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SEX AND DIVERSITY IN THE INVASIVE PLANT *HIERACIUM AURANTIACUM*

By

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B.S., Western Washington University, 2003

Thesis

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for the degree of

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Sex and diversity in the invasive plant *Hieracium aurantiacum*

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Sexual reproduction is generally thought to provide long-term fitness advantages over asexual reproduction in the form of increased genetic diversity. Some work, however, suggests that asexual reproduction can also be advantageous. One situation when asexual reproduction provides an advantage is when colonizing a new range (Baker's law), as in plant invasions. This study investigated the population structure of the invasive plant *Hieracium aurantiacum*. *H. aurantiacum* is an apomict – producing much of its seed asexually – and has become a common invasive in North America and New Zealand. The genetic diversity of *H. aurantiacum* was assessed over its invasive ranges in the Eastern and Western North America, as well as one location from its native range in the Czech Republic. Using AFLP analysis (with 45 loci), I generated genetic profiles of 225 *H. aurantiacum* and 60 individuals from 6 other *Hieracium* species (some native to and some introduced to North America) for comparison. Virtually no genetic variability was found in *H. aurantiacum* (clonal diversity was 0.035). Other *Hieracium* species, however, showed a range of diversity, showing clonal diversities from 0.154 to 1.0. One *H. aurantiacum* genotype dominated the sampled range (G1, in 51 of 53 sampled locations) and was identical to the sample from the Czech Republic. Two other genotypes were found in restricted ranges (G2 and G3). One was a population recently derived from nursery stock, and the other may represent another introduction or a mutated clonal line – each differed from each other and G1 at only two loci. It is quite possible that virtually all *H. aurantiacum* worldwide are clones. Despite this plant's lack of genetic variation, it is able to grow over a wide invaded range, which may be due to phenotypic plasticity in fitness-related traits. Many theories about invasion success involve genetic diversity in invading populations to provide the necessary flexibility to flourish in a variety of habitats in an invaded range. In asexual invaders tending towards low genetic diversity, however, phenotypic plasticity of fitness-related traits is a more likely possibility.

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CHAPTER 1

APOMIXIS, PLANT INVASIONS, AND *HIERACIUM AURANTIACUM*

Understanding the genetic variation within groups of organisms can lead to important insights into the history and evolutionary potential of those groups. For example, human migration history has been estimated using genetic techniques (e.g. Cavalli-Sforza & Feldman 2003) and evolutionary potential is often estimated in conservation genetics studies on organisms from grizzly bears (e.g. Miller & Waits 2003) to coral (e.g. Van Oppen & Gates 2006). Genetic variation in groups of organisms is affected by evolutionary processes such as drift and selection, but also by the organism's mating system. Asexual mating systems in particular tend to reduce genetic variation.

Apomixis is a form of parthenogenesis (asexual production of offspring without genetic input from a male) in plants, which allows them to make seeds without sex such that each seed grows up into a clone of its mother (Koltunow et al. 1995). Apomixis is quite common in plants, but usually only accounts for a portion of the seed produced (the rest is produced sexually) (e.g. Richards 2003). When plants produce seed asexually like this, there is no need for pollination. Because pollination becomes less likely when population density is low, asexual reproduction is advantageous at the edge of a plant's range and when founding new populations in distant ranges. In the extreme case, a single seed transported far beyond its species' range could found a new population. Plants that reproduce exclusively by apomixis, however, are rare. Most apomictic species reproduce partially by sexual and partially by asexual means (Novak & Mack 2000, Richards 2003, Krahulková et al. 2004). Some of those species maintain the sexual/asexual balance within individuals (some sexual and some apomictic seed) (e.g. Krahulcová et al. 2004, Paun et al. 2006) while some maintain the balance over their distributional range: sexual and asexual populations of these species tend to exhibit a predictable spatial pattern, with asexual populations generally found at higher

latitudes than their sexual counterparts. This is referred to as “geographic parthenogenesis” (van Dijk 2003, Hörandl 2006).

Sex maintains variability in populations by recombination, independent assortment, and the mixing of genes from different individuals (Weismann, 1886 referenced in Hoekstra, 2005; West et al, 1999). Lineages of plants that circumvent sex entirely, according to commonly accepted ideas, should be short-lived: sex leads to variation which leads to adaptation which is necessary for persistence in a changing environment. Despite the advantages of sex, however, asexual breeding systems are disproportionately represented among invasive plants, probably because they are unaffected by low population density (e.g. Baker 1955, Webb & Kelly 1993, Rambuda & Johnson 2004).

Asexual invasives are widespread and show an array of genetic diversities in their invaded ranges (e.g. Amsellem et al. 2000, Chapman et al. 2000, Novak & Mack 2000, Van Der Hulst et al. 2003, Chapman et al. 2004, Maron et al. 2004, Edwards et al. 2006). Out of the many studies of the genetic diversity of asexual invaders, only two have shown no genetic variability (Poulin et al. 2005, Wang et al. 2005). These invaders with little diversity presumably have a way besides rapid adaptation to maintain their fitness across varying habitats. A possible mechanism for this is phenotypic plasticity (Baker 1965, Schweitzer & Larson 1999, Weber & D’Antonio 1999, Sakai et al. 2001, reviewed in Daehler 2003). Phenotypic plasticity is basically the range of phenotypic variation that can be produced by a single genotype, usually in response to the environment (Parker et al. 2003). Plasticity is, of course, present in some characteristics and to some degree in all plants – any response of a plant trait to its environment represents plasticity. Invasive plants with little genetic variability, and little opportunity to generate it, could rely on plasticity in ecologically relevant traits to ensure their survival across varying environments.

Hieracium aurantiacum – commonly known as orange hawkweed – is an invasive plant in North America and New Zealand. It originally came from central and northern Europe, and has since spread over a large range in North America, from Florida to

Alaska (USDA 2006). What makes this plant and its invasion particularly interesting is its reproductive strategy. Like many invasives, *H. aurantiacum* reproduces asexually. The proportion of asexual offspring produced, however, is unusually high (Bicknell et al. 2003). Not only does it clone itself vegetatively by sending out stolons (which grow into new plants) but also produces clonal seed via apomixis.

The degree of sexuality and genetic diversity in most invasive species remains unknown. My main question is whether or not my study species, *H. aurantiacum*, has any variation across its invaded range here in the United States. In this thesis, I first review the background information on apomixis and invasion, then present my research in which I tested samples collected from this widespread invader from across its North American range for genetic diversity and the genetic signal of sex. Finally I present my research in an informal narrative form that is more accessible and interesting to a non-scientific audience.

Apomixis

Among asexual breeding systems, one of the most common is parthenogenesis, in which a mother produces offspring without fertilization by a male. Parthenogenesis has been observed to some degree in ~60% of British plants (Richards 2003), and as White (1973) emphasized, virtually all well studied animals (including humans) show the potential for it. Apomixis is a form of parthenogenesis whereby a mother plant, by one of a variety of mechanisms, produces seeds that are clones of herself (Koltunow et al. 1995). This system is not subject to the theoretical disadvantages of sex (see Lloyd 1980, Barton & Charlesworth 1998, Howard & Lively 2002) and has the potential to produce large numbers of identical organisms (van Dijk 2003, Richards 2003) which can be disseminated as seeds. Apomixis has great potential application in crop plants, where it could fix heterozygosity (which often leads to increased vigor and production) and infinitely propagate desirable genotypes (Hanna & Bashaw 1987, van Dijk & van

Damme 2000, Spillane et al. 2001) as well as preventing the spread of GM genes (foreign genes inserted into the genome of an organism using genetic modification techniques, Daniell 2002). Apomixis, combined with phenotypic plasticity, can also be advantageous for invasive plants (Parker et al. 2003).

Mechanisms of apomixis vary (Bicknell & Koltunow 2004), but all share three basic processes: (1) the formation of a cell that can generate an embryo without undergoing meiosis (apomeiosis), (2) the fertilization-independent development of an embryo (parthenogenesis), and (3) the ability to either generate endosperm without fertilization or use endosperm generated by fertilization, but not double-fertilization (Koltunow 1993). Beyond these three basic steps, however, there is a lot of mechanistic diversity – e.g. the cell destined to become the clonal embryo can come from different places (megaspore mother cell, mother's somatic cell, etc.) and the new seed's endosperm can sometimes require an input of pollen to jumpstart its formation (see Bicknell & Koltunow 2004), etc. This multitude of mechanisms is probably due to the many independent emergences of apomixis in different plant lineages (Bicknell & Koltunow 2004).

Most apomicts, in addition to their clonal offspring, also produce offspring via sex (facultative apomicts, e.g. Richards 2003). These facultative apomicts are able to maintain variation through sex as well as take advantage of the colonization advantage and reproductive assurance provided by apomixis. In fact, facultative sexual reproduction maintains variability almost, if not equally as well as exclusively sexual reproduction (Green & Noakes 1995). Apomictic species can also act as pollen donors to close sexual relatives (as noted by Mendel, reviewed in Nogler 2006, Bicknell et al. 2000). Apomicts could possibly pass the apomixis gene to the resulting offspring (Bicknell et al. 1999), generating multiple apomictic lineages sprouting like branches from the trunk of the sexual line.

Until recently, many speculated that apomixis is only beneficial in the short run, and that fully apomictic lineages are destined for extinction (Darlington 1939, Maynard

Smith 1978). This idea has come under scrutiny as more attention is paid to organisms coming from asexual lineages that are quite old (Judson & Normark 1996).

Invasion

Invasive species are becoming increasingly prominent in the scientific literature as well as the public eye. They pose one of the most serious threats to native ecosystems and biodiversity (Heywood 1989), and are also of serious economic concern (Vitousek et al. 1997, Pimentel et al. 2005). Pimentel et al. (2005) estimated a total cost of almost \$120 billion per year in the USA alone due to loss, damage, and the price of attempting to control invasive plants, animals, and microbes (the projected cost of plants alone is \$34.6 billion). Scientific interest ranges from the study of the process of invasion and which species might become invasive if given the chance to methods of controlling invaders, while the public's eye is often on species that affect commercial enterprise or recreation (e.g. *Didemnum* sp. in Wacker, 2006).

In order to become invasive, a species must first be transported to an area in which it is not native (sensu Elton 1958, Sakai et al. 2001). Although this process is not always mediated by human activity, in the modern world it typically is – these introductions have increased dramatically with the historically recent rise of transport and commerce (di Castri, 1989). Introductions occur in various ways: many plant species that become invasive are introduced as horticultural imports (Reichard & White, 2001), and species also can be transported in foodstuffs, the ballast water of large ships, the luggage of travelers, etc. (Kolar & Lodge 2001, Sakai et al. 2001). Once in a new area, most foreign species simply die, unsuited for survival there (Mack et al. 2000, Kolar & Lodge 2001). Of the few that do survive the initial colonization, most of die out after only a few generations. Only very rarely does an introduced species flourish to the extent of becoming invasive (Mack et al. 2000, Kolar & Lodge 2001). Before the population explosion that begins an invasion there is usually a lag period which lasts

many generations (Cousens & Mortimer 1995, Sakai et al. 2001), during which the invader persists unobtrusively.

Because so much damage is caused by invasives, and not all alien species become invasive, there is great interest in predicting which species, when transported to a new environment, could become invaders. A logical prediction might be that future invaders would be close relatives of already successful invaders, which should share many relevant traits. But though there are a few examples of groups of closely related invaders (e.g. melastomes in Hawaii, see Mack et al. 2000), relation to an invasive species is not generally a good predictor of potential invasiveness (Mack et al. 2000). Other attempts to compile lists of “invasive attributes” have only recently proved somewhat successful (see Kolar & Lodge 2001, Richardson & Rejmánek 2004, McIntyre et al. 2005, Cadotte et al. 2006). Invasion, however, is not just a function of the potential invaders’ characteristics, but also those of the environments they could potentially invade (Crawley 1987, Sakai et al. 2001, Richardson & Pyšek 2006).

The idea of ecosystems being more or less “invasible” has received increasing attention (e.g., Usher et al. 1988, Alpert et al. 2000, Richardson & Pyšek 2006), and is often linked to native species richness – invasibility declines with increasing richness (e.g. Burke & Grime 1996, Tilman 1997, Kennedy et al. 2002). This proposition has been controversial, however, and others have found that in the wild, invasibility can *increase* with native species richness, presumably because both natives and invasives are responding to another factor such as nutrient availability (e.g. Lonsdale 1999, Levine 2000, McKinney 2001). Other factors shown to be predictive of invasibility include latitude, land form (island or mainland), and whether or not the area in question is a nature reserve: temperate ecosystems are generally more invasible than tropical, islands are, in general, more invasible than the mainland, and nature reserves are less invasible than areas that encounter more human traffic, probably because humans are the main transporter of exotic species (Lonsdale, 1999).

Even if a foreign plant (incipient invader) *is* particularly well suited to life in a new environment, it seems unlikely that it could compete with the locally adapted

native species. But invaders are able to thrive and often even out-compete natives. This discrepancy has led to a handful of theories. Two of the most prevalent are that foreigners coming to a new location are escaping from the biotic constraints of their homeland such as herbivores, parasites, and disease (the enemy release hypothesis or ERH) (see Torchin et al. 2003, but also see Colautti et al. 2004 for an argument against), and that nascent invasives, faced with new and often strong selective pressure, can undergo rapid evolution (e.g. Pritchard 1960, Reznick 2001, Müller-Schärer & Steinger 2004), making them more competitive in their new environment. These two mechanisms may also work in concert, with a new invader evolving to commit more resources to competitiveness than defense from predators and pathogens it no longer encounters (known as the evolution of increased competitive ability or EICA hypothesis) (see Blossey & Nötzold 1995, Joshi & Vrieling 2005, Bossdorf et al. 2005, but also see Maron et al. 2004 for a negative finding).

Invaders often live in a wide range of new habitats as well as their native one (where, in fact, they are not always particularly common, see Hierro et al. 2004). This could be attributed to the rapid evolution mechanism mentioned above. It is also possible, however, that this widespread success is due to phenotypic/developmental plasticity (Parker et al. 2003, Richards et al. 2006), or both evolution and plasticity (Sexton et al. 2002). Plasticity has been one of many explanations of why invaders do so well in their recipient communities (e.g. Williams et al. 1995, Richards et al. 2006), and hypothesizes that some genotypes allow organisms to exhibit a wider range of phenotypes in response to their environment than others (Pigliucci 2005). This makes them more plastic – and thus likely to thrive – when facing different or differing environments. Asexual or selfing plants could tolerate a wide range of conditions by being phenotypically plastic (Parker et al. 2003).

There are many other promising hypotheses and findings about invasion beyond these, such as the novel weapons hypothesis. This hypothesis suggests that plants' weapons that are relatively ineffective against its native competitors due to adaptation by those competitors could be extremely effective against novel competitors in a new

range (Callaway & Aschehoug 2000). There is probably no single reason for all invasions.

Apomixis in invasion

Asexual species (such as apomicts) are generally more successful as colonists than entirely sexual species, which gives them more opportunities to become invasive. Baker's law hypothesizes that this is because asexuals can establish populations from single propagules (Baker 1955, 1967). New, small populations of asexuals would also avoid the effects of pollen limitation, be indifferent to pollinator availability and, by definition, avoid Allee effects (see Asmussen 1979). Baker's law is well supported by the fact that the proportion of invasive species that exhibit some degree of asexuality is higher than that of plants in general (e.g. Baker 1955, Webb & Kelly 1993, Rambuda & Johnson 2004). Not only do asexuals have a long distance colonization advantage, but are also unaffected by low population density – as when expanding their range – because they are able to reproduce in the absence of other members of their species. Perhaps because of this colonization and range expansion advantage, apomictic members of a species generally have a much wider range than their sexual conspecifics (see van Dijk 2003, Hörandl 2006).

Although apomicts would have all of the colonization advantages mentioned above, newly founded populations would contain little, if any, genetic variation. A study of *Pennisetum setaceum* in three invaded ranges using 122 ISSR fragments (Inter Simple Sequence Repeats) showed no variation at all (Poulin et al. 2005), while a study of *Rubus alceifolius* in its native and several introduced ranges showed some populations with one clone and some with more (Ansekken et al. 2000), and other studies have shown even more variation. For example, *Pilosella officinarum* (now known as *Hieracium pilosella*) in its invaded range in New Zealand showed a total of 39 clones using ISSRs, only 13 of which were sampled more than once (Chapman et al. 2000). Apomictic

invaders can exist as one or many clonal lineages within their invaded range, probably as a result of multiple introductions from a more genetically diverse home range. Without genetic variation, or with little variation and no sex, these populations may evolve relatively slowly. However, apomicts and invaders with low levels of genetic variation may be able to colonize different environments by being phenotypically plastic in relevant traits, making local adaptation less of a factor (Marshall & Jain 1968, Baker 1974, Rice & Mack 1991, Parker et al. 2003). Parker et al. (2003) even suggested that for clonally reproducing invaders with general-purpose genotypes (those that are phenotypically plastic), recombination could be detrimental.

Hieracium aurantiacum

The apomict *Hieracium aurantiacum* (orange hawkweed) is native to central and northern Europe (Czech Republic, Germany, Slovakia, Poland, Romania, Ukraine, Norway, Finland) and has become an invasive alien in North America as well as in New Zealand. In North America, this member of the Asteraceae can be found east of eastern Minnesota and west of western Montana, generally occurs north of 40 degrees latitude, and can be found all the way north into Alaska (USDA, 2006). It appears to have been introduced first on the east coast of North America, where herbarium records trace it back to an 1884 specimen from Rhode Island (Leland, 1884). There it may have escaped into the wild from gardens where it was cultivated as an ornamental. On the west coast, the first herbarium specimen was collected in coastal Oregon in 1927, near the Hood River Gorge (Leach, 1927), and in New Zealand, *H. aurantiacum* was naturalized around 1911 (NZPCN 2005), probably arriving as a contaminant in grass seed imported from Europe. It grows mostly in disturbed environments, such as roadsides, lawns, abandoned fields, and newly burned areas. It is also, however, growing in some undisturbed areas such as alpine meadows, and has recently been seen to be pushing into forest understory in western Montana (Mark VanDermeer, personal

communication). It is a management concern because it often grows so thickly that it can completely exclude the colonization and growth of other plants.

H. aurantiacum is a perennial with a hairy basal rosette and flower heads with clusters of orange flowers. Flower stalks are 8-24 inches tall, and often flower many times per season. It is an autonomous apomict (Koltunow et al. 1998), meaning that it does not require pollen input to initiate endosperm formation when forming clonal seed (although it does *produce* pollen). Koltunow et al. (1998) reported that *H. aurantiacum* produces 93.8% clonal seed. Besides producing apomictic seed, *H. aurantiacum* also reproduces clonally via stolons, often creating dense, impenetrable mats. Although apomixis has traditionally been associated with polyploidy (Asker & Jerling 1992) and *H. aurantiacum* has been seen to be anywhere from triploid to octoploid, an individual in New Zealand was identified to be diploid and apomictic (Bicknell 1997).

H. aurantiacum is part of a group of 14 non-native hawkweeds in the northwestern United States, and is listed as invasive in Washington, Oregon, Idaho, Montana, and Colorado (USDA 2006). Aside from the non-native hawkweeds, all of which are stoloniferous and apomictic to some degree, there are 25 native hawkweeds (which are not stoloniferous or apomictic). Despite the fact that *H. aurantiacum* is apomictic, it still produces pollen and can hybridize with *H. pilosella* (meadow hawkweed), a partially sexual relative native to Eastern Europe (Houliston & Chapman 2001), possibly even passing the apomixis gene on to the resulting offspring (Bicknell et al. 2000, Chapman & Bicknell 2000). *H. aurantiacum*'s pollen may also function allelopathically – Murphy & Aarssen (1995) showed that it inhibited the germination of pollen collected from sympatric species of Fabaceae. Although this specific interaction was determined not to be an ecologically important phenomenon, they showed that allelopathic interactions *are* possible, and further investigation is needed to determine whether or not this could be significant in communities containing *H. aurantiacum*, and potentially in its invasion of new environments.

H. aurantiacum is a good system with which to ask questions about the extent to which genetically depauperate invasive species can spread. Also, deeper knowledge of

the genetic diversity (or lack thereof) in the species could have important management applications.

Study Objectives

This study seeks to determine the genetic diversity and population structure in *Hieracium aurantiacum*, an invasive species throughout North America. This study will lead to insights into asexual invaders, and lay the groundwork for future studies on phenotypic plasticity and its role in invasion. Because invasive species are of such concern, I hope that this knowledge can also be used in the management of this and other asexually reproducing invaders.

Chapter 2 presents this research in journal format, laying out a more concise background, a methodology, results, and conclusions. Chapter 3 tells the story of my research in a narrative form. In this chapter I bring myself into the story in order to make it more digestible and interesting to a non-scientific audience.

CHAPTER 2

GENETIC DIVERSITY IN THE APOMICT *HIERACIUM AURANTIACUM*

Introduction

Understanding genetic variation often leads to important insights into the history and evolutionary potential of populations and species (e.g. Cavalli-Sforza & Feldman 2003, Miller & Waits 2003, Van Oppen & Gates 2006). Genetic variation in groups of organisms is affected by factors such as drift and selection, but also by mating system. One fundamental difference in mating system that can lead to large differences in genetic variation is that between sexual and asexual reproduction.

Sexual reproduction is so widespread in eukaryotes that it is generally assumed to provide a large fitness advantage. Sex increases the heritable variation in populations, providing raw material for selection, thereby increasing the rate of adaptation (Weismann, 1886 referenced in Hoekstra, 2005; West et al, 1999). When analyzed from a theoretical vantage, however, many *disadvantages* of sex become apparent (Barton & Charlesworth 1998, Howard & Lively 2002). These include recombination breaking up favorable groups of genes and the 2:1 advantage that a female should have if she started producing only female offspring (Lloyd 1980). Despite these disadvantages, sex is still commonly thought to be advantageous. One of the most common arguments for why sex is advantageous is the Red Queen Hypothesis (Hamilton 1980), which posits that organisms and their parasites are in a perpetual evolutionary arms race, with the parasites evolving to better attack the host, and the host evolving to avoid being attacked.

Asexual reproduction is common in plants, and is accomplished either vegetatively or by seed. Vegetative reproduction is simply the growth of a new plant from some piece of the parent, such as a fallen leaf or a specialized organ like a stolon.

Asexual reproduction by seed, a form of parthenogenesis, is called apomixis. Apomicts circumvent meiosis and sex, and produce seeds that are clones of the mother (Koltunow et al. 1995). Apomixis, though avoiding theoretical disadvantages of sex, also avoids the genetic mixing and diversity that sex produces. Because of this, apomicts have, until recently (Judson & Normark 1996), been considered evolutionary dead ends (Darlington 1939, Maynard Smith 1978).

Despite the theoretical disadvantages of skipping sex, apomicts and other asexuals make up a disproportionate number of invasive species (e.g. Baker 1955, Webb & Kelly 1993, Rambuda & Johnson 2004). This is perhaps because apomixis could confer a large colonization advantage (Baker 1955, Baker 1967, Hörandl 2006) as a result of the ability to establish populations from single propagules which can often be dispersed a great distance. This should be advantageous both in new (i.e. invaded) ranges, as well as at the edge of current ranges where population density is low. Indeed, apomicts often have wider distributions than sexual members of the same species (geographic parthenogenesis; van Dijk 2003). The combination of clonal reproduction and founder effects in new populations, however, often leads to lower genetic diversity. In general, when clonal diversity in introduced ranges is compared to the plants' native ranges, the introduced range has less genetic diversity than the native (Amsellem et al. 2000, Edwards et al. 2006). Studies on invasive apomicts in their invaded range have generally found two or more different clonal lines (Novak & Mack 2000, Chapman et al. 2000, Amsellem et al. 2000, Edwards et al. 2006, Chapman et al. 2004). Two studies, however, have shown no genetic diversity in a plant's invaded range. Poulin et al. (2005) showed that in three invaded areas, fountaingrass (*Pennisetum*) showed no genotypic diversity, and Wang et al. (2005) showed no genetic diversity in *Alternanthera* in southern China, inviting further investigation into the genetics of highly clonal invaders by suggesting that it is possible for a completely clonal invasive to colonize a wide geographic area.

Genetically depauperate invaders often colonize a variety of environments. Without sex (or with very little sex, as in facultative apomicts) to generate and maintain

variation, it is possible that phenotypic plasticity – the ability of an organism to produce different phenotypes in response to different or differing stimuli – allows invasive asexuals to flourish over a wide range (Parker et al. 2003). In fact, Williams et al. (1995) proposed that invaders in general are more plastic. If an invader were sufficiently plastic in ecologically relevant traits, it is possible that it could thrive in a wide variety of environments without *any* genetic variation. In contrast, invasives that are at least partially sexual are thought to adapt to different environments by rapid evolution (Maron et al. 2004).

In this study, I will investigate the population structure of the invasive plant *Hieracium aurantiacum*. To do so, I looked at the genetic diversity of *Hieracium aurantiacum* in its invaded range in North America. *H. aurantiacum* is a widely invasive apomict that originated in central and northern Europe (Czech Republic, Germany, Slovakia, Poland, Romania, Ukraine, Norway, Finland) and can now be found in western and eastern North America (from Florida to Alaska) and New Zealand. Not only will this research address questions about the invasion potential of apomicts, but also set the stage for experiments regarding the role of phenotypic plasticity in plant invasion and provide information valuable for management of an invasive plant.

Materials and Methods

Collections

To cover the largest possible portion of *Hieracium aurantiacum*'s North American distribution, I obtained samples from both western and eastern North America (Figure 1, for precise locations see appendix A). For western North American populations, half of the locations were collected as whole plants in the summer of 2005 using a stratified sampling scheme. The plants were brought back and kept in a greenhouse in Missoula, MT. The other half of the western range as well as the eastern range were sampled in 2006 and 2007. For those locations, seeds were taken from plants and brought to either

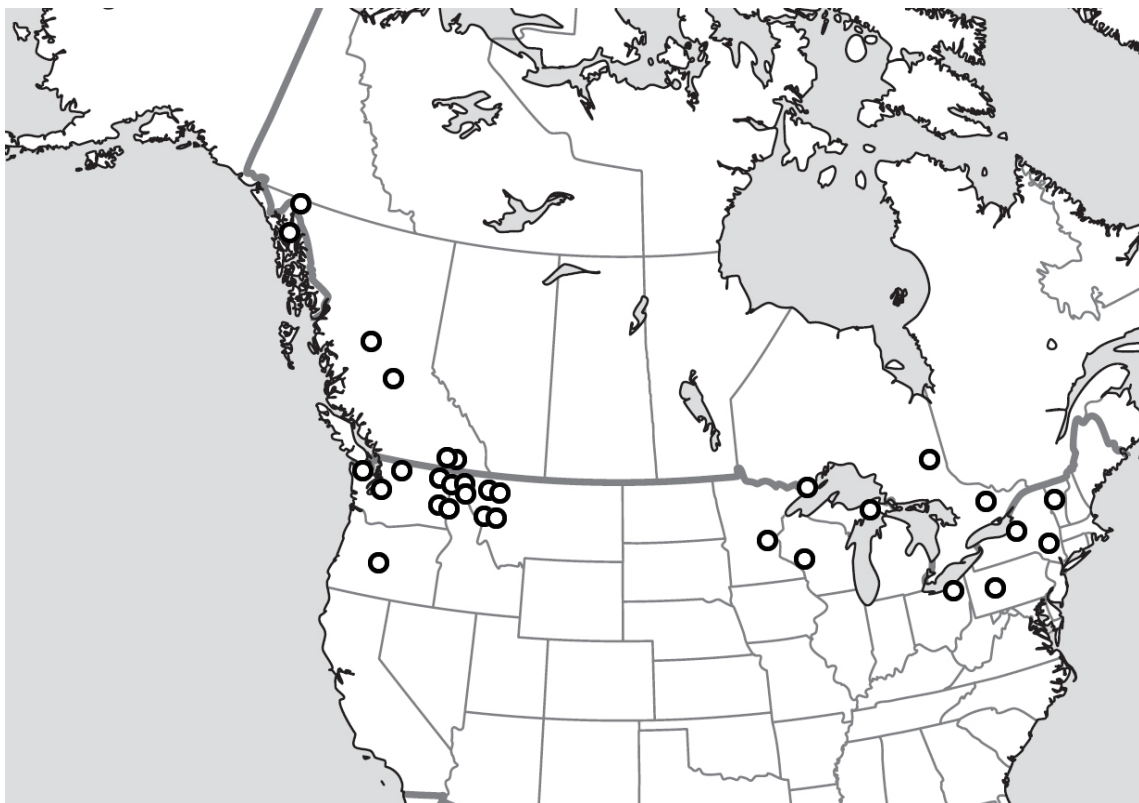


Figure 1. Collection locations for *Hieracium aurantiacum* samples. Each circle represents one sampling location. The one location sampled from the Czech Republic is not included in this map, but was included in all analyses.

Missoula, MT or Moscow, ID for cultivation. For comparison with *H. aurantiacum*'s native range, seeds were sent from one location in Eastern Europe by Dr. Anna Krahulcová (see appendix A, location name "Europe"). One location, referred to as G3, was found in Bend, OR and was composed of individuals planted directly from nursery stock. Another location, referred to as G2, was found in Homer, AK and represents a group of individuals that has resided in the area for at least 23 years. These individuals may represent clonal lines that escaped from the gardens of immigrants from Russia, who have lived in the area for more than 100 years.

Seeds were also obtained from populations of *H. albertinum*, *H. albiflorum*, *H. caespitosum*, *H. floribundum*, *H. glomeratum*, and *H. piloselloides*. *H. albertinum* and *H. albiflorum* are native species, and the others are introduced in North America. *H. caespitosum* and the other *Hieracium* species introduced to North America (Table 1) are closely related to *H. aurantiacum* (for sampling locations see appendix A). Some of these species are at least partially sexual, and served to ensure that these methods were able to detect the diversity sex generates in their genotypes.

Plant care

Germination was accomplished for collected seeds by placing approximately 16 seeds from each plant on wet filter paper in petri dishes (Stergios 1976), which were sealed with parafilm to prevent moisture loss. As these seeds were collected relatively late in the season (July-August), germination probability was increased by placing the sealed dishes in a lighted (24 hours per day under standard fluorescent bulbs) 4°C refrigerator for 30 days to simulate winter. They were then placed in a greenhouse in full sun (12 hours of light per day from overhead sodium vapor lamps) at ambient temperature (temperature ranged from 16 to 27°C) for 14 days. All seedlings were transplanted onto soil (Sunshine Mix #1) in 4 inch pots and grown under the same light and temperature conditions as above. Plants collected whole were grown under the same conditions.

Table 1. Summary of collection and genotypic diversity comparison information for all species studied. N is the total number of individuals of each species used for analysis. The letter after the species name indicates the origin of the species: (I) indicates that it is introduced, and (N) indicates that it is native. Sampling locations indicates the number of distinct locations sampled for each species. Number of loci indicates the number of AFLP loci that were scored to generate genetic profiles for individuals in each species. Number of polymorphic loci is the number of those loci that showed any variation in each species. Clonal diversity was calculated by dividing the number of genotypes by the number of individuals. A clonal diversity of 1 shows that all individuals sampled were different.

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Species	N	Sampling locations	Number of loci	Number of genotypes	Clonal diversity	Average clonal diversity per sampling location
<i>H. aurantiacum</i> (I)	225	53	45	8	0.035	0.0007
<i>H. albertinum</i> (N)	5	1	52	5	1.000	1.0000
<i>H. albiflorum</i> (N)	4	1	46	2	0.500	0.5000
<i>H. caespitosum</i> (I)	13	2	58	8	0.615	0.3077
<i>H. floribundum</i> (I)	13	2	63	2	0.154	0.0769
<i>H. glomeratum</i> (I)	19	2	87	5	0.263	0.1316
<i>H. piloselloides</i> (I)	6	1	77	2	0.333	0.3333

DNA isolation

Tissue samples (~200mg of tissue from the newest leaves) were taken from one of the progeny from each of five plants per collection location (if available). Each sample was immediately frozen on dry ice. Samples were then powdered by deep-freezing them in liquid nitrogen and bead-beating them at ~1000 beats per minute with a 5mm stainless steel ball. DNA was extracted from the powdered tissue using a CTAB/chloroform extraction protocol modified from Doyle & Doyle (1990), and the DNA was finally suspended in 50ul of autoclaved Milli-Q® water for storage at 4°C.

To further clean the DNA, samples were run through Qiagen Plant Mini 96-well kits (Qiagen Inc., Hilden, Germany). Successful extraction was verified and DNA concentrations determined by spot-checking the samples with Hoechst 33258 fluorescent dye and a UV fluorometer (Turner Biosystems TBS-380).

AFLP analysis

I performed AFLP analysis using a protocol adapted from Papa et al. (2005) with the following modifications. Restriction/ligation was performed on 8-400ng of DNA in a reaction mixture containing 4ul 5x T4 DNA Ligase buffer (Invitrogen Corporation, Carlsbad, California, USA), 2ul 0.5M NaCl, 0.55ul 10x BSA, 5U MseI, 4U EcoRI, 1ul each of MseI and EcoRI adapter pairs, and sterile distilled H₂O to a total volume of 20ul. These reactions were conducted at room temperature (~23°C) for approximately 16 hours. Adapter sequences were the same as described in Xu et al. (2000). Restriction/ligation products were diluted 1:10 in sterile distilled H₂O. Pre-selective amplification used 5ul of diluted restriction-ligation product in 4.0ul 5x colorless GoTAQ flexi buffer (Promega Corporation, Madison, Wisconsin, USA), 1.2ul 25mM MgCl₂, 1.6ul 2.5mM dNTPs, 2ul 10x BSA, 0.4ul each of 10uM EcoRI+A and MseI+C primers, 1.25U of GoTAQ flexi (Promega Corporation), and water to a total volume of 20ul. The pre-amplification PCR was conducted as in Papa et al. (2005) with the addition of a 3 minute 94°C denaturation step before the cycling, and six extra cycles (to

a total of 36). The pre-amplification product was then diluted 1:20 for final amplification. For final amplification, the primers designed for the EcoR1 cut sites were labeled with the FAM dye from Invitrogen (Invitrogen Corporation). Twelve primer pairs were used in preliminary tests, and two showed multiple strong peaks (E-AGG/M-CAC and E-CAA/M-CAC). These two primer pairs were used with 3ul of the diluted PCR product from the pre-amplification in a reaction with the following ingredients: 4.0ul 5x colorless GoTAQ flexi buffer (Promega Corporation), 1.2ul 25mM MgCl₂, 1.6ul 2.5mM dNTPs, 2ul 10x BSA, 0.2ul 10uM labeled Mse1 primer, 0.1ul 10uM labeled EcoR1 primer, 1.25U GoTAQ flexi (Promega Corporation), and sterile distilled water to a final volume of 20ul. The final amplification was completed using the following program: 3 minutes at 94°C, 10 cycles of [30 seconds at 94°C, 30 seconds at 66°C (reduced by 1°C each cycle), 2 minutes at 72°C], 36 cycles of [30 seconds at 94°C, 30 seconds at 56°C, and 2 minutes at 72°C], followed by a final extension for 10 minutes at 72°C. For visualization of the PCR products, 1ul of the product of this reaction was run through an ABI 3130xl Genetic Analyzer capillary electrophoresis machine (Applied Biosystems, Foster City, California, USA) with Genescan 500 ROX size standard (Applied Biosystems).

These reactions generated genetic profiles of all samples with 45 total loci for *H. aurantiacum* (each locus here being a DNA fragment). Separate profiles were developed for each of the other *Hieracium* species (Table 1). The number of loci used for each species is shown in Table 1. Using the Genemapper software package (Applied Biosystems, Foster City, California, USA), profiles were generated for each individual and peaks were scored as binary data (1=presence, 0=absence). Each peak was then hand checked by looking at the profile in Genemapper. Only individuals showing strong and unambiguous peak profiles were used in the final analysis, and sampling locations within 25km were combined into one location for analysis. During data analysis and subsequent cross-checking, collections from two locations plus one individual were discovered to be mistakenly collected *H. caespitosum*, and were not included in the final analysis. A total of 225 *H. aurantiacum* individuals from 54

locations were used. The numbers of individuals for the other six species are reported in Table 1.

Power Analysis

To be sure that my sample size and number of loci were sufficient to detect genetic diversity, I conducted likelihood profiling to determine the maximum amount of heterogeneity that could be present in a genome that would go undetected using 45 markers with a 95% confidence. I also calculated the proportion of heterogenous individuals that could be present over the sampled range and go undetected by 225 sampled individuals with a 95% confidence (see Hilborn & Mangel 1997). Both of these calculations assume random sampling, the first of markers from the genome and the second of individuals from the sampled range.

Results

H. aurantiacum

One dominant genotype of *H. aurantiacum* (G1) was found in 51 of the 53 locations sampled (Figure 2). Two other genotypes were found in extremely limited ranges (G2 in Homer, AK: one location spread over ~20km, and G3 in Bend, OR: one point location) (Figure 2). While G3 was intentionally planted nursery stock, G2 was an established group of individuals which may have escaped from cultivation in the gardens of local immigrants from Russia, who have been growing them for longer than 23 years. Each of these three genotypes differed at two loci, showing a loss and a gain of one peak relative to the others (Figure 3). It is also possible that the differences between G2 and G3, due to the nature of AFLPs and the similarity in height of these peaks (peak height of ~50 at the loci: 102bp and 155bp) that this wasn't a gain *and* a loss, but simply an insertion/deletion mutation that caused the movement of one peak.

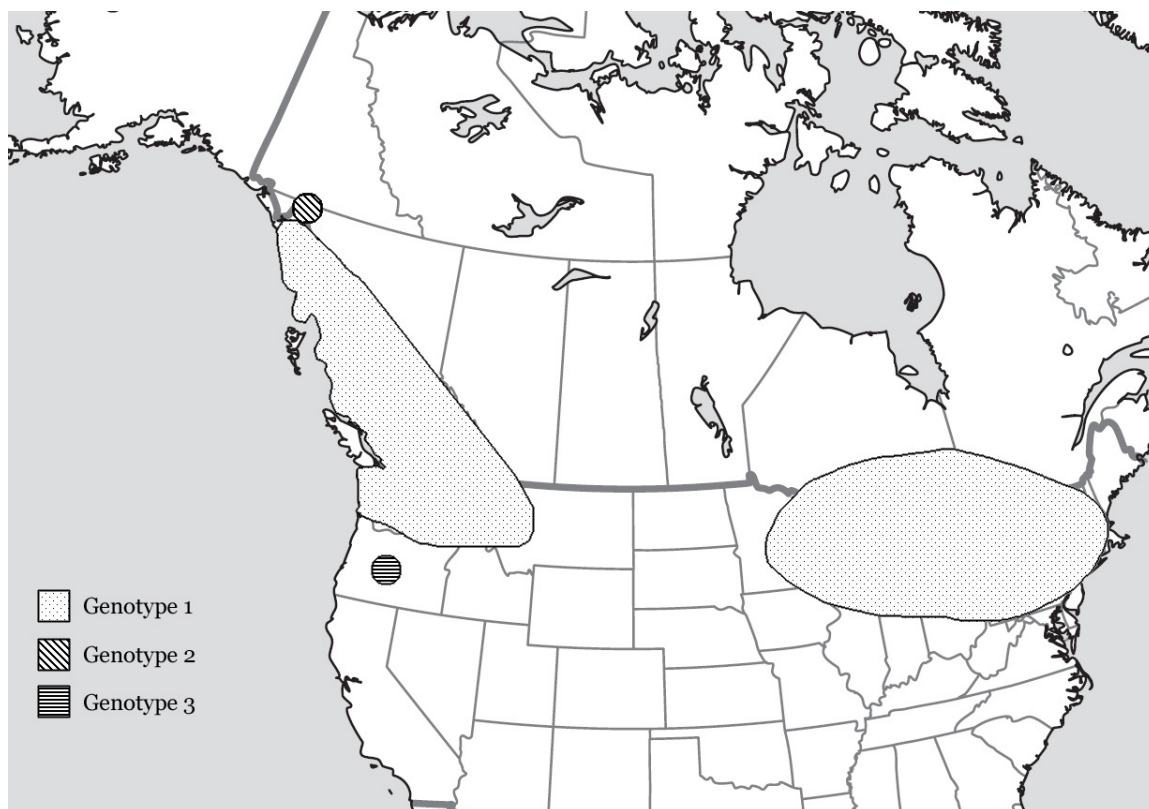


Figure 2. *Hieracium aurantiacum* genotypes as determined by AFLP analysis. Not included is the one location sampled in the Czech Republic, which was assigned to genotype 1. Genotypes 2 and 3 are each composed of only one sampling location – their ranges are disproportionately large in this figure to illustrate their locations.

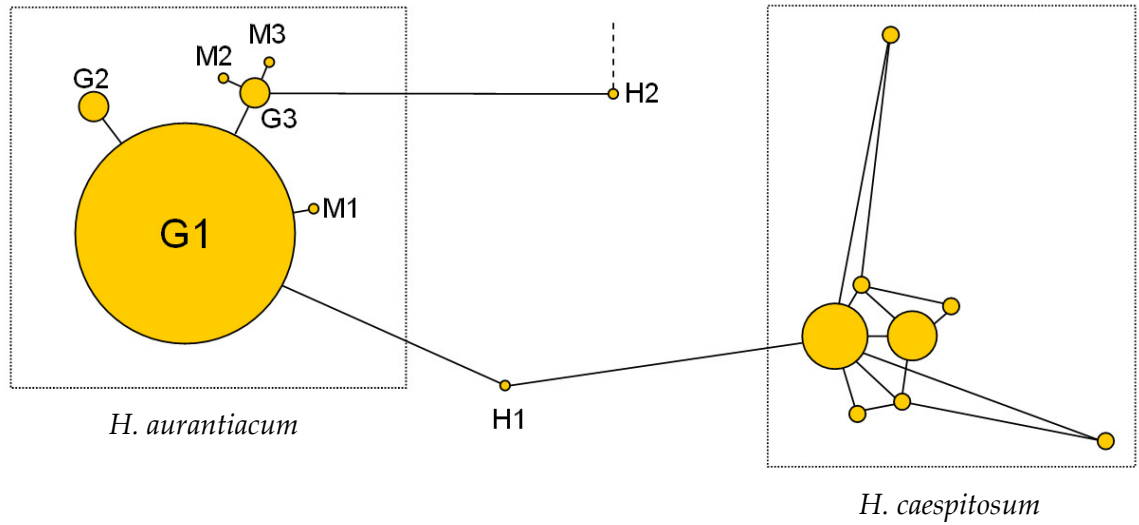


Figure 3. Genotypic difference map of *H. aurantiacum* and its closest related species in this study, *H. caespitosum*. Line lengths are proportionate to number of loci different between groups. Circle size in *H. aurantiacum* is proportionate to the number of study sites where a clone was found, and for H1, H2, M1, M2, and M3 represent a single individual. Circle size in *H. caespitosum* represents the number of individuals found with that genotype. N for *H. aurantiacum* is 225, and N for *H. caespitosum* is 13.

Individuals not in the three main groups

Five *Hieracium aurantiacum* individuals differed from the three main genotypes. Three of these only differed at one locus from the locally dominant genotype, and were considered mutants (see Figure 3, M1-M3). Two others differed at 10 or more loci. The first of these, referred to as hybrid 1 (see Figure 3, H1), was collected in Barry's Bay, Ontario, and had extra peaks at eight loci and missing peaks at two. Of these ten differences, eight could be explained by hybridization with *H. piloselloides*, as represented by the samples that were genotyped in this study. The other plant that differed at more than one locus was collected in Homer, AK and showed a gain of seven peaks and a loss of seven. The gain and loss of peaks did not correspond with profiles from any of the six other *Hieracium* species genotyped here (Figure 3), but may represent hybridization with a species of *Hieracium* not sampled in this study.

The three individuals that showed differences at only one locus from the locally dominant genotype differed from that dominant genotype at different loci (Figure 3). Two of these three came from the single location where G3 was found, and one came from a location dominated by G1.

Other *Hieracium* species

The six other *Hieracium* species examined showed a range of diversity (Table 1), from a clonal diversity (number of genotypes/number of individuals genotyped) of 0.154 in *H. floribundum* to 1.0 in *H. albertinum*. None was as low as *H. aurantiacum*, which showed a clonal diversity of 0.035. Average clonal diversity per sampled location shows an even greater difference between *H. aurantiacum* (0.0007) and the other sampled *Hieracium* species (1.0 – 0.0769).

Power Analysis

Calculations showed an ability to detect anything more than 6.4% genetic heterogeneity between genotypes with 95% confidence. In the distribution of *H.*

aurantiacum sampled, I have 95% confidence that I would have sampled individuals with heterogeneous genetic signatures unless their frequency was below 1.3%.

Discussion

Diversity in *H. aurantiacum*

Hieracium aurantiacum shows remarkable genetic homogeneity over its invaded range in North America. The vast majority of individuals sampled had identical genotypes, indicating obligate asexuality on a large scale. The genotype that dominated the sampled range (G1) was also identical to the specimen obtained from the Czech Republic, where *H. aurantiacum* was found to be identical along a transect from the German to Polish border (Fehrer et al. 2002). This implies that with few exceptions, *H. aurantiacum* may be composed of only one clone over its entire world-wide range. The collector that obtained individuals with the G3 genotype indicated that those individuals were currently being cultivated. The collector of individuals with the G2 genotype indicated that *H. aurantiacum* had been growing in the area for at least 23 years, but that a Russian Orthodox community in the area has been cultivating it for much longer (Linda Wilson, personal communication). These two genotypes, G2 and G3, may show differences because they represent separate introductions from Europe, because of artificial selection while being cultivated, or simply because mutations were fixed in the clonal line. Samples from the seed gardens that supply nurseries, as well as over *H. aurantiacum*'s range in Europe would be fascinating additions to this study. Although multiple introductions to North America are likely, because of the genetic homogeneity in this species it is impossible to infer their number or origins at this time.

Low diversity in invasive populations has been seen before, but not to the extent reported here. The majority of studies showing low diversity in clonal invasive species found some genetic diversity in the range studied, usually as multiple clonal lines (e.g. Hollingsworth & Bailey 2000 – *Fallopia japonica* (Polygonaceae), Baumel et al. 2001 –

Spartina angelica (Poaceae)). Two previous studies, however, found no diversity in the studied portions of the invaded range (Poulin et al. 2005 – *Pennisetum setaceum* (Poaceae), Wang et al. 2005 – *Alternanthera philoxeroides* (Amaranthaceae)). This study verifies and extends these results, showing the genetic homogeneity of *H. aurantiacum* over a range larger than that considered in studies of other species. Although sex may be advantageous in many situations, it may not be necessary to colonize and inhabit a wide geographical range.

Genetically distinct individuals

The power analysis suggests a small probability of missing variation within the sampled range based on the number of samples and loci used. Also, the variation seen in other *Hieracium* species confirms that the methods employed in this study would be able to detect variation in *H. aurantiacum* if it was there. One form of genetic diversity that these methods could not detect is differing ploidies. A study of the ploidy level across this plant's range would be informative, although it is likely that all plants are tetraploid, the common ploidy in Eastern Europe (Fehrer et al. 2002).

The two individuals with differences at ten or more loci from the rest of the samples may be hybrids. *H. aurantiacum* has been shown to donate pollen to closely related *Hieracium* species and form viable offspring (e.g. Krahulcová et al. 2004, Houliston et al. 2006, Nogler 2006), and allelic discrepancies in one of these individuals (H1) matched the genetic profile of *H. piloselloides*, which has been shown to hybridize with *H. aurantiacum* (Bicknell et al. 2000), and showed a phenotype consistent with hybridization. The other (H2) could be a hybrid between *H. aurantiacum* and a closely related species that wasn't sampled in this study. Hybridization, and the resulting creation of novel genotypes, could be a source of new invasive apomictic lines – the combination of previously separate genetic material can increase the invasiveness of plants (Lavergne & Molofsky 2007).

The three individuals with two alleles differing from the individuals surrounding them were most likely simple mutants. The rate of mutation this would imply (three

mutations out of 10,215 scored loci) is reasonable. There is little data on expected AFLP mutation rates, except one preliminary finding in whitefish (Campbell and Bernatchez 2004), which proposes a rate of 10^{-4} . This tentative rate would also easily allow the level of variation observed.

Compared to *H. aurantiacum*, the other *Hieracium* species in this study showed a great deal of genetic diversity. Figure 3 shows the *H. aurantiacum* complex in comparison to the closely related *H. caespitosum*, which showed an intermediate clonal diversity among the sampled *Hieracium* species. *H. aurantiacum*'s genetic homogeneity indicates that it is probably an obligate apomict, and the genetic diversity in these other species is most likely attributable to sex.

Invasion mechanism

Because *H. aurantiacum* has essentially no genetic variability over the vast majority of its North American range, phenotypic plasticity may be important in allowing it to grow in a variety of habitats. With low genetic variation, invasive species might rely on plasticity as a mechanism for invasion (Baker 1965, Schweitzer & Larson 1999, Weber & D'Antonio 1999, Sakai et al. 2001, reviewed in Daehler 2003). Phenotypic plasticity may even be a causal trait in the invasion of specific species (Williams et al. 1995 – *Pennisetum setaceum*, Annapurna & Singh 2003 – *Parthenium hysterophorus*, Price & Morgan 2006 – *Leptospermum scoparium*, Pan et al. 2006 – *Alternanthera philoxeroides*). Although some think plasticity is less important than genetic diversity and its associated adaptive opportunities (Lee 2002), in species with very little or no genetic diversity, phenotypic plasticity in relevant traits may be crucial for tolerating varied or varying conditions (see Richards et al. 2006). *H. aurantiacum*, with no genetic variability, may rely on plasticity to tolerate such a wide range. Plasticity alone, however, probably cannot wholly explain this plant's invasion.

The invaded environments' susceptibility to invasion, or invasibility, probably also plays a role. Biotic and abiotic properties of the invaded environment can be important in invasion (e.g. Usher et al. 1988, Alpert et al. 2000, Richardson & Pyšek 2006). Often

the diversity of native species is important, and also the availability of resources. *H. aurantiacum* preferentially grows along roadsides, in abandoned fields, and in lawns – all disturbed habitats. Many conceptual models state that increases in resources (such as space in disturbed habitats) increase the invasibility of a habitat (e.g. Davis et al. 2000, Shea & Chesson 2002). *H. aurantiacum* probably depends almost entirely on disturbed habitats to establish itself – it has been shown that *H. aurantiacum* in Montana is not an effective competitor with local grasses unless it can establish before the grasses (Elizabeth Crone, unpublished data). It is this plant's ability to exclude recolonization by natives once it does establish that is cause for worry: it could be establishing in remote ecosystems disturbance by disturbance, making a mountain of *Hieracium* one molehill at a time.

Invasives may often grow in environments for which they are “pre-adapted” (Neuffer & Hurka 1999, Ayala et al. 2000, Maron et al. 2004), meaning those that are similar to their native environment. Although this may play a role for *H. aurantiacum*, it is unlikely that conditions are similar enough over its wide invaded range for pre-adaptation alone to be the driving force in its invasion.

Conclusion

From its introduction to North America, probably in the late 1800s, *H. aurantiacum* has spread across a wide range despite being almost completely genetically homogenous. It is generally thought that asexual lineages like this are doomed (Darlington 1939, Maynard Smith 1978). Recently, however, people have been paying more attention to asexual lineages that have been around for a long time – for example, species of bdelloid rotifers, ferns in the family Vittareaceae, and some species of mycorrhizae (reviewed in Judson & Normark 1996). The reported persistence of these species indicates that being asexual doesn't prevent lineages (genotypes) from persisting. If anything, these long-lived asexual lines point back to questions about the value of sex (see Barton & Charlesworth 1998, Howard & Lively 2002, Otto & Gerstein 2006). In fact, the main theories suggesting the demise of asexual lineages are

considered and refuted in Judson & Normark (1996). One of the most prominent of these theories is the Red Queen Hypothesis (Hamilton 1980), which states that organisms and their parasites are in a constant evolutionary arms race. If an organism couldn't evolve, the theory goes, its parasites would quickly evolve to efficiently parasitize it, which could lead to its eradication. Ladle et al. (1993), however, proposes dispersal as a substitute for sex. Instead of evolving to compete, asexual species could simply out-disperse their parasites, essentially living on the run. *H. aurantiacum*, as an essentially monoclonal species, could very well persist in just this way. If *H. aurantiacum*'s primary defense against parasites was dispersal, an effective biocontrol effort would have to ensure almost total coverage of its range, such that there would be no escape. Because virtually all individuals of *H. aurantiacum* are genetically identical – that most are, in fact, only *one* individual – their ability to adapt to control measures should be very limited. This, combined with the basic lack of genetic variability, could ensure that bio-control agents or pesticides affect *H. aurantiacum* uniformly (Nissen et al. 1995).

CHAPTER 3

SEND IN THE CLONES

The French-Canadian border-guard has jowls and full, pouty lips. He holds my driver's license, registration, and proof of insurance and glares at me with one raised eyebrow. I'm belted in to my rental Toyota, which sits next to his little border-guard hut here on a lazy two lane highway in the hills spanning the border between Vermont and Quebec. Four day old stubble roughens my cheeks and my passenger seat is a mess of food, cds, maps. I thought I could just smile and nod my way through the border without trying to explain my research, so I, well, withheld some information. It is true that I was visiting my friend in Vermont and I am travelling back to Minnesota through Canada, but jowly here has smelled my omission. Backpedalling now, I try to explain that I'm on a research trip, driving around Eastern North America collecting seeds from orange hawkweed — an invasive plant — for genetic analysis.

"You are collecting weeds?" His glare sharpens.

"Ah, well, no, yes, they're an invasive plant here. I'm doing research for my Master's degree."

"You have many small envelopes in the trunk of your car." Oh crap, does he really think I'm a drug dealer?

"I'm collecting seeds from the plants? To analyze? Population genetics?" What, I wonder, will French-Canadian jail be like?

"Pull ahead, go under the roof. Do not get out of your car, except you may go to the toilet."

He walks into his hut, glancing at me over his shoulder. I notice he has a stripe running down the outside of each leg of his green slacks.

Orange hawkweed grows low to the ground — a hairy little jumble of leaves — and sends up sprays of rich orange flowers on hairy stalks. It is originally from Eastern

Europe and was brought to the United States about 100 years ago, probably by immigrants who brought it for their gardens. They have since escaped from those gardens. Now you can see that orange flooding abandoned fields and roadsides, poking through underbrush, and popping out of lawns throughout Eastern and Western North America (it doesn't grow in the center), from Florida to Alaska. It is a beautiful plant, and it's been here so long that many people applaud it as a wildflower.

The problem is that orange hawkweed grows so well here that it's out-competing native species. Like other invasives — zebra mussels in the Great Lakes, Kudzu in the Eastern United States, spotted knapweed in the mountain West — orange Hawkweed is pushing into our ecosystems and growing our native species out of house and home. This is driving some of these natives towards extinction, and even affecting commercial enterprise like ranching, fishing, and farming. One study showed a cost of \$137 billion per year caused by invasive species, due to crop losses, damage to property, and the cost of controlling the invasives. Even though orange hawkweed usually only grows in non-natural habitats (like ditches), people have started to see it covering the bare ground left by wildfires in Montana and creeping its way into pristine alpine meadows. If this plant can get a foothold in our wild spaces, it could spread, threatening both native species and local economies.

Lots of foreign species find their way into our country, but very few of them become invasive. In fact, most simply wither and die. So, out of all of the foreign plants that end up in the United States, how has this one become so successful? There are two main ideas about how foreigners can become successful invaders. One says that a new invader can evolve and adapt to its new habitat really quickly. It's a matter of pressure. Plants in their own habitats don't have much pressure to change because they are adapted to their environment well enough to get by. They can make good enough use of the available nutrients, get enough water and light, and pretty much avoid getting eaten. But in new environments, environments to which plants aren't adapted, there is great pressure to adapt. If they don't change to efficiently use the local resources, light regime, etc, they die.

The other theory says that the invasive is far away from its home range, and so doesn't suffer from the diseases and predators that normally attack it — things like parasitic fungi, root-eating worms, and leaf-eating insects. Because most diseases and plant eaters are pretty specific in the plants that they attack, a foreign plant will often go unnoticed by the parasites, pathogens, and herbivores in its new environment. Invasives, then, could escape from their natural enemies and grow un-harassed.

I roll the window down and turn up the music. The border-guard finally let me into Quebec, and I'm heading North towards Montreal. I can't blame him for giving me a hard time — my trunk *is* filled with little paper envelopes, also vials of blue powder (for drying out pieces of leaf if I can't find seeds). It probably does look pretty suspicious. The country road falls long and rope-like between the steep slopes of a valley the color of moss and honey. I squint at my speedometer, trying to pick out how fast 50 kilometers per hour is as I whoosh through the tiny towns that nestle around the road. Hand-painted signs hang from rough stone buildings, swinging lazily in the cool summer breeze. Everything is printed in French. I could easily have just crossed the German border into France to pick up a couple loaves of bread. Wind from the open window roars in my ears as I round a sweeping corner, and I see a dot of just the right shade of orange. My stomach jumps — I swerve to the side of the road, braking hard. There, swaying with the tall grass. I grab a handful of envelopes and my notebook and stomp out through the grass to find my plant.

I'm hunched over, looking for seeds, and it takes the mosquitoes a few minutes to find me, but soon I am engulfed by their ululating whine. Okay: seeds in the envelope, number the envelope, write which envelope came from where in the notebook. Crap! I wave my arms around my head and do a quick sprint, hoping to outrun the little bastards. I earn about two seconds of silence. How do they do that? There's no way they can fly that fast. Okay, shoot: run back and get another sample. I grab hand-fulls of seeds and run in circles, cramming them into envelopes. I can

imagine the curious, dubious, and frightened stares that must be coming from the passing cars.

Finally I lick the last envelope and press it shut. I dash to the car, fling open the door, and *slam*, I'm safe. The air is hot and thick in here but that's just fine — a ravenous swarm churns outside the car, throwing themselves against the glass in frustration. Back where I collected, I can see that the hawkweed here is growing like it usually does — in thick, impenetrable mats. Flat masses of those hairy leaves form a blotchy patchwork with the grass. All of the little plants together in clusters like that are just shoots off of one parent, one founder plant, born as a limb reaching from the founder. All of them are genetically identical.

Plants have been cloning themselves for a long time, probably almost as long as they've been around. Someone did a count and found that 60% of British plants made at least some of their offspring clonally. That simply doesn't happen in the animal world, where cloning is usually done in a lab, and not easily. Plants can clone themselves in many ways, but basically there are two paths: clones by vegetation and clones by seed. Vegetative clones are like the little orange hawkweed plants that grow out of the big ones, just offshoots from a mother plant breaking away to grow on its own. This is the most common way plants clone themselves, and if you have a spider plant or blackberries growing in your yard you can see it in action — rhizomes or stems snake out from the mother, grow roots, and go for it.

Making clonal seeds is more complicated. Plants make seeds in the same way that we make babies. Each one generally needs an egg from the mother — in plants you can find these at the base of flowers — and a sperm from the father, which is delivered by pollen. Making a clonal seed, however, is different: the mother plant skips sex, ignores both egg and sperm, and just uses one of her own cells to make an embryo. This would be like a human mother spontaneously getting pregnant because one of the cells in her uterus just popped out and grew into a baby. The seeds that plants get from this process

look like any other seeds, but will grow into a plant that's genetically identical to its mother – a clone.

Making seeds like this works out great for plants that can do it because then they can always make seeds, even when they can't get pollen to fertilize their eggs. This is handy when a plant is out of its home range, away from other members of its species. Because it doesn't need pollen to make seeds, a single plant can just start a whole population by itself. Imagine a single seed from a European plant that's never seen the Americas. Joe traveler runs through a nice little cute-as-a-button park to catch a bus to the London Heathrow airport, and gets this seed stuck on his brown and gray argyle sock. After a relaxing flight – sinuses dry from canned air, plate of limp zucchini and baby carrot, knees pressed against the the vomit bag in front of him – he pulls his wheeled luggage out of the airport in Seattle. Brow furrowed and fingers tapping impatiently on his gray slacks, he hails a taxi, and as he jerks the car door shut it knocks his foot, jarring the seed onto the worn and oily blacktop. A puff of exhaust from the leaving taxi blows it into the moist ditch, where it quickly puts down roots, pokes its flowers up above the grass, and spreads clonal seeds out on the wind. This scenario is perhaps a bit dramatic, but by no means is it far fetched. Invasive species are mostly introduced by humans, and most of the introductions are accidental.

I crouch in my little clearing, hands scrambling to lock poles onto my limp tent. The rain started when I was shoving the last pieces of gathered wood into the brown metal box next to the fire pit, and it stops as I yank the zipper down the front of my tent to jump inside. I look to the sky and see an improbably straight line dividing the storm that's blowing out over Lake superior and the clear evening sky it's uncovered. I can smell what is almost the ocean as I weave through the shrubs to the edge of the cliff – each envelope-sized leaf is the color of a ripe lime, shiny from the rain. I stand over the water – as clear as the air – and the storm roils and retreats over Lake Superior, lighting arcing like broken glass from heaped almost-black clouds down to the lake. I walk back to camp and lay damp logs and splintered sticks on crumpled newspaper, get the third

match to light, and start a nice smoky fire. After half an hour of fussing and poking it with a stick it's built up some heat. I put my can of soup (President's Choice minestrone, thank you Canada) on the crusty black grill that swings out over the fire pit.

When I tried to explain my project to the bored, gun-toting American border-guards this morning, they hastily waved me through into Minnesota. I love the Midwestern friendliness I always associate with this place: the girl at the gas station chatting to me about the merits of different energy drinks, "Just one can of Rockstar and I'm just fine all day, for sure," and the Ranger here at Split-Rock Lighthouse state park laughing at her own jokes and giving me a pack of matches out of her own purse for my fire. My sampling trip is almost done: a loop from Minnesota to Maryland to Ontario, and now back to Minnesota. My car is a mile back through weaving mowed grass paths, stairs scaling the rock with a little bench to rest on half way up. In the back seat a folding plastic organizer bulges with little brown envelopes of seeds. They'll come with me on the bus from Minneapolis back to Montana, where I'll grow them up and extract their DNA. Then I'll generate genetic fingerprints (DNA patterns that should be unique to every individual), and figure out how clonal orange hawkweed really is. The ground is damp so I sit on a log to eat my soup and the crusty end of a loaf of bread. I click on my headlamp, crawl into my dark tent, open my book, and fall asleep.

I'm in my office back in Montana and am staring at my monitor, which sits on a long black desk I share with a lab tech named Aaron. Out the shoulder to ceiling windows next to me I can see a ponderosa pine going white with snow. Aaron has his headphones on and is singing softly along to the music—right hand on the mouse, left drumming out the beat on the black desk top. I push my shoes off under the desk and fidget with my mouse, waiting for my email to come up. Today, if everything went well, I will get the results from the genetic fingerprinting I did on my plants – orange hawkweed I collected on the East Coast, and an amalgamation of them that other people gave me from the West Coast, Alaska, the Czech Republic. And there they are. I have it. A little formatting, copy, paste. They're all the same. Aside from a couple mutants,

which *have* to exist in a group of plants this big, they are all genetically identical. No one has ever seen a set of clones that occupies such a large range – from Pennsylvania to Kodiak Island to the Czech republic, totally the same. Like genetically identical. Like a whole worldwide species that's all one clone. Like wow. According to commonly accepted ideas about sex and why it's a good idea, this shouldn't happen.

Asexual species can't adapt and evolve as fast as sexual ones, which leads many people to the conclusion that they're just random freaks of nature, doomed to quick extinction. Evolution basically lets a lineage of organisms change over many generations to adapt to a changing environment. The process (evolution by natural selection) requires some things to work in a group of organisms: variation in that group, heritability of that variation, and that some of the organisms, because of the variation, are able to survive and reproduce better than the others. To illustrate this, imagine a group of little red blobs on Mars. These blobs are anywhere from raisin to marble size, with lots of variation in between. The blobs reproduce just like Earth's animals (that is to say by having sex), and the child blob of two parent blobs is generally about as big as the average of its two parents. Sometimes, however, mutations happen, and a child blob can be either bigger or smaller than its parents. Mars is a pretty harsh place to live, and not all of the blobs can survive – because of the increasingly strong wind storms that sweep across the Martian plains, in fact, the bigger blobs are more likely to survive than the little ones. Because of this, over the generations, the smaller blobs die more than the bigger ones, and any mutant blob that's bigger than other blobs survives better than smaller blobs. Because the larger blobs are more likely to survive (and reproduce), and big mutants do better, the blob community, over many generations, will be composed of bigger and bigger blobs. That's evolution. That's also why people think that asexual species won't do well – they can't generate the variation that this process needs as quickly as sexual species.

Most organisms can also change a little bit *without* evolving though, just using the flexibility built in to their genomes. Like if you take me and put me in the sun for a month, I will turn brown (or at least bright red). Or when a house plant gets less light

than it wants, it gets long and tall. The ability to change physical form without genetic change is called phenotypic plasticity – this would be like one of the martian blobs getting bigger if it sensed the wind picking up. Every organism is plastic like this to some degree – whenever something changes its form or function in response to its surroundings, that's plasticity. So some plants, like orange hawkweed perhaps, could be able to grow in a whole bunch of different environments – from ditches in Alaska to abandoned lots in Washington DC – just by being plastic.

Some people argue that no, plasticity isn't enough, you need sex and constant evolution because organisms are always evolving to escape from their predators and parasites, which are evolving to attack the organisms. It's like an evolutionary treadmill: lots of work to just maintain a balance with your predators and parasites. Some plants, however, could skip out on the battle and just run away, sending their seeds out farther than their parasites can go.

A life on the run and phenotypic plasticity could easily work together too. So orange hawkweed, even though it doesn't have sex, might be just fine.

I can feel the sun on my shoulders as I follow a dirt path down from the base of the rock face towards Kootenai Creek. The air is thick with the musky smell of sun-baked ponderosa pine needles and my holey argyle sweater is getting stuffy on what is becoming a warm spring day. My house-mate Mike, strolling easily in front of me, kicks up little clouds of dust that float on the air, and the grey, spindly branches that stuck it out all winter are pushing leaves out their tips - each light-green bundle swinging open from the center. Green sprouts of annuals poke between rocks and cured pine needles all along the descending path. Wait. Those ones. Hairy leaves, little rosette, that certain *look*. I think they must be. I stop there to look down at the hopeful little cluster of baby hawkweed plants. I know they're horrible invasives, but I can't stir up any ire. They look so hopeful and alive. Funny to think that these are clones, *twins*, of the plants that I pulled out of a ditch in Ontario, the ones that popped defiantly through the close cropped grass of an ancient graveyard in Pennsylvania. Are all of the plants together

just one individual? One entity spread in a spotty, thin layer over two continents? Does it get lonely being the only member of its species? Or are these just a whole bunch of hermaphroditic sisters and brothers, one happy, ambitious, genetically identical family? I leave the hawkweed babies to their business and continue down the path.

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Appendix A

Sampling location details by species.

<i>H. aurantiacum</i>			
Location Name	Number of individuals in final analysis	Latitude	Longitude
St. Regis North	6	47°18'19.47"N	115° 5'42.83"W
St. Regis South	6	47°18'14.46"N	115° 5'41.33"W
Troy	3	48°26'11.91"N	115°52'53.87"W
Kern Rd.	3	48°23'45.14"N	115°52'50.40"W
Hayed	5	48°25'37.97"N	115°48'46.10"W
Highway 2	5	48°20'43.45"N	115°31'3.56"W
Marshall Mountain	5	46°53'18.80"N	113°56'41.00"W
Columbia Falls	5	48°21'21.97"N	114°10'48.17"W
North Hilltop Rd.	6	48°23'4.63"N	114°13'15.57"W
Lake Blaine Rd.	3	48°14'8.27"N	114° 6'23.58"W
River Road	6	48° 2'57.77"N	114° 0'37.34"W
Ferndale	4	48° 4'27.15"N	113°59'53.99"W
Granite Ck. Rd. #1	4	48°37'2.98"N	120°33'44.93"W
Granite Ck. Rd. #2	5	48°35'30.02"N	120°30'11.45"W
Granite Ck. Rd. #3	4	48°39'39.15"N	120°33'32.87"W
K diamond K Ranch	4	48°35'47.92"N	118°44'25.10"W
Republic	3	48°43'2.10"N	118°39'12.21"W
Forest Capital	4	48°37'11.73"N	118°33'35.00"W
Garfield	5	47° 0'18.30"N	117° 8'27.08"W
Clallam	5	48° 5'59.80"N	123°45'36.61"W
BLB	2	54°13'46.39"N	125°45'30.26"W
CBC	2	49°18'15.85"N	117°40'6.25"W
NBC	2	49°29'27.49"N	117°17'13.91"W
PRI	2	48°10'2.62"N	116°54'24.90"W
QBC	2	52°58'52.20"N	122°29'9.96"W
SPW	2	47°25'27.38"N	121°24'40.62"W
Cedar Creek	4	45°23'38.80"N	93°13'59.14"W
Black River Falls	1	44°14'15.17"N	90°42'41.09"W
Kirtland	4	41°37'44.00"N	81°21'42.00"W
Luthersburg	4	41° 3'12.00"N	78°43'10.00"W
Shandaken	5	42° 6'53.51"N	74°25'8.69"W
Pulaski	2	43°34'3.62"N	76° 3'12.37"W

Location Name	Number of individuals in final analysis	Latitude	Longitude
Waterbury	2	44°20'56.21"N	72°44'40.53"W
Barry's Bay	2	45°29'32.15"N	77°41'22.19"W
Matheson	1	48°31'36.90"N	80°27'1.67"W
Seney	1	46°20'37.53"N	86° 0'8.58"W
Grand Portage	2	47°57'28.62"N	89°41'25.19"W
Emida	4	47° 7'12.97"N	116°36'0.04"W
Bend	13	44° 1'28.97"N	121°16'39.01"W
Homer EER	8	59°43'45.26"N	133°16'34.06"W
Homer HC	9	59°40'52.11"N	133°39'18.28"W
Kodiak 4	14	57°49'4.30"N	134°21'17.12"W
Kodiak 1	13	57°47'15.79"N	134°24'52.97"W
Kodiak 2	11	57°47'23.92"N	134°23'53.32"W
Homer HC	5	59°40'52.11"N	133°39'18.28"W
Homer HAJ	11	59°39'21.65"N	133°35'4.11"W
Homer BCH	6	59°38'55.91"N	133°36'3.64"W
Europe	1	49°11'5.40"N	13°20'44.64"E

H. albertinum

Location Name	Number of individuals in final analysis	Latitude	Longitude
HALBERT	6	47° 1'16.99"N	122° 1'53.03"W

H. albiflorum

Location Name	Number of individuals in final analysis	Latitude	Longitude
HALBIF	4	47° 1'16.99"N	122° 1'53.03"W

H. caespitosum

Location Name	Number of individuals in final analysis	Latitude	Longitude
HCA #1	10	47° 7'18.99"N	122°35'40.02"W
HCA #2	3	47° 8'32.00"N	116°26'51.02"W

H. floribundum

Location Name	Number of individuals in final analysis	Latitude	Longitude
HFL #1	9	55°24'37.62"N	127°41'33.87"W
HFL #2	4	55°23'52.86"N	127°41'49.84"W

H. glomeratum

Location Name	Number of individuals in final analysis	Latitude	Longitude
HGL #1	7	49°33'24.98"N	117°15'0.01"W
HGL #2	12	49°26'5.94"N	119° 5'16.00"W

H. piloselloides

Location Name	Number of individuals in final analysis	Latitude	Longitude
HPI	6	55° 9'16.44"N	127°46'0.44"W