the invaded range than other native populations that were not involved in the initial colonization. Such an approach has been successfully used to trace invasion trajectories of several invasive species, e.g. *Capsella bursa-pastoris* (Neuffer, 1996), *Bromus tectorum* (Novak & Mack, 2001), or *Cenchrus biflorus* (Bond *et al.*, 2002).

Prior to the Giant Alien project no information was available on patterns of genetic diversity of *H. mantegazzianum* across a wide geographical scale although some data were available at a local scale. Walker *et al.* (2003) investigated populations of *H. mantegazzianum* in several catchments in England using microsatellite markers. Their results suggested that the genetic structure of these populations was influenced by drift during the relatively short time since the initial introduction.

In the present study, the genetic diversity of *Heracleum* species has been investigated using a biogeographical approach (Hierro *et al.*, 2005). Plants of *H. mantegazzianum*, *H. sosnowskyi*, and *H. persicum* were sampled from across a wide geographical range in the native (Caucasus and south-west Asia) and invaded (Europe) distribution ranges. In addition, in order to provide a broader picture of the relationships among taxa within *Heracleum*, five other representatives of *Heracleum* were sampled.

Amplified fragment length polymorphism (AFLP; Vos *et al.*, 1995) was used to investigate patterns of genetic similarities as this molecular fingerprinting technique can be successfully applied to: (i) analysis of relationships among populations (Amsellem *et al.*, 2000; Cresswell *et al.*, 2001; Jorgensen & Mauricio, 2004); (ii) analysis of the relationships among closely related species and delimitation of species boundaries (Bruneau *et al.*, 2005; Milla *et al.*, 2005; Perny *et al.*, 2005); and (iii) phylogenetic inferences (Despres *et al.*, 2003; Cervera *et al.*, 2005; Koopman, 2005).

The study aimed to answer the following questions: (i) Which tall *Heracleum* species are invasive in Europe, and what are their genetic relationships? (ii) What is the relationship between the populations of *H. mantegazzianum* in the native range of the Caucasus and the invaded distribution range in Europe? (iii) By using this approach, can insight into invasion history be obtained and source populations from the native range be located?

STUDY SPECIES

The genus *Heracleum* (Apiaceae) includes 60–70 species of perennial or biennial herbs, distributed in the temperate northern hemisphere and in high mountains as far south as Ethiopia. Centres of the highest species diversity are the Caucasus Mountains (26 species) and China (29 species), particularly Hengduan Mountains (Mandenova, 1950; Fading & Watson, 2005). From what is known, *Heracleum* species are diploid with $2n = 22$ (Bell & Constance, 1966; Grossheim, 1967; Weimarck *et al.*, 1979). The nomenclature used in this paper follows Mandenova (1950, 1987).

Heracleum mantegazzianum Sommier & Levier is a monocarpic perennial native to the Western Greater Caucasus (Russia, Georgia), where it grows in species-rich, tall-herb mountain meadows, clearings, and in forest margins up to the treeline of *c.* 2000 m a.s.l. (Nielsen *et al.*, 2005). It was introduced as a garden ornamental plant around 1817 and the first naturalized population was documented in Cambridgeshire in 1828 (Jahodová *et al.*, 2007). At present, the species is recorded in at least 19 European countries, in 14 of which, it was first noted before 1900 (Nielsen *et al.*, 2005). It is also naturalized in Canada and the United States (Morton, 1978; Kartesz & Meacham, 1999). Recently, solid information on its biology and ecology has accumulated (Krinke *et al.*, 2005; Moravcová *et al.*, 2005, 2006; Pergl *et al.*, 2006; Perglová *et al.*, 2006).

Heracleum sosnowskyi Manden is a monocarpic perennial native to eastern and central Caucasus, Transcaucasia, and north-east Turkey. It was first introduced to Russia in 1947 as a highly productive fodder crop for livestock. Later it was introduced to other countries such as Belarus, Ukraine, Baltic countries, and former Eastern Germany. The planting schemes have been largely abandoned, although they still continue in parts of Russia (Nielsen *et al.*, 2005).

Heracleum persicum Desf. ex Fischer is a polycarpic perennial native to Turkey, Iran, and Iraq. The status of this invasive species in Europe is still not fully resolved. In Scandinavia, the species with the common name 'Tromsø palm' has been frequently referred to as *H. persicum* or *H. laciniatum* auct. scand. Although studies that have compared this species with *H. mantegazzianum*, which is also invasive in Nordic countries, clearly indicated that they are two distinct taxa (Often & Graff, 1994), the conspecificity of Tromsø palm with *H. persicum* has only recently been examined (L. Fröberg, unpublished data). The history of introduction of *H. persicum* is unclear. It was the first species to be described as early as 1829 (Fischer *et al.*, 1841) and it is likely that some other large hogweeds were misidentified as *H. persicum*. This makes the reconstruction of *H. persicum* invasion into Europe difficult. Compared to the other two species, the distribution of *H. persicum* in Europe is restricted to Scandinavia (Nielsen *et al*., 2005; Jahodová *et al*., 2007).

METHODS

Sampling

Samples for DNA analysis were collected from single leaves of individual plants. One to four plants were sampled per population, taking care not to collect from possible siblings. A population was defined as a discreet stand in a specific locality. Localities were at least 2 km apart.

The most intensive sampling was carried out for *H. mantegazzianum* (Table 1, Fig. 1). Samples from 29 individuals were collected from 11 natural populations in the north-western Caucasus, covering a large area of the Russian part of the Caucasus between the Black Sea, Elbrus Range, and Stavropol. In addition, samples were obtained from two populations in Abkhazia, close to the *locus clasicus* of this species (Sommier & Levier, 1895). In the invaded area of Europe, 47 individuals were sampled from 18 populations, covering a large part of the species known distribution.

For *H. sosnowskyi*, 11 individuals were sampled from four populations in the native range in Armenia. In the introduced range, 27 individuals collected from 10 populations in five countries of Eastern Europe and in Germany were analysed (Table 1, Fig. 1).

The samples of *H. persicum* (including samples identified as *H. laciniatum*) representing the invaded range were collected from 23 individuals from eight Scandinavian populations. A total of four individuals from two native populations (Iran) were also analysed. A sample from the National Botanic Garden of Belgium in Meise, grown from a seed originally received from the Centre de Recherche et de l'Application Pl. Ind. & Medical in Teheran, was also obtained (Table 1, Fig. 1).

In addition, the following *Heracleum* species were included in the analysis: *H. sphondylium* L., *H. sibiricum* L. (native to parts of Europe), *H. antasiaticum* Manden., *H. leskovii* Grossh., and *H. ponticum* (Lipsky) Schischk. ex Grossh. (native to Caucasus and/or Transcaucasia). Some samples of unresolved taxonomic identity or putative hybrids sent by external collectors were also analysed. In total, 189 samples from 72 populations of eight *Heracleum* species were used in the study. Samples from Apiaceae family, *Daucus carota* (native to Europe) and *Ferulago* sp. (native to the Caucasus), were used as outgroups (Table 1).

Sample preservation and DNA isolation

The majority of samples were preserved using silica gel (Chase & Hills, 1991) and stored at room temperature. Some samples were preserved in NaCl-CTAB (saturated brine containing cetyltrimethylammonium bromide) solution (Rogstad, 1992) in the field and stored in a fridge prior to DNA isolation. DNA from samples Hx-Uk-02 and Xx-Uk-01 was isolated using fresh material. A sample of *H. persicum* (Hp-Ir-01–1) was provided as a herbarium material.

Total genomic DNA from all samples was isolated using commercially available DNeasy 96 Plant Kit or DNeasy Mini Plant Kit (both from QIAGEN, Valencia, CA, USA). The quality and quantity of the extracted DNA were assessed by agarose gel electrophoresis with uncut lambda DNA standards (Sigma, St Louis, MO, USA). DNA samples were stored at –20 °C.

AFLP protocol

Fluorescent AFLP analysis was performed as described by Trybush *et al*. (2006). *Eco*RI and *Mse*I were used for digestion of DNA. Out of 55 AFLP primer combinations screened on a representative reference sample set, nine primer combinations

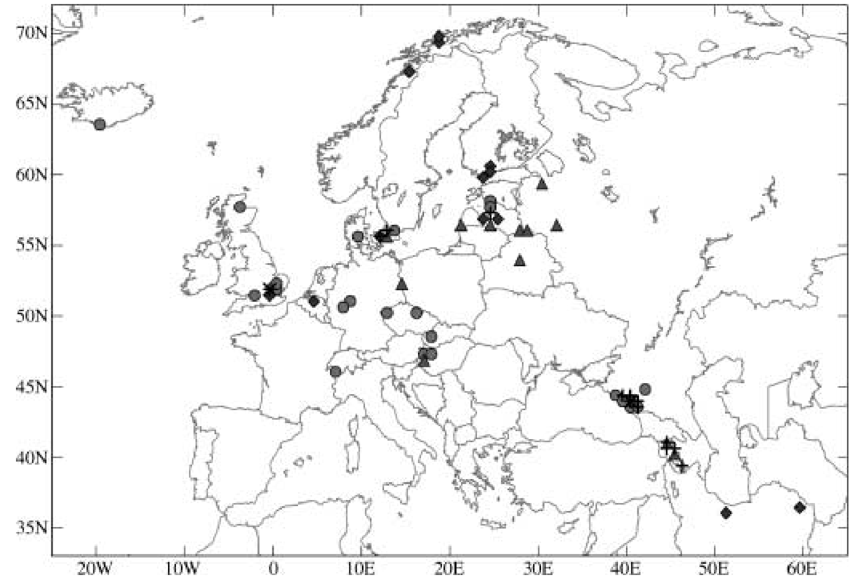

Figure 1 Map of sampled populations. ● — *Heracleum mantegazzianum*; ◆ — *Heracleum persicum*; ▲ — *Heracleum sosnowskyi*; + — other *Heracleum* species; ✳ — outgroup species.

Table 1 Information about analysed populations of *Heracleum* species

Code	N_i	Original determination	New species assignment based on AFLP results*	Range	Locality (country)	Latitude	Longitude	Altitude [m a.s.l.]	Preservation method	Collector	Date of collection
Hm-Cz-06	3	*H. mantegazzianum*		I	Žitný (Czech Rep.)	50°03′43″	12°37′33″	787	SG	J. Pergl, I. Perglová	17.6. 2004
Hm-Cz-14	4	*H. mantegazzianum*		I	Kostelec n. Orlicí — near the castle (Czech Rep.)	50°07′23″	16°11′52″	290	SG	J. Pergl, I. Perglová	9.7. 2004
Hm-Dk-08	3	*H. mantegazzianum*		I	Vejle (Denmark)	55°42′00″	09°32′00″	20	SG	Ch. Vibe Nissen	27.5. 2004
Hm-De-03	2	*H. mantegazzianum*		I	Burgwald (Germany)	51°01′37″	08°45′03″	330	SG	J. Hüls, J. Thiele	18.9. 2003
Hm-De-07	3	*H. mantegazzianum*		I	Helmeroth (Germany)	50°43′00″	07°45′00″	200	SG	J. Thiele	11.7. 2004
Hm-Hu-01	2	*H. mantegazzianum*		I	Bozzai village (Hungary)	47°12′25″	16°45′14″	190	SG	I. Dancza	13.7. 2004
Hm-Hu-02a	3	*H. mantegazzianum*		I	Cuha-valley; Zirc (Hungary)	47°17′28″	17°51′54″	375	SG	I. Dancza	19.7. 2004
Hm-Hu-02b	1	*H. mantegazzianum*		I	Arboretum, Zirc (Hungary)	47°15′48″	17°52′37″	395	SG	I. Dancza	19.7. 2004
Hm-Is-02	3	*H. mantegazzianum*		I	Mulakot Farm (Iceland)	63°43′00″	W19°53′00″	100	SG	B. Magnusson	14.7. 2004
Hm-Sk-01	3	*H. mantegazzianum*		I	Vel'ké Záluž̌ie (Slovakia)	48°25′60″	18°00′00″	180	SG	A. Fehér, L. Končeková	21.9. 2004
Hm-Se-01	3	*H. mantegazzianum*		I	Linderöd (Sweden)	55°57′00″	13°50′00″	n.g.	SG	L. Fröberg	27.7. 2003
Hm-Ch-02	3	*H. mantegazzianum*		I	Champex (Switzerland)	46°02′01″	07°06′51″	1520	SG	S. Hansen, J. Hattendorf	17.5. 2004
Hm-Uk-19	2	*H. mantegazzianum*		I	Ferneaux Pelham (UK)	51°55′52″	00°04′50″	119	SG	O. Booy	7.7. 2004
Hm-Uk-22	2	*H. mantegazzianum*		I	Cawdor (UK)	57°32′27″	W03°50′20″	n.g.	SG	O. Booy	19.5. 2004
Hm-Uk-33	3	*H. mantegazzianum*		I	Hotron Court (UK)	51°33′50″	W02°20′17″	145	SG	Š. Jahodová	31.5. 2004
Hm-Uk-45	2	*H. mantegazzianum*		I	Great Shelford (UK)	52°09′31″	00°09′22″	n.g.	SG	O. Booy	24.8. 2004
Hs-Ee-01	3	*H. sosnowskyi*	*H. mantegazzianum*	I	Tali (Estonia)	58°02′60″	24°48′00″	n.g.	SG	I. Roze	15.6. 2004
Hs-Lv-18	2	*H. sosnowskyi*	*H. mantegazzianum*	I	Kirbizi (Latvia)	57°38′00″	24°27′00″	35	SG	I. Roze	17.6. 2004
Hm-Ru-16	3	*H. mantegazzianum*		N	Nizhnyj Archyz (Russia)	43°39′22″	41°25′07″	1800	SG	O. Booy, Š. Jahodová	6.6. 2004
Hm-Ru-20	3	*H. mantegazzianum*		N	Chamishki Guzeripl (Russia)	44°00′37″	40°02′45″	1363	SG	O. Booy, Š. Jahodová	9.6. 2004
Hm-Ru-29	2	*H. mantegazzianum*		N	Between Archipo-Osipovka and Tekos (Russia)	44°24′49″	38°30′17″	20	SG	O. Booy, Š. Jahodová	11.6. 2004
Hm-Ru-30	2	*H. mantegazzianum*		N	Archipo-Osipovka — tourist camp next to the Black sea (Russia)	44°21′35″	38°31′24″	1	SG	Š. Jahodová, O. Booy	12.6. 2004
Hm-Ru-31	3	*H. mantegazzianum*		N	Between Novomichailovsky and Psebe (Russia)	44°17′15″	38°54′04″	30	SG	Š. Jahodová, O. Booy	12.6. 2004
Hm-Ru-33	2	*H. mantegazzianum*		N	Tichonovka — valley of river Ashe (Russia)	43°57′58″	39°16′42″	16	SG	Š. Jahodová, O. Booy	15.6. 2004
Hm-Ru-34	4	*H. mantegazzianum*		N	Bolshoe Pseushko (Russia)	44°04′34″	39°20′47″	269	SG	Š. Jahodová, O. Booy	15.6. 2004
Hm-Ru-36	1	*H. mantegazzianum*		N	Novoekaterinovskaja — Mt. Strizhament (Russia)	44°46′40″	42°00′55″	800	SG	O. Booy, Š. Jahodová	16.6. 2004
Hx-Ru-02	3	*H. ponticum* × *H. mantegazzianum*	*H. mantegazzianum*	N	9 km north of Lago-Naki (Russia)	44°08′60″	40°04′00″	1260	CTAB	R. Wittenberg	7.7. 2003
Hm-Ab-01	3	*H. mantegazzianum*		N	Upper reaches of the river Bovyu (Abkhasia)	43°28′17″	40°44′50″	1290	SG	D. Geltman	24.7. 2004
Hm-Ab-02	3	*H. mantegazzianum*		N	Avadkhara (Abkhasia)	43°30′23″	40°39′13″	1475	SG	D. Geltman	26.7. 2004

Table 1 *Continued*

Code	N_i	Original determination	New species assignment based on AFLP results*	Range	Locality (country)	Latitude	Longitude	Altitude [m a.s.l.]	Preservation method	Collector	Date of collection
Hm-Lv-01	3	*H. mantegazzianum*	*H. persicum* or hybrid	I	Riga — Park Bastejkalns (Latvia)	56°58′00″	24°03′60″	n.g.	CTAB	G. Gavrilova	8.7. 2003
Hm-Lv-07	4	*H. mantegazzianum*	*H. persicum* or hybrid	I	Ergli (Latvia)	56°53′60″	25°38′00″	190	SG	G. Gavrilova	3.9. 2003
Hm-Uk-34	2	*H.* cf. *mantegazzianum*	*H. persicum*	I	London — Kensington Gardens (UK)	51°30′28″	W00°10′26″	50	SG	O. Booy	2.7. 2004
Hm-Uk-44	2	*H.* cf. *mantegazzianum*	*H. persicum*	I	London — Buckingham Palace (UK)	51°29′54″	W00°08′44″	n.g.	SG	O. Booy	31.8. 2004
Hp-Dk-01	3	*H. persicum*		I	Roskilde, E part of Bognæs (Denmark)	55°40′60″	12°01′60″	n.g.	SG	L. Fröberg	26.7. 2003
Hx-Dk-03	3	*H. laciniatum*	*H. persicum*	I	Bognæs, Roskilde (Denmark)	55°41′01″	12°02′12″	n.g.	CTAB	Ch. Nielsen	22.7. 2003
Hm-Fi-01	4	*H. persicum*		I	Karkkila (Finland)	60°31′16″	24°20′54″	100	SG	P. Uotila	21.7. 2004
Hp-Fi-03	3	*H. persicum*		I	Helsinki (Finland)	60°15′21″	24°58′16″	25	SG	P. Uotila	11.8. 2004
Hp-No-01	3	*H. persicum*		I	Balsfjord (Norway)	69°13′00″	19°04′60″	n.g.	SG	L. Fröberg	10.8. 2003
Hp-No-02	3	*H. laciniatum*	*H. persicum*	I	Tromsø city on island Tromsøya (Norway)	69°39′00″	18°57′00″	7	SG	A. Often	6.7. 2004
Hp-No-03	1	*H. laciniatum*	*H. persicum*	I	Fauske — along a main road to Bodø (Norway)	67°15′35″	15°23′04″	30	SG	A. Often	15.7. 2004
Hm-Fi-02	3	*H. persicum*		I	Tammisaari (Finland)	59°59′01″	23°24′55″	3	SG	P. Uotila	21.7. 2004
Hp-Be-01	1	*H. persicum*		n.g.	Meise — National Botanic Garden of Belgium (Accession no. 19773309) (Belgium)	50°55′29″	04°20′05″	n.g.	SG	D. De Meyere	8.7. 2005
Hp-Ir-01	1	*H. persicum*		N	Near Hassanakdar — Karaj River valley (Iran)	36°00′60″	51°17′60″	2060	Herb	M.G. Pimenov, Mozaffarian Kljuykov, Sutin	29.6. 2001
Hp-Ir-03	4	*H. persicum*		N	25 km North of Mashad (Iran)	36°21′40″	59°23′00″	1405	SG	R. Sobhian	20.6. 2005
Hs-By-01	3	*H. sosnowskyi*		I	Minsk — Botanical Garden (Belorussia)	53°54′30″	27°37′07″	203	SG	D. Geltman	23.8. 2004
Hs-De-01	3	*H. sosnowskyi*		I	Steinhöfel-Heinersdorf (Germany)	52°25′60″	14°14′00″	75	SG	J. Thiele	14.5. 2004
Hs-Hu-01	3	*H. sosnowskyi*		I	Keszthely (Hungary)	46°44′55″	17°13′23″	126	SG	I. Dancza	15.7. 2004
Hs-Lv-06a	3	*H. sosnowskyi*		I	Kekava (Latvia)	56°19′00″	24°15′60″	11	SG	G. Gavrilova	21.8. 2003
Hs-Lv-15	3	*H. sosnowskyi*		I	Krote (Latvia)	56°32′60″	21°34′00″	100	SG	I. Roze	3.6. 2004
Hs-Lv-21	3	*H. sosnowskyi*		I	Spilukalns (Latvia)	56°04′00″	27°42′00″	170	SG	I. Roze	1.7. 2004
Hs-Ru-01	2	*H. sosnowskyi*		I	Vicinity of Dvorishehe vilage, Sebezh National Park (Russia)	56°14′25″	28°24′44″	n.g.	SG	G. Konechnaya, I. Illarionova	7.5. 2004
Hs-Ru-03	2	*H. sosnowskyi*		I	Andreapol (Russia)	56°33′27″	32°15′24″	196	SG	G. Konechnaya	3.8. 2004
Hs-Ru-04	2	*H. sosnowskyi*		I	Gatchina (Russia)	59°32′37″	30°09′48″	75	SG	D. Geltman, G. Konechnaya	31.8. 2004
Hx-Dk-04	3	*H.* cf. *stevenii*	*H. sosnowskyi*	I	Copenhagen — Ryvangen Naturpark (Denmark)	55°43′18″	12°34′00″	14	CTAB	Ch. Nielsen	4.8. 2003
Hs-Ar-01	3	*H. sosnowskyi*		N	Between Tashir and Mikhaylovka (Armenia)	41°08′56″	44°17′25″	1522	CTAB	R. Wittenberg	19.7. 2003
Hs-Ar-02	3	*H. sosnowskyi*		N	Between Saratovka and Stepanavan (Armenia)	41°04′02″	44°19′41″	1504	CTAB	R. Wittenberg	19.7. 2003

Table 1 *Continued*

Code	N_i	Original determination	New species assignment based on AFLP results*	Range	Locality (country)	Latitude	Longitude	Altitude [m a.s.l.]	Preservation method	Collector	Date of collection
Hs-Ar-03	2	*H. sosnowskyi*		N	Lori Berd Fortress (Armenia)	41°00′11″	44°25′54″	1315	CTAB	R. Wittenberg	19.7. 2003
Hx-Ar-05	3	*H. trachyloma*	*H. sosnowskyi*	N	Near Lermakert (Armenia)	40°03′52″	45°16′03″	2169	CTAB	R. Wittenberg	21.7. 2003
Hx-Dk-01	3	*H. sibiricum*		I	Bognæs, Roskilde (Denmark)	55°41′05″	11°59′45″	n.g.	CTAB	Ch. Nielsen	22.7. 2003
Hx-Lv-01	3	*H. sibiricum*		I	vicinity of Allaži, Stiveri (Latvia)	57°07′60″	24°49′00″	n.g.	CTAB	G. Gavrilova	10.7. 2003
Hm-Ru-24–16	1	*H. sibiricum*		I	Mirny — 10 km SW of Majkop (Russia)	44°33′04″	40°00′10″	256	SG	Š. Jahodová, O. Booy	10.6. 2004
Hm-Ru-25–16	1	*H. sibiricum*		I	NW of Chadyzhensk (Russia)	44°28′04″	39°26′18″	148	SG	O. Booy, Š. Jahodová	10.6. 2004
Hx-Dk-02	3	*H. sphondylium*		I	Arboretet, Hørsholm (Denmark)	55°52′00″	12°30′29″	n.g.	CTAB	Ch. Nielsen	23.7. 2003
Hx-Uk-02	1	*H. sphondylium*		I	Luton (UK)	51°52′46″	W00°26′49′	n.g.	fresh	Š. Jahodová	19.7. 2004
Hx-Uk-04	3	*H. sphondylium*		I	Smug Oak (UK)	51°41′60″	W00°20′23′	n.g.	SG	O. Booy	16.7. 2004
Hm-Ru-19a-15	1	*H. ponticum*		N	Grushevaya Poljana (10–20 km S of Kurdzhinovo) (Russia)	43°53′47″	40°56′39″	920	SG	Š. Jahodová, O. Booy	7.6. 2004
Hx-Ru-03	3	*H. ponticum*		N	Lago-Naki — tourist camp (Russia)	44°05′51″	40°00′59″	1670	CTAB	R. Wittenberg	7.7. 2003
Hx-Ar-01	3	*H. antasiaticum*		N	North of Bazum (Armenia)	40°53′06″	44°26′10″	1591	CTAB	R. Wittenberg	20.7. 2003
Hx-Ar-02	3	*H. antasiaticum*		N	Near Lermontovo (Armenia)	40°45′03″	44°38′59″	1804	CTAB	R. Wittenberg	20.7. 2003
Hx-Ar-04	3	*H. antasiaticum*		N	Near Aygut (Armenia)	40°40′26″	45°12′34″	1480	CTAB	R. Wittenberg	20.7. 2003
Hx-Ar-10	4	*H. antasiaticum*		N	Near Tatev (Armenia)	39°23′10″	46°14′57″	1472	CTAB	R. Wittenberg	23.7. 2003
Hx-Ru-04	3	*H. leskovii*		N	Archyz — Observatory (Russia)	43°39′41″	41°24′59″	1782	CTAB	R. Wittenberg	10.7. 2003
Hx-Ru-01	3	*Ferulago* sp.		N	Lago-Naki — 9 km north of tourist base (Russia)	44°08′60″	40°04′00″	1260	CTAB	R. Wittenberg	7.7. 2003
Xx-Uk-01	1	*Daucus carotta*		N	Luton (UK)	51°52′46″	W00°26′49′	n.g.	Fresh	Š. Jahodová	19.7. 2004

N_i, number of individuals analysed per population; *if different from the original determination; I, invaded; N, native; n.g., not given; SG, silica gel; herb, herbarium.

that produced clear, polymorphic and reproducible patterns were selected for use in the survey. In order to achieve high throughput the primer combinations were pooled into three following multiplexes: (i) *Mse* CAA/*Eco* ACGG, *Eco* ACA, *Eco* AAG; (ii) *Mse* CTA/*Eco* ACGG, *Eco* AGC, *Eco* AGG; and (iii) *Mse* CTT/*Eco* ACAG, *Eco* AGC, *Eco* AAG. The selective PCR was performed in 5 μL. Reproducibility of the AFLP procedure was assessed by analysing DNA from two separate extractions of a randomly chosen sample (Hm-Ru-36–1) using all nine primer combinations.

Data analysis

Unambiguous polymorphic and monomorphic fragments were scored as present (1) or absent (0) using GENOTYPER® 3.7 software (Applied Biosystems, Foster City, CA, USA). Some ambiguous peaks were coded as missing values; however, markers with more than 5% of ambiguous peaks were discarded. In total, 439 (0.34%) out of 129 117 possible states in the binary matrix were coded as missing values. Genetic similarity among individuals was estimated from the number of shared amplified fragments using Dice's (1945) and Jaccard's (1908) similarity coefficients as this approach is appropriate for analysis of dominant markers (Meyer *et al*., 2004). To asses the hierarchical relationships between samples, unweighted pair group mean (UPGMA; Sneath & Sokal, 1973) and neighbour-joining (NJ; Saitou & Nei, 1987) analyses were performed based on the similarity (for UPGMA) or distance (for NJ) matrices. A sample of *D. carota* was used for rooting of the dendrogram in the NJ analysis. To test the goodness of fit of the cluster analysis to the similarity matrix, cophenetic correlation and Mantel (1967) tests with 1000 replicates were conducted. All calculations were performed using NTSYS-pc software package, versions 2.11 and 2.2 (Rohlf, 2005).

Robustness of nodes of the dendrogram was estimated using a bootstrap method as implemented in program TreeCon (Van de Peer & De Wachter, 1994). Principal coordinate analysis (PCO) was performed by double-centring the Dice's similarity matrix, and computing eigen-vectors using the Dcentre and Eigen modules of the NTSYS-pc.

To test for a relationship between geographical distance and genetic similarity, Mantel tests (1967) with 1000 permutations were performed. Dice's similarity matrix of pooled data per populations was tested against a matrix of geographical distances between populations as calculated from geographical coordinates using GIS-Arcview (Jenness, 2005).

Regression analysis was used to explore the relationship between the genetic and geographical distance of populations in native and invaded distribution ranges.

Partitioning of the genetic variation among or within taxon groups, as revealed by NJ analysis, and among or within regions (native or invaded) for *H. mantegazzianum* and *H. sosnowskyi* was assessed by an analysis of molecular variance (AMOVA; Excoffier *et al*., 1992). The statistical significance of the variance components was determined by 1000 permutations as implemented in ARLEQUIN (version 3.01) (Excoffier *et al*., 2005). Regional variation was not tested for *H. persicum* as not enough samples from the native range was available to permit statistically sound analysis.

The binary data matrices were checked for markers that may represent introgression between *H. mantegazzianum* and other *Heracleum* species in the invaded region. Those would be present in *H. mantegazzianum* and *H. shondylium* or *H. sibiricum* from Europe and missing in samples of *H. mantegazzianum* from the Caucasus.

RESULTS

The nine primer combinations were used to screen the total sample of 189 *Heracleum* individuals from 72 populations and four samples from the two outgroup species. A total of 669 AFLP markers ranging in size from 50 to 458 bp were scored. On average, 74 markers were produced per primer combination. The reproducibility between the duplicated samples was 99.7%, which is well within the acceptable average AFLP error range (Bonin *et al*., 2004; Cervera *et al*., 2005; Milla *et al*., 2005). Thirty-eight fragments were unique to the two outgroup taxa. In the 189 *Heracleum* samples, 92.6% of markers were polymorphic. The pairwise genetic similarity coefficients between *Heracleum* samples ranged from 0.47 to 0.99 for Dice coefficient (Table 2) and from 0.31 to 0.98 for Jaccard coefficient.

In the limited number of samples studied here there was no clear evidence for species-specific markers. However, some interesting discrepancies in marker frequency were noted. One marker with 100% frequency in European populations of *H. sibiricum* and *H. sphondylium* and one marker with 100% frequency in European *H. sphondylium* populations were found with 4.3% and 2.1% frequency, respectively, in European *H. mantegazzianum* (in populations Hm-Hu-1, Hm-Hu-2a). Five markers with at least 70% frequency in European *H. sphondylium* or *H. sibiricum* were present in *H. mantegazzianum* in Europe with frequencies 2.15–10.6% (populations Hm-Cz-14, Hm-De-03, Hm-Hu-2b, Hm-Se-1, Hm-Uk-22, Hm-Uk-33, Hm-Uk-45). Additionally, five more markers were present in *H. mantegazzianum* in Europe with frequencies higher than 15%. None of these markers were present in any population of *H. mantegazzianum* from the Caucasus.

Cluster analyses

The UPGMA analysis using Dice and Jaccard similarity matrices produced similar clusters (data not shown) with a high correlation to their co-phenetic matrices (0.98 and 0.976, respectively). Relationships obtained using the NJ method were similar to those obtained with UPGMA but the correlation between cophenetic matrices was greater than for UPGMA (0.99 using Dice and 0.984 using Jaccard coefficients), therefore the NJ method based on Dice similarity was considered the most suitable for determining phenetic relationships in this study.

Several clusters were apparent in the dendrogram resulting from the NJ analysis (Fig. 2). The analysis separated individuals of *H. sphondylium*, *H. sibiricum*, and *H. ponticum* into one discrete grouping (Cluster 1, Fig. 2). The remaining individuals formed a much larger cluster, which was subdivided into a discrete subcluster (Cluster 2.1, Fig. 2) containing *H. antasiaticum* and *H. leskovii*

Table 2 Pairwise Dice's similarity coefficient among selected samples

Compared samples	N*	Min	Max	Mean	SD
All *Heracleum* samples	17,766	0.471	0.990	0.809	0.082
H. mantegazzianum	2850	0.794	0.990	0.887	0.025
H. sosnowskyi	666	0.841	0.979	0.891	0.021
H. persicum	780	0.728	0.990	0.836	0.037
H. antasiaticum	78	0.798	0.954	0.856	0.035
H. sibiricum	28	0.545	0.983	0.715	0.107
H. sphondylium	21	0.809	0.899	0.854	0.026
H. mantegazzianum (Europe)	1081	0.794	0.990	0.884	0.030
H. mantegazzianum (native range)	531	0.872	0.941	0.905	0.013
H. mantegazzianum (Europe vs. native range)	1238	0.800	0.926	0.881	0.021
H. sosnowskyi (Europe)	351	0.845	0.979	0.901	0.021
H. sosnowskyi (native range)	55	0.870	0.943	0.908	0.016
H. sosnowskyi (Europe vs. native range)	297	0.841	0.906	0.876	0.012
H. persicum (Europe)	578	0.766	0.990	0.847	0.034
H. mantegazzianum vs. *H. sosnowskyi*	2888	0.786	0.921	0.871	0.020
H. mantegazzianum vs. *H. persicum*	3040	0.726	0.893	0.824	0.026
H. sosnowskyi vs. *H. persicum*	1520	0.753	0.886	0.828	0.023
H. mantegazzianum vs. *H. sosnowskyi* vs. *H. persicum*	7448	0.726	0.921	0.843	0.032
Average for 11 *H. mantegazzianum* populations in Europe[1]	n/a	0.891	0.984	0.947	0.026

N*, number of pairwise comparisons; [1]only populations where at least three samples were analysed are considered; n/a, not applicable.

(bootstrap support 100%) and a larger subcluster (Cluster 2.2, Fig. 2) combining the three invasive species (bootstrap 98%) and itself made up of several smaller clusters. The first corresponded to a subgrouping of *H. persicum* (Cluster 2.2.1, Fig. 2), which combined samples from Iran and Europe (bootstrap support 59%). A second subcluster (Cluster 2.2.2, bootstrap 69%, Fig. 2) contained distinct subgroupings of *H. sosnowskyi* and *H. mantegazzianum*, in which, for both species, the majority of samples from Europe were separated from those from the Caucasus. One of the *H. sosnowskyi* clusters comprised exclusively samples from Europe, whereas another one combined samples from Europe with those from native Armenia (bootstrap support 60%). One *H. ponticum* (Hm-Ru-19 A-15) was not grouped into any of the above-mentioned clusters and remained separated, like the outgroup taxa *Ferulago* sp. and *D. carota*.

The AFLP analysis revealed possible mislabellings or misidentifications (Fig. 2) during sample collection: (a) samples from two locations in Europe labelled as *H. sosnowskyi* were found to be genetically closer to *H. mantegazzianum* from the Caucasus than to *H. sosnowskyi*; (b) putative hybrids of *H. ponticum* × *H. mantegazzianum*, which had been classified as such on the basis of its intermediate morphology, clustered closely with other *H. mantegazzianum* samples from native Caucasus; (c) samples labelled as *H. stevenii* clustered together with *H. sosnowskyi* samples from Armenia; (d) samples from one Armenian population labelled as *H. trachyloma* were found very close to *H. sosnowskyi* samples from Armenia; (e) samples from two parks in London (UK) provisionally identified as *H.* cf. *mantegazzianum* were found to be closer to *H. persicum* than to *H. mantegazzianum*; (f) samples from three different locations labelled as *H. laciniatum* clustered with *H. persicum*; and (g) samples taken from individuals in two parks in Latvia and labelled as *H. mantegazzianum* showed great genetic similarity to *H. persicum*. In all of the above cases, except the last, the position of the samples was consistent for UPGMA, NJ, and PCO analyses and also when individual primer combinations were analysed separately (data not shown).

Separate NJ analyses for samples from the native and the invaded ranges were also conducted. Figure 3 shows a dendrogram resulting from the analysis of 85 samples from native areas representing all 10 species. Overall, the clustering pattern was the same as for the previous analysis. However, a very strong bootstrap support (above 95%) was detected for *H. mantegazzianum*, *H. sosnowskyi*, and *H. persicum*. In contrast, analysis of samples of these three species from the invaded area lacked bootstrap support for the majority of clusters, including species clusters (data not shown).

Principal coordinate analysis

The results of the PCO analysis of all 189 *Heracleum* samples revealed similar groupings to that of the NJ analysis, except that all samples of *H. ponticum* including the sample Hm-Ru-19a-15 were grouped together. Four axes (explaining 30.1% of the total variance) were needed to separate all species except *H. sphondylium* from *H. sibiricum* (data not shown).

Similarly, PCO analysis of the invasive species (*H. persicum*, *H. sosnowskyi*, and *H. mantegazzianum*) from both native and invaded ranges (Fig. 4) segregated the samples into three groups corresponding to the three species. Species separation was more pronounced between samples from native areas. Moreover,

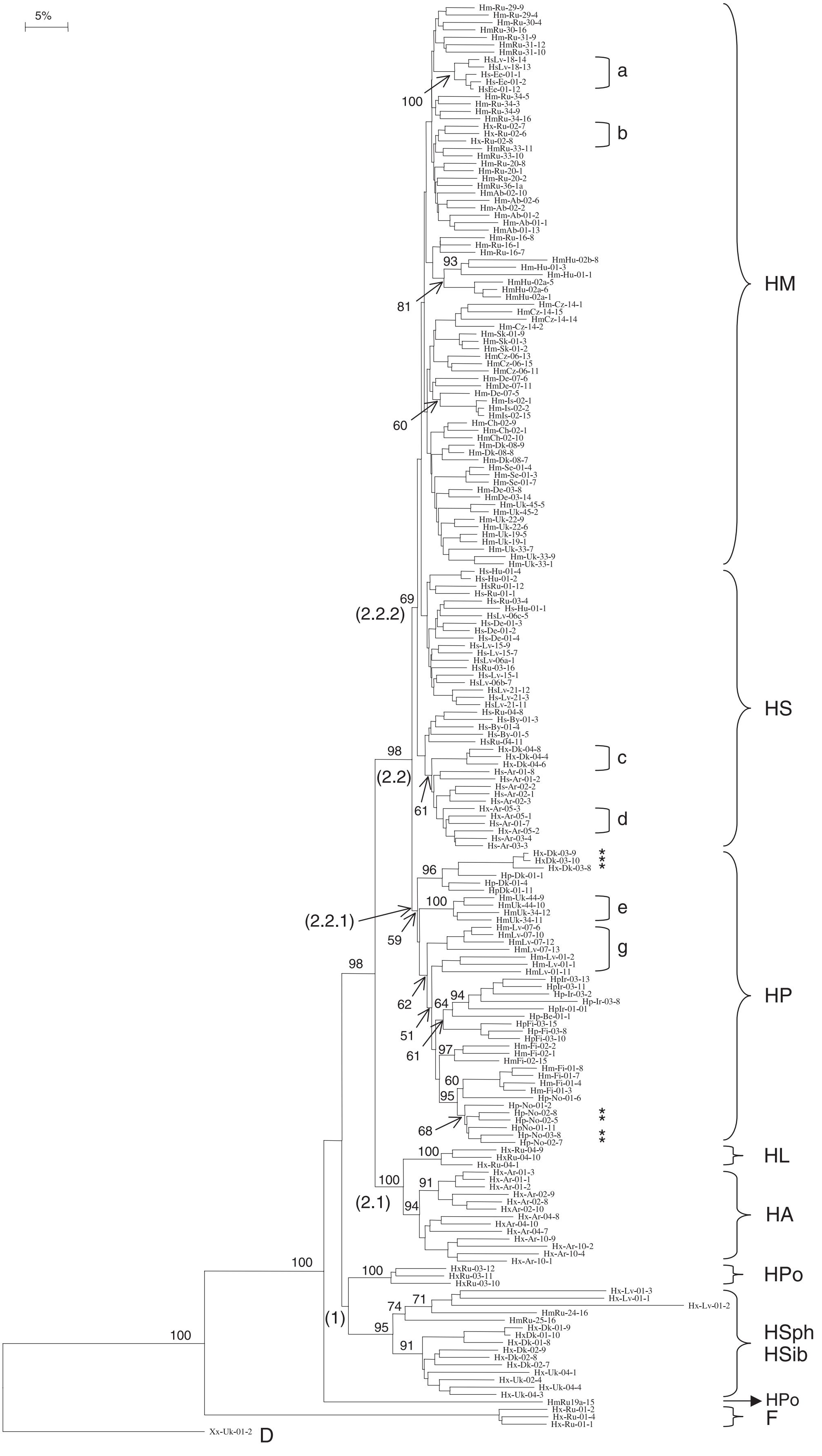

Figure 2 Neighbour-joining analysis of AFLP data showing relationships between all samples. The dendrogram was rooted with a sample of *Daucus carota*. Bootstrap values above 50% (500 replicates) are given for higher than population clusters (numbers at nodes; arrows have been added to indicate the position of values in places where there is insufficient space to enter them directly). Numbers in brackets indicate well-defined clusters, letters a–g indicate misidentified samples (see Results). Asterisks indicate the position of samples labelled as *H. laciniatum*. HM — *Heracleum mantegazzianum*; HS — *Heracleum sosnowskyi*; HP — *Heracleum persicum*; HL — *Heracleum leskovii*; HA — *Heracleum antasiaticum*; HPo — *Heracleum ponticum*; HSph — *Heracleum sphondylium*; HSib — *Heracleum sibiricum*; F — *Ferulago* sp.; D — *Daucus carota* (for sample codes see Table 1).

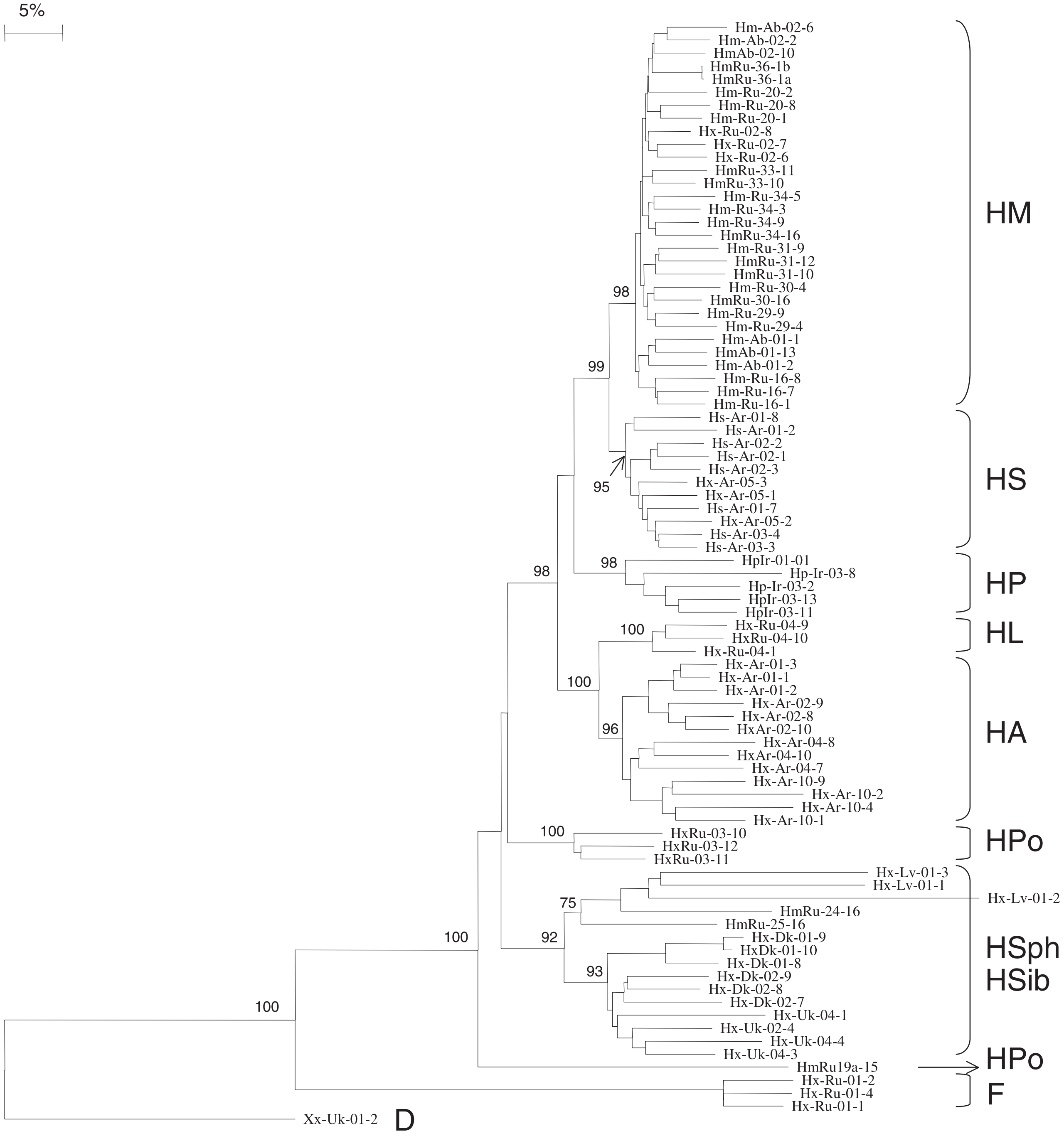

Figure 3 Neighbour-joining analysis of samples from native distribution areas. The dendrogram was rooted with a sample of *Daucus carota*. Bootstrap values above 50% (300 replicates) are given for clusters at species' or higher level (numbers at nodes) (for legend see Figure 2).

for *H. mantegazzianum* and partly also for *H. sosnowskyi*, the PCO analysis separated samples from their native and invaded ranges. Considerable variation was detected in samples from the invaded range, especially in the case of *H. mantegazzianum* and *H. persicum*.

Correlation between genetic and geographical distance

Mantel tests revealed a significant positive correlation between geographical and genetic distance for *H. mantegazzianum* populations sampled in the native range of the Caucasus ($r = 0.561$, $t = 3.681$, $P = 0.001$). Regression based on all possible pairwise combinations also indicated isolation by distance (genetic distance = 0.0579 geographical distance$^{0.0917}$; $F = 37.72$, d.f. = 1, 53; $P < 0.0001$, $R^2 = 0.416$) (Fig. 5). In contrast, the genetic distance of populations from the invaded range was not correlated with their geographical distance in *H. mantegazzianum* ($r = 0.146$, $t = 0.877$, $P = 0.158$; with outlying population from Iceland excluded: $r = 0.198$, $t = 1.468$, $P = 0.087$), *H. sosnowskyi* ($r = 0.16$, $t = 0.653$, $P = 0.217$), and *H. persicum* ($r = 0.172$, $t = 1.089$, $P = 0.165$).

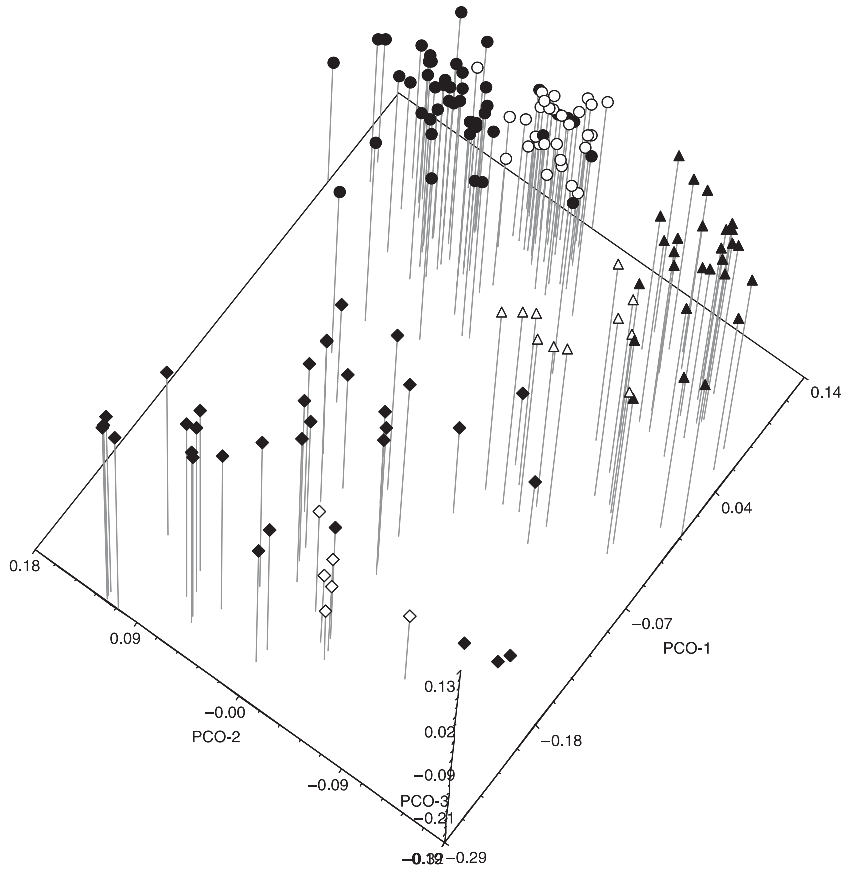

Figure 4 Principal coordinate analysis (PCO) diagram showing the position of samples of three invasive *Heracleum* species from both native (Caucasus, Armenia, and Iran, respectively) and invaded (Europe) distribution ranges in a three-dimensional space. The first three axes together explain 20.7% of variability. Circles — *Heracleum mantegazzianum*; triangles — *Heracleum sosnowskyi*; diamonds — *Heracleum persicum*; open symbols — samples from native ranges; closed symbols — samples from Europe.

AMOVA

The results of AMOVA analyses are summarized in Table 3. Approximately 36% of variation in the data set were distributed among species when all eight *Heracleum* species were considered as separate groups. For the three invasive species alone it was 19%, i.e. less than for the five non-invasive species (43%). When populations of *H. mantegazzianum*, *H. sosnowskyi*, and *H. persicum* from native ranges were analysed separately from those invading in Europe, the proportion of variation attributable to the differences among species was higher in the native (39%) than invaded range (23%). This result is in a perfect agreement with the pattern revealed by NJ and PCO analyses. AMOVA between native and invaded regions for *H. mantegazzianum* and *H. sosnowskyi* showed that most of variation (90% and 77%, respectively) is distributed within regions for both species.

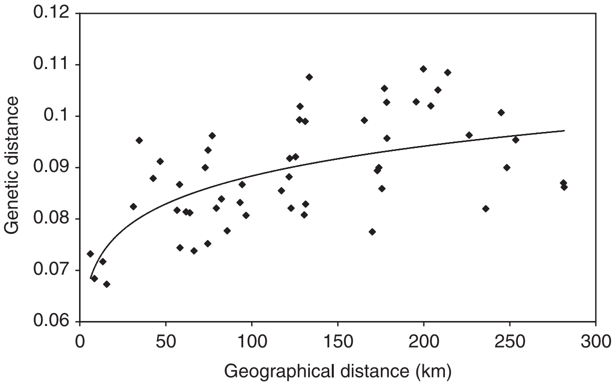

Figure 5 Genetic distance between *Heracleum mantegazzianum* populations (calculated as 1 – Dice similarity coefficient) in their native range in the Caucasus plotted against the geographical distance between populations. Eleven populations were sampled and the plot is based on all possible pairwise combinations. GENETIC DISTANCE = 0.0579 GEOGRAPHICAL DISTANCE$^{0.0917}$; $F = 37.72$, d.f. = 1, 53; $P < 0.0001$, $R^2 = 0.416$.

DISCUSSION

General pattern of genetic relatedness between and within species

Neighbour-joining and principal coordinate analyses revealed higher genetic similarity between samples of *H. mantegazzianum*, *H. sosnowskyi*, and *H. persicum* than between those species and *H. sphondylium*, *H. sibiricum*, *H. ponticum*, *H. antasiaticum*, and *H. leskovii*. A close genetic relationship between *H. mantegazzianum* and *H. persicum* was also inferred from a recent phylogenetic study (M. Zych, unpublished data). Our results show that invasive plants sampled in the European distribution range can be assigned to three taxa: *H. mantegazzianum*, *H. sosnowskyi*, and *H. persicum*. The conspecificity of European samples with the respective

Table 3 The results of analyses of molecular variance (AMOVA) of AFLP data for selected sample groups. All estimations are significant at $P < 0.001$

Grouping	Number of samples	Number of species groups	Source of variation	d.f.	Sum of squares	Variance components	% variation
All *Heracleum* samples	189	8	Among species	7	2981.52	19.46	35.95
			Within species	181	6275.94	34.67	64.05
H. mantegazzianum, *H. sosnowskyi*, and *H. persicum* (Europe and native ranges)	154	3	Among species	2	828.35	7.87	19.06
			Within species	151	5046.63	33.42	80.94
H. sphondylium, *H. sibiricum*, *H. ponticum*, *H. antasiaticum*, and *H. leskovii*	35	5	Among species	4	896.09	28.48	43.31
			Within species	30	1118.17	37.27	56.69
H. mantegazzianum, *H. sosnowskyi*, and *H. persicum* (Europe)	92	3	Among species	2	664.74	9.28	22.65
			Within species	95	3010.54	31.69	77.35
H. mantegazzianum, *H. sosnowskyi*, and *H. persicum* (native ranges)	45	3	Among species	2	429.42	16.39	39.01
			Within species	42	1076.58	25.63	60.99
	76	1	Among regions	1	140.03	3.12	9.92
H. mantegazzianum (Europe vs. Caucasus)			Within regions	74	2092.69	28.28	90.08
H. sosnowskyi (Europe vs. Armenia)	38	1	Among regions	1	144.57	7.61	22.97
			Within regions	36	919.33	25.54	77.03

species from native areas was evident from the pattern of clustering on the dendrograms and PCO plots (Figs 2 & 4).

For all three invasive *Heracleum* species, more within-taxon variation was detected in the invaded range (Europe) than in the region of native distribution. It has been previously suggested that hybridization and inbreeding may be important incentives in evolution of invasive species during their initial establishment and subsequent range expansion (Barrett & Richardson, 1986; Ellstrand & Schierenbeck, 2000; Tsutsui *et al*., 2000). It is likely that there were opportunities for intraspecific hybridization following the introduction of the *Heracleum* species because they were introduced to Europe as garden ornamentals, and seed exchange was very popular in the 19th and at the beginning of 20th century not only between botanical gardens, but also between private landlords (Nielsen *et al*., 2005). Existence of putative hybrids between the native *H. sphondylium* and the invasive *H. mantegazzianum* was recorded in Britain (McClintock, 1973; Grace & Stewart, 1978) and Germany (Ochsmann, 1996) and confirmed by experimental crossing (Weimarck *et al*., 1979; Stewart & Grace, 1984). However, Weimarck *et al*. (1979) noted that backcrossing is rare. In a study of pollen loads on a hybrid *Heracleum* site, Grace & Nelson (1981) concluded that selective foraging behaviour of insect was a main barrier to gene flow between *H. sphondylium* and *H. mantegazzianum* in Britain. Interspecific hybridization was also studied between several other species of *Heracleum* in Russia as a part of a breeding programme of hogweeds as fodder plants (Boodiak *et al*., 1981).

In the present study, only a low number of individuals of *H. mantegazzianum* from Europe carried markers that were absent in *H. mantegazzianum* from Caucasus. To find out whether these are due to introgression or new polymorphism requires further study.

A small proportion of the total variance was found between continents for *H. mantegazzianum* and *H. sosnowskyi* (9.92% and 22.97%, respectively), which is in general concordance with the previously reported variation for other invasive species, e.g. 3.74% for selfing biennial *Alliaria petiolata* (Durka *et al*., 2005) and 6.4% for apomictic *Erigeron annuus* (Edwards *et al*., 2006). The values may differ due to differences in breeding system, introduction history and also due to markers used (Woodhead *et al*., 2005).

Variation between native and introduced regions was lower for *H. mantegazzianum* compared to *H. sosnowskyi* and indicates that *H. mantegazzianum* has higher gene flow between the ranges, possibly due to more frequent independent introductions, thus reducing the bottleneck on the continental scale.

Implications for the taxonomy of tall *Heracleum* species

In the present study, although the three invasive species showed a high overall genetic similarity and significant intraspecific variation, they could be distinguished using AFLPs. Several cases of mislabelling or misidentification were revealed between *H. mantegazzianum* and *H. sosnowskyi* and between *H. mantegazzianum* and *H. persicum*. The putative hybrids, *H. ponticum* × *H. mantegazzianum,* were clustered closely with samples of *H. mantegazzianum* from native Caucasus. The consistent positioning of samples obtained by using different analyses supports the view that the molecular data presented in our study reveal the true identity of the taxa studied.

A sample labelled as *H. stevenii* clustered together with *H. sosnowskyi* samples from Armenia. *Heracleum stevenii* is closely related to *H. antasiaticum* and *H. leskovii* (Mandenova, 1950), which were also analysed in the present study. It was assumed that *H. stevenii* would be genetically closer to *H. antasiaticum* and *H. leskovii*, than to *H. sosnowskyi* or any other species. As this was not the case, it is quite likely that this sample of *H. stevenii* is in fact *H. sosnowskyi*. Furthermore, plants from two parks in

Latvia labelled as *H. mantegazzianum* showed a great genetic similarity to *H. persicum*. The inconsistent positioning of these plants on the dendrograms suggests that they are of hybrid origin.

AFLP also helped to resolve the status of the Scandinavian invasive species with the common name 'Tromsø palm', frequently referred to as *H. persicum* or *H. laciniatum* auct. scand. Samples from different locations labelled as *H. laciniatum* did not form a genetically distinct group; instead, they clustered with *H. persicum*. This, together with lacking formal description for *H. laciniatum*, implies that *H. laciniatum* auct. scand. is likely to be synonymous with *H. persicum*. The identity of *H. persicum* has now also been confirmed morphologically by comparison with herbarium material from Turkey (L. Fröberg, unpublished data).

None of the statistical methods employed to analyse the AFLP data distinguished between the two closely related species: *H. sphondylium* and *H. sibiricum*. These are considered as separate species in the Flora of Caucasus (Mandenova, 1950), whereas Flora Europea (Brummitt, 1968) treats *H. sibiricum* as a subspecies of *H. sphondylium*. Our results accord with the latter treatment.

Reconstructing the invasion history of *Heracleum* in Europe

The general pattern of clustering of *H. mantegazzianum* individuals sampled from the native and invaded ranges into separate groups prevents us from direct speculation about the origin of the invading plants. However, it unveils substantial genetic differentiation between the invading and native populations which may be caused by genetic drift during founding events (Hawley *et al*., 2006) and may indicate rapid evolution in the new range (Bossdorf *et al*., 2005). An exception to the general pattern is the genetic proximity of two populations from Europe (Tali, Estonia and Kirbizi, Latria) to those from the caucasus. This may indicate a distinct introduction event.

The genetic proximity of samples from three Hungarian (Hm-Hu-01, Hm-Hu-02a, Hm-Hu-02b) and one Caucasian population (Hm-Ru-16; Fig. 2) indicates another possible separate introduction event of *H. mantegazzianum* into Europe.

Like in *H. mantegazzianum*, the populations of *H. sosnowskyi* sampled in Europe were distinct from those from the native distribution range. *Heracleum sosnowskyi* was introduced to Eastern Europe as a crop plant; seeds from the Republic of Kabardino-Balkaria (Russian Caucasus) and Dagestan were used in plant breeding programs in NW Russia (Murmansk and Leningrad) (Marchenko, 1953). The main goal of these programmes was to produce a high-yielding variety with the minimal content of furanocoumarins, a substance responsible for photodermatitis. Selection for these compounds may well have resulted in distinct European genotypes. However, the samples of *H. sosnowskyi* from Denmark (Hx-Dk-04, originally determined as *H.* cf. *stevenii*, see above) were clustered with those from Armenia, suggesting that this is an example of a separate introduction event.

The samples of *H. persicum* collected in Europe were not clearly separated from the few samples obtained from Iran. Samples of *H. persicum* collected in Denmark, on the other hand, clustered separately from those collected in Norway and Finland, suggesting that multiple introductions were responsible for invasion of *H. persicum* to Nordic countries.

The lack of correlation between genetic and geographical distance for all three invasive *Heracleum* taxa in Europe indicates predominantly human-mediated dispersion at the continental scale (Ward, 2006). In the native range, however, the genetic distance between populations of *H. mantegazzianum* showed a highly significant increase with increasing geographical distance, indicating isolation by distance. The increase in dissimilarity with geographical distance was not found to be a linear relationship, suggesting that there is a threshold beyond which geographical isolation no longer promotes increase in dissimilarity between populations of *H. mantegazzianum.* Isolation by distance was not possible to test for the other two taxa in the native range due to insufficient numbers of sampled populations and this would be an interesting topic for further study.

Conclusions

This study is the first to demonstrate that within the taxonomically complex genus *Heracleum*, there are three distinct, but genetically close species, that invade in Europe. *Heracleum laciniatum* is synonymous with *H. persicum* in Nordic countries. The high overall genetic variability in the invaded range suggests that, as far as the continental scale is concerned, the species were not affected by genetic bottleneck upon their introduction to Europe. The results indicate that multiple introductions of all three species are likely to have occurred. Following the introduction, rapid evolution, drift, or hybridization are assumed to have played a role in genetic structuring of these invading populations.

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