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A Continent-Wide Clone: Population Genetic Variation of the Invasive Plant Hieracium Aurantiacum (Orange Hawkweed; Asteraceae) in North America

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A CONTINENT-WIDE CLONE: POPULATION GENETIC VARIATION OF THE INVASIVE PLANT *HIERACIUM AURANTIACUM* (ORANGE HAWKWEED; ASTERACEAE) IN NORTH AMERICA

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We investigated the population genetic structure of the invasive plant *Hieracium aurantiacum* (Asteraceae), a facultative apomict. We generated amplified fragment length polymorphism fingerprints for *H. aurantiacum* samples from across its invasive range in North America ($N = 226$) and from six other North American native and invasive *Hieracium* species ($N = 60$). Almost no genetic variability was found in the North American *H. aurantiacum* across locations from Alaska and Oregon to Pennsylvania and Ontario (clonal diversity = 0.035). In contrast, other *Hieracium* species showed a range of clonal diversities (range = 0.154–1.0). The single *H. aurantiacum* genotype that dominated the North American invaded range was identical to a sample from the native range (Czech Republic), where low genetic diversity has also been reported. However, we did find evidence of hybridization between *H. aurantiacum* and at least one other nonnative *Hieracium* species in North America, indicating that the generation of novel hybrid genetic combinations may be an important factor in this invasive group of *Hieracium* taxa. Our findings suggest that sexual recombination and genetic diversity are not essential for successful plant invasion and that phenotypic plasticity alone may provide the flexibility necessary for the establishment of *H. aurantiacum* in diverse habitats.

Keywords: apomixis, clonal reproduction, genetic variation, hawkweed, invasive plant.

Online enhancement: appendix table.

Introduction

Biological invasions present a serious economic and environmental challenge worldwide (reviewed by Pimentel et al. [2005]) and are also a scientific puzzle. What is it about the invading taxa and the invaded communities that promotes successful invasion? Recent work suggests that genetic diversity, including novel diversity generated by hybridization (Ellstrand and Schierenbeck 2000), and rapid adaptation to nonnative habitats is a key factor in successful invasions (reviewed by Lee [2002]). However, both genetic bottlenecks during invasion and the strong selective filter of initial establishment may often reduce genetic diversity in introduced species (Dlugosch and Parker 2008). Understanding the distribution of genetic variation within and among introduced populations remains a key step in understanding the biology of invasion (Sakai et al. 2001).

The mode of reproduction (sexual vs. asexual, outcrossing vs. selfing) is a major factor influencing both the ecology of invasion and patterns of genetic variation in introduced populations (Barrett et al. 2008). Many invaders depend at least in part on asexual reproduction (Kolar and Lodge 2001). In particular, apomictic plant taxa, which produce seeds that are clones of the mother plant (Koltunow et al. 1995), make up a disproportionate fraction of the invasive flora (e.g., Baker 1965; Webb and Kelly 1993; Rambuda and Johnson 2004; Silvertown 2008). This is probably due to the colonization advantage conferred by the production of long-distance propagules (seeds) capable of establishing populations from a single founder (Baker 1967; Hörandl 2006).

While the production of apomictic seeds avoids the theoretical costs of sex (Barton and Charlesworth 1998) and need for a mate or pollinators (Lloyd 1980), strictly clonal reproduction should limit heritable variation and the rate of adaptive evolution (e.g., Goddard et al. 2005). However, because even a small amount of sexual reproduction can allow the spread of advantageous alleles, even highly asexual taxa may be quite diverse. Although a few studies of apomictic plants have found only a single genotype in an introduced range (Poulin et al. 2005; Wang et al. 2005), most such studies have found multiple clonal lines (Amsellem et al. 2000; Chapman et al. 2000, 2004; Novak and Mack 2000; Edwards et al. 2006). Furthermore, some facultatively apomictic or largely clonal invaders appear to possess the genetic diversity necessary to adapt locally along ecological gradients in their invaded range (Maron et al. 2004; Facon et al. 2008).

In the short term, the benefits of apomixis may often outweigh its disadvantages, particularly at range edges where population densities are low (Judson and Normark 1996). Indeed, apomicts often have wider distributions than sexual members of the same species or genera (so-called geographic parthenogenesis; van Dijk 2003). This suggests that phenotypic plasticity—the ability of an organism to produce different phenotypes under different growth conditions—may allow invasive asexuals to flourish over a wide range despite low genetic diversity (Parker et al. 2003). If a history of selection in their home ranges has

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made apomictic taxa particularly plastic in ecologically relevant traits, they may be uniquely predisposed to perform well in novel invaded environments as well (Williams et al. 1995). In general, however, it remains an open question whether apomictic invaders have low genetic diversity and, if so, how they cope with the novel environments of their invaded ranges.

In this study, we investigate genetic variation in the facultatively apomictic plant *Hieracium aurantiacum* (orange hawkweed, subgenus *Pilosella*, Asteraceae) across its nonnative range in North America. *Hieracium aurantiacum* has its center of origin in central Europe, was introduced into North America more than a century ago, and is now considered invasive in North America from Florida to Alaska (USDA PLANTS Database; USDA, NRCS 2008, http://plants.usda.gov) and in New Zealand and Australia. *Hieracium aurantiacum* spread quickly throughout southeastern Canada and the northeastern United States after its initial introduction into Vermont as a garden plant in 1875 (Voss and Bohlke 1978). It was first recorded in western North America in 1927 at the Crown Point overlook of the Columbia Gorge in Oregon (Rice 2009; http://invader.dbs.umt.edu), where it was probably also grown as an ornamental, and in vacant lots in Spokane, Washington, in 1945. Because orange hawkweed continued to be cultivated as an ornamental until quite recently, its broad geographical range within a short time of first detection is likely to reflect independent escapes from cultivation rather than endogenous spread (Wilson et al. 1997).

Orange hawkweed is commonly found in pastures, lawns, and other cultivated or disturbed habitats. However, because *H. aurantiacum* also invades diverse natural habitats, from old fields to alpine meadows to postburn forest understories (Wilson et al. 1997), it is listed as a noxious weed in five western states. Furthermore, *H. aurantiacum* is one of more than a dozen closely related and potentially hybridizing nonnative hawkweeds in western North America (Wilson et al. 2006; Gaskin and Wilson 2007). *Hieracium aurantiacum* and close relatives have become model systems for understanding the genetic and developmental basis of apomixis (Bicknell 1997; Koltunow et al. 1998, 2000; Bicknell et al. 2000; Catanach et al. 2006), but it is not clear what role apomixis plays in its spread in the nonnative range. Understanding patterns of genetic diversity in North American populations of *H. aurantiacum* and other hawkweeds will address general questions about the role of reproductive mode and genetic diversity in plant invaders and also provide baseline information for the management of this important group of invasive species.

Material and Methods

Plants

We obtained *Hieracium aurantiacum* samples from both western and eastern North America (fig. 1; see appendix table A1 in the online edition of the *International Journal of Plant Sciences* for location names, sample numbers, and coordinates of each population). For western North America, samples from about half of the sites were collected as whole plants in the summer of 2005. Additional western populations and all eastern populations were sampled in 2006 and 2007 as seeds and plants cultivated in greenhouses at the University of Montana or the University of Idaho. For comparisons with

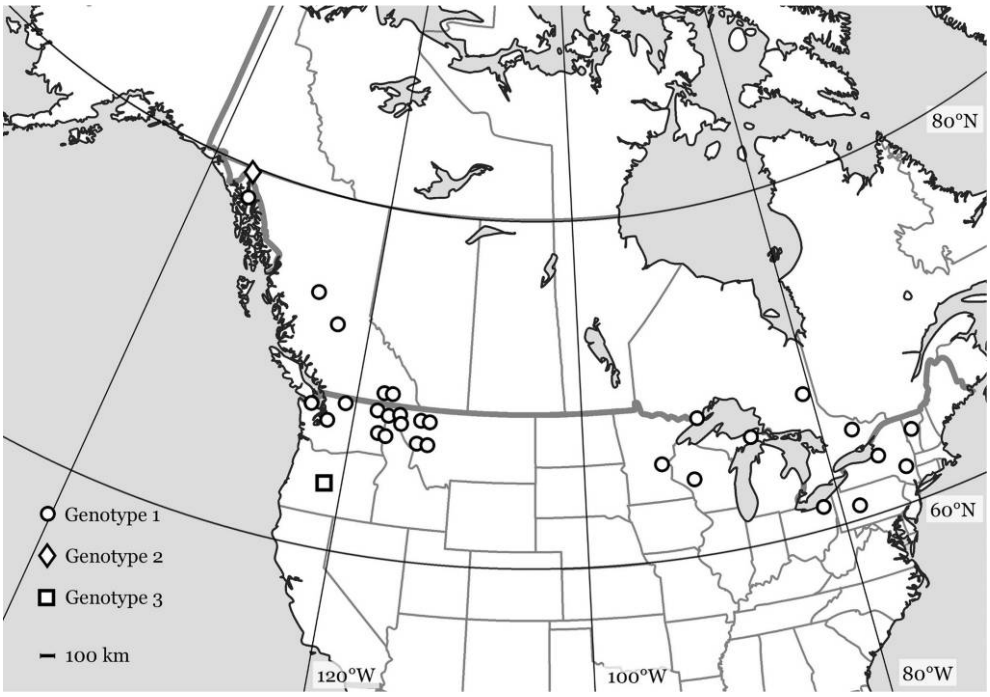

Fig. 1 Collection locations and major genotypes as determined by amplified fragment length polymorphism analysis of *Hieracium aurantiacum* samples. Not included is the one location sampled in the Czech Republic, which was assigned to genotype 1.

the native range, we obtained seeds from one location in eastern Europe (provided by Dr. Anna Krahulcová; see table A1). For all collections, an effort was made to sample broadly across each site. For seed collections, a single seedling from each maternal family was used in the analyses.

Seeds were also obtained from populations of *H. albertinum*, *H. albiflorum*, *H. caespitosum*, *H. floribundum*, *H. glomeratum*, and *H. piloselloides* (for sampling locations, see table A1). *Hieracium albertinum* and *H. albiflorum* are native species, whereas the others are introduced in North America. Because the native species are nonapomictic and the other nonnatives are at least partially sexual (Wilson et al. 2006; Gaskin and Wilson 2007), they serve as a positive control for our ability to detect genetic diversity and sex in the *H. aurantiacum* samples using amplified fragment length polymorphism (AFLP) fingerprinting.

Plant Care

Seed collections were germinated on wet filter paper in petri dishes sealed with Parafilm, which were stratified at 4°C in a refrigerator for 30 d and then transferred to a greenhouse at ambient temperature (range: 16°–27°C) with supplemental light from HPS lamps (12 h/d). Both transplants and plants grown from seed were maintained in soilless potting mix (4-inch pots) under the above greenhouse conditions until tissue was harvested for DNA isolation.

DNA Isolation

Tissue samples (~200 mg of tissue from young leaves) were taken from up to five plants (field-collected plants or single progeny of five maternal seed families) per site. Genomic DNA was extracted following a CTAB/chloroform extraction protocol modified for 96-well format and using a bead beater (Geno/Grinder 2000, Spex Certiprep, Metuchen, NJ) for tissue homogenization (Fishman and Willis 2005). Qiagen Plant DNEAsy Mini 96-well kits (Qiagen, Hilden, Germany) were used to further clean the DNA samples.

AFLP Analysis

AFLP fingerprints were generated following Papa et al. (2005) with the following modifications. Restriction/ligation was performed at room temperature (~23°C) for ~16 h. The 20-μL restriction/ligation reactions included ~400 ng of DNA, 4 μL 5× T4 DNA Ligase buffer (Invitrogen, Carlsbad, CA), 2 μL 0.5 M NaCl, 0.55 μL 10× BSA, 5 U Mse1, 4 U EcoR1, and 1 μL each of Mse1 and EcoR1 adapter pairs (Xu et al. 2000). Preselective amplifications used 5 μL of 1 : 10 diluted restriction-ligation product in 4.0 μL 5× GoTaq Flexi buffer (Promega, Madison, WI), 1.2 μL 25 mM $MgCl_2$, 1.6 μL 2.5 mM dNTPs, 2 μL 10× BSA, 0.4 μL each of 10 μM EcoR1+A and Mse1+C primers, 1.25 U of GoTaq Flexi and water to a total volume of 20 μL. The preamplification thermocycler program followed Papa et al. (2005), with the addition of an initial 3-min 94°C denaturation step and six amplification cycles. We tested 12 selective primer pairs (EcoRI + 3 and MseI + 3; with 5′ fluorescent labeling of the EcoRI primers) and chose two with consistently strong peak profiles for fingerprinting (E-AGG/M-CAC and E-CAA/M-CAC). Selective amplification reactions used 3 μL of the 1 : 20 diluted preamplification product as template in the following reaction mix: 4.0 μL 5× GoTAQ Flexi buffer, 1.2 μL 25 mM $MgCl_2$, 1.6 μL 2.5 mM dNTPs, 2 μL 10× BSA, 0.2 μL 10 μM Mse+3 primer, 0.1 μL 10 μM EcoRI+3 primer, 1.25 U GoTAQ Flexi in a total volume of 20 μL. The final amplification used a touch down PCR program: 3 min at 94°C, 10 touchdown amplification cycles (30 s at 94°C, 30 s at 66°C, 2 min at 72°C) with the annealing temperature decremented by 1°C each cycle, 36 amplification cycles (30 s at 94°C, 30 s at 56°C, 2 min at 72°C), with a 10-min final extension (72°C). AFLP profiles were generated using automated capillary electrophoresis (ABI 3130xl Genetic Analyzer, Applied Biosystems, Foster City, CA) with an in-lane size standard and visualized using GeneMapper software (Applied Biosystems).

The two primer pairs generated AFLP profiles with 45 total loci for *H. aurantiacum*. Separate profiles were developed for each of the other *Hieracium* species (table 1). For each individual, we used GeneMapper to score peak (fragment) presence or absence with a standard threshold, then verified each genotype call by eye. Only individuals showing strong and unambiguous peak profiles across the full size range were used in the final analysis. About 30% of individuals were analyzed twice (from restriction ligation through final amplification and scoring) to verify replicability of the analysis.

Results

A single genotype of *Hieracium aurantiacum* (G1) was found in 46 of the 48 locations sampled, ranging from Alaska to Pennsylvania (fig. 1). Two other genotypes were found in multiple individuals at single locations (G2 in Homer, AK; G3

Table 1

Genotypic diversity of *Hieracium aurantiacum* and native (N) and introduced (I) congeners in North America

Species	Total no. plants sampled	No. populations sampled	Loci[a]	Unique genotypes	Clonal diversity	Mean clonal diversity per site
H. aurantiacum (I)	226	48	45	8	.035	.0007
H. albertinum (N)	5	1	52	5	1.000	1.0000
H. albiflorum (N)	4	1	46	2	.500	.5000
H. caespitosum (I)	13	2	58	8	.615	.3077
H. floribundum (I)	13	2	63	2	.154	.0769
H. glomeratum (I)	19	2	87	5	.263	.1316
H. piloselloides (I)	6	1	77	2	.333	.3333

[a] Number of consistently scorable peak positions summed across all individuals in each species.

in Bend, OR). These three genotypes are closely related, with each showing one fragment loss and one fragment gain relative to the others (fig. 2). These differences may represent either single insertion/deletion polymorphisms or sequence changes at two loci. Both G2 and G3 were found in relatively local ranges (compared to G1) and in populations with a history of recent cultivation. The G2 genotype (which occurred in plants up to 20 km apart) was found in an area where *H. aurantiacum* is thought to have established from plants cultivated by the Russian Orthodox community in Homer, Alaska, and the population with the G3 genotype was established from plants purchased at a local nursery in Bend, Oregon (L. Wilson, personal communication).

Five other *H. aurantiacum* individuals differed from the three main genotypes. Three of these (G4–G6; fig. 2) differed at a single locus (different in each case) from the locally dominant genotype and were considered mutants or rare recombinants, as their replicability was confirmed by rerunning and rescoring of the variant individuals' AFLP profiles. Two of these plants came from the location where G3 was found, and one came from a location dominated by G1.

Two additional plants differed at 10 or more loci. The first of these, referred to as hybrid 1 (see fig. 2, H1), was collected in Barry's Bay, Ontario, and had novel peaks at eight loci and missing peaks at two. Of these 10 differences from all other *H. aurantiacum*, eight could be explained by hybridization with *H. piloselloides*, as represented by the few samples ($N = 2$) genotyped in this study that came from British Columbia (table A1), which is probably not the actual parent population of H1. The other plant that differed at more than one locus was collected in Homer, Alaska, and showed a gain of seven peaks and a loss of seven relative to the dominant G3 genotype at that location. The gained and lost peaks did not correspond to profiles from any of the six other *Hieracium* species genotyped here (fig. 2) but may represent hybridization with a species of *Hieracium* not sampled in this study. Further elucidation of the extent and local history (e.g., reproductive mode and generation of hybrids) of hybridization among nonnative *Hieracium* will require more intensive sampling and more informative markers such as microsatellites.

Clonal diversity (number of genotypes/number of individuals genotyped; table 1) in *H. aurantiacum* was extremely low (0.035), as was average clonal diversity per sampled location (0.0007). *Hieracium aurantiacum* was much more clonal than any of the six other species sampled, which had clonal diversities ranging from 0.154 in nonnative *H. floribundum* to 1.0 in native, obligately sexual *H. albertinum* (table 1). The extreme genetic uniformity of *H. aurantiacum* is underlined by our finding of eight distinct genotypes in just two populations of *H. caespitosum*, which is closely related and also considered to be facultatively apomictic (fig. 2).

Discussion

Genetic Diversity in North American Hieracium aurantiacum

Hieracium aurantiacum shows genetic homogeneity over its invaded range in North America. The vast majority of individuals sampled were genotypically identical, presumably because of a combination of exclusively clonal reproduction (via apomictic seeds and vegetative shoots) in the introduced range and low genetic diversity in the introduced material. Furthermore, the genotype that dominated the sampled range (G1) was also identical to a specimen obtained from the Czech Republic, where *H. aurantiacum* was found to be identical along a transect from the German to the Polish borders (Fehrer et al. 2002). The two distinct genotypes found in multiple individuals are associated with recent cultivation and may differ because they represent separate introductions from geographically distinct origins, because of

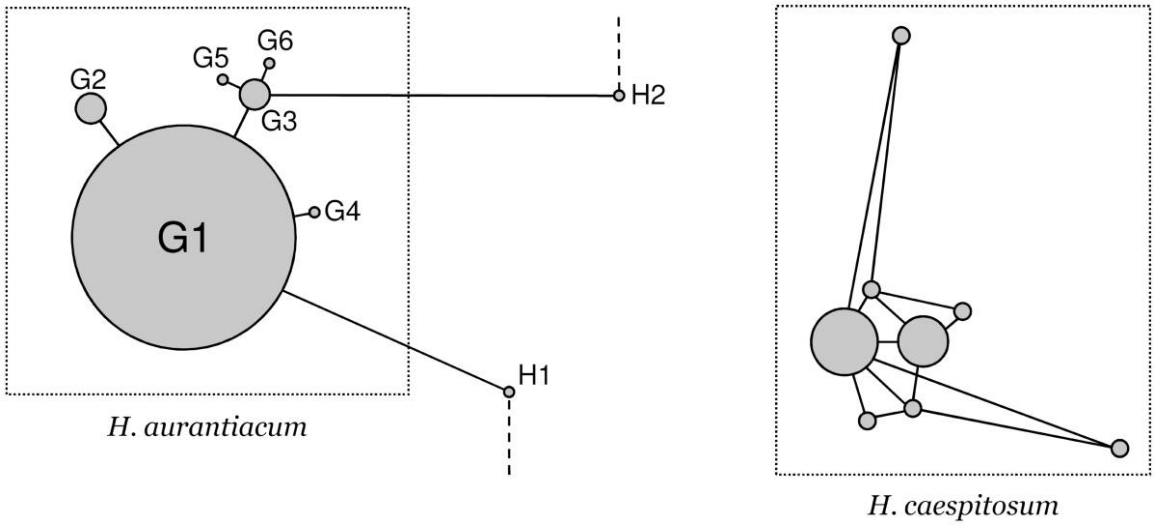

Fig. 2 Genotypic difference map of *Hieracium aurantiacum* and its closest related species in this study, *Hieracium caespitosum*. Line lengths are proportionate to number of loci different between groups. Circle size in *H. aurantiacum* is proportionate to the number of study sites where a clone was found, and for H1, H2, G4, G5, and G6, it represents a single individual. Circle size in *H. caespitosum* represents the number of individuals found with that genotype. For *H. aurantiacum*, $N = 225$, and for *H. caespitosum*, $N = 13$.

hybridization and/or selection in cultivation, or simply because rare mutations or recombinants spread clonally in a local area. Although multiple introductions of *H. aurantiacum* to North America are likely given cultivation and its long history on this continent, the lack of variation in AFLP fingerprints does not allow determination of the number of origins. Indeed, even multiple origins may not have represented genetically distinct entities. Further sampling in the native and other introduced ranges will be necessary to confirm that this pattern represents the species-wide diversity. However, the G1 genotype of our single Czech accession, combined with genetic homogeneity across the Czech Republic (Fehrer et al. 2002), suggests that the dominant North American genotype may also be common in Europe.

The rare genotypes represented by single samples (G4–G6) in this study are likely to represent new mutations rather than sexual recombination. The rate of mutation this would imply (three mutations out of 10,215 total loci assessed) is reasonable. Although there are few data on AFLP mutation rates, a proposed mutation rate of 10^{-4} per locus (Campbell and Bernatchez 2004) is consistent with the level of variation observed.

The extent of homogeneity in *H. aurantiacum* is unusual even for highly asexual invasive species. Previous studies have found no diversity in the studied portions of the invaded range of *Pennisetum setaceum* (Poulin et al. 2005) and *Alternanthera philoxeroides* (Wang et al. 2005), but both were studied across smaller and less latitudinally and altitudinally diverse ranges. The majority of studies of clonal invasive species have found at least some genetic diversity in the range studied, usually as multiple clonal lines (e.g., Hollingsworth and Bailey 2000). These results suggest that *H. aurantiacum* reproduces almost entirely through apomictic seed production and vegetative cloning in its invaded range and is capable of invading diverse habitats in the absence of the genetic diversity that sex and recombination generate. Further study of the phenotypic variation within and among clones will be necessary to determine whether heritable variation in quantitative traits follows the same pattern.

Hieracium aurantiacum is described as a facultative apomict, as are most other members of the subgenus *Pilosella* (Krahulcová et al. 2000). However, the other nonnative, facultatively apomictic *Hieracium* in this study displayed a relatively large amount of genetic diversity, despite more geographically restricted sampling. These results confirm that our markers and methods have the power to detect sex and clonal variation and highlight the extreme homogeneity of *H. aurantiacum*. In addition, studies of introduced *Hieracium* in New Zealand have found relatively high levels of genetic diversity and suggest multiple genetically distinct introductions as well as hybridization (Chapman et al. 2004; Trewick et al. 2004). Because *H. aurantiacum* also occurs in New Zealand and Australia but has not been genetically characterized there, it would be interesting to compare the genetic fingerprints of plants in that southern invasive range to those in our study.

We found evidence of hybridization in plants initially identified as *H. aurantiacum*, with two individuals differing from the dominant genotype at a large number of loci. *Hieracium aurantiacum* has been shown to form viable experimental hybrids with related *Hieracium* species as both a pollen and ovule donor (e.g., Houliston and Chapman 2004; Krahulcová et al. 2004; Nogler 2006), but natural hybrids have not previously been reported in North America. Novel alleles in one of putative hybrids (H1) matched the genetic profile of *H. piloselloides*, which has been shown to experimentally hybridize with *H. aurantiacum* (Bicknell et al. 2000). The H1 individual also had an intermediate flower color consistent with introgression from one of the yellow-flowered hawkweeds. The other putative hybrid (H2) may be a hybrid between *H. aurantiacum* and a congener not sampled in this study. Studies of related species in both native and introduced ranges have also found hybridization and ploidy variation (Krahulcová et al. 2000; Houliston and Chapman 2001; Chapman et al. 2004; Trewick et al. 2004), suggesting that interspecific hybridization may be a common feature of the facultatively apomictic *Hieracium* taxa (Fehrer et al. 2007). Hybridization, and the resulting creation of novel genotypes, has been proposed as an important driver of plant invasions, particularly in clonal or apomictic taxa (e.g., Ellstrand and Schierenbeck 2000; Lavergne and Molofsky 2007). Although such hybridization does not appear to have been a major component in the past invasive history of *H. aurantiacum* in North America, evidence of gene flow with other (apparently more sexual) species may be cause for management concern. In particular, given its success as an invader of diverse habitats, *H. aurantiacum* may be an important source of novel adaptive variation for introduced hawkweeds in North America.

Implications

The ability to reproduce in the absence of mates or pollinators, both vegetatively and via apomictic seeds, may predispose a plant to a successful long-distance colonizer (Baker 1965). Our results suggest that, for *H. aurantiacum*, this advantage may outweigh any costs associated with a lack of genetic diversity over the short term. Given the recent focus on rapid evolution as an engine of invasion (e.g., Lee 2002), this is an important reminder that a lack of genetic diversity need not preclude invasion. Indeed, taxa with a long history of low genetic diversity (e.g., apomictic species, selfers) may be particularly good colonizers of novel ranges during both initial establishment (due to assured reproduction) and later spread (due to lack of inbreeding depression and high phenotypic plasticity).

Phenotypic plasticity has been proposed as a major contributor to the success of invasive species. In particular, plasticity in traits that leads to the maintenance of uniformly high fitness across diverse environments (the "master and jack" strategy) may increase the potential for invasiveness (Richards et al. 2006). Several studies have examined evidence that invasive taxa are more phenotypically plastic than taxonomically paired noninvasives (Daehler 2003; Richards et al. 2006 and references therein). Although experimental studies of plasticity in limited environments are always subject to caveats (Hulme 2008) and some taxa show no pattern, this growing evidence does suggest that unusual phenotypic variability may be a common feature of invaders. In sexual taxa, plasticity may allow invaders to persist in marginal habitats long enough for local selection to act on genetic variation. In highly asexual taxa such as *H. aurantiacum*, phenotypic plasticity alone may underlie the ability to invade diverse habitats. Our finding that *H. aurantiacum* from across North America share a single clonal genotype provides the opportunity to assess whether this invader shows particularly high plasticity. If so, we would predict that the dominant *H. aurantiacum* genotype exhibits high

mean fitness across relevant ecological gradients (water availability, growing season length, etc.) relative to clones of more sexual relatives, as has been shown for asexual versus sexual lines of *Antennaria* (Bierzychudek 1989).

Our data also have implications for the management of *H. aurantiacum* and related taxa in North America. Although hybridization with other nonnative *Hieracium* is cause for concern and careful monitoring, the genetic uniformity of *H. aurantiacum* itself may be good news from a management perspective. For highly asexual taxa, dispersal to new sites (Ladle et al. 1993) rather than evolving new defenses (Hamilton 1980) may be the key to long-term persistence. *Hieracium aurantiacum*, a highly dispersive but genetically depauperate species, may escape coevolving predators and parasites through constantly colonizing new habitats. If this is the case, this species may be particularly vulnerable to specific biocontrols from its native range (Nissen et al. 1995). Together with recent phylogenetic analyses confirming that the nonnative *Hieracium* (subgenus *Pilosella*) in western North America form a clade distinct from native congeners (Gaskin and Wilson 2007), our data suggest that orange hawkweed is a promisingly distinct and narrow target for biological control despite its broad range.

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