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Patterns of Genetic Diversity in the Globally Invasive Species Wild Parsnip (*Pastinaca sativa*)

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Wild parsnip is an invasive species with a global distribution in temperate climates. Parsnips are native to Eurasia and have been cultivated for more than five centuries. It is unclear whether the global invasion of this species is a consequence of escape from cultivation or the accidental introduction of a Eurasian wild subspecies. In this study, we used nuclear ribosomal DNA internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) markers to evaluate the genetic structure of wild parsnip in its native range (Europe) and in three distinct geographic regions where it is considered invasive: eastern North America, western North America, and New Zealand. We also compared wild and cultivated parsnips to determine whether they are genetically distinct. From 112 individuals, we recovered 14 ITS and 27 cpDNA haplotypes. One ITS haplotype was widespread; few haplotypes were rare singletons. In contrast, at least two lineages of cpDNA haplotypes were recovered, with several novel haplotypes restricted to Europe. Cultivated parsnips were not genetically distinct from wild parsnips, and numerous wild parsnip populations shared haplotypes with cultivars. High genetic diversity was recovered in all three regions, suggesting multiple introductions.

Nomenclature: Wild parsnip, *Pastinaca sativa* L. PASA2.

Key words: Chloroplast haplotypes, colonization history, cultivars, invasion genetics, New Zealand.

A major consequence of international trade is the recurrent establishment of invasive species on a global scale. Invasive species can be extremely detrimental to native ecosystems and are considered a leading threat to biodiversity worldwide (Pysek et al. 2012; Pysek and Richardson 2011; Vilà et al. 2011). Tracking the source population and invasion routes is the first step in designing effective management strategies (Schaal et al. 2003). Historical documentation and real-time observations of invasions are rare, but advances in molecular methods and tools for population genetics have made it possible to reconstruct the colonization history of invasive populations (Lombaert et al. 2011). These techniques have been used with increasing frequency to analyze the genetic structure of introduced populations, to make inferences about their origins, and to determine routes of establishment (reviewed in Estoup and Guillmaud 2010). Recent studies that have examined the genetic relationship between closely related, wild and cultivated species suggest that cultivation has an important role in influencing the spread of invasive weeds. Naturalized cultivars might become invasive or introgressive with closely related wild and cultivated species and might produce invasive hybrids. For example, invasive orange wattle [*Acacia saligna* (Labill.) Wendl. f.] (Fabaceae) in South Africa is more closely related to a cultivated variety in Western Australia than it is to its wild Australian counterpart (Thompson et al. 2012). Invasive wild radish (*Raphanus raphanistrum* L.) (Brassicaceae) frequently hybridizes with cultivated radish (*Raphanus sativus* L.) in California (Ridley et al. 2008) and potentially in South Africa (Barnaud et al. 2013). Nineteen percent of wild grape [*Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi] (Vitaceae) comprises naturalized cultivars in Spain (DeAndreas et al. 2012). Cultivars are likely associated with the origin and spread of many invasive species.

Wild parsnip (*Pastinaca sativa* L.) (Apiaceae) is an herbaceous, biennial native of Europe that now occurs on every continent, except Antarctica (Averill and DiTommaso 2007). Parsnips are believed to have originated in the Caucasus

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Management Implications

The availability of molecular data and advances in population genetic analysis have made it possible to estimate the patterns of species migrations with considerable precision. Findings from historical colonization and dispersal studies can shed light on human-mediated transport of biological materials and provide a broad understanding of how humans may have contributed to the introduction and spread of an invasive species. Evidence obtained in this study indicates that escape from cultivation led to the globalization of wild parsnips and introduced populations have high genetic diversity. Increased levels of diversity in invasive populations can act as a primer for rapid adaptive evolution of this noxious invasive species. Future studies with increased sampling and additional loci have the potential to elucidate specific patterns of colonization and admixture in this agriculturally entwined invasive plant and may serve as a model for investigating the natural and agricultural history of plant species that exist in wild, cultivated, and feral forms.

Mountains, a center of diversity for the genus *Pastinaca* (Rubatzky et al. 1999). However, *P. sativa* is the only species in the genus to occur outside Europe, and its spread throughout the world may have been linked to its cultivation as a food crop. The long, carbohydrate-rich taproot produced by cultivated varieties of this species is frequently consumed in many parts of the world. Parsnips are currently cultivated commercially on at least three continents (North America, Europe, and Oceania) and, in all three regions, wild parsnips occur as an invasive species (Averill and DiTommaso 2007). The distinction between a wild and a cultivated parsnip is unclear. Some sources cite the cultivated parsnip *Pastinaca sativa* subsp. *sativa* as a distinct subspecies from its wild counterpart, *Pastinaca sativa* subsp. *sylvestris* (Mill.) Rouy & E.G. Camus, because of physical and chemical differences (Averill and DiTommaso 2007; Berenbaum et al. 1984). Wild parsnips are considered invasive because they are a nuisance to humans and livestock; they also present an aggressive threat to a variety of natural communities once they become established and can displace native species (Averill and DiTommaso 2007). Roots of wild parsnips are tough and can be poisonous because of the production of large quantities of myristicin, a hallucinogenic phenylpropanoid (Stahl 1981). Aboveground structures of wild parsnips also contain higher concentrations of the photodermatitis-inducing furanocoumarins compared with cultivated varieties (Berenbaum et al. 1984). These furanocoumarins are demonstrably allelopathic in related umbellifers and may allow wild parsnips to grow in large monocultures and outcompete other species (Juntilla 1976). In spite of the phenotypic differences, the evolutionary origins of, and taxonomic distinctions between, the two forms have never, to our knowledge, been fully examined.

The history of parsnip domestication from its wild progenitor is obscure. Historically, the name *Pastinaca* may have referred to parsnip or to carrot (*Daucus carota* L. var. *sativus* Hoffm.), and there is some evidence that both were cultivated and consumed in ancient Greece (Hedrick and Sturtevant 1972). Parsnip seeds have been recovered in a Neolithic Swiss lake dwelling, dating back 4000 years, possibly the earliest evidence of parsnip cultivation (Weaver 1997). The cultivation of parsnips, as distinct from carrots, has been documented with some certainty throughout Europe in the 14th century, in South America (Venezuela) in 1564, and in 1604, in Peru (Averill and DiTommaso 2007). In North America, Native Americans purportedly used the roots for medicinal purposes as early as 1606 (Hedrick and Sturtevant 1972). Modern varieties of cultivated parsnips can be traced to the *P. sativa* 'Student' cultivar selectively bred from wild parsnips in 1849 by Professor James Buckman at the Royal Agricultural College, Cirencester, England (Buckman 1865). At least one parsnip cultivar from Surrey, England, *P. sativa* 'Gladiator', is a known hybrid between a cultivated parsnip and a wild parsnip.

In eastern North America, cultivars may have escaped soon after introduction in the early 17th century (Sturtevant 1890). The earliest herbarium record for wild parsnips in the United States dates back to 1822, and the specimen was collected near Hamden, CT (GBIF data portal, 2015). In the western United States, although no historical information is available on dates of arrival, earliest herbarium records of *P. sativa* date back to 1879. Herbarium records indicate that by 1930 wild parsnips occurred throughout the lower 48 U.S. states (GBIF data portal, 2015; NANSH, 2015). Compared with the eastern United States, wild parsnip is uncommon in the western United States, with most populations occurring in disturbed agricultural settings (P. Ode, personal communication). In North America, wild parsnips currently occur in 45 of the 50 U.S. states and in all Canadian provinces and territories, except Nunavut and the Northwest Territories (Averill and DiTommaso 2007). It also occurs as an invasive species in southern South America, South Africa, China, Australia, and New Zealand. In New Zealand, the first written record of wild parsnip can be dated to 1867 (Webb 1978).

In this study, we used molecular markers to examine the relationship between wild parsnips and modern parsnip cultivars, as well as to evaluate patterns of genetic diversity among wild parsnips in eastern North America, western North America, and New Zealand—regions that have been colonized by this invasive plant. Two loci were analyzed: the nuclear ribosomal DNA internal transcribed spacer (ITS) region and the chloroplast DNA (cpDNA) *psbM–trnT* region. Specifically, we wanted to identify (1) whether the genetic diversity of wild parsnips in the three study regions has been influenced by founder effects (alteration or reduction or both in genetic diversity) during

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introduction and invasion, (2) whether native European wild parsnips (*P. sativa* subsp. *sylvestris*) are genetically distinct from modern cultivars (*P. sativa* subsp. *sativa*), and (3) whether North American and New Zealand invasive populations are more closely related to modern cultivars or European wild parsnips

Materials and Methods

Sample Collection. Samples for DNA analysis were obtained from single leaves of individual plants. *Pastinaca sativa* samples were obtained from 58 sites in Europe, 27 sites in eastern North America, 8 sites in western North America, and 9 sites in the South Island of New Zealand (Table 1).

Each site consisted of a wild parsnip population isolated from other populations by either anthropogenic/geographic barriers or at least 20 km distance. Ten samples were collected at each site, but a preliminary analysis showed no variation in samples within sites for both loci, so only one sample per site was analyzed. Both ITS and cpDNA were included in the preliminary analysis, and we examined four individuals from the TOW population and three from the OCE population in New Zealand, three from the SWISS3 population in Europe, four from the IOW1 population, and three from the MAU2 population in the eastern United States (population information in Table 1). We found no variation at both loci in all these populations. All regions except the western United States were analyzed. The sampling effort was in proportion to available populations, with more samples in Europe and eastern North America, and fewer samples in western North America and New Zealand. One leaf per plant was collected and placed in a standard (10.2 cm by 22.9 cm) paper envelope and dried in a field press for 60 d. In New Zealand, dried seeds were collected by Dr. Margaret Stanley (University of Auckland) and shipped to the University of Illinois at Urbana-Champaign (UIUC). New Zealand seeds were planted in the UIUC Department of Entomology greenhouse, and leaf material was collected approximately 4 wk after germination and dried in a field press. Field collections were supplemented by leaf material obtained from *P. sativa* herbarium specimens (Illinois Plant Biology Herbarium and Illinois Natural History Survey Herbarium) and previously isolated DNA available in the Downie laboratory (Table 1). We also included previously isolated DNA from *Pastinaca sativa* subsp. *urens* (Godron) Čelak, *Pastinaca sativa* subsp. *divaricata* (Desf.) Rouy & Camus, and *Pastinaca pimpinellifolia* M. Bieb. In addition, we planted six varieties of cultivated parsnips in the UIUC Department of Entomology greenhouse and collected leaf tissue from rosettes approximately 1 mo after germination. Parsnip cultivars analyzed in this study were acquired from the United States and New Zealand; the cultivars 'All-American Organic' (Todds Seeds, Novi, MI), 'US Hollowcrown' (Burpee Seeds, Warminster, PA), 'All-American Heirloom' (Botanical Interest, Broomfield, CO), and 'Excalibur' (Thompson and Morgan, Ipswich, England) were purchased in the United States, and cultivars 'Melbourne Whiteskin' (McGregor's, Auckland, New Zealand) and 'New Zealand Supersnip' (McGregor's) were purchased in New Zealand.

Molecular Markers. Two loci, ITS and *psbM-trnT*, were used to determine the diversity and distribution of haplotypes across geographic regions and cultivars. The ITS locus consists of two segments: ITS1, which is located between the 18S and 5.8S ribosomal genes, and ITS2, between the 5.8S and 28S ribosomal genes. Together, ITS1 and ITS2 are approximately 483 base pairs (bp) long in *P. sativa* (Downie and Katz-Downie 1996).

The nuclear rDNA ITS region has been used frequently in lower-level phylogenetic analyses in plants because of its ease of amplification and high mutation rate. ITS is also useful as a species barcode for angiosperms (Li et al. 2011), and adequate intraspecific and interspecific variation exists at this locus to delineate species within the family Apiaceae (Liu et al. 2014). Although ITS has been useful at infrageneric-level analyses, it can also be effective in providing information about population genetic structure (e.g., Besnard et al. 2007; Gao et al. 2012; Lorenz-Lemke et al. 2005). Because it occurs in the nuclear genome, ITS is biparentally inherited and recombines, so it has the potential to reveal recent gene flow and hybridization events.

The cpDNA *psbM-trnT* locus is a highly variable noncoding region, approximately 1,400 bp long in Apiaceae (Downie and Jansen 2015). The chloroplast genome is haploid, nonrecombinant, and maternally inherited and can be informative for intraspecific phylogenetic analysis, especially over a large geographic area (Ouborg et al. 1999). cpDNA has a different evolutionary rate compared with nuclear DNA and, because it is maternally inherited, measures of gene flow are not confounded by reticulation (McCauley 1995). Both regions have been previously used together (Gao et al. 2010) and have the potential to elucidate the genetic structure of globally invasive plants.

DNA Extraction, Amplification, and Sequencing. Whole-genomic DNA was amplified from approximately 20 mg of dried leaf material using a Qiagen DNeasy Plant Mini kit according to the manufacturer's directions (Qiagen Inc., Valencia, CA). ITS1 (18S to 5.8S) and ITS2 (5.8S to 28S) were polymerase chain reaction (PCR)–amplified separately using previously published primers (Table 2). The 5.8S region that joins ITS1 and ITS2 is highly conserved and, therefore, was not completely sequenced. The cpDNA *psbM-trnT* locus contains intervening genes *trnD*, *trnY*, and *trnE*, and the spacers between *psbM* and *trnE* and between

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Table 1. Sampling locations and haplotype information for all *Pastinaca sativa* individuals genotyped.[a]

Species	Origin	Country/cultivar	Site code	DNA accession	Collection location/voucher information	ITS haplotype	Chloroplast haplotype
P. sativa	Cultivar	All American Heirloom	AAH	4012	Greenhouse-grown cultivar	H1	C6
P. sativa	Cultivar	All American Organic	AAO	4007	Greenhouse-grown cultivar	H12	C13
P. sativa	Cultivar	Excalibur	Ex	4010	Greenhouse-grown cultivar	H1	C27
P. sativa	Cultivar	Melbourne Whiteskin	Mel	4009	Greenhouse-grown cultivar	H1	C1
P. sativa	Cultivar	New Zealand Supersnip	SS	4008	Greenhouse-grown cultivar	H8	C5
P. sativa	Cultivar	US Hollowcrown	USHol	4011	Greenhouse-grown cultivar		C6
P. sativa	Europe	Netherlands	NETH2	3947	51.596683°N, 4.335817°E	H1	C13
P. sativa	Europe	Netherlands	NETH3	3948	51.629183°N, 4.36405°E	H1	C6
P. sativa	Europe	Netherlands	NETH4	3959	52.0902°N, 4.968183°E	H1	C1
P. sativa	Europe	Netherlands	NL-D	3956	52.500683°N, 6.084317°E	H9	C24
P. sativa	Europe	Netherlands	NL-H	3957	51.94705°N, 5.75605°E	H1	C25
P. sativa	Europe	Netherlands	NL-I	3986	52.061883°N, 5.1959°E		C1
P. sativa	Europe	Netherlands	NL-M	3958	53.0919°N, 5.376883°E	H1	C11
P. sativa	Europe	Austria	AUS1	3924	47.063617°N, 10.38475°E	H1	C3
P. sativa	Europe	Austria	AUS2	3938	47.134133°N, 10.516367°E	H1	
P. sativa	Europe	Austria	AUS3	3961	47.076483°N, 10.6619°E	H1	C14
P. sativa	Europe	Austria	AUS4	3950	47.230033°N, 10.854983°E	H1	C21
P. sativa	Europe	Austria	P7	4145	Austria, Schwertberg, *Keck s.n.* (ILL)	H1	
P. sativa	Europe	Belgium	BE-B	3962	50.870133°N, 4.546217°E	H1	C7
P. sativa	Europe	Belgium	BE-C	3951	51.119483°N, 4.4504°E	H1	C22
P. sativa	Europe	Belgium	BELG1	3919	51.079655°N, 3.445499°E	H1	C8
P. sativa	Europe	Denmark	DK-A	3963	55.774983°N, 9.707017°E	H1	C23
P. sativa	Europe	Denmark	DK-B	3932	55.817883°N, 9.789017°E	H1	C2
P. sativa	Europe	Denmark	DK-C	3964	55.88345°N, 9.826067°E	H8	C16
P. sativa	Europe	Denmark	DK-D	3960	56.0964°N, 10.0525°E	H1	C5
P. sativa	Europe	France	244	244	France, cultivar UIUC from seeds obtained from Conservatoire et Jardins Botaniques de Nancy, France, *Downie 244* (ILL)	H1	C2
P. sativa	Europe	France	FRAN1	3921	48.547733°N, 2.484917°E	H1	
P. sativa	Europe	France	FRAN2	3975	48.339683°N, 2.594768°E	H1	C7
P. sativa	Europe	France	FRAN3	3976	47.850983°N, 3.549317°E	H1	C13
P. sativa	Europe	France	FRAN4	3977	45.921683°N, 5.268833°E	H1	C13
P. sativa	Europe	France	FRAN6	3936	45.821783°N, 4.991933°E	H1	C7
P. sativa	Europe	France	FRAN7	3937	45.863867°N, 5.144867°E	H1	C12

Table 1. Continued.

Species	Origin	Country/cultivar	Site code	DNA accession	Collection location/voucher information	ITS haplotype	Chloroplast haplotype
P. sativa	Europe	Germany	166	166	Germany, cultivar UIUC from seeds obtained from Botanischer Garten Oldenburg, Germany, *Downie 166* (ILL)	H1	C1
P. sativa	Europe	Germany	P6	4144	Germany, Grossheirath, Bavaria, Coburg District, *Siegel s.n.* (ILL)	H1	
P. sativa	Europe	Germany	P8	4146	Herbarium	H1	C4
P. sativa	Europe	Hungary	P4	4143	Hungary, *Steinitz 15904* (ILL)	H1	C3
P. sativa	Europe	Germany	70	70	Germany, cultivar UIUC from seeds obtained from Botanischer Garten Mainz, Germany, *Downie* 70 (ILL)	H1	
P. sativa	Europe	Germany	GE-B	3953	53.3288°N, 8.141233°E	H1	C2
P. sativa	Europe	Germany	GE-C	3954	53.381883°N, 8.27085°E	H1	C3
P. sativa	Europe	Germany	GE-E	3966	53.736767°N, 9.9673°E		C1
P. sativa	Europe	Germany	GE-G	3952	53.8442°N, 11.880233°E		C4
P. sativa	Europe	Germany	GE-J	3929	51.626233°N, 12.179883°E	H1	C5
P. sativa	Europe	Germany	GE-M	3930	50.933583°N, 10.2337°E		C13
P. sativa	Europe	Germany	GER10	3931	50.21385°N, 7.550217°E	H1	
P. sativa	Europe	Germany	GER11	3955	50.46985°N, 7.228867°E	H1	C3
P. sativa	Europe	Germany	GER12	3968	50.9421°N, 6.927433°E	H1	C2
P. sativa	Europe	Germany	GER13	3969	51.130233°N, 6.587567°E	H1	C18
P. sativa	Europe	Germany	GER15	3982	52.317733°N, 13.063133°E	H1	
P. sativa	Europe	Germany	GER2	3939	47.7758°N, 9.174917°E		C4
P. sativa	Europe	Germany	GER3	3940	47.907667°N, 8.9662°E	H1	C3
P. sativa	Europe	Germany	GER4	3941	48.226983°N, 8.84695°E	H1	C11
P. sativa	Europe	Germany	GER5	3942	48.146267°N, 8.902383°E	H6	C3
P. sativa	Europe	Germany	GER6	3943	48.595°N, 9.068117°E	H1	C19
P. sativa	Europe	Germany	GER7	3944	48.89475°N, 8.704067°E	H1	C7
P. sativa	Europe	Germany	GER8	3945	48.996767°N, 9.04525°E	H1	
P. sativa	Europe	Germany	GER9	3946	49.05965°N, 9.161433°E	H7	C20
P. sativa	Europe	Switzerland	SWIS1	3970	46.483267°N, 7.180317°E	H1	C5
P. sativa	Europe	Switzerland	SWIS2	3933	46.466617°N, 7.125267°E		C3
P. sativa	Europe	Switzerland	SWIS3	3985	823.6°N, 7.118755°E	H11	C14
P. sativa	Europe	Switzerland	SWIS4	3935	46.460967°N, 7.105517°E	H1	C15
P. sativa	Europe	Switzerland	SWIS5	3920	46.4664°N, 7.070217°E		C3
P. sativa	Europe	Switzerland	SWIS6	3971	46.539283°N, 7.0685°E	H1	C13
P. sativa	Europe	Switzerland	SWIS7	3983	46.808167°N, 7.1281°E	H11	

Table 1. Continued.

Species	Origin	Country/cultivar	Site code	DNA accession	Collection location/voucher information	ITS haplotype	Chloroplast haplotype
P. sativa	Europe	Switzerland	SWIS8	3972	46.5865°N, 7.08805°E	H1	
P. sativa	Europe	Switzerland	SWIS9	3984	47.0332°N, 7.535933°E	H1	C13
P. sativa	New Zealand	New Zealand	COT3	3997	45.678391°S, 170.625991°E		C6
P. sativa	New Zealand	New Zealand	KANE9	3990	45.899857°S, 170.441315°E	H1	
P. sativa	New Zealand	New Zealand	KARI20	3994	45.639728°S, 170.665741°E	H1	
P. sativa	New Zealand	New Zealand	MOER13	3993	45.365655°S, 170.860748°E		C5
P. sativa	New Zealand	New Zealand	OCE19	3998	45.699938°S, 170.603256°E	H1	C6
P. sativa	New Zealand	New Zealand	ROC1	3992	45.658185°S, 170.641987°E	H13	
P. sativa	New Zealand	New Zealand	ROC9	3991	45.658185°S, 170.641987°E	H11	
P. sativa	New Zealand	New Zealand	TOW2C	3996	45.893159°S, 170.459071°E	H14	
P. sativa	New Zealand	New Zealand	TOW8B	3995	45.893159°S, 170.459071°E	H1	
P. sativa	E. North America	Canada, ON	P11	4138	Canada, Ontario, Carleton Co., March Twp., South March, *Cody et al. 13178* (ILL)	H1	
P. sativa	E. North America	USA, IA	IOW1	3988	41.69493°N, 91.68002°W	H1	
P. sativa	E. North America	USA, IA	IOW14	4005	41.69493°N, 91.68002°W	H1	
P. sativa	E. North America	USA, IA	IOW2	4015	42.22875°N, 96.25153°W		C13
P. sativa	E. North America	USA, IL	543	543	Illinois, Champaign Co., Urbana, *Downie 543* (ILL)	H1	C3
P. sativa	E. North America	USA, IL	734	734	Illinois, Champaign Co., Urbana, Hart Woods, *Downie 734* (ILL)	H1	
P. sativa	E. North America	USA, IL	742	742	Illinois, Champaign Co., Urbana, Phillips Tract, *Downie 742* (ILL)		C4
P. sativa	E. North America	USA, IL	FAR	3949	38.823427°N, 88.782921°W	H5	
P. sativa	E. North America	USA, IL	MON	4001	41.39215°N, 87.77428°W	H1	C6
P. sativa	E. North America	USA, IL	NEO	3926	38.946593°N, 88.599243°W		C3
P. sativa	E. North America	USA, IL	PEOR	4002	40.77622°N, 89.75208°W		C5
P. sativa	E. North America	USA, IL	ROCH	4019	41.92747°N, 89.0026°W	H13	
P. sativa	E. North America	USA, IL	ROCK1	4014	43.50299°N, 89.949°W	H1	
P. sativa	E. North America	USA, IN	IND1	3927	40.136366°N, 87.385941°W	H1	C10
P. sativa	E. North America	USA, IN	IND2	4020	40.105387°N, 87.372465°W	H1	C5
P. sativa	E. North America	USA, MN	MIN	4004	43.65555°N, 93.72836°W	H1	C1
P. sativa	E. North America	USA, NC	P19	4142	North Carolina, Ashe Co., Jefferson, *Radford and Pence 44875* (ILLS)	H1	
P. sativa	E. North America	USA, OH	HEB	4006	39.94041°N, 82.47897°W	H1	
P. sativa	E. North America	USA, PA	ADD	4018	40.11343°N, 80.42863°W		C13
P. sativa	E. North America	USA, PA	CLAY	4017	40.11343°N, 80.42863°W	H11	C26

Table 1. Continued.

Species	Origin	Country/cultivar	Site code	DNA accession	Collection location/voucher information	ITS haplotype	Chloroplast haplotype
P. sativa	E. North America	USA, TN	P18	4141	Tennessee, Knox Co., Knoxville, *Patrick 80764* (ILLS)	H1	
P. sativa	E. North America	USA, WI	ARE	3989	43.74992°N, 90.08477	H10	
P. sativa	E. North America	USA, WI	JANE	3987	42.58098°N, 88.98255°W	H1	C7
P. sativa	E. North America	USA, WI	MAU2	4003	43.75779°N, 89.96798°W	H11	C6
P. sativa	E. North America	USA, WI	MAU3	4013	39.94041°N, 82.47897°W	H16	C6
P. sativa	E. North America	USA, WI	SCH	4021	43.349477°N, 90.431471°W		C11
P. sativa	E. North America	USA, WV	MORG	4016	39.6855°N, 79.83334°W	H11	C6
P. sativa	W. North America	USA, AZ	AL	3917	34.99095°N, 111.742517°W	H1	C6
P. sativa	W. North America	USA, CO	AC	3916	40.407117°N, 106.80665°W		C5
P. sativa	W. North America	USA, CO	G	3915	39.380267°N, 107.0784°W		C9
P. sativa	W. North America	USA, NM	AE	3914	36.991667°N, 106.781333°W	H1	
P. sativa	W. North America	USA,NM	T	3913	36.84335°N, 106.570383°W	H1	C6
P. sativa	W. North America	USA,UT	AM	3918	37.245117°N, 112.667083°W	H1	C7
P. sativa	W. North America	USA,WA	P13	4139	Washington, King Co., Seattle, *Jones s.n.* (ILL)	H1	
P. sativa	W. North America	USA,WY	P17	4140	Wyoming, Washakie Co., Old Main Gulch, Tensleep Preserve, 10 mi of Ten Sleep, *Phillippe et al. 36942* (ILLS)	H1	
P. sativa subsp. *divaricata*	Europe	France	P1	4137	France, Corsica, Col de Capronale, *Lambinon and Duvigneaud 76/Co/576* (ILL)	H3	C5
P. sativa subsp. *urens*	Europe	France	1878	1878	France, cultivar Ville de Mulhouse Conservatoire Botanique, no. 99138A, Hildenbrand et al. s.n.; *Downie 1878* (ILL)	H2	C4
P. pimpinellifolia	Europe	Turkey	665	665	*Pimenov et al. 665* (MW)	H4	

[a] Abbreviations: E., eastern; ITS, internal transcribed spacer, Twp., Township; UIUC, University of Illinois at Urbana-Champaign; W., western; ILL, Illinois Plant Biology Herbarium; ILLS, Illinois Natural History Survey Herbarium; MW, Moscow University Herbarium.

Table 2. Polymerase chain reaction primers for internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) *psbM–trnT*.

Locus	Primer and location	Reference	Primers
ITS	18S F 5′	Feist and Downie 2008	TAG AGG AAG GAG AAG TCG TAA
	5.8S R 3′	Feist and Downie 2008	ATA CTT GGT GTG AAT TGC AGA A
	5.8S (ITS-3N) F 5′	Spalik and Downie 2006	CGA TGA AGA ACG TAG CGA AAT
	28S (C26A) R 3′	Wen and Zimmer 1996	AGC GGA GGA AAA GAA AC
cpDNA	*psbM* F 5′	Modified from Shaw et al. (2005)	AGC AAT AAA TGC RAG AAT ATT TAC TTC CAT
	trnE R 3′	Modified from Shaw et al. (2005)	TCC TGT AGA GAG AAA GTT CCT G
	trnD F 5′	Modified from Shaw et al. (2005)	ACC AAT TGA ACT ACA ATC CC
	trnT R 3′	Shaw et al. (2005)	CTA CCG CTG AGT TAA AAG GG

trnE and *trnT* were amplified separately and used to assemble a complete *psbM–trnT* contig after sequencing. PCR primers for the chloroplast region are listed in Table 2.

Each 25-µl PCR reaction included sterile water (9.5 µl in ITS reactions; 10.75 µl in cpDNA reactions), 5.0 µl of 5 × of colorless GoTaq Flexi buffer (Promega Corp., Madison, WI), 4.0 µl of deoxynucleotides (dNTPs; each dNTP at 1.25 µM; Invitrogen Corp., Carlsbad, CA), 3.0 µl of $MgCl_2$ (25 mM), 0.5 µl of each primer (20 µM), GoTaq Flexi DNA polymerase (0.5 µl in ITS reactions; 0.25 µl in cpDNA reactions; Promega Corp.), and 1.0 µl of unquantified template DNA. DMSO (dimethyl sulfoxide; 1.25 µl) was added to the ITS reactions to relax secondary structures. For samples that were difficult to amplify, template DNA was diluted 1:10 or 1:100 to reduce contaminant concentration. The PCR protocol is outlined in Downie and Katz-Downie (1996) for ITS and Shaw et al. (2005) for *psbM–trnT*. For some cpDNA amplifications, Taq polymerase was replaced by a high-fidelity Phusion polymerase (New England BioLabs Inc., Ipswich, MA) in 20-µl reactions with protocols as specified by the manufacturer. The Phusion PCR protocol is as follows: initial denaturation for 30 s at 98 C, followed by 35 cycles of 10 s at 98 C, 10 s at 58 C, and 30 s at 72 C.

PCR products were purified using the Exo-Sap method: 2.25 µl sterile water, 0.25 µl exonuclease I (Exo; 20 units/µl, New England BioLabs), and 0.50 µl shrimp alkaline phosphatase (SAP; 1 unit/µl, Promega Corp.) were combined and added directly to each 25-µl reaction tube, then incubated for 30 min at 37 C followed by 15 min at 80 C to inactivate the enzymes. For some samples, SAP was replaced with Antarctic Phosphatase (New England BioLabs).

Sequencing reactions were performed using the ABI Prism BigDye Terminator ver. 3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Primers used for PCR amplification were also used for forward and reverse sequencing. Each 10-µl reaction included 1 µl ultrapure water, 2 µl sequencing buffer, 4 µl glycerol, 1.5 µl of the forward or reverse primer (10 pM), and 0.5 µl of BigDye. The sequencing reaction included initial denaturation for 1 min at 95 C, followed by 35 cycles of 15 s at 95 C, 5 s at 45 C, and 4 min at 60 C. Visualization of sequences was carried out using an ABI 3730XL high-throughput DNA capillary sequencer at the UIUC Keck Biotechnology Center.

Data Analysis. Forward and reverse sequences were assembled, and disagreements were manually corrected in Sequencher ver. 5.2 (Gene Codes Corporation, Ann Arbor, MI, http://www.genecodes.com). All ambiguous nucleotide positions (double chromatogram peaks) that might have been heterozygous alleles were coded with an N. It is unlikely that our treatment of ITS heterozygotes influences our analyses because ITS heterozygotes were relatively uncommon. Chloroplast sequences *psbM–trnE* and *trnE–trnT* were assembled into one contig using default parameters. Exported sequences from all samples were aligned with MUSCLE ver. 3.5 (Edgar 2004) using the default parameters. Sequences for *P. sativa* subsp. *divaricata*, *P. sativa* subsp. *urens*, and *P. pimpinellifolia* (Downie and Katz-Downie 1996; Logacheva et al. 2008) were included in the data matrices for comparison to wild parsnip sequences.

Because the complete 5.8S region that separates ITS1 and ITS2 was not sequenced, data matrices for ITS1 and ITS2 were concatenated in MESQUITE (Maddison and Maddison 2011; http://mesquiteproject.org) after sequence alignment.

Standard molecular diversity indices were calculated for both loci using DnaSP (Rozas 2009; http://www.ub.edu/dnasp/). Diversity calculations included the number of haplotypes, the number of segregating (polymorphic) sites, the nucleotide diversity, and haplotype diversity of all variable nucleotide sites in each locus. Sequence divergence between geographic regions was inferred using pairwise exact tests with 10,000 Markov chain Monte Carlo (MCMC) chains implemented in ARLEQUIN ver. 3.5 (Excoffier and Lischer 2010; http://cmpg.unibe.ch/software/arlequin35/). Exact tests for population differentiation are based on the nonparametric Fisher's exact test, which is used to determine whether an association exists between the counts of two variables. In the case of populations, exact tests compare geographic regions based on the occurrence of haplotypes

Table 3. Molecular diversity indices estimated from internal transcribed spacer (ITS) and chloroplast sequences from wild parsnips on three continents and cultivated parsnips.

Sequence region	Geographic origin	No. sequences	No. haplotypes	Segregating sites (S)	Nucleotide diversity	Haplotype diversity
ITS	Europe	52	6	6	0.0095	0.537
	Eastern North America	21	5	7	0.0353	0.333
	Western North America	6	1	0	0.000	0
	New Zealand	7	4	4	0.0476	0.387
	Cultivars	5	3	2	0.0285	0.440
psbM–trnT	Europe	52	22	58	0.0139	0.941
	Eastern North America	15	9	49	0.0131	0.914
	Western North America	5	4	17	0.0067	0.900
	New Zealand	3	2	15	0.0068	0.555
	Cultivars	6	5	45	0.0082	0.933

(Raymond and Rousset 1995). The exact test is a useful measure of differentiation when sample sizes are unbalanced (Goudet et al. 1996). Haplotype networks were constructed in HapStar (Teacher and Griffiths 2011) from minimum spanning trees obtained in ARLEQUIN.

Results and Discussion

We successfully amplified and sequenced the ITS locus for 91 individuals (GenBank accession numbers: KT766558-KT766731) and the *psbM–trnT* locus for 81 individuals. Alignment of concatenated ITS1 and ITS2 sequences contained 483 nucleotide positions, of which 28 were polymorphic in all sequences analyzed. The polymorphisms were all single-nucleotide polymorphisms (SNPs), with seven SNPs parsimony-informative. In wild parsnips, 19 sites were polymorphic, and four of these were parsimony-informative. In cultivated parsnips, two sites were polymorphic, and none were parsimony-informative. Individuals were separated into 14 haplotypes (hereby denoted as H1 to H14; Table 1).

Alignment of the concatenated *psbM–trnT* region contained 1,464 nucleotide positions and 14 SNPs, and all SNPs were parsimony-informative. Most of the variation in this region is a result of nine multiallelic insertion–deletion polymorphisms with an average indel length of 9.4 bp. Wild parsnips had all of the 14 SNP and 9 indel polymorphisms, whereas cultivars had six SNP and three indel polymorphisms. Combined indel and SNPs polymorphisms separated all 81 individuals into 27 haplotypes (hereby denoted as C1 to C27; Table 1).

Most individuals (79%) shared ITS haplotype H1. H11 was the second most-common haplotype, shared by 5% of all individuals, and all other observed ITS haplotypes were unique to one or two individuals. Europe had the highest number of ITS haplotypes (Table 3). Eastern North America had two unique haplotypes and two shared with New Zealand (H11 and H13; Figure 1) in addition to the most-common haplotype, H1.

All six western North American parsnips samples were H1. The two subspecies of wild parsnip from Europe, *P. sativa* subsp. *urens* and *P. sativa* subsp. *divaricata*, had unique ITS haplotypes and, as expected, the ITS sequence for *P. pimpinellifolia* was different from all *P. sativa* sequences. Three cultivars, *P. sativa* 'All-American Heirloom', *P. sativa* 'Excalibur', and *P. sativa* 'Melbourne Whiteskin', belonged to the ubiquitous H1. One New Zealand cultivar (*P. sativa* 'New Zealand Supersnip') shared haplotype H8 with the European wild parsnips. European parsnips consisted predominantly of the H1 haplotype but had more unique haplotypes (n = 6) than any other region.

ITS has been successfully used as a molecular marker for intraspecific studies of many plants, but for some, it has shown very little variation. For example, Gao et al. (2012) reported 19 distinct ITS haplotypes in the endemic Tibetan species stonecrop [*Rhodiola alsia* (Fröed.) S.H. Fu] (Crassulaceae) with strong branch support for the evolutionary relationship between haplotypes, whereas Soltis and Kuzoff (1993) found very little variation in ITS1 in populations of Gray's biscuitroot [*Lomatium grayi* (J.M. Coult. & Rose) J.M. Coult. & Rose] and Slickrock biscuitroot [*Lomatium laevigatum* (Nutt.) J.M. Coult. & Rose] (Apiaceae). ITS is used more frequently for inferring infrageneric relationships, such as within the Apiaceae genera cowparsnip (*Heracleum* spp.) (Yu et al. 2011), burnet saxifrage (*Pimpinella* spp.) (Magee et al. 2010), and angelica (*Angelica* spp.) (Feng et al. 2009). In this study, ITS clearly differentiated between *P. sativa* and *P. pimpinellifolia* and among the subspecies of *P. sativa* (*P. sativa* subsp. *urens* and *P. sativa* subsp. *divaricata*), but variation among *P. sativa* individuals was low. These findings suggest that ITS in *P. sativa* might evolve too slowly to differentiate cultivated and wild parsnips, and the introduction and cultivation of *P. sativa* might have occurred too recently for ITS to be a useful marker in this system.

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Table 4. Pair-wise comparisons of differentiation (exact tests) among geographic regions calculated from divergence at the ribosomal internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) locus. P values derived from 10,000 Monte chain Monte Carlo runs are depicted in the table and significant differences at $P < 0.05$ are denoted by an asterisk (*). The two subspecies are *Pastinaca sativa* ssp. *divaricata* and *Pastinaca sativa* ssp. *urens*.[a]

		Europe	Eastern NA	Western NA	New Zealand	Cultivar
ITS	Eastern NA	0.038*				
	Western NA	1.000	0.799			
	New Zealand	0.050	0.529	0.560		
	Cultivar	0.109	0.259	0.180	1.000	
	Subsp.	0.002*	0.011*	0.012*	0.167	0.186
cpDNA	Eastern NA	0.423				
	Western NA	0.195	0.927			
	New Zealand	0.231	1.000	1.000		
	Cultivar	0.311	0.977	1.000	1.000	
	Subsp.	0.595	0.439	0.806	0.606	0.864

[a] NA, North America; subsp., subspecies.

There were substantially more chloroplast haplotypes (chlorotypes) than ITS haplotypes; 27 chlorotypes were present in 81 samples. Chloroplast markers separated individuals into at least two distinct lineages, groups A and B (Figure 2). Of the 22 chlorotypes found in Europe, 15 occurred nowhere else. Eastern North American parsnips shared 86% of their haplotype diversity (seven chlorotypes) with European parsnips and had two unique chlorotypes (Figure 2). Western North American parsnips had one unique chlorotype (C9) and shared three chlorotypes (C5, C6, and C7) with Europe and eastern North America. Two chlorotypes (C5 and C6) were recovered from New Zealand parsnips, both of which also occurred in Europe, North America, and cultivars. Cultivated parsnips shared 80% of their chlorotype diversity with Europe and eastern North America. One cultivar, *P. sativa* 'Excalibur', had a unique chlorotype (C27). *Pastinaca sativa* subsp. *divaricata* had the ubiquitous C5 chlorotype and *P. sativa* subsp. *urens* shared C4 with European wild parsnips.

High levels of haplotype diversity were found in all introduced geographic regions, even though western North America and New Zealand provided disproportionally fewer samples from fewer populations (Table 3). Nucleotide diversity was also similar between all regions (Table 3), indicating no loss of genetic variation after introduction. Numerous studies comparing neutral genetic variation between native and invasive populations suggest that genetic diversity in the invasive range is often the same as or higher than that in the native range (Bossdorf et al. 2005). Higher genetic variation is often a consequence of multiple introductions from genetically diverse native populations. For example, the population genetic structure of the brown anole lizard *Anolis sagrei* in Florida shows that at least eight different introductions from all over the world are responsible for the extant diversity in its invasive range (Kolbe et al. 2004). Similarly, Genton et al. (2005) found that the invasive common ragweed (*Ambrosia artemisiifolia* L.) (Asteraceae) in France originated from multiple sources in North America. For wild parsnips, two possibilities exist in terms of multiple introductions: the plant may have been introduced from what has been traditionally considered the European wild parsnip, *P. sativa* subsp. *sylvestris*, or invasive populations may consist of individuals that have escaped from multiple cultivars. Few studies have examined invasive plants that are garden or agricultural escapees, but one such study found that high genetic diversity of invasive European olive (*Olea europaea* L. ssp. *europaea*) (Oleaceae) in Australia is a consequence of the introduction of multiple cultivars in the invasive range (Besnard et al. 2007).

Exact tests showed no overall geographic differentiation at the cpDNA locus among geographic regions and no differentiation between cultivars and wild parsnips (exact test for overall differentiation, $P = 0.79$, pairwise tests in Table 4). However, the ITS locus showed that plants in Europe may be genetically distinct from those in eastern North America (exact test for overall differentiation, $P = 0.0007$, pairwise tests in Table 4). In spite of the extremely high occurrence of haplotype H1 in both regions, eastern North America had two haplotypes, H11 and H13, which were not found in European samples in this study. The lack of genetic divergence in introduced populations suggests rapid range expansion, which is typical for many weedy species.

Even though there is no evidence to suggest that cultivars are genetically distinct from wild parsnips, the distribution of haplotypes suggests that some chlorotypes (group B; Figure 2) are unique to wild parsnip populations. These chlorotypes may represent the original wild form, *P. sativa* subsp. *sylvestris*, whereas group A might represent *P. sativa* subsp. *sativa*, the cultivated parsnip. European parsnips had a much greater diversity of haplotypes, with at least 15 haplotypes unique to this geographic region, which is expected for

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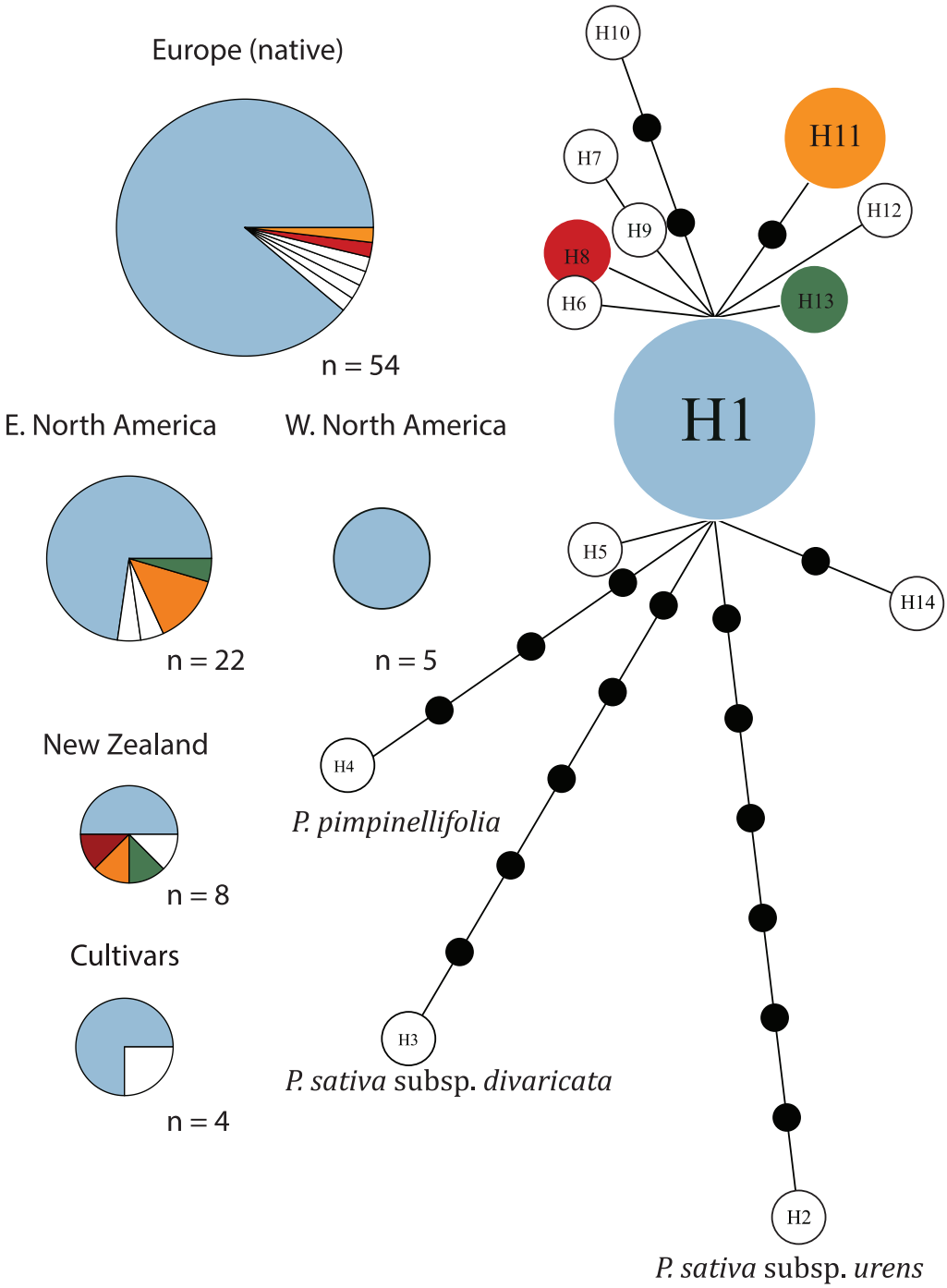

Figure 1. The relationship between internal transcribed spacer haplotypes and the distribution of haplotypes in Europe, North America, New Zealand, and in cultivars. The size of each circle is proportional to the number of individuals. Branches between haplotypes are proportional to the number of mutational steps. Black circles depict missing haplotypes. Only haplotypes shared among regions are assigned a color. (Color for this figure is available in the online version of this article.)

the native range. Interestingly, nearly half of Europe's wild parsnips have chlorotypes shared with cultivars, suggesting that, even in the native range, wild parsnip populations are a combination of escaped cultivars and originally wild *P. sativa* subsp. *sylvestris* chlorotypes. At least two of these noncultivar chlorotypes (C3 [blue] and C7 [purple] Figure 2) also occurred with some frequency in North American populations, suggesting that either escape from cultivation or introduction from Europe or both may have been responsible for the invasion of wild parsnips in North America. In

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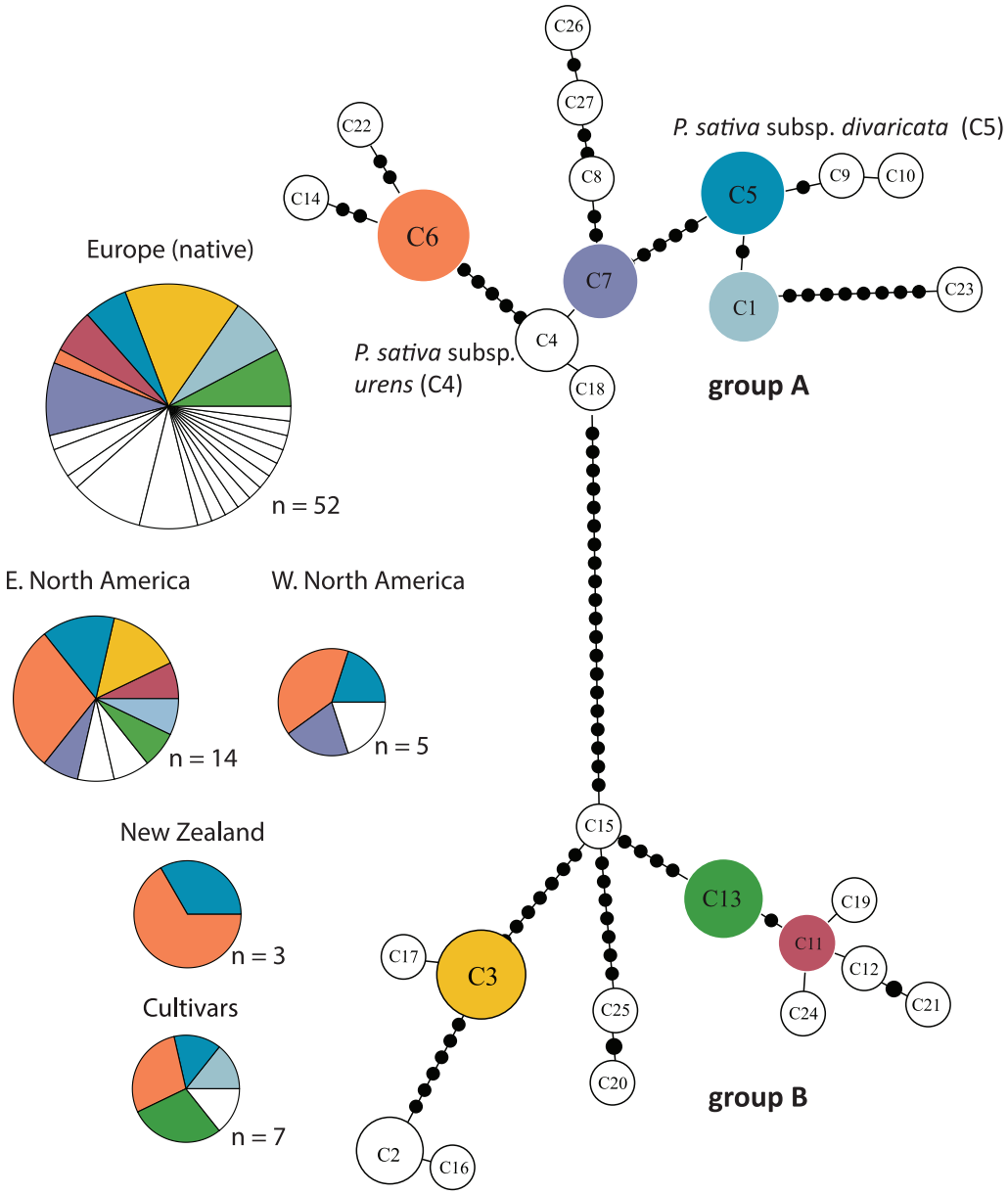

Figure 2. The relationship among *psbM–trnT* haplotypes and the distribution of haplotypes in Europe, North America, New Zealand, and in cultivars. The size of each circle is proportional to the number of individuals. Branches between haplotypes are proportional to the number of mutational steps. Black circles depict missing haplotypes. Only haplotypes shared among regions are assigned a color. (Color for this figure is available in the online version of this article.)

New Zealand, both haplotypes were shared with cultivars (i.e., 'New Zealand Supersnip,' 'US Hollow Crown' and 'All-American Heirloom'), suggesting that New Zealand populations are primarily escapees from cultivation.

The differences in morphology and chemistry between wild and cultivated parsnips are likely maintained by strong herbivore selection for increased defenses, even though wild and cultivated parsnips do not appear to be genetically distinct for the markers used in this study. At least in North America, parsnips are not commercially cultivated on a large scale, and these biennial plants are usually harvested for the root before flowering, making recent hybrids unlikely in most of the sampled wild populations. Herbivore-mediated selection for chemical traits can act rapidly in wild parsnip populations (Jogesh et al. 2014; Zangerl and Berenbaum 2005; Zangerl et al. 2008), so it is likely that strong selection maintains the apparent morphological differences between wild and cultivated parsnips.

The closely related wild carrot, *Daucus carota*, represents a similar system where the species has been cultivated

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throughout the world and wild forms occur as a globally invasive species. In comparison to parsnips, wild carrots are genetically distinct from cultivated carrots (Bradeen et al. 2002; Iorizzo et al. 2013; Shim and Jorgensen 2000). In view of the fact that the two species were probably domesticated at the same time, the clear genetic distinction between wild and cultivated forms of carrot in contrast to the lack of differentiation in parsnips may reflect differences in the history and manner of domestication. Based on recent evidence from a large SNP data set, carrots appear to have been domesticated from yellow- and purple-rooted wild carrots in central Asia, which are genetically distinct from European wild carrots (Iorizzo et al. 2013). The modern cultivars of parsnips, on the other hand, are known to have been bred from European wild parsnips and hybridizations between the two may have occurred at a higher frequency in the process of cultivation (e.g., the cultivar *P. sativa* 'Gladiator' is a hybrid), reducing the genetic distance between wild and cultivated forms. Although the morphological and chemical distinction between wild and cultivated parsnips is a consequence of herbivore-mediated natural selection, the phenotypic differences between wild and cultivated carrot are a result of selection for specific cultivar genotypes (Grebenstein et al. 2011). A recent large-scale study comparing wild and domesticated carrots showed diversifying selection in at least 27 markers (Grezebelus 2014). Wild carrots in North America are more closely related to wild carrots in Europe, suggesting that introduction from Europe, and not escape from cultivation, is primarily responsible for the colonization of this invasive plant. In wild parsnips as well, it appears that accidental introduction form Europe is at least partly responsible for the high diversity of chlorotypes observed in North America.

The lack of genetic differentiation among isolated geographic regions suggests that the historical source of all invasive populations is the same, and not enough time has passed for the fixation of alleles at this locus**.** It is also plausible, although less likely, that contemporary gene flow among geographic regions occurs with some frequency. Gene flow among parsnip populations within a geographic region is expected to be high because of the predominantly roadside distribution of this plant and the ease with which its seeds can be carried long distances by vehicles. Pickering and Mount (2010) found that seeds from 372 exotic plant species have been collected from clothing, equipment, or vehicles, indicating that long-distance, human-mediated dispersal can occur on a regular basis. In some species, admixture is associated with global trade. For example, levels of admixture in the Chinese mitten-crab (*Eriocheir sinensis*) were strongly correlated with shipping volume, an association expected if human-mediated dispersal was its primary means of gene flow between continental Europe and the United Kingdom (Herborg et al. 2007). Thus, it is reasonable to hypothesize that high rates of gene flow via multiple contemporary introductions of *P. sativa* in its invasive range may contribute to the lack of genetic differentiation between continents.

This study provides preliminary evidence suggesting that wild and cultivated parsnips are not genetically distinct. Invasive populations in all geographic regions harbor high genetic diversity, indicative of multiple introductions or high rates of gene flow. Only two loci were analyzed with limited sampling in this study and analyses of multiple loci, especially diploid loci with higher mutation rates, such as microsatellites, can help elucidate the source or sources and routes of invasion and the extent of gene flow and admixture within and among continents.

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