Population genetics of Giant Hogweed (*Heracleum mantegazzianum:* Apiaceae) **in the western Swiss Alps: evidence for high genetic drift and loss of genetic diversity in invasive populations**

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Abstract

The Giant Hogweed (*Heracleum mantegazzianum*) has successfully invaded 20 European countries as well as some parts of Canada and the USA. It has become a problematic species as it is able to displace native biodiversity and is also regarded as a health problem. In the present study we used a set of eight nuclear microsatellites together with four plastid DNA markers to analyze the invasion history and population genetics of *H. mantegazzianum* in the western Swiss Alps. Additionally samples from the native range were included to have some basis for comparison of genetic diversity between populations from the two ranges. The molecular markers used exhibited substantial variation, and identified strong isolation of invasive populations, highlighting the role of genetic drift during the invasion process. Native populations exhibited substantially higher levels of genetic diversity compared to invasive populations, confirming a founder effect during the invasion process. Although invasive population went through an initial founder event, relatively high genetic diversity was observed in invasive populations, indicating that rapid population expansion coupled with occasional gene flow between populations possibly helped maintain substantial levels of genetic diversity within invasive populations.

Key words: Biological invasions, Population Genetics, Microsatellites, Plastid DNA, Assignment tests.

Résumé

La Berce du Caucase (*Heracleum mantegazzianum*) a envahis 20 pays Européens ainsi que certaines parties du Canada et des Etats-Unis D'Amérique. Cette espèce est devenue problématique car sa présence s'accompagne d'une réduction de la biodiversité native. Elle peut aussi être nocive pour l'homme et constitue donc un problème de santé publique. Dans la présente étude, nous avons utilisés huit microsatellites nucléaires ainsi que quatre loci chloroplastiques pour caractériser l'historique d'invasion et la génétique de populations de Berce du Caucase dans les Préalpes Vaudoises. De plus, un échantillonage dans l'aire d'origine de la plante a été inclus pour avoir une base de comparaison de la diversité génétique se trouvant dans les populations invasives et natives. Les marqueurs moléculaires utilisés démontrent une variabilité importante, et ont identifiés une forte isolation entre les populations invasives, probablement dû au à de la dérive génétique pendant le processus d'invasion. Les populations natives sont caractérisées par une diversité génétique significativement plus élevée que dans les populations invasives. Ceci confirme l'hypothèse d'un effet fondateur lors de l'introduction de la Berce du Caucase en Suisse. Toutefois, les populations invasive contiennent un niveau de diversité génétique relativement élevé, indiquant qu'une expansion rapide de ces populations couplé avec du flux de gènes occasionnel ont probablement aidé a maintenir une diversité importante à l'intérieur des populations invasives.

Mots clés : Invasions Biologiques, Génétique des populations, Microsatellites, ADN chloroplastique, Tests d'assignements.

Introduction

Biological invasions are natural phenomena (Sauer, 1988; Mack *et al.,* 2000; Brown & Sax, 2004) that have occurred throughout geological times, and have contributed to shaping the diversity of life on earth (Vermeij, 1991; Overpeck *et al.,* 1992). During the past 500 years, humans developed efficient means of transportation on a global scale and often transported species along with them (Baker, 1974; Crosby, 1986; Sykora, 1990), thus breaching main biogeographical barriers and leading to homogenization of global biotas (Lodge, 1993; Olden *et al.,* 2004). Invasive Alien Species (IAS; sensu Richardson *et al.,* 2000; Pyšek *et al.,* 2004) are now considered a significant component of human induced global environmental change (Elton, 1958; D'Antonio & Vitousek, 1992; Lodge, 1993; Vitousek *et al.,* 1996, 1997a, 1997b; Mack *et al.,* 2000; Cassey *et al.,* 2005), and are one of the most important threats to biodiversity worldwide (Heywood, 1989) second in impact only to the destruction and fragmentation of habitats (Walker & Steffen, 1997; Wilcove *et al.,* 1998; Sala *et al.,* 2000; Genovesi & Shine, 2002). Indeed, IAS can profoundly alter ecosystem structure and processes (e.g. Hobbs & Mooney, 1986; Vitousek & Walker, 1989; Vitousek, 1990; Dukes & Mooney, 2004; Hawkes *et al.,* 2005), compete with native species for limited resources (e.g. Williams, 1994; and references therein) and increase disturbance in invaded ecosystems (Williamson, 1996; Schmitz *et al.,* 1997; but see Mack *et al.,* 2000 for further examples).

Human mediated alteration of ecosystems has further promoted the establishment of IAS as pristine habitats are less easily reached and invaded than disturbed communities found in proximity of ports and main cities (Williamson, 1996; Lozon & MacIsaac, 1997;

Pyšek & Richardson, 2006). IAS can also indirectly interact with one another to enable each other to become invasive, i.e. invasion meltdown (Grosholz, 2005). Furthermore, other elements of global change can partly interact with biological invasions and increase the prevalence of invaders (Dukes & Mooney, 1999; Benning *et al.,* 2002). Apart from the damages done to ecosystems and biodiversity, IAS are also responsible for severe negative impacts on economy (e.g. Nalepa & Schloesser, 1993), particularly in forestry, agriculture, fisheries and management costs of invasive species (Pimentel *et al.,* 2000; Perrings *et al.,* 2002; Allendorf & Lundquist, 2003; Pimentel *et al.,* 2005). Additionally, certain invasive species are the causes of some serious health hazards (Soulé, 1992).

Much of the research done on IAS has focused on their ecology and attempted to identify traits common to successful invaders and invaded communities (Lambrinos, 2004; Richardson & Pysek, 2006). However, unsatisfactory answers were often found (e.g. Baker & Stebbins, 1965; Drake *et al.,* 1989; Roy, 1990; Groves & di Castri, 1992; Lodge, 1993; Kolar & Lodge, 2002; Shaffner, 2005). For instance Rejmanek and Richardson (1996) studied Pine species introduced to South Africa and identified small seed mass, short juvenile period and short intervals between seed sets as the best predictors of invasiveness. They also highlight taxonomic affinities as important predictors of species' invasiveness. Forcella *et al.* (1986) identified that the native latitudinal range of herbaceous species is the best predictor of their invasive potential. As yet, no universal predictor has been found to explain species' invasiveness (Kolar & Lodge, 2001), but taxonomic affinities with known invasive species (Reichard & Hamilton, 1997) and features likely to endow species with higher relative fitness (Roy, 1990) are often attributes of invasive species (i.e. large number of seeds, ecological dominance, etc). One can assume that combinations of appropriate life

history traits, favorable environmental conditions (i.e. similar conditions in native and invaded range) as well as anthropogenic changes to the environment (e.g. disturbance) may all contribute to species' invasiveness (Thuiller *et al.,* 2006).

Population and evolutionary genetics (reviewed in Sakai *et al.,* 2001; Lee, 2002) hold promise to better understanding species' invasiveness, as well as bringing insight into novel management practices (Hufbauer, 2004). The recent development of molecular tools such as microsatellites, coupled with the development of powerful statistical analyses and user-friendly computer packages have proven to be highly pertinent to the study of population biology of IAS.

Upon introduction, the sub-samples of original (native) populations are often faced with genetic processes that may reduce the amount of genetic variation within populations and increase differentiation between populations (e.g. Thulin *et al.,* 2006). Bottlenecks and genetic drift (arising through the introduction of a limited amount of individuals isolated from source populations) have often been identified as reasons for the low level of genetic diversity observed in invasive populations (e.g. Husband & Barrett, 1991; Amsellem *et al.,* 2000; Abdelkrim *et al.,* 2005; Colautti *et al.,* 2005; Grapputo *et al.,* 2005; Lindholm *et al.,* 2005; Parisod *et al.,* 2005). This reduced genetic diversity in introduced populations (Nei *et al.,* 1975) might limit local adaptations to the novel environment (Marron *et al.,* 2004). On the other hand, multiple introductions (e.g. Genton *et al.,* 2005; Bossdorf *et al.,* 2005; Durka *et al.,* 2005; Williams *et al.,* 2005; Facon *et al.* 2006; Kelly *et al.,* 2006) of previously isolated native populations of the same species will tend to increase the amount of genetic variance lost during the founder event (by converting inter population genetic variance in the native range into intra population variance in the

invaded range; c.f. Kolbe *et al.,* 2004), thus leading to an increased potential for evolutionary change (Marron *et al.,* 2004). Rapid population expansion may also help maintain high levels of genetic variance within populations (e.g. Zenger *et al.,* 2003; Bousset *et al.,* 2004). Moreover, additive variance has been shown to be released from epistatic interactions among a group of neutral traits during population bottlenecks (Naciri-Graven & Goudet, 2003), thus increasing the raw material for evolutionary change to occur. Finally, hybridization between individuals from two previously separated populations or even different sub-species or species in the invaded range may act as a stimulus for the evolution of invasiveness by producing novel genotypes which, by chance may be highly adapted to local conditions (Ellstrand & Schierenbeck, 2000; Abbott *et al.,* 2003). All these factors will act separately or to a certain extent together to shape the fate of an invading species (Marron *et al.,* 2004; Bossdorf *et al.,* 2005).

Heracleum mantegazzianum originates from the Caucasus Mountains of south-west Asia where it occurs in clearings, meadows and forest margins of the upper forest belts up to 2000 m (Mandenova, 1950). It has successfully established invasive populations in 20 Central and Western European countries (Tiley *et al.,* 1996; and references therein) as well as Canada and the USA (Morton, 1978; Dawe & White, 1979) and is known to have significantly increased its geographical range in recent years (Pyšek, 1991, 1994; Tyley *et al.,* 1996; Caffrey, 1999; Jeanmonod, 2005). In Switzerland, *H. mantegazzianum* is one of the 20 species in the Swiss Commission for Wild Plant Conservation (CPS/SKEW) black list. It has become a problematic species as it is able to reduce native biodiversity (Pyšek & Pyšek, 1995), increases erosion in river banks (which may affect salmonid spawning; e.g. Caffrey, 1999) and may cause a health hazard when its sap comes in contact with human skin

subsequently exposed to UV rays (Drever & Hunter, 1970; Tyley *et al.,* 1996; and references therein). A large seed set, large size, the ability to germinate early in spring (prior to native species) and its undeniable link with man (through horticulture and apiculture) are among the many characteristics that have promoted the establishment of the species and endowed it with its great invasive potential.

Most recent studies carried out on *H. mantegazzianum* concerned its biology, ecology or related management practices (Tiley *et al.,* 1996; Pyšek *et al.,* 2007). More particularly, certain studies focused on the spread of the species in invaded areas (Pyšek, 1991; Pyšek & Pyšek, 1995; Pyšek *et al.,* 1998; Müllerova *et al.,* 2005). All together these studies highlight the role of rivers and anthropogenic means (roads, railways, etc) as dispersal vectors of diaspores. Other studies focused on the reproductive behavior and population age structure of the species (Pyšek *et al.,* 1995; Caffrey, 1999; Krinke *et al.,* 2005; Pergl *et al.,* 2006). The first two studies focused on the effect of different management efforts and the response of the plants to the control measures. Both studies identified control of seed input and long-term follow-up as most efficient management measures. Krinke *et al.* (2005) provided the first study on seed bank in *H. mantegazzianum.* They once again highlight the enormous reproduction potential of the plant, and identify seasonal dynamics in the composition of the persistent seed bank. Pergl *et al.* (2006) identified that in unmanaged sites of the invaded range, plants flowered earlier than in the native range, and that flowering was delayed in managed sites. They also highlight that the favorable climate in the invaded range and increased chances for dispersal due to high human population density are possible factors enabling invasion of *H. mantegazzianum.*

To this date, only two studies have used genetics to study the invasion of

H. mantegazzianum. Walker *et al.* (2003) used four nuclear microsatellite loci and one plastid loci to investigate the population structure of *H. mantegazzianum* in north-east England. They found high genetic diversity, and attributed it to a large initial founding population or multiple introductions. They also suggested strong population sub-structuring due to genetic drift occurring during the initial founding, but had no means of comparison with populations from the native range. Jahodová *et al.* (2007) used AFLP makers to characterize the genetic similarity of invasive *Heracleum* species found in Europe and compared it to samples from the native range. They also found high levels of genetic diversity in the invaded range, and suggested that those populations were not affected by bottlenecks and possibly resulted from multiple introductions.

In the present study, we investigate the population genetic structure and genetic variability of *H. mantegazzianum* in western Switzerland and compare them with samples from the native range (Fig. 1; Appendix. 1). The objectives of the present study can be summarized in the following questions: (i) What is the genetic relationship between *H. mantegazzianum* populations from the native and invaded ranges? (ii) Do invasive populations have less genetic diversity than native populations? (iii) What is the underlying genetic structure of invasive populations from the western Swiss Alps? (iv) Which populations (in the western Swiss Alps) may have served as initial sources from which all other invasive populations are issued?

Material and Methods

Study species

Historical accounts suggest that *Heracleum mantegazzianum* was first discovered by S. Sommier and E. Levier in the early 1890's in the Kliutsch Valley, Abkhazia (Sommier & Levier, 1895), but the species is known to occur throughout western Caucasus (Nielsen *et al.,* 2005). Seeds of the plant were brought to H. Correvon in 1892 at the Botanical Garden of the Horticultural Society for Plant Acclimation in Plainplais, Geneva, Switzerland (Jeanmonod, 1999). The plant was subsequently described as a new species of *Heracleum* (Sommier & Levier, 1895). H. Correvon then greatly contributed to the dissemination of the plant in botanical gardens throughout Europe (Perrier, 2001) as well as alpine botanical gardens in western Switzerland (Dessimoz, 2006). However, earlier records of *H. mantegazzianum* introduction derive from Great Britain, as the plant was listed in 1817 at Kew Botanical Gardens in London (Tyley *et al.,* 1996; Nielsen *et al.,* 2005) as well as from the Czech Republic where the species is said to have been introduced in 1862 (e.g. Müllerova *et al.,* 2005). The fact that the historical records of the introduction of *H. mantegazzianum* in Europe are divergent according to the countries may suggest that the species has been introduced multiple times in Europe, possibly from disparate source populations (c.f. Pyšek, 1991; Jahodová *et al.* 2007).

Heracleum mantegazzianum is characterized by many attributes common to invaders: it is a tall, herbaceous, short-lived perennial which is often dominant where established and is dispersed mainly by anthropogenic means (Nielsen *et al.,* 2005). Its common name, "Giant Hogweed", illustrates the dimensions of the plant: its stout stem can sometimes

reach heights of 5.5 m, its alternate leaves can measure 2.5 m in length and its inflorescences (compound umbels) can reach a diameter of 50 cm (Tyley *et al.,* 1996). The typical life cycle of *H. mantegazzianum* lasts four years, with reproduction occurring in the last year (Tyley *et al.,* 1996; Caffrey, 1999). In western Switzerland, *H. mantegazzianum* germinates in spring (February to April), flowering starts at the end of June and continues until August. Fruits are shed in September. There, it occurs from an altitude of 380 meters to 1980 meters.

Flowers are hermaphrodite, protandrous, insect pollinated and self-compatible. Up to ten umbels can be present on one stem, with the largest being at the center. Each umbel can bear thousands of flowers. The plant reproduces exclusively by seeds and a single individual can produce between 5'000 and 100'000 seeds with an average of 20'000 seeds per plant (Pyšek *et al.*, 1995; and references therein). Seeds require a period of moist followed by frost in order to successfully germinate (Tyley *et al.,* 1996), this in turn results in a short-term persistent seed bank (Krinke *et al.,* 2005) containing seeds, that if under favorable conditions will almost all germinate (Tyley *et al.,* 1996). The bulk of the seed set is dispersed around the plant's stalk by wind to a distance of about ten meters (Neiland *et al.,* 1987), although some seeds may be found at distances of 50 meters from mother plants (Caffrey, 1994). In addition, long distance dispersal can occur naturally along water courses, as seeds can float for up to three days (Clegg & Grace, 1974; Dawe & White, 1979). Long distance anthropogenic dispersal can be mediated by vehicles and trains along roads and railways, as well as by direct collection of seed heads by people who sometimes used them for decorative purposes (Lündstorm, 1984).

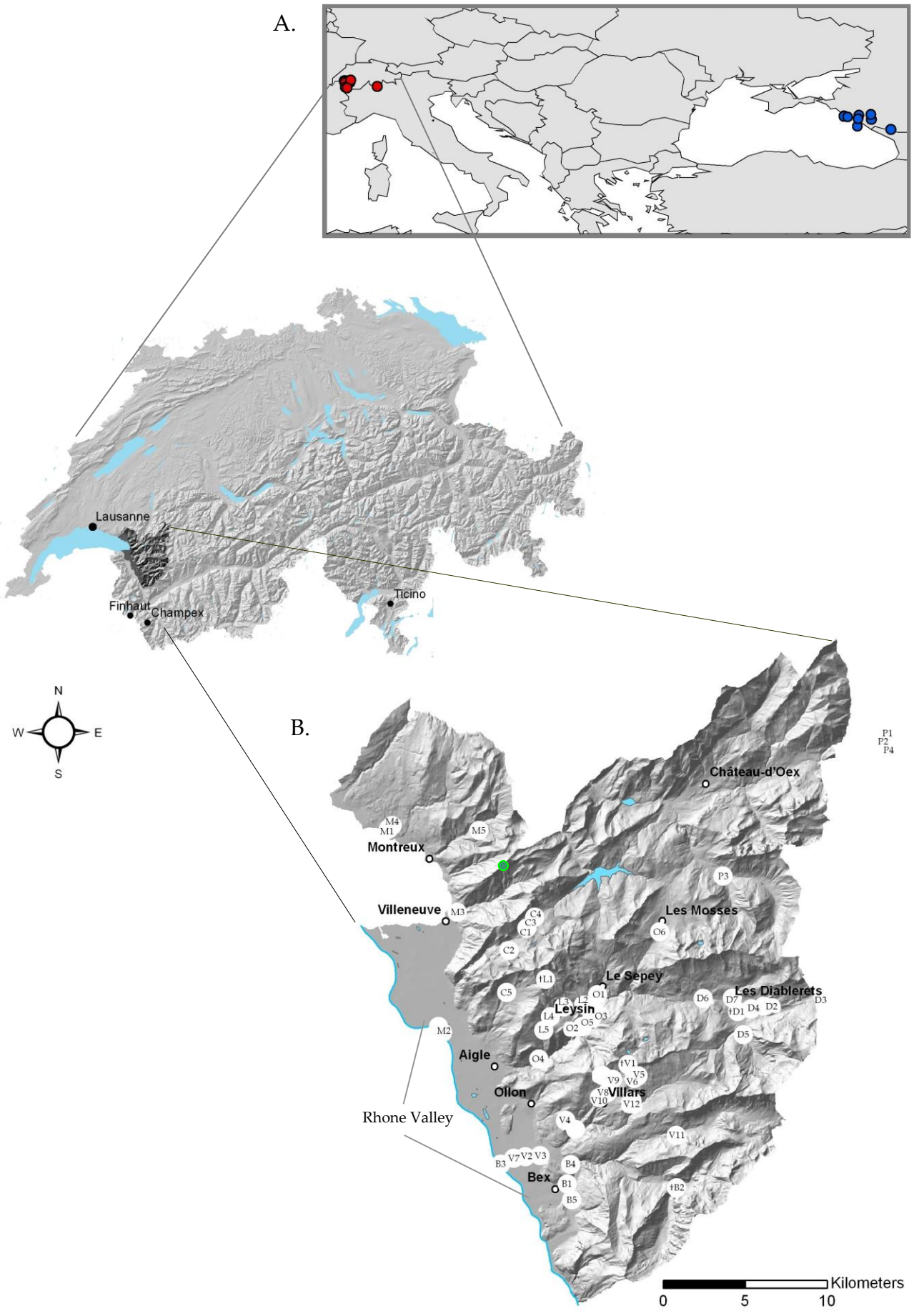
Heracleum mantegazzianum's ornamental peculiarities were the major cause of its far-

flung dissemination (Nielsen *et al.*, 2005). Beekeepers are said to have helped to spread the plant further as their flowers were thought to produce prolific amounts of nectar and were thus used to increase honey yield (Reinhardt *et al.*, 2003). This has apparently been the case in the study area (Dessimoz, 2006; E. Mottier, personal communication). *Heracleum mantegazzianum* has repeatedly escaped from the foci of introduction and can currently be found in villages, roadsides, railway embankments, farmlands, meadows and riparian habitats at considerable distances from human settlements (Jeanmonod, 1999, 2005).

A congeneric species, *Heracleum sphondylium* is also present in western Switzerland. This plant is easily recognizable from *H. mantegazzianum* as it rarely exceeds 1,50m in height, has much smaller inflorescences, and contains numerous hairs on both sides of leaves and on the stems.

Study area

The western Swiss Alps (Fig. 1) are a low lying mountain range made of calcareous bedrock and of a total surface area of 704 km^2. Elevation of the study area ranges from 372 m in the Rhone Valley in the south-western edge, to 3210 m, at the summit of the Diablerets chain. The climate is cool and wet, with abundant rainfall (from 1000 mm to 2400 mm per year) that increases with elevation. In winter, snowfalls are frequent and abundant, shielding vegetation against extreme colds. The average yearly temperatures, which are dependent on elevation and exposure, range from -3˚C to 10˚C. This area was chosen as it is heavily invaded by *H. mantegazzianum*, and the plant is starting to become a hazard for rural economy, tourism, public health and native biodiversity.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Les Diablerets
Leysin
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Villars
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Figure 1. A) Map of sampled populations of Heracleum mantegazzianum; invasive populations are represented by red circles, native populations are represented by blue circles.

B) Location and IDs of sampled populations in the western Swiss Alps. IDs represent different regions as follows: D-Diablerets, L-Leysin, O-Ormont-dessous, V-Villars, C-Corbeyrier, M-Montreux, P-Pays d'enhaut, B-Bex, † indicates putative source populations (see below). The green circle corresponds to an ancient and extinct population at the Rochers-de-Naye botanical garden.

Sample collection and DNA extraction

A total of 734 individuals coming from a representative sample of 49 *H. mantegazzianum* populations from the western Swiss Alps were collected in summer 2005 and 2006 (Fig. 1; Appendix 1). A horizontal transect was run through each sampled population (in order to minimize biasing sampled towards descendents of the same individuals), and about 10 cm^2 of leaf material was collected from individual plants at a minimum interval of two meters. Leaves were desiccated in silica gel until completely dried. Additionally, 55 individuals coming from four populations were sampled in Lausanne (Loz), Champex (CHA) and Finhaut (FIN) as well as Ticino (Ti) (Fig. 1; Appendix 1) as well as 36 individuals from five populations of native *H. sphondylium* (Appendix 1). In order to compare the genetic variability of invasive and native populations, a sample of 129 individuals originating from 11 populations collected in the native range of the species (the Caucasus mountains) were also included (Fig. 1; Appendix 1).

Twenty mg of leaf material from all individuals collected was ground in 2-ml Eppendorf tubes using a Tissuelyzer (QIAGEN) with two tungsten carbide beads in each tube. DNA was extracted using Dneasy Plant mini kits (QIAGEN) according to the

manufacturer's protocol. Purified DNA extracts were stored at -20°C until subsequent manipulations.

Molecular markers and plastid DNA sequencing

All individuals included in the study were characterized by means of eight nuclear microsatellites (SSR): *A43, C52* (Walker *et al.,* 2003), *HMNSSR-131*, *HMNSSR-132A, HMNSSR-132B, HMNSSR-140, HMNSSR-206* and *HMNSSR-211* (Henry *et al.* in prep. Appendix 2). Polymerase Chain Reaction (PCR) was performed in a total volume of 25 µl containing 1X reaction buffer (Colorless GoTaq® reaction buffer, Promega; 1.5 mM $MgCl_2$, pH 8.5), 0.2 mM of each dNTP, 0.4 µM of forward and reverse primers, template DNA (about 20 - 100 ng) and 1 u GoTaq® DNA polymerase. Each forward primer was labeled with one fluorescent dye (HEX, NED or FAM). Cycling was performed in a T3 Thermocycler (Biometra). Cycling parameters were as follows: one cycle at 94°C followed by 35 cycles of 45 s at 94°C, 1 min at annealing temperature (50 – 58°C; see Walker *et al.,* 2003; Henry *et al.* in prep. Appendix 2 for further info), 2 min at 72°C, and a final cycle at 72°C for 10 min. PCR products were multiplexed on an ABI PRISM 3100 with GENESCAN 350 size standard, and alleles were called using GENEMAPPER version 3.7 (Applied Biosystems). The software MICRO-CHECKER version 2.2.1 (van Oosterhout *et al.,* 2004) was used to check for the presence of null-alleles, stuttering and large allele drop-outs for all loci in all populations prior to further analysis.

In order to investigate maternal origin of populations, four plastid loci were screened on all samples, *ccmp5*, *ccmp10* (Weising & Gardner 1999), *Trn-TL-indel1* and *Trn-TL-indel2*

(Henry *et al.,* in prep. Appendix 2). Genotyping procedures were identical to those previously described for nuclear loci. These markers were combined to obtain a mutli-locus plastid DNA (ptDNA) chlorotype for each individual. For each different multi-locus genotype found in distant populations (e.g. from both the native and invasive ranges), the *Trn*T-*Trn*L intergenic spacer was PCR amplified (following Taberlet *et al.,* 1991) and then sequenced in order to confirm chlorotype identification or detect possible additional polymorphisms. This method enables rapid and cheap screening of chlorotypes in different populations, as sequencing is not necessary for all individuals (e.g. Parisod & Besnard, in press). Sequencing was performed using the Big Dye 3.1 Terminator cycle sequencing kit (Applied Biosystems) according to manufacturer's instructions and an ABI PRISM 3100 genetic analyser (Applied Biosystems).

Data analyses

Deviation from Hardy-Weinberg equilibrium (HWE) within each sample and over all samples as well as linkage disequilibrium (LD) among all pairwise combinations of loci in the entire dataset were calculated using the software FSTAT version 2.9.4. (Goudet, 2005a; unless otherwise stated, all following calculations were conducted using FSTAT). All tests implemented in FSTAT are randomization-based, where data fitting the null-hypothesis are generated by randomizing the appropriate units (e.g. alleles randomized within samples), the statistic obtained is then compared to the observed data. The proportion of statistics from the randomized data giving larger or equal to the observed data provides an unbiased estimator of the p-value (Goudet, 1995). Sequential Bonferroni corrections were

applied to adjust the p-value when multiple tests were conducted according to Rice (1989). The inbreeding coefficient (F_{IS}) was calculated over all loci, in order to measure heterozygote deficit within populations, as this metric is useful to quantify departure from HWE within each sampled populations. Significance of F_{IS} was assessed using 10'000 permutations.

The genetic relationship between native and invasive *H. mantegazzianum* as well as *H. sphondylium* populations was assessed using Principal Component Analysis (PCA) implemented in the freeware PCAGEN 2.0β (Goudet, 2002). The software NETWORK (Bandelt *et al.*, 1999) was used to construct a median-joining haplotype network illustrating the phylogenetic relationship between the divergent chlorotypes existing in the *H. mantegazzianum* taxon.

To estimate genetic diversity at the population level in native and invasive ranges, observed and expected heterozygosities (H_o and H_s; Nei, 1987), allelic richness (**Rs;** El Mousadik & Petit, 1996), and number of alleles (N_a) were calculated for all populations independently. The comparisons among groups of samples tab-sheet implemented in FSTAT was used to test the significant differences of H_s and R_s between native and invasive populations as well as between suspected sources and derived populations from the western Swiss Alps with 1000 randomizations of individuals among groups tested (Fig. 1 and Appendix 1).

Population genetic structure was investigated by overall and pair-wise F_{ST} (Θ; Weir & Cockerham, 1984). Significance of pair-wise F_{ST} was assessed using 5000 permutations. Due to the relatively large size of the sample used, mean values of pairwise F_{ST} was calculated for each population for the sake of data presentation. To shed light on the

underlying population sub-structure of *H. mantegazzianum* populations in the western Swiss Alps, isolation by distance (IBD) was tested in a sub-sample consisting of the 49 invasive populations from the study area, by regressing genetic distances [in the form of F_{ST} / **(1 -** F_{ST}**)**] against Euclidian geographical distances between all pair-wise combinations of populations. Significance was tested by a Mantel test as implemented in FSTAT with 2000 permutations. Estimates of variance components and hierarchical F-statistics over all loci were calculated on all populations from the western Swiss Alps using the HIERFSTAT package for R (Goudet, 2005b). This calculation was undertaken in order to separate the different variance components residing within sampled populations and among valleys in the western Swiss Alps. For this analysis, populations from Diablerets (D), Ormont-dessous (O) and Leysin (L) were pooled together as they form part of a single valley (all other previously used groups of populations, M, C, P, B are considered as part of separate valleys).

Additionally, and in order to gain an insight in the invasion history of *H. mantegazzianum* in the western Swiss Alps, the software GENECLASS version 2.0 (Piry *et al.*, 2004) was used to assign individuals from western Swiss Alps to putative source populations (alpine botanical gardens and beehives; Fig. 1, Appendix 1) from which other populations may be derived. The standard criterion described by Rannala & Mountain (1997) which applies Bayesian statistics to compute probabilities of assignment of individuals to putative source populations was used. Moreover, we used the simulation algorithm for population assignment described by Paetkau *et al* (2004). Simulation of 10'000 genotypes for each population with an arbitrary threshold probability value of 0.05 or greater was used to determine origin of derived individuals from invasive population.

If the individual's probability was lower than the decided threshold, it was considered to be unassigned (i.e. the source population had probably not been sampled). Derived populations were considered to be correctly assigned to a putative source population only if at least half the individuals in the population were assigned to the source.

Results

Nuclear microsatellites, linkage disequilibrium and Hardy-Weinberg equilibrium

The eight nuclear microsatellite loci used in this study produced between one and 14 alleles per population. A total of 164 alleles for all loci, (ranging from nine to 56 alleles per locus), was detected in the entire dataset (53 *H. mantegazzianum* and five *H. sphondylium* populations from Switzerland as well as 11 *H. mantegazzianum* populations from Caucasus). The mean number of alleles per locus and per population ranged from 1.6, in one invasive population (P3) to 6.6 in a native population (S25; Appendix 1). Observed and expected heterozygosities (H_O and H_S) overall loci and populations were 0.448 and 0.458 respectively. All F_{IS} values did not differ significantly from zero.

The software MICRO-CHECKER did not detect null alleles, large allele dropouts or stuttering in any of the markers used (data not shown). Tests for linkage disequilibrium based on 5600 permutations were all non-significant at the 5% nominal level (data not shown), indicating that all loci used are unlinked. Tests of deviation from Hardy-Weinberg equilibrium (HWE) based on 10000 permutations were all non-significant after standard Bonferroni corrections (Appendix 1).

Genetic relationship between sampled populations

The genetic relationships between invasive (Switzerland) and native (Caucasus, south-west Russia) populations of *H. mantegazzianum* as well as their mutual relationships with *H. sphondylium* from western Switzerland is illustrated below (Fig. 2). Percentage inertia explained by each of the two axes were 17% and 13% respectively, with the first axis

(PCA1) being marginally significant at 5% nominal level, and the second axis (PCA2) being highly significant even at 1% nominal level. This analysis identified two main clusters corresponding to the two *Heracleum* species (verified on the basis of morphological features), which are mainly separated by the second axis. A single population (FIN) did not cluster within any of the two species, yet as the axis separating this population from the others (PCA1) is not significant and, on the basis of morphological traits, this population was considered as part of the *H. mantegazzianum* cluster in further analyses. Within the *H. mantegazzianum* cluster, native populations clustered at a central position. Important genetic differentiation between invasive forms from the western Swiss Alps is observed, as population scatter along the first axis. Populations from the western Swiss Alps generally cluster in proximity of other populations sampled in the same region, although a certain overlap is observed.

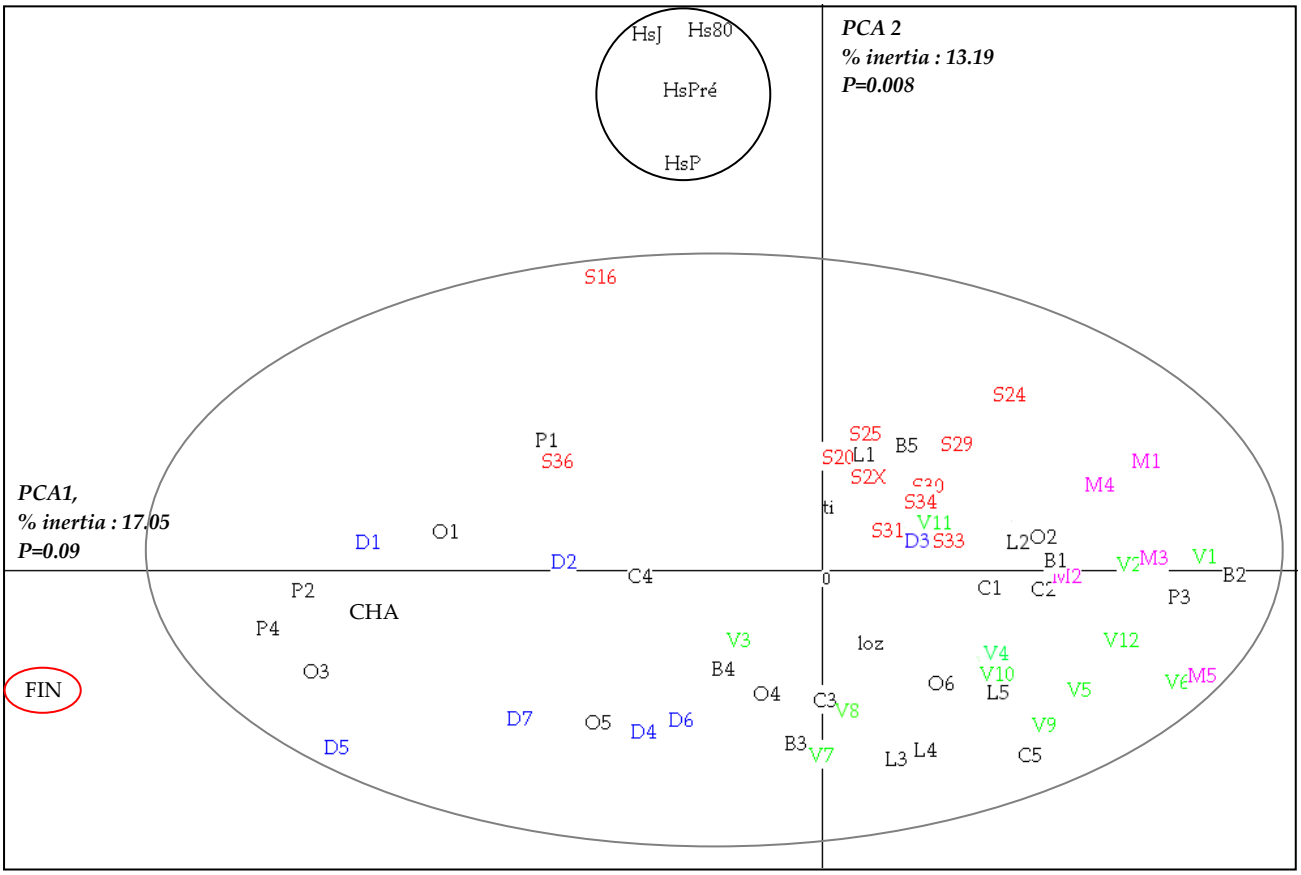

Figure 2. Principal Component Analysis (PCA) of entire dataset consisting of 53 invasive (Switzerland; black, blue, green and pink where, ID illustrates locations of collection: D-Diablerets, L-Leysin, O-Ormont-dessous, V-Villars, C-Corbeyrier, M-Montreux, P-Pays d'enhaut, B-Bex), and 11 native (Caucasus,SW Russia; red, ID starts with "S") H. mantegazzianum populations. Four native H. sphondylium were also included in order to ascertain the relationship between the two species. Note that the Finhaut (FIN) population did not cluster within any of the two species (red circle). Significance of inertia explained by the two axes was tested using 5000 permutations of the dataset in PCAGEN 2β (Goudet, 2002).

Evidence for a founder event in the invasion of the western Swiss Alps

Mean gene diversity and allelic richness values were significantly higher in native populations from Caucasus than invasive populations from Switzerland: $\boldsymbol{H}_S$ = 0.58 (SD = 0.1) and 0.43 (SD = 0.09) and $\boldsymbol{R}_S$ = 4.69 (SD = 0.91) and 2.69 (SD = 0.53) for native and invasive populations respectively. Both comparisons were highly significant, with p-values of 0.001 (Fig. 3), indicating an important decrease of genetic diversity in invasive populations compared to natives.

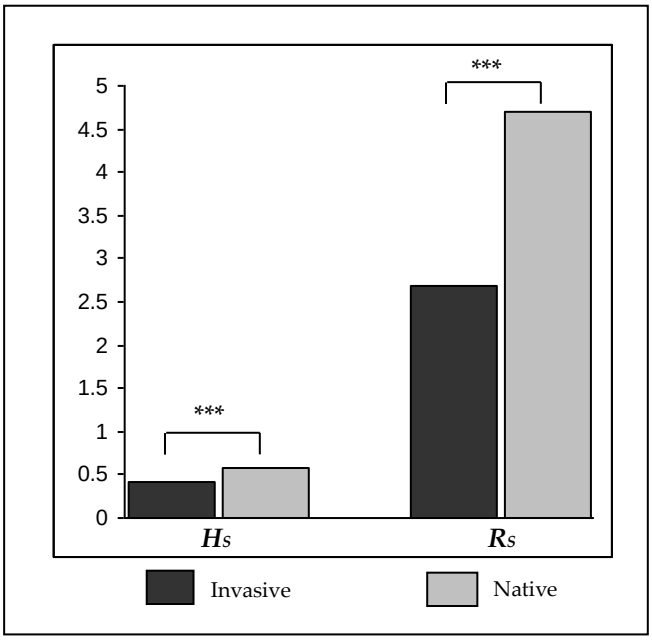

*Figure 3. Mean gene diversity (**H**s) and mean allelic richness (**R**s) between native and invasive* H. mantegazzianum

*populations. Significance level of permutation test implemented in FSTAT are indicated for each comparison (***p<0.005).*

Plastid markers confirmed this tendency as only five chlorotypes were found in invasive populations from Switzerland compared to ten in the sample from Caucasus. Two chlorotypes were commonly found in invasive Swiss populations, A*1 represented by 82% and B*1 represented by 27% of all individuals sampled in Switzerland. The three other chlorotypes found in the Switzerland were rare: A*2, A*3 and AB*3 represented by 0.4%, 0.5% and 0.1% of individuals respectively (Fig. 4). Of these rare chlorotypes, A*3 and AB*3 did not occurred in the sample from the native range. Invasive Swiss populations were mostly made up of a single chlorotype, although some populations had two chlorotypes (Appendix 1). In the Caucasus populations, chlorotype B*1 was the most common (48%). Native populations were made up of one, two or three chlorotypes. On the basis of sequence data, these chlorotypes grouped into two main groups (A* and B*) with intermediate forms (AB*). The phylogenetic relationship among chlorotypes is illustrated in figure 5.

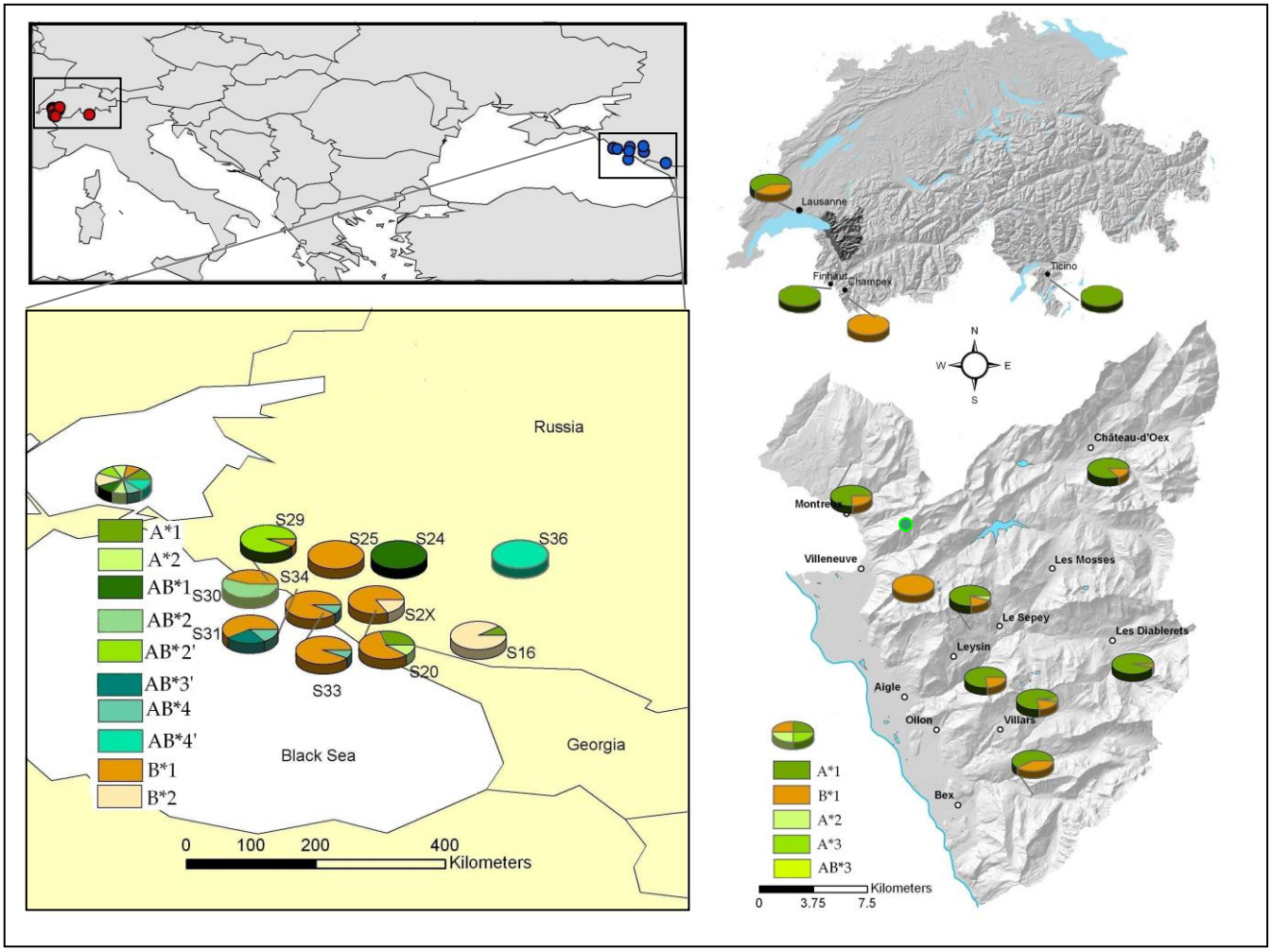

Figure 4. Geographical location of the different chlorotypes revealed in the Heracleum mantegazzianum *taxon. Blue circles correspond to native populations, red circles correspond to invasive populations. Five chlorotypes are found in invasive Swiss populations, while ten are found in native Caucasus populations. The green circle corresponds to an ancient, extinct population at the Rochers-de-Naye botanical garden.*

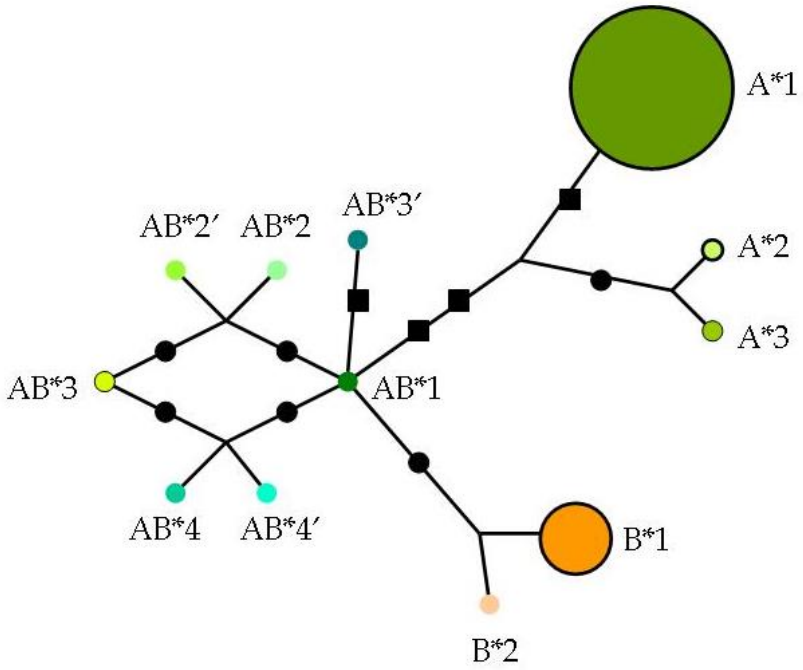

Figure 5. Median-joining haplotype network (Bandelt et al.*, 1999) constructed using sequences from the* Trn-*T*-Trn*L intergenic spacer. Black squares indicate substitutions while black circles indicate indels. Chlorotypes found only in invasive Swiss populations are indicated by a black outline. Chlorotypes found in both native and invasive populations are indicated by a large black outline. Chlorotypes with no outlines are only found in native populations from Caucasus. The size of the circles corresponds to the frequency of each chlorotype in the invasive Swiss sample only.*

Population genetic structure in the western Swiss Alps

Population genetic structure in the sample from the western Swiss Alps was investigated by quantifying genetic differentiation among population by means of pairwise ***F*ST**. Geographical locations and mean pairwise ***F*ST** of sampled populations (Fig. 6), and pairwise ***F*ST** values for all combinations of populations from the western Swiss Alps (Fig. 7) are shown below. Pairwise ***F*ST** values range from 0.017 (D4-D7) to 0.625 (M4-D5) with a mean overall value of 0.286 (SD 0.097).

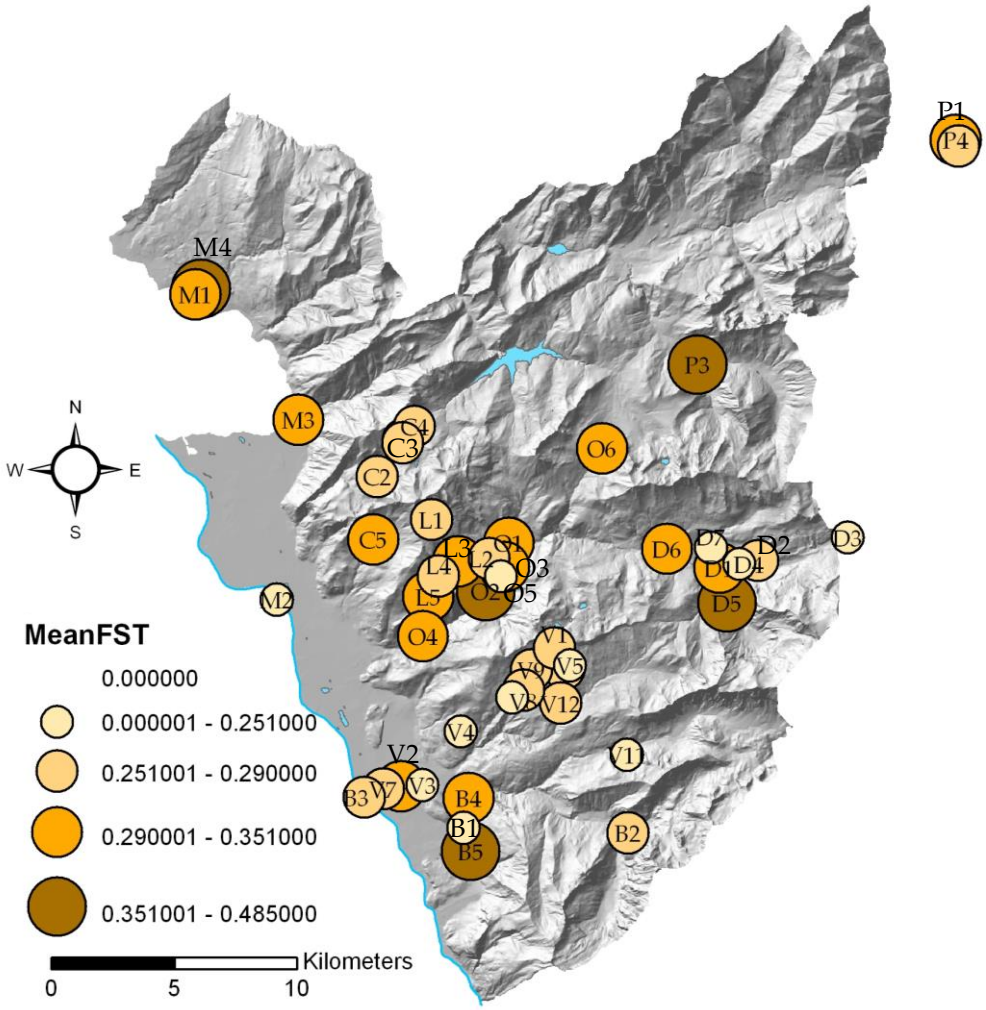

Figure 6. Mean values of pairwise ***F****ST. Sampling locations are indicated by circles of width corresponding to their mean pairwise* ***F****ST values. (D-Diablerets, L-Leysin, O-Ormont-dessous, V-Villars, C-Corbeyrier, M-Montreux, P-Pays d'enhaut, B-Bex, S-Caucasus).*

Both figures show that population M4 is highly differentiated from all other populations (except population M1), indicating strong differentiation of these two populations compared to the rest of the populations from the western Swiss Alps. Population D5, P3, O2 and B5 also portray relatively high pairwise ***F****ST* values, yet these populations are located in highly isolated areas and have small effective sizes, (less than 50 individuals). Figure 7 also shows that populations from Diablerets (D), Villars (V) and Corbeyrier (C) are genetically more similar to populations sampled in the same area. On

the other hand, populations from all other regions (O, L, M, P, B) are not more similar to populations from the same area than other populations. Globally, all populations sampled were highly differentiated and showed strong evidence for genetic isolation. The pairwise comparisons were all significant except for pairwise F_{ST} values of population P3 which only consist of six individuals (data not shown). Interestingly, the population from Finhaut (FIN) which had an outlying position in the PCA (Fig. 2) has the highest mean value of differentiation from of all populations sampled (F_{ST} = 0.514).

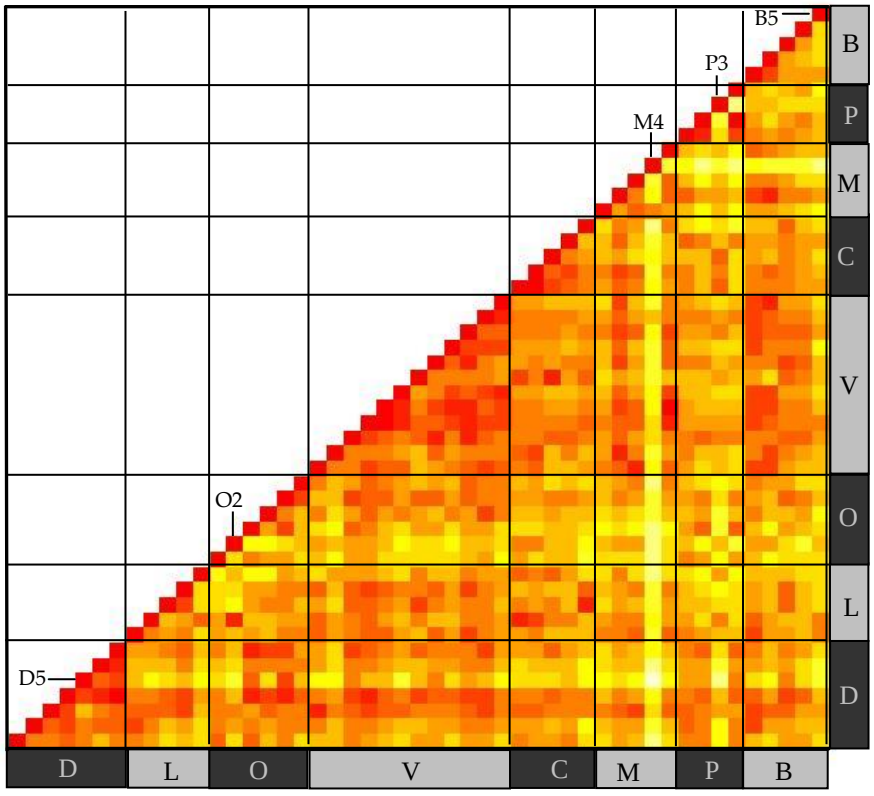

Figure 7. Pairwise F_{ST} *values for all combination of populations from the western Swiss Alps. Each population is represented by a column and its corresponding line,* F_{ST} *values range from 0 (red) to 0.7 (light yellow), intermediate values are indicated by corresponding colours. Populations are arranged in the standard order (D-Diablerets, L-Leysin, O-Ormont-dessous, V-Villars, C-Corbeyrier, M-Montreux, P-Pays d'enhaut, B-Bex).*

A clear and highly significant pattern of isolation by distance is observed in populations from the western Swiss Alps (Fig. 8; 6.25% of the total variance is explained by the model, P = 0.0002). This analysis illustrates that gene flow occurs in populations in close geographical proximity. Those populations are thus less genetically isolated than populations at greater geographical distances.

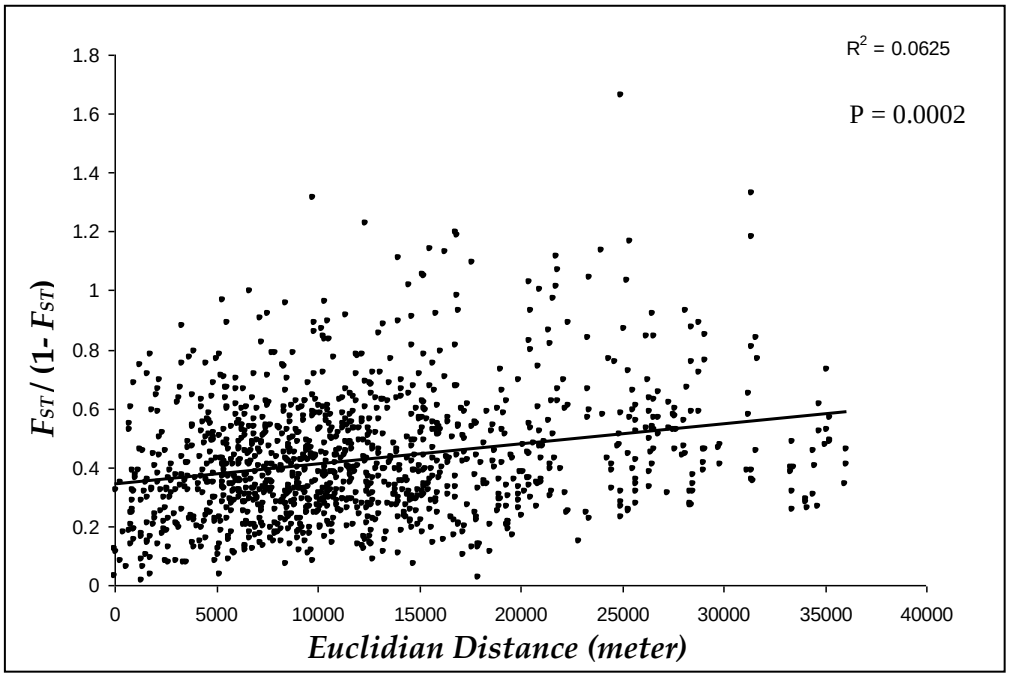

Figure 8. Isolation by distance in the invasive populations from the western Swiss Alps illustrated by a regression of genetic distance against Euclidian geographical distance. P-value of Mantel test undertaken in FSTAT *is 0.0002.*

Table 1 shows the estimates of variance components over all eight microsatellite loci used in this study. This analysis was undertaken in order to get an additional insight in the hierarchical organisation of genetic variance between valleys and within populations among valleys. Five percent of the total variance component is attributed to the effect pf valleys (i.e. populations being sampled in different valleys). Most genetic variance resided within populations among valleys (26%), once more highlighting the strong isolation

between sampled populations.

Table 1. Hierarchical components of genetic variance within valleys, populations and individuals. Significant values are indicated in bold (P = 0.001).

	Valleys	Populations
Total	**0.05**	0.3
Valleys	0	**0.26**
Populations	0	0

Identification of putative source populations in the invasion of the western Swiss Alps

In order to unfold the pathway of invasion of *H. mantegazzianum* in the western Swiss Alps, five populations corresponding to sites of possible early introductions (alpine botanical gardens, private gardens and beehives) were tested as being putative sources from which *H. mantegazzianum* invaded the western Swiss Alps. Levels of genetic diversity were significantly higher in these putative sources than in other populations from the western Swiss Alps, thus confirming that these populations are possible sources [Fig. 9; $\boldsymbol{H}_s$ = 0.53 (SD = 0.09) and 0.41 (SD = 0.03), $\boldsymbol{R}_s$ = 3.3 (SD = 0.64) and 2.63 (SD = 0.23) for source and derived populations respectively]. Both comparisons were significant with p-values of 0.003 and 0.01 for $\boldsymbol{R}_s$ and $\boldsymbol{H}_s$ respectively.

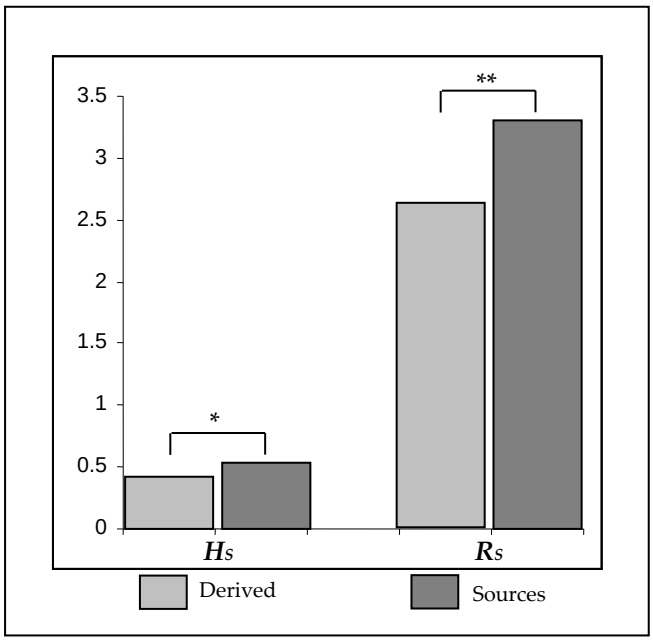

*Figure 9. Mean gene diversity (**H**s) and mean allelic richness (**R**s) between putative source and derived populations from the western Swiss Alps. Significance level of permutation test implemented in FSTAT are indicated for each comparison (*p<0.05, **p<0.01).*

Assignment tests were then conducted by using the five putative sources as references. Twenty-four out of 49 populations were not assigned to any of the putative sources. It is interesting to note that these unassigned populations are mostly made up of individuals displaying chlorotype B*1 (Table. 2; Appendix. 1), thus indicating that the possible source of these populations had not been sampled (see below). The population from the ancient botanical garden of Tour d'aï (L1) and the population from the Lac de Bretaye recreational area (V1) are identified as the most likely source populations (both with 13 populations assigned to them) of all invasive populations displaying chlorotype A*1 and the rare chlorotypes (A*2, A*3 and AB* 3). Some populations are also sometimes assigned to both of these populations simultaneously, (as well as to other source populations) indicating close genetic affinities between the two sources and possibly a common origin. It is interesting to note that Pont-de-Nant botanical garden (B2) is only designated as the

possible source of a single population. Populations P2 and P4 were both assigned to the beehive site P1, which is not surprising due to the close geographical distance separating the three populations.

Table 2. Population assignments performed in GENECLASS 2 (Piry et al., 2004) using the standard criteria of Rannala & Mountain (1997), the simulation algorithm of Paetkau et al. (2004) and 10000 simulated individuals per populations. A threshold P value of 0.05 was applied for assignments, moreover, derived populations were considered assigned to putative source populations only if more than 50% of the individuals that make up the population are assigned to the source population (indicated in bold). Thus some populations may be assigned to more than one source population (indicating close genetic affinities), other may not be assigned at all (indicated in grey).

	†D1	†L1	†V1	†P1	†B2		†D1	†L1	†V1	†P1	†B2
D2 (16)	2	**10**	2	3	1	V8 (16)	2	7	6	2	-
D3 (16)	1	**7**	**9**	**8**	4	V9 (16)	-	**9**	**12**	6	-
D4 (16)	5	5	3	5	-	V10 (16)	1	**11**	**8**	2	-
D5 (16)	**14**	**11**	3	**12**	-	V11 (16)	-	-	7	3	1
D6 (16)	6	**11**	**11**	**14**	2	V12 (16)	-	4	**8**	-	6
D7 (16)	6	4	3	7	-	C1 (16)	1	-	-	1	-
L2 (16)	1	4	3	-	-	C2 (16)	2	2	1	2	-
L3 (15)	-	3	5	-	-	C3 (16)	-	-	-	-	-
L4 (15)	2	**13**	**12**	-	-	C4 (16)	2	1	1	2	-
L5 (16)	-	1	2	-	-	C5 (16)	-	6	4	2	-
O1 (16)	6	2	2	6	-	M1 (16)	-	4	1	-	1
O2 (16)	-	**7**	**9**	-	-	M2 (16)	-	3	4	-	1
O3 (16)	**9**	6	-	1	-	M3 (16)	3	**13**	**14**	2	2
O4 (10)	1	-	4	4	1	M4 (16)	-	-	-	-	-
O5 (16)	2	6	2	-	-	M5 (16)	-	**11**	**13**		5
O6 (16)	-	**14**	**13**	-	-	P2 (10)	1	-	-	**8**	-
V2 (16)	-	1	6	2	3	P3 (6)	-	-	-	-	-
V3 (16)	-	2	-	1	-	P4 (8)	2	-	-	**6**	-
V4 (16)	1	6	6	-	1	B1 (16)	-	6	**8**		5
V5 (16)	-	**9**	**11**	1	2	B3 (16)	1	6	5	3	-
V6 (16)	-	4	**13**	-	**8**	B4 (16)	-	2	3	1	-
V7 (16)	-	1	2	1	-	B5 (16)	-	-	-	-	-

Discussion

The present study revealed that: (i) Invasive populations from the western Swiss Alps are highly differentiated from native populations from Caucasus. (ii) Invasive populations underwent a founder event upon introduction to Switzerland as they contained significantly less genetic diversity and chlorotypes than their native counterparts. (iii) Invasive populations are highly structured and display a pattern of isolation by distance at the regional scale investigated here. And, (iv) although at least four independent introductions are evidenced in Switzerland, invasive populations in the western Swiss Alps were most likely founded by two separate introductions from populations introduced to alpine botanical gardens.

Genetic relationship between native and invasive populations

Invasive populations from the western Swiss Alps were highly differentiated from the native populations from Caucasus, constituting a first line of evidence for an initial founder event at introduction and important differentiation of isolated invasive populations through drift. The presence of five chlorotypes in invasive populations from Switzerland compared to ten chlorotypes found in native populations constitutes a second line evidence for the founder event that has occurred in the introduction of *H. mantegazzianum* to Switzerland.

Invasive populations often exhibit substantially lower levels of genetic diversity compared to native populations (Baker, 1974) this being often attributed to a founder event. Yet examples ranging from two extremes can be found in the literature, where a

single clone has successfully invaded large areas (e.g. Amsellem *et al.* 2000; Hollingsworth & Bailey, 2000), where invasive populations exhibit similar levels of genetic diversity than natives (Genton *et al.,* 2005) or where invasive populations display substantially higher levels of genetic diversity than native populations (Kolbe *et al.,* 2004). In the case of Giant Hogweed, Walker *et al.* (2003) and Jahodová *et al.* (2007) both identified high levels of genetic diversity in invasive populations of *H. mantegazzianum* in Europe, and attributed it to possible multiple introductions or to a large initial founding population. Although we reveal important levels of genetic diversity within invasive populations in Switzerland (equivalent to that found by Walker and colleagues), these values were significantly reduced compared to that observed in native populations from Caucasus (Fig. 8). This third line of evidence confirms a possibly important founder event occurring at the initial introduction of the species to Switzerland.

Evidence for more than a single introduction in Switzerland is revealed by the presence of two main chlorotypes groups found in invasive populations (which were never found to occur together in native populations). Populations displaying highly differentiated genotypes (M1/4, Ti and FIN) are also suspected to originate from independent introductions. Furthermore, it is interesting to note that the population from Finhaut (FIN) is characterized by unusually high pairwise F_{ST} values with all other populations, including native samples. This population is suspected to have been introduced from Poland (S. Nicoud, personal communication), and due to the extreme genetic divergence from all populations (even higher than that observed between *H. sphondylium* and *H. mantegazzianum*) the taxonomic status of this population should be reviewed as it may belong to another giant *Heracleum* taxon (c.f. Jahodová *et al.,* 2007). Although at least four

independent introductions of invasive *Heracleum* taxa are suspected in Switzerland, those do not seem to have contributed to enhancing genetic variability within populations, (at least not at the scale investigated here), as the strong isolation between populations has probably prevented extensive genetic exchanges. In turn, rapid population expansion after the initial founding of populations (owing to the outstanding reproduction capacity of *H. mamtegazzianum*) as well as limited gene flow between populations in close geographical locations (c.f. Lacy, 1987) seem to have been the major factors for the high inter-population genetic diversity observed between invasive populations.

Population genetic structure of H. mantegazzianum *in the western Swiss Alps*

High population differentiation is observed in invasive populations from the western Swiss Alps. The observed values of pairwise F_{ST} are much higher than those observed by Walker and colleagues for a region larger than the one studied here. The mean F_{ST} values observed here are comparable to short-lived perennials, characterized by mixed-mating, with regionally, wind dispersed seeds (Hamrick & Godt, 1996). The latter description indeed summarizes well the life-history traits of *H. mantegazzianum,* yet it is important to bear in mind the scale at which such metrics are investigated. Comparable F_{ST} values were observed for the samples from Caucasus and the sample from the western Swiss Alps, yet the geographical scale was ten times smaller in Switzerland than in Caucasus (for a comparison of scales see Fig. 2), highlighting the extreme differentiation among invasive populations in the western Swiss Alps.

A clear pattern of isolation by distance supports this point. Populations of

H. mantegazzianum in the western Swiss Alps are fairly small (maximum of 350 individuals; F. Dessimoz, pers. comm.), and are represented by isolated patches in the landscape (P. Henry *et al.*, unpubl. data). Pollinators would thus be restricted to fertilizing flowers on individuals from the same patch (populations), thus inflating the differentiation of populations through limited gene flow (as noted by Walker *et al.*, 2003).

Identification of source populations in the invasion of the western Swiss Alps

Putative source populations (Alpine botanical gardens, private gardens and beehives) exhibited substantially higher levels of genetic diversity than other populations in the western Swiss Alps, thus suggesting that they were indeed sites of early introduction, and likely sources of the invasion of the western Swiss Alps by *H. mantegazzianum.* Nearly half of the invasive populations were not assigned to any of the possible foci of introduction. Other populations were assigned to two or even three source populations, indicating close genetic affinities of identified sources.

The total unassignment of populations from the Corbeyrier (C) region, and more generally all populations with a high frequency of chlorotype B*1 suggest that the original source of these populations had not been sampled. Indeed, the source population from which these populations may have been founded may be an ancient, extinct population found at the Rochers-de-Naye botanical garden (Fig.1 & 2) which disappeared only a few years after it was founded as the climatic conditions at that site were unsuitable (E. Mottier, personal communications). This population is suspected to be the origin of all populations displaying the B*1 chlorotype in the western Swiss Alps, which constitutes a

first, independent introduction.

Two populations, namely Tour d'Aï botanical garden (L1) and the Lac de Bretaye recreational area (V1) are identified as the most likely sources of all other invasive populations from the western Swiss Alps, (i.e. populations displaying chlorotype A*1 and the three rare chlorotypes (A*2, A*3, and AB*3). These two populations are highly likely to have originated from a second independent introduction. They are both attractive sites, situated at high altitudes (1967 and 1798 m.a.s.l respectively) where visitors could have easily have had access to *H. mantegazzianum* seeds, and transport them lower down in valleys to found new populations. These populations are thus highly likely to have contributed to the genetic pool of all other invasive populations.

Interestingly, the population from the Pont-de-Nant botanical garden (B2) was only poorly designated as a possible source (only one population is partially assigned to it). The poor assignment of derived populations to this putative source may be due to the fact that an intensive eradication campaign has taken place in and around the botanical garden and this selection regime may have biased our sample and thus prevented us from capturing the entire genetic pool of the original population. Yet this populations was probably introduced together with the L1 and V1 populations due to genetic affinities and common chlorotypes.

We thus suspect two successive waves of introduction of *H. mantegazzianum* in the western Swiss Alps, (both originating from Alpine botanical gardens). Other independent introductions are identified, notably in Montreux (M1/4) and Finhaut (FIN), yet these two introductions are probably more recent, are only very localized and have thus not contributed to the genetic pool of the invasive *H. mantegazzianum* populations in the

western Swiss Alps. If a larger geographical scale is chosen for a similar study, one is expecting to find an even more important number of independent introductions. As times goes by, and the species' range expansion increases, chances for genetic exchanges between populations originating from independent introduction will also increase, thus admixture events may possibly further contribute to the success of an already very successful invader.

The present study identified a possible invasion scenario and genetic characteristics of *H. mantegazzianum* in the western Swiss Alps, yet, a clearer vision could be attained if herbarium specimens from botanical gardens could be used to infer origins and sequences of introductions to Switzerland. A study delineating the taxonomy of giant *Heracleum* species could facilitate the interpretation of observed phenomena. Furthermore, GIS-based dispersal models could also be powerful tools to evaluate the likelihood of the invasion scenario put forth here, and to shed light on dispersal behaviour of *H. mantegazzianum* in both its native and invaded ranges.

Appendices

Appendix 1.

Information concerning sampled populations with measures of genetic diversityand inbreeding coefficient at eight microsatellite loci: ***ID****, full name of sample population,* ***Alt.****, altitude in metres above sea level,* ***N****, number of individual sampled;* N_A*, mean number of alleles overall loci;* H_O*, mean observed heterozygosity overall loci;* H_S*, average gene diversity overall loci;* R_S*, mean allelic richness;* ***Haplo.****, number of Chlorotypes in population with name of corresponding Chlorotype in brackets;* F_{IS}*, inbreeding coefficient;* ***HWE****, deviation from Hardy-Weinberg Equilibrium as calculated using 10'000 permutations in* FSTAT 2.94 (Goudet, 2005a).

ID	*Country/ Canton*	*Locality*	*Alt.*	*Latitude*	*Longitude*	*N*	N_A	H_O	H_S	R_S	*Haplo. N*	F_{IS}	*HWE*
D1 †	Vaud	Les Diablerets	1169	N 46°35'55.3''	E 7°13'11.3''	16	3.125	0.398	0.479	2.452	1 (A*1)	0.168	no
D2	Vaud	Les Diablerets, Pont Bourquin	1343	N 46°35'13.1''	E 7°17'91.3''	16	2.875	0.430	0.523	2.497	1 (A*1)	0.179	no
D3	Vaud	Col du Pillon	1424	N 46°26'86.4''	E 7°00'22.4''	16	3.375	0.547	0.534	2.681	1 (A*1)	-0.024	no
D4	Vaud	Les Diablerets	1263	N 46°33'57.3''	E 7°16'31.3''	16	2.875	0.477	0.46	2.392	2 (A*1/B*1)	-0.036	no
D5	Vaud	Les Diablerets, Lederrey	1217	N 46°25'14.4''	E 7°11'08.7''	16	2.125	0.352	0.282	1.773	1 (A*1)	-0.247	no
D6	Vaud	Les Diablerets, Les Granges	1129	N 46°30'08.4''	E 7°04'93.7''	16	2	0.255	0.301	1.832	1 (A*1)	0.153	no
D7	Vaud	Les Diablerets, Les Torneys	1197	N 46°31'90.6''	E 7°07'16.2''	16	2.5	0.391	0.413	2.218	1 (A*1)	0.054	no
L1 †	Vaud	Tour d'Aï, Jardin Alpin	1967	N 46°35'16.9''	E 7°03'67.9''	16	4.75	0.398	0.589	3.262	2 (A*1/2)	0.323	no
L2	Vaud	Cergnats, Les Noisetiers	1040	N 46°34'99.0''	E 7°16'94.9''	16	3	0.484	0.464	2.488	2 (A*1/B*1)	-0.043	no
L3	Vaud	Leysin, Les Esserts	1316	N 46°35'98.0''	E 7°22'66.6''	15	2.125	0.425	0.337	1.914	1 (A*1)	-0.261	no
L4	Vaud	Leysin, Le Fedey	1406	N 46°33'75.9''	E 7°00'46.9''	15	2.5	0.417	0.318	1.963	1 (A*1)	-0.312	no
L5	Vaud	Leysin, Gare du Village	1232	N 46°35'83.6''	E 6°97'51.7''	16	2.625	0.297	0.306	1.943	1 (A*1)	0.029	no
O1	Vaud	Le Sepey	948	N 46°28'81.7''	E 7°02'22.5''	16	2.25	0.422	0.435	2.098	2 (A*1/B*1)	0.029	no
O2	Vaud	Pont de la Tine	856	N 46°33'97.7''	E 7°03'52.7''	16	2.375	0.383	0.341	1.971	2 (A*1/B*1)	-0.123	no
O3	Vaud	Pont de la Tine	803	N 46°34'51.2''	E 7°04'31.1''	16	2.5	0.391	0.382	2.118	1 (A*1)	-0.023	no
O4	Vaud	Aigle, Les Afforets	619	N 46°35'04.7''	E 7°02'02.9''	10	1.875	0.350	0.329	1.749	1 (B*1)	-0.063	no
O5	Vaud	Pont de la Tine	807	N 46°44'97.1''	E 6°88'30.6''	16	2.5	0.469	0.441	2.347	2 (A*1/B*1)	-0.063	no
O6	Vaud	Les Mosses	1414	N 46°34'85.6''	E 7°04'49.7''	16	2.875	0.352	0.353	2.131	1 (A*1)	0.002	no
V1 †	Vaud	Col de Bretaye	1798	N 46°31'14.7''	E 7°07'69.4''	16	3.375	0.400	0.466	2.688	1 (A*1)	0.141	no
V2	Vaud	Ollon, Praille les Glareys	444	N 46°26'80.6''	E 6°99'08.5''	16	3	0.453	0.454	2.538	2 (A*1/B*1)	0.002	no
V3	Vaud	Ollon, Prés de la Grange	478	N 46°26'39.2''	E 6°97'12.8''	16	3.625	0.469	0.521	2.847	2 (A*1/B*1)	0.1	no
V4	Vaud	Huémoz	1004	N 46°28'82.1''	E 7°02'26.6''	16	3.375	0.484	0.487	2.725	1 (A*1)	0.004	no
V5	Vaud	Villars-sur-Ollon, Golf	1595	N 46°30'35.6''	E 7°05'54.2''	16	3	0.422	0.429	2.417	1 (A*1)	0.017	no
V6	Vaud	Villars-sur-Ollon, Golf	1654	N 46°31'27.5''	E 7°07'98.9''	16	2.625	0.438	0.395	2.241	1 (A*1)	-0.108	no
V7	Vaud	Ollon, La Praille	405	N 46°34'85.5''	E 7°15'86.7''	16	2.375	0.422	0.368	2.014	1 (B*1)	-0.147	no
V8	Vaud	Villars-sur-Ollon	1265	N 46°31'09.3''	E 7°05'95.1''	16	3	0.453	0.451	2.411	1 (A*1)	-0.006	no
V9	Vaud	Villars-sur-Ollon	142	N 46°40'21.8''	E 6°93'53.7''	16	3	0.359	0.35	2.20	1 (A*1)	-0.027	no
V10	Vaud	Villars-sur-Ollon, Chesière	1202	N 46°35'73.9''	E 7°04'73.4''	16	2.875	0.344	0.460	2.494	1 (A*1)	0.253	no
V11	Vaud	Gryon, Cergnement	1262	N 46°24'44.5''	E 7°02'77.6''	16	4.5	0.406	0.578	3.30	2 (A*1/3)	0.297	no
V12	Vaud	Villars-sur-Ollon, Plan Meunier	1389	N 46°29'87.7''	E 7°07'50.1''	16	3.625	0.359	0.444	2.617	2 (A*1/AB*3)	0.191	no
C1	Vaud	Les Plans d'Enhaut	1440	N 46°39'39.2''	E 6°99'08.2''	16	3.125	0.508	0.479	2.512	1 (B*1)	-0.062	no
C2	Vaud	Les Plans d'Enhaut	1489	N 46°38'13.9''	E 6°97'70.9''	16	2.75	0.425	0.468	2.35	1 (B*1)	0.092	no
C3	Vaud	Grand Ayerne	1459	N 46°39'48.6''	E 6°99'10.8''	16	2.125	0.375	0.359	2.022	1 (B*1)	-0.044	no
C4	Vaud	Grand Ayerne, Tank	1426	N 46°39'98.9''	E 6°99'69.2''	16	3.625	0.450	0.505	2.858	1 (B*1)	0.109	no
C5	Vaud	Luan	1196	N 46°39'21.5''	E 7°09'64.9''	16	2.25	0.317	0.292	1.939	1 (B*1)	-0.084	no
M1	Vaud	Clarens, La Maladaire	393	N 46°26'71.3''	E 6°98'14.9''	16	3.375	0.358	0.415	2.484	1 (A*1)	0.137	no
M2	Vaud	Roche, Iles de Clous	387	N 46°35'59.4''	E 7°15'43.9''	16	3.375	0.517	0.546	2.881	1 (B*1)	0.054	no

ID	*Country/ Canton*	*Locality*	*Alt.*	*Latitude*	*Longitude*	*N*	N_A	H_O	H_S	R_S	*Haplo.*	F_{IS}	*HWE*
M3	Vaud	Villeneuve, La Rive	437	N 46°36′57.6′′	E 7°00′60.1′′	16	3.125	0.400	0.38	2.486	2 (A*1/B*1)	-0.052	no
M4	Vaud	Clarens, La Maladaire	417	N 46°44′77.6′′	E 6°88′00.3′′	16	2.25	0.242	0.203	1.669	1 (A*1)	-0.191	no
M5	Vaud	Les Avants, Nermont	984	N 46°44′74.3′′	E 6°95′21.6′′	16	2.375	0.325	0.359	2.045	1 (A*1)	0.094	no
P1	Bern	Saanen, ruches	1218	N 46°50′65.5′′	E 7°28′43.6′′	16	4.25	0.583	0.658	3.389	1 (A*1)	0.113	no
P2	Bern	Saanen, chemin	1278	N 46°50′32.8′′	E 7°28′50.1′′	10	3	0.588	0.526	2.699	1 (A*1)	-0.118	no
P3	Vaud	L'Etivaz	1158	N 46°42′29.4′′	E 7°14′67.2′′	6	1.625	0.375	0.238	1.625	1 (B*1)	-0.579	no
P4	Bern	Saanen, route	1250	N 46°50′55.9′′	E 7°28′34.3′′	8	2.5	0.339	0.467	2.393	1 (A*1)	0.274	no
B1	Vaud	Bex	453	N 46°26′38.3′′	E 7°02′66.5′′	16	3	0.482	0.551	2.610	1 (A*1)	0.125	no
B2 †	Vaud	Pont de Nant	1255	N 46°34′52.2′′	E 7°00′98.5′′	16	2.75	0.400	0.455	2.435	1 (A*1)	0.121	no
B3	Vaud	Bex, Grandes Iles d'Amont	404	N 46°25′30.5′′	E 7°02′39.9′′	16	2.625	0.451	0.388	2.189	1 (B*1)	-0.162	no
B4	Vaud	Bex, Les Luisances	515	N 46°28′01.1′′	E 7°11′02.6′′	16	2.375	0.458	0.433	2.104	1 (A*1)	-0.06	no
B5	Vaud	Bex	472	N 46°33′61.5′′	E 6°92′44.2′′	16	3	0.417	0.451	2.412	1 (B*1)	0.075	no
FIN	Valais	Finhaut	NA	N 46°23′77.6′′	E 6°56′79.9′′	16	2	0.267	0.199	1.558	1 (A*1)	-0.339	no
CHA †	Valais	Champex	NA	N 46°02′05.2′′	E 7°41′59.9′′	16	3.625	0.406	0.468	2.776	1 (B*1)	0.132	no
Loz	Vaud	Lausanne Vidy	320	N 46°50′93.7′′	E 6°61′66.6′′	16	3	0.547	0.488	2.656	2 (A*1/B*1)	-0.121	no
Ti	Ticino	Sant'Abbondio	NA	N 46°07′51.3′′	E 8°46′11.6′′	8	2.25	0.552	0.409	2.136	1 (A*1)	-0.35	no
S20	**Russia**	**Chamishki - Guzeripl**	**1363**	**N44°00'36.5"**	**E40°02'44.6"**	**7**	**5.625**	**0.725**	**0.699**	**4.22**	**3 (A*1/2/B*1)**	**-0.038**	**no**
Sx02	**Russia**	**Lago-Naki**	**1260**	**N44°08'60.0"**	**E40°04'00.0"**	**6**	**4.625**	**0.500**	**0.608**	**4.248**	**2 (A*1/B*2)**	**0.178**	**no**
S25	**Russia**	**NW of Chadyzhensk**	**148**	**N44°28'04.1"**	**E39°26'17.7"**	**12**	**6.625**	**0.740**	**0.684**	**4.402**	**1 (B*1)**	**-0.08**	**no**
S29	**Russia**	**Archipo-Osipovka**	**20**	**N44°24'48.8"**	**E38°30'17.4"**	**12**	**5.25**	**0.652**	**0.626**	**3.744**	**2 (B*1/AB*2′)**	**-0.042**	**no**
S16	**Russia**	**Nizhnyj Archyz**	**1800**	**N43°39'22.0"**	**E41°25'07.4**	**8**	**4.25**	**0.489**	**0.579**	**3.314**	**2 (A*1/B*2)**	**0.157**	**no**
S24	**Russia**	**Mirny**	**256**	**N44°33'04.4"**	**E40°00'09.7"**	**12**	**3.375**	**0.386**	**0.389**	**2.585**	**1 (AB*1)**	**0.007**	**no**
S30	**Russia**	**Archipo-Osipovka**	**0**	**N44°21'34.6"**	**E38°31'24.4"**	**10**	**4.875**	**0.602**	**0.585**	**3.556**	**2 (B*1/AB*2)**	**-0.03**	**no**
S31	**Russia**	**Novomichailovsky**	**30**	**N44°17'14.9"**	**E38°54'03.5"**	**14**	**5**	**0.464**	**0.516**	**3.37**	**3 (B*1/AB*3′/4)**	**0.101**	**no**
S33	**Russia**	**Tichonovka**	**16**	**N43°57'57.7"**	**E39°16'41.7"**	**12**	**5**	**0.464**	**0.475**	**3.279**	**2 (B*1/AB*4)**	**0.022**	**no**
S34	**Russia**	**Bolshoe Pseushko**	**269**	**N44°04'33.9"**	**E39°20'47.0"**	**10**	**6.5**	**0.510**	**0.614**	**4.014**	**2 (B*1/AB*4)**	**0.17**	**no**
S36	**Russia**	**Mt. Strizhament**	**800**	**N44°46'40.1"**	**E42°00'54.8"**	**14**	**3.875**	**0.673**	**0.635**	**3.258**	**1 (AB*4′)**	**-0.06**	**no**
Hspr	*Vaud*	*Leysin, En Prélan*	*1350*	*N 46°33′56.3′′*	*E 6°99′16.7′′*	*8*	*5.375*	*0.625*	*0.572*	*3.608*	*NA*	*-0.093*	*no*
Hs80	*Vaud*	*Les Avants, Nermont*	*NA*	*N 46°44′70.1′′*	*E 6°94′85.5′′*	*6*	*4.25*	*0.396*	*0.6*	*3.935*	*NA*	*0.34*	*no*
HsJ	*Vaud*	*Col de Jaman*	*1512*	*N 46°45′17.2′′*	*E 6°97′78.0′′*	*6*	*4.75*	*0.500*	*0.592*	*4.316*	*NA*	*0.155*	*no*
Hsp	*Vaud*	*Chessel*	*374*	*N 46°33′94.2′′*	*E 6°92′10.1′′*	*6*	*3.375*	*0.729*	*0.552*	*3.176*	*NA*	*-0.321*	*no*

† indicates putative source populations (i.e. alpine botanical gardens or beehives). Native populations are indicated in bold, H. sphondylium *populations are indicated in italic.*

Appendix 2. [Draft of PRIMER NOTE]

Isolation of nuclear microsatellites and plastid indels in the invasive plant *Heracleum mantegazzianum* (Apiaceae)

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Abstract

This study reports the isolation and characterisation of six highly polymorphic nuclear SSR loci and four plastid indels in the invasive plant *Heracleum mantegazzianum* (Apiaceae). The application of these markers was tested in 30 individuals from two populations from both native and invaded range. The number of alleles ranged from five to eight per microsatellite loci with substantial heterozygote deficiencies, especially in the invasive population. On the other hand, plastid indels revealed the presence of five divergent chlorotypes. Applications of these markers include population genetics and phylogeography of *H. mantegazzianum*.

Heracleum mantegazzianum (Sommier et Levier, 1895), the Giant Hogweed, is a perennial monocarpic diploid weed that has successfully invaded over 20 European countries (Tiley *et al.*, 1996; and references therein) and is also reported as an invader in Canada and the USA (Morton, 1978; Dawe & White, 1979). It reproduces exclusively sexually and a single plant can produce up to 100'000 seeds (Pyšek *et al.*, 1995; and references therein). The ecology and management practices of Giant Hogweed have been thoroughly studied (e.g. Nielsen *et al.*, 2005; Pyšek *et al.*, 2007), yet its population genetics has lagged behind with only two studies up to date (Walker *et al.*, 2003; Jahodová *et al.*, 2007). Here we describe the development of six new polymorphic nuclear microsatellite loci and three plastid indels and one plastid microsatellite which may be useful to study the population genetic, population structure, and phylogeography of the species in both native and invaded ranges.

Individual ISSR-PCR was carried out using six 5'-anchored microsatellite primers that gave clear multi-band products in an initial screen in *Heracleum mantegazzianum*: GGCC$(AG)_8$, GGCC$(AC)_8$, CCGG$(AG)_8$, CCGG$(AC)_8$, GCGC$(AG)_8$ and GCGC$(AC)_8$. Reactions were carried out in a total volume of 20 µl containing 100 ng genomic DNA, 20 pmol primer, 1x PCR reaction buffer (5 mM Tris-HCl [pH9.1], 1.6 mM $[NH_4]_2SO_4$, 15 µg/µl BSA), 200 µM each dNTP, 2.5 mM $MgCl_2$ and 1.0 U *Taq* polymerase (Genetix) using the following parameters: initial denaturation at 94 °C for 3 min followed by 35 cycles of denaturation at 94 °C, annealing at 60 °C for 1 min, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. 10 µl PCR product were resolved on a 2% agarose gel and visualised by ethidium bromide staining to ensure clarity of fragments. The remaining 10

μl from each reaction were purified, pooled and ligated into pGEM-T (Promega) according to manufacturer's instructions. Following transformation into *Escherichia coli* JM109, 48 positive (white) clones were sequenced. All clones had inserts containing flanking microsatellite motifs corresponding to the anchored primer used to generate the ISSR fragment. Nine unique clones contained one internal microsatellite motif. For these, pairs of flanking primers were designed to amplify the repeated regions.

Plastid indels were developed by sequencing the *Trn*T-*Trn*L intergenic spacer (following Taberlet *et al.,* 1991). Primer sequences were then defined in regions flanking the indels of interest. Sequencing was performed using the Big Dye 3.1 Terminator cycle sequencing kit (Applied Biosystems) according to manufacturer's instructions and an ABI PRISM 3100 genetic analyser (Applied Biosystems).

Thirty individuals sampled in two geographically distinct locations: the western Swiss Alps (N 46°35'16.9'', E 7°03'67.9'') and the Caucasus Mountains of south-west Russia (N44°17'14.9", E38°54'03.5") were used to test the newly developed markers. Polymerase Chain Reaction (PCR) was performed in a total volume of 25 μl containing 1X reaction buffer (Colorless GoTaq® reaction buffer, Promega; 1.5 mM $MgCl_2$, pH 8.5), 0.2 mM of each dNTP, 0.4 μM of forward and reverse primers, template DNA (about 20 - 100 ng) and 1 u GoTaq® DNA polymerase. Each forward primer was labeled with one fluorescent dye (HEX, NED or FAM). Cycling was performed in a T3 Thermocycler (Biometra). Cycling parameters were as follows: one cycle at 94°C followed by 35 cycles of 45 s at 94°C, 1 min at annealing temperature (Table 1, 2), 2 min at 72°C, and a final cycle at 72°C for 10 min.

PCR products were multiplexed on an ABI PRISM 3100 with GENESCAN 350 size standard, and alleles were called using GENEMAPPER v3.7 (Applied Biosystems).

The software FSTAT version 2.9.4. (Goudet, 2005) was used to test for linkage disequilibrium (LD) and deviation from Hardy-Weinberg equilibrium (HWE) in the entire sample. In addition, the software MICRO-CHECKER (van Oosterhout *et al.*, 2004) was used to check for the presence of null-alleles, stuttering, and large allele dropouts in the dataset. FSTAT was also used to estimate observed and expected heterozygosities (H_o, H_s), number of alleles (N_a) and allele size range (Table 1).

Table 1. Characteristics of *Heracleum mantegazzianum* nuclear SSR for one population in both its native and invaded range, including loci names (Loc.) and GenBank accession numbers (GenBank), core sequences (Primers), annealing temperature (Anneal.), Size range, number of alleles (N_a), average observed (H_o) and expected (H_s) heterozygosities.

Loci names	GenBank	Seq.	Primers	Anneal.(°C)	Size range (bp)	N_a	H_o	H_s
HMNSSR131		$(TA)_8$	(*)GCGATTCTCGATCTGTAAGCTT TACTATAATTCTGAACCCTAGTT	57	121-131	5	0.469	0.714
HMNSSR132A		$(CT)_7$	(*)CGATTGCTCTTCTTTTGAGCAT AGGGTTTTGATAAAGTTAGGAAT	58	104-122	5	0.134	0.568
HMNSSR132B		$(CT)_7$	(*)ATTCCTAACTTTATCAAAACCCT AGAGAGCCAGGTTTTGTATAAC	58	84-98	8	0.388	0.602
HMNSSR140		$(TC)_8$	(*)GTATCCGGATCTGTACCTGTA GCCTACAAAATCAAACAACTGA	58	117-143	6	0.170	0.274
HMNSSR206		$(TC)_{10}$	(*)GCGATTGCTCTTCTTTTGAGCA TTGGGGTTTTGATAAAGTTAGGAA	58	106-116	5	0.424	0.516
HMNSSR211		$(CT)_8$	(*)CAGCCTTCTTGTTGTTATCACCA TGGGTGTTAGAGTTTTGAAAAAGA	58	103-119	6	0.469	0.345

Of the nine unique nuclear microsatellite clones isolated, seven produced reliable

amplifications and six were polymorphic in *H. mantegazzianum*. The number of alleles ranged from five to eight and average observed and expected heterozygosities (***H****o*, ***H****s*) are 0.431 and 0.553 respectively. The software MICRO-CHECKER detected the possible presence of null alleles in three loci (*HMNSSR132a, HMNSSR132b,* and *HMNSSR140*) as is suggested by the heterozygote deficiencies at these three loci (Table 1). This heterozygote deficiency may also be due to a wahlund effect observed due to the disparate nature of the populations sampled in the native and invaded ranges of the species. The native population is in Hardy-Weinberg equilibrium after Bonferroni corrections, but the invasive population is significantly out of HWE. Recent colonization (less than a 100 years) may be responsible for the observed pattern. All loci were independent as attested by non-significant tests for LD (data not shown).

When combined with *ccmp5* and *ccmp10* (Weising & Grardner, 1999), the four plastid loci developed here enable the identification of five divergent chlorotypes. Three of these were found in the native population, and an additional two in the invasive population. Interestingly, not chlorotypes were shared between the two populations, confirming strong differentiation among the two samples.

Table 2. Characteristics of *Heracleum mantegazzianum* plastid indels for one population in both its native and invaded range, including loci names (Loc.) and Genbank accession numbers (GenBank), core sequences (Primers), annealing temperature (Anneal.) and allele sizes.

Loci names	GenBank	Primers	Anneal.(°C)	Allele sizes (bp)
TrnTL indel1		(*)AGATAAATTCTACCTGCAAGG	56	84, 90
		TGACTAGCTAATAGTAATCGC		
TrnTL indel2		(*)TTAGTTTTTTTCTCACATCAC	53	98, 107
		GATTTAATCTAAAAATAGAAC		
TrnTL indel3		(*)ATTACACTTCTATATTTTATTGC	50	110, 112
		TCCATCTTTACGAATCAAAG		
TrnTL indel4		(*)TTCTGATTGGACCAAATGCG	53	147, 214
		TCTACCGATTTCGCCATATC		

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