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INVASION AND ITS EFFECTS ON BIODIVERSITY IN NATIVE AREAS

Exotic species are those which have been dispersed, either by chance or human action, to an environment outside of their native range. Only a small portion of these exotics are able to become well established in the invaded population and even expand beyond it. The ones that can are termed invasive species (Muller-Scharer, Schaffner & Steinger, 2004; Bais et al., 2003). Broz et al. (2007) estimates that 25,000 species of invasive plants are in the U.S. invading 700,000 hectares of United States wildlife habitat per year and tallies the economic burden of invasive plant species to be around $35 billion dollars annually. Most pressingly, invasive species pose a significant threat to biodiversity. They threaten biodiversity by outcompeting native plants and becoming dense in the populations they invade. This presents a real threat to the economy and environment (Prentis et al., 2008). In fact, biological invasions are thought to be the second leading cause of biodiversity loss in today's world, after habitat fragmentation (Keane & Crawley, 2002). For example, 50% of Hawaii's native plant species are endangered and 200 have already gone extinct, largely driven out by introduced invasive species (Broz & Vivanco, 2009b). There is even some concern in the scientific community that invasive species may have the potential to instigate evolutionary changes in native species (Lee, 2002).

The disruptive effects of invasions are facilitated by human action. Anthropogenic actions concerning dispersal of species and habitat disturbance have made biological invasions by angiosperms a bigger problem (Muller-Scharer, Schaffner & Steinger, 2004). As humans continue to make our environment susceptible to invasives, it is becoming ever more important to understand how invasives are able to be successful so that we can combat them. This topic review will examine some methods exotic species use to go on to become established and spread, becoming invasive in their introduced area.

INTRODUCTION TO THE STUDY SPECIES, *CENTAUREA STOEBE*

Since every invasion and every exotic species presents a new challenge with novel invasion strategies, the invasion of *Centaurea stoebe* (synonymously referred to as *C. maculosa*) into North America will be used as a case study to deeply examine invasion strategies. *Centaurea stoebe* is a short-lived (approximately 3 years) perennial member of the aster family (Broz et al., 2007). It occurs in two different cytotypes, or with two different chromosomal factors. In its home range, it exists in both diploid and tetraploid cytotypes, but only the tetraploid version is present in introduced ranges in North America (See Figure 1; Treier et al., 2009). *C. stoebe*, commonly called spotted knapweed, is native to Eurasia and was introduced to North America in the late 1800s or early 1900s in several distinct introduction events (Hahn and Muller-Scharer, 2013; Marrs, Sforza, & Hufbauer, 2008). According to the U.S. Department of Agriculture and the Natural Resources Conservation Service (2001), *C. stoebe* is now found in all but four of the continental United States. The detrimental effects of its invasion are most clearly seen in the northwestern United States (See Figure 1). In Montana alone, C. stoebe covers nearly 4.5 million acres (Broz et al., 2007). Bais et al. (2003) introduce spotted knapweed as one of the most economically damaging invasive species in North America. It can take over in a variety of conditions and is especially common in disturbed areas in pastures and prairies. In its native range in Eurasia, it is not problematic, but in North America it has formed monocultures and replaced many native plants. On top of all this, it degrades the infrastructure of communities by increasing runoff that leads to erosion and causing decreases in forage for wildlife (Broz & Vivanco, 2009b).

It is important to understand the mechanisms of invasion for specific invasive species, so that conservation efforts can be most tailored and effective. There are two major hypotheses in the literature as to why *Centaurea stoebe* is a successful invader. One theory assumes that there is pre-existing genetic variation and adaptation in C. stoebe that provide them with success in novel environments. The other postulates that rapid evolutionary change occurs in the invasive species in its new habitat (Muller- Scharer, Schaffner & Steinger, 2004).

RAPID EVOLUTION HYPOTHESES

Some researchers believe that there is considerable opportunity for studying adaptation in invasives, since their invaded environments are certain to have different abiotic and biotic characters than their native range which offer new selective pressures (Colautti & Lau, 2015). This first major

school of thought posits that invasive species quickly evolve and adapt to introduced areas, giving them the ability to dominate and spread.

Evolution of Increased Competitive Ability

The first theory that falls under the rapid evolution hypothesis is the evolution of increased competitive ability (EICA), also known as the release from natural enemies hypothesis. Until the mid- 2000's, this was the sole credible theory for the invasive capabilities of plants. The release from natural enemies hypothesis predicts that exotic plants experience a decrease in regulation by herbivores and other natural enemies, which allows them to quickly spread and increase in abundance (Keane & Crawley, 2002). The exotic plants are able to excel competitively because former restrictions on their resource allocation change in the invaded range. Colautti and Lau (2015) use optimal defense theory to explain this change in resource allocation. Due to the invasive's release from specialist/generalist herbivores in the introduced areas, plants can allocate resources to growth and reproduction that would previously have gone to defense (Colautti & Lau, 2015).

Support for this theory is seen in research on a variety of invasive plants. Plant invaders have been shown to suffer less damage from herbivores and are attacked less by herbivores in their invaded range when compared against conspecifics in the native range (Wolfe, 2002; Keane & Crawley, 2002). To test the escape from natural enemies idea, Wolfe (2002) took samples from populations of the herbaceous invasive plant Silene latifolia (bladder campion) from both its European home range and North American invaded area. Wolfe measured herbivorous attacks on the phloem, flowers, and seeds of the samples and looked for fungal infection. He found that 60% of the sampled S. latifolia in North America experienced no damage, while only 16% of samples from the home range escaped predation, and the majority of the home range S. latifolia suffered herbivory from more than one type of enemy. Wolfe's experiment showed that plants in Europe were 17 times more likely to suffer herbivory damage, from both generalists and specialists (See Figure 2). To test an invasive plant's response to decreased natural herbivory, Blossey and Notzold (1995) compared the growth of Lythrum salicaria (purple loosestrife) in areas with and without its natural herbivorous enemies. The plants without natural herbivory were shown to grow significantly taller and experience more vegetative growth (See Figure 3).

Experiments done on *Centaurea stoebe* specifically have added support to EICA and the release from natural enemies hypothesis. Broz et al. (2009a) provided support at the genomic level. In this study, seeds from C. stoebe diploids, European tetraploids and invasive tetraploids were grown in a common garden greenhouse. They used quantitative PCR to see patterns of gene expression related to defense and other plant performance areas. At the molecular level, Broz et al. (2009a) showed that invasive populations of tetraploid spotted knapweed showed less expression of gene transcripts related to defenses than native tetraploids did (Figure 4). This could have fueled the rapid spread in range and ability of species to colonize invaded areas with increased competitive ability. Not all researchers fully support this hypothesis. Research reveals mixed results for this theory. Some have found that invasive populations grow larger (Blossey & Notzold, 1995; Joshi & Vrieling, 2005) while others are smaller (Bossdorf et al., 2004) However, introduced populations on average do have significantly larger plants compared to native conspecifics over a range of different species (Colautti & Lau, 2015).

Novel Weapons Hypothesis

Within the broad category of rapid evolution hypotheses, there is another theory for how invasives are able to take over in novel environments. The novel weapons hypothesis offers an alternate rapid evolution hypothesis that varies from evolution of increased competitive abilities and release from natural enemies proponents. The novel weapons hypothesis describes the proposed allelopathic capability of invasive species to release phytotoxins into the soil to displace native species in their vicinity (Bais et al., 2003). This is explained as a means of decreasing the threat of competition from other plants rather than outcompeting them.

The phytochemical used by spotted knapweed is negatively charged racemic catechin, which it exudes from its roots (Bais et al., 2003). To test the effects of this phytochemical, Bais et al. (2002) extracted negatively charged racemic catechin from invasive spotted knapweed and added it to the soil of North American crop plants and weeds such as tomatoes, wheat and diffuse knapweed. The response and

germination capacity of the North American species were measured. All of the North American plants tested died within fourteen days after the racemic catechin was added. In a later study, Bais et al. (2003) nailed down the details of how catechin kills competitors. The toxin stimulates root reactive oxygen species, starting a calcium signaling cascade in the targeted plant that interferes with gene expression and ultimately leads to root system death. To further test the novel weapons hypothesis to see if invasive spotted knapweed differed from Eurasian spotted knapweed, Bais et al. (2003) compared concentrations of racemic catechin in the soil around spotted knapweed roots in Europe versus North America. Results indicate that concentrations of these phytotoxins are over two times greater in invaded areas than home ranges. Further, when grown in pots of negatively charged catechin, native European grasses are more resistant than North American native grasses; their germination and growth are not as affected, demonstrating that the toxins and defenses coevolved in the spotted knapweed's native area and the native North American plants are ill-prepared to survive the allelochemicals.

Perhaps the two hypotheses previously discussed can be simultaneously true. Callaway and Ridenour (2004) explain the connection between novel weapons hypothesis and EICA, and discuss that the possession of novel weapons may well function as a mechanism for EICA. The authors explain that the advantageous trait of having a novel weapon in an invaded area can provide invasives with a competitive advantage over native species that will then be selected on to improve fitness and allow the invasive to grow and reproduce more successfully. Basically, possessing a novel weapon can lead to the rapid evolution of that weapon or chemical defense. Callaway and Ridenour term this mechanism "allelopathic advantages against resident species" (p. 441).

Stress-induced modification of the genome

A newer and less studied hypothesis of rapid evolution during plant invasion is that when invasive species encounter a novel environment and attempt to colonize it, the invasive species are under a lot of stress. This environmental stress has been shown to decrease the stability of the genome in some invasive species (Prentis et al., 2008). This stress can alter the genome and transcriptome resulting in mutations that may lead to new phenotypes. This can work in an invasive plant's favor because increasing

phenotypic variation within a population gives selection more to act on, potentially facilitating rapid evolution. Although evidence of this genome destabilization has been documented in economically important crop plants such as flax and rice (Prentis et al., 2008), future research should be conducted to find evidence for stress-induced modification of the genome in Centaurea stoebe. While providing examples of rapid evolution in invasive species is important, knowing the rate and direction of adaptive evolution would help us in planning more successful invasive control strategies (Muller-Scharer, Schaffner & Steinger, 2004).

PRE-ADAPTATION HYPOTHESES

Pre-adaptation hypotheses represent the second major school of thought concerning invasion success. Proponents of this hypothesis would argue that the invasive plants do not undergo rapid evolution, they simply have pre-existing characteristics that confer them success in novel areas.

Effect of climatic niche on invasion

Research has given credibility to the idea that invasive species can be pre-adapted or predisposed to their new ranges based on their climatic niche. "Climate-matching" refers to this approach of predicting the invasive range of an introduced species by considering the constraints of their ecological niche to be long-term and stable (Peterson, 2003). The basic concept of niche is used in this hypothesis to assume that invasives will establish best in areas that have comparable ecological conditions to the areas they are confined to in their home range. Models have been developed that predict the range and distribution of invasive plants with high accuracy (See Figure 5 for the technique; Peterson, 2003). Proponents of this theory would say that invasive plants are pre-adapted to their invaded areas because they are following ecological distributions based on their native range.

It has in fact been established that spotted knapweed thrives best in regions with climatic conditions similar to those in the native niche (Broennimann et al., 2014). By using climate-matching techniques and comparing the actual range of *Centaurea stoebe* to the predicted range in North America, it was determined that the range was as expected based on the native occurrence in Eurasia. Further studies have suggested that there are slight niche differences between the European tetraploid and diploid.

There is increased drought tolerance in European tetraploid plants compared to European diploid plants which could explain why only the tetraploid has become successful in North America, supporting the pre-adaptation hypothesis. Enhanced tolerance to drought may give tetraploids a selection advantage in Europe which could have aided in their invasion of the U.S (Mraz, Tarbush & Muller-Scharer, 2014; Treier et al., 2009). Tetraploid plants showed thicker leaves and reduced specific leaf area compared with diploid plants grown in a greenhouse experiment (Mraz, Tarbush & Muller-Scharer, 2014). Proponents of this theory suggest that by being pre-adapted to a drier range, invasive tetraploid spotted knapweed was able to occupy a similarly dry niche in North America.

Phenotypic plasticity

Phenotypic plasticity, the ability of a single genotype to express a range of phenotypes in response to different environmental conditions, confers many organisms the ability to adapt well to new environments. Since invading novel territory is key to an invader's success, phenotypic plasticity may play an especially crucial role in invasive establishment when the invaded range selects for a more generalist, plastic strategy (Turner, Freville, & Rieseberg, 2015). Mraz, Tarbus, and Muller-Scharer (2014) provide evidence that disturbed, invaded ranges may select for more plastic genotypes (Figure 6). To test if the invasive tetraploid was pre-adapted to non-ideal drought conditions in the American northwest because of greater phenotypic plasticity, plants from three *Centaurea stoebe* geocytotypes (diploid, European tetraploid, N. American tetraploid) were analyzed in response to three conditions of varying water availability. Results revealed that the European tetraploid was less affected by drought than the diploid cytotype was. The European tetraploid actually grew better in some morphological characteristics than the N. American tetraploid, supporting a pre-adaptation hypothesis. The North American tetraploid, though, had the highest levels of phenotypic plasticity, in line with what is required of colonization.

In a different experiment done by Hahn, van Kleunen, and Muller-

Scharer (2012) demonstrated increased levels of phenotypic plasticity in traits associated with rapid growth and development such as lead area, number of shoots, and overall biomass in European tetraploids compared to European diploids

when the cytotypes were grown in a common garden experiment. Further, they showed plasticity to be adaptive. This may have helped the tetraploid become successful in invasion and not the diploid. This study did not find significant increases in plasticity in North American tetraploids over their European conspecifics, supporting the pre-adaptation theory.

Life history hypotheses

Evolving adaptive and optimal life history traits is crucial to an individual's fitness in a given area (Hahn & Muller-Scharer, 2013). Therefore, some researchers have suggested that the utilization of optimal life history strategies gives invasives a selective advantage in invaded ranges. Recall that only tetraploid spotted knapweed is found in North America even though distributions are about even in the home range. This may suggest that tetraploids have more favorable life history strategies than diploids do in the North America ecosystems. Interestingly, Prentis et al. (2008) has commented that polyploids have been shown to occur more frequently in invasive plants than among angiosperms in general. Further, among invasive species, Lee (2002) noted that polyploid hybrids have been shown to have greater fitness than diploids. In other common invasive plants such as sweet vernal grass, the tetraploid cytotype possesses higher fitness and competitive ability in a range of environments and is more successful at colonization (Prentis et al., 2008).

Recall that spotted knapweed polyploids are short-lived, perennial polycarps, as opposed to the diploids which are annual/biennial monocarps. A monocarpic strategy has been shown to be favorable when herbivory affects survival, since herbivory decreases gains from future reproduction (Muller- Scharer, Schaffner & Steinger, 2004). It makes sense that this strategy might be favorable in the native range of spotted knapweed that is full of specialist herbivores. However, the invasive North American cytotype has a polycarpic strategy and fruits many times in its lifetime. Muller-Scharer, Schaffner, and Steinger (2004) propose that this trend is explained by removal from specialized natural enemies that can kill plants that overwinter after seed set. It is favorable to put more effort towards future reproduction when there is no survival threat from specialist herbivores. The ability for tetraploids to be able to produce seeds longer than the diploids most likely helped the tetraploids become dominant in North America (Treier et al., 2009). Broz et al. (2009a) found that due to this polycarpic strategy, North American C. stoebe tetraploids have greater fecundity than diploids do (See Figure 7). Since tetraploid cytotypes do not direct all their resources to one big reproductive event, they have been shown to have a competitive advantage over the diploid cytotypes, which is revealed in their superior growth in dense vegetation (Broz et al., 2007).

Hybridization

Research shows that hybrid plants may have inherent benefits for colonization. Blair, Blumenthal, and Hufbauer (2011) studied the colonization advantages of hybrids. They concluded that simply having DNA from more than one parental species could explain the superb invasive qualities of some species. As has already been clearly established, when invasives colonize a new range, they encounter novel species and conditions. Having increased amounts of phenotypic variation from two parental species can help an invader cope with these new conditions. Adding further support to this theory, Blair, Blumenthal, and Hufbauer (2011) mention that *C. stoebe* only became invasive after hybridizing.

Furthering this line of research with *Centaurea stoebe* specifically, Mraz et al. (2012) conducted a molecular study using phylogenetic and network analyses of over 40 samples of populations of C. stoebe and related groups to trace the polyploid cytotype back to its origin. Results indicated with high certainty that the tetraploid spotted knapweed is an allopolyploid, resulting from a hybridization of two species. Polyploidization can increase genetic variation by increasing the allelic combinations per locus (Mraz et al., 2012). Therefore, the hybridization and polyploidization history of C. stoebe may contribute to its invasion success by increasing its adaptation potential by offering more variation up to favorable selection.

FUTURE RESEARCH

Means of invasion have been thoroughly studied, and it is clear that invasive *Centaurea stoebe* uses multiple techniques to establish dominance in invaded areas. In their review paper, Colautii and Lau (2015) point out that few studies measure the specific effect of invasive species on the native species in invaded areas. I will analyze native plant communities in the presence and absence of invaders in areas with similar population structures. I hypothesize that in areas with invasive species, native species will be experiencing selection from the introduced invasive *Centaurea stoebe* that favors traits related to their competitive ability. The ability to predict the future impacts of invasives in native communities will help conservationists plan biological control techniques more specifically and effectively.

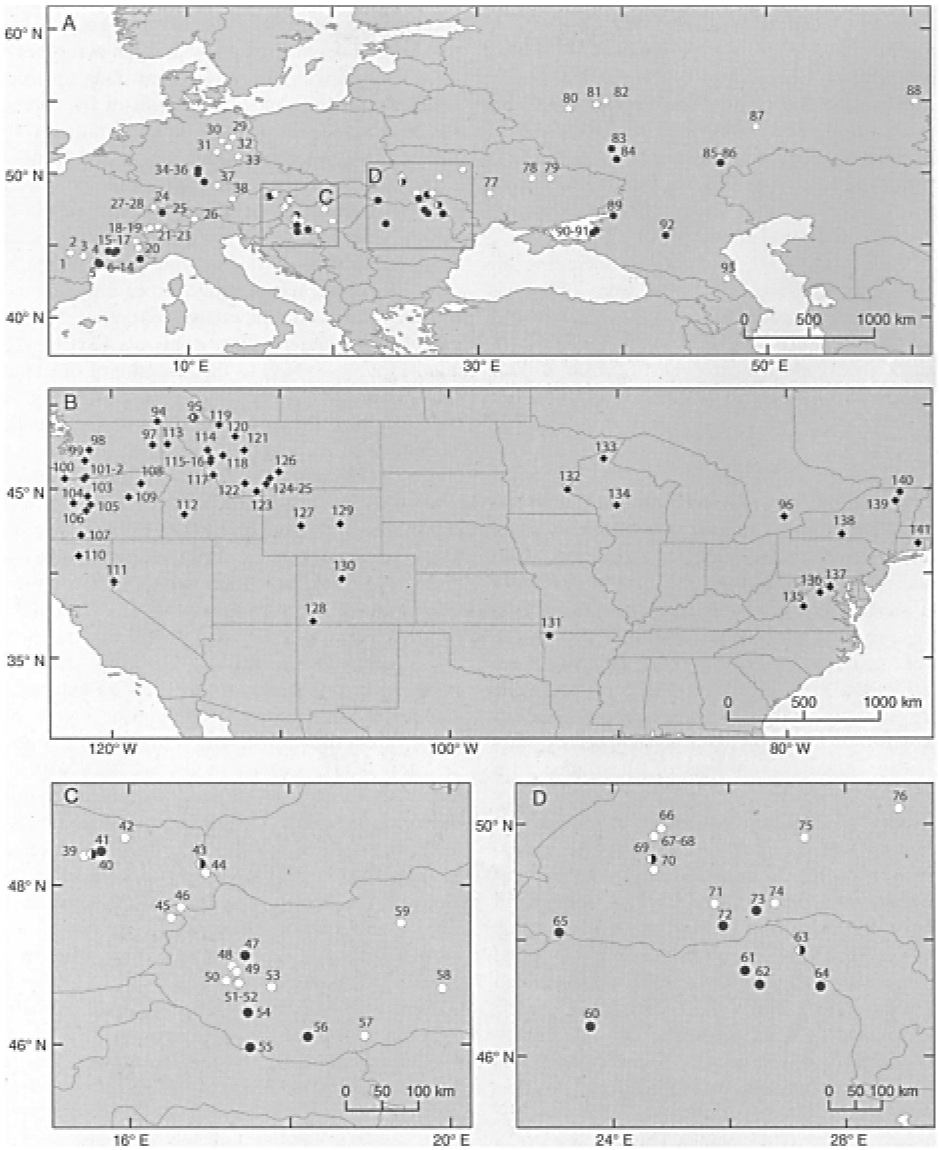

Figure 1. Open symbols represent diploid cytotypes, while closed symbols represent tetraploids (A) Distribution of a mixed-cytotype population of *Centaurea stoebe* in its home range. (B)Distribution of *Centaurea stoebe* in its invaded range in the United States showing that only tetraploids are present. (C&D) Closer view of mixed cytotype populations in Europe (Treier et al., 2009).

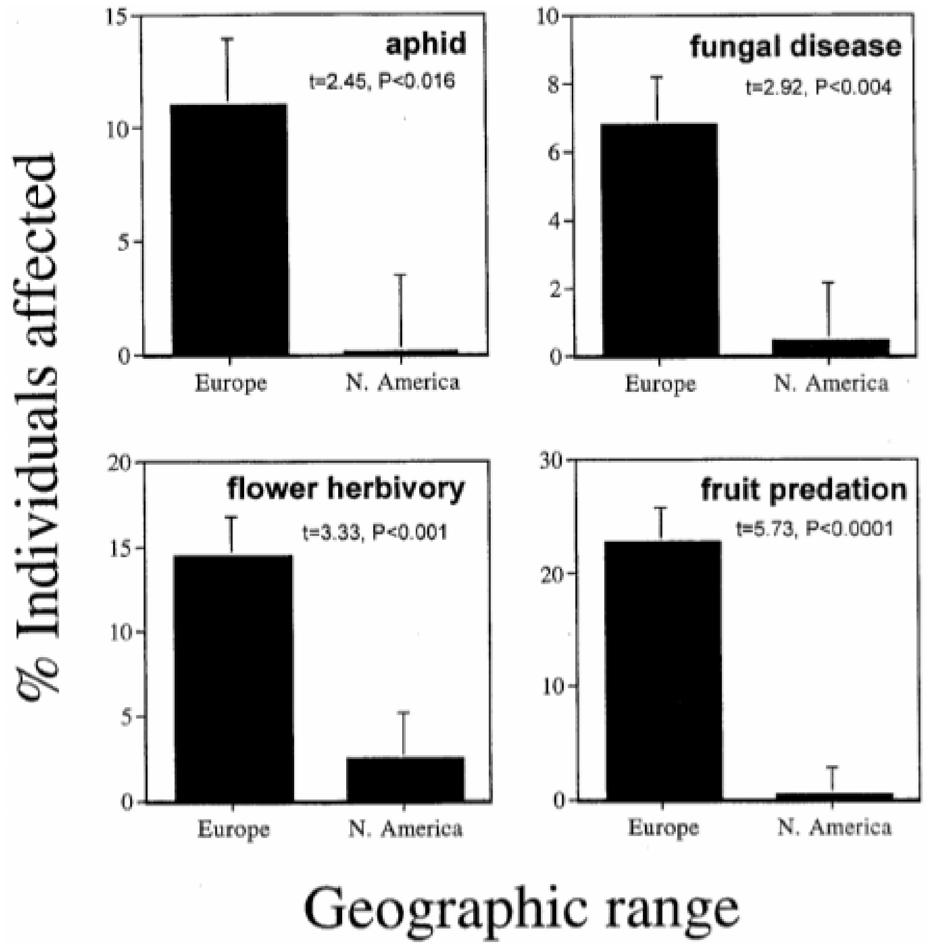

Figure 2. Samples taken from populations of the herbaceous invasive plant *Silene latifolia* (bladder campion) from both its European home range and North American invaded area. Herbivorous attacks on the phloem (from aphids), flowers, and seeds/fruit as well as fungal infection were measured. The majority of the home range *S. latifolia* suffered herbivory from more than one type of enemy. Plants in Europe were 17 times more likely to suffer herbivory damage, from both generalists and specialists (Wolfe, 2002).

Table 1 Mean (± SE) dry biomass and height at the end of the growing season (*N* = 20 replicates per group) of purple loosestrife plants from Ithaca (US) and Lucelle (Switzerland). Plants were grown in a common garden under identical conditions

	Ithaca	Lucelle	*P**
Dry biomass (g)			
1991	29.3 ± 1.9	16.3 ± 1.4	< 0.001
1992	96.0 ± 12.3	28.7 ± 4.3	< 0.001
Plant height (cm)			
1991	99.7 ± 4.4	82.6 ± 2.0	0.001
1992	177.8 ± 4.8	109.7 ± 7.4	< 0.001

*Probability value (*t*-test).

Figure 3. To test an invasive plant's response to decreased natural herbivory, Blossey and Notzold (1995) compared the growth of *Lythrum salicaria* (purple loosestrife) in areas with and without its natural herbivorous enemies. The plants without natural herbivory were shown to grow significantly taller and experience more vegetative growth.

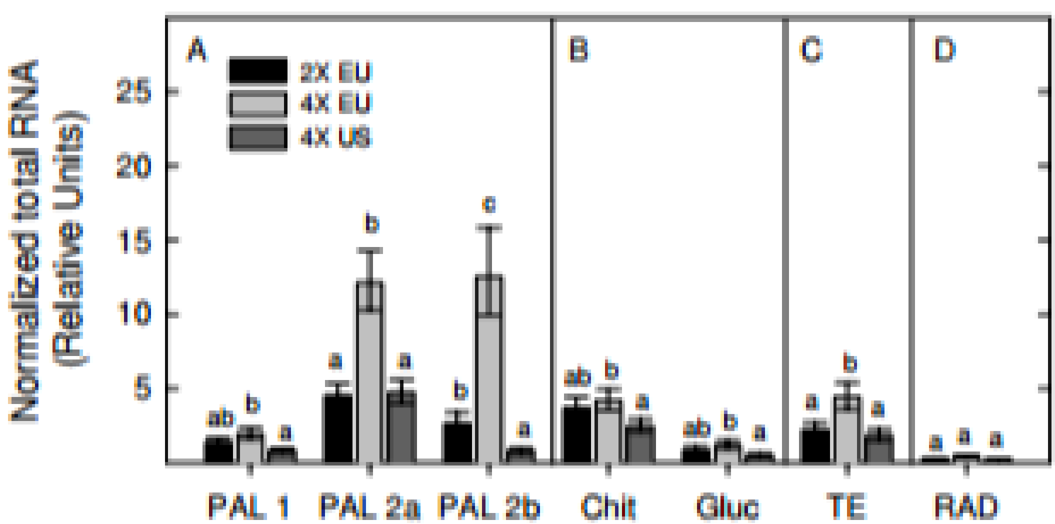

Figure 4. Seeds from *C. stoebe* diploids, European tetraploids and invasive tetraploids were grown in a common garden greenhouse. Quantitative PCR was used to see patterns of gene expression related to defense and other plant performance areas. At the molecular level, Broz et al. (2009a) showed that invasive populations of tetraploid spotted knapweed showed less expression of gene transcripts related to defenses than native tetraploids did (Broz et al., 2009a).

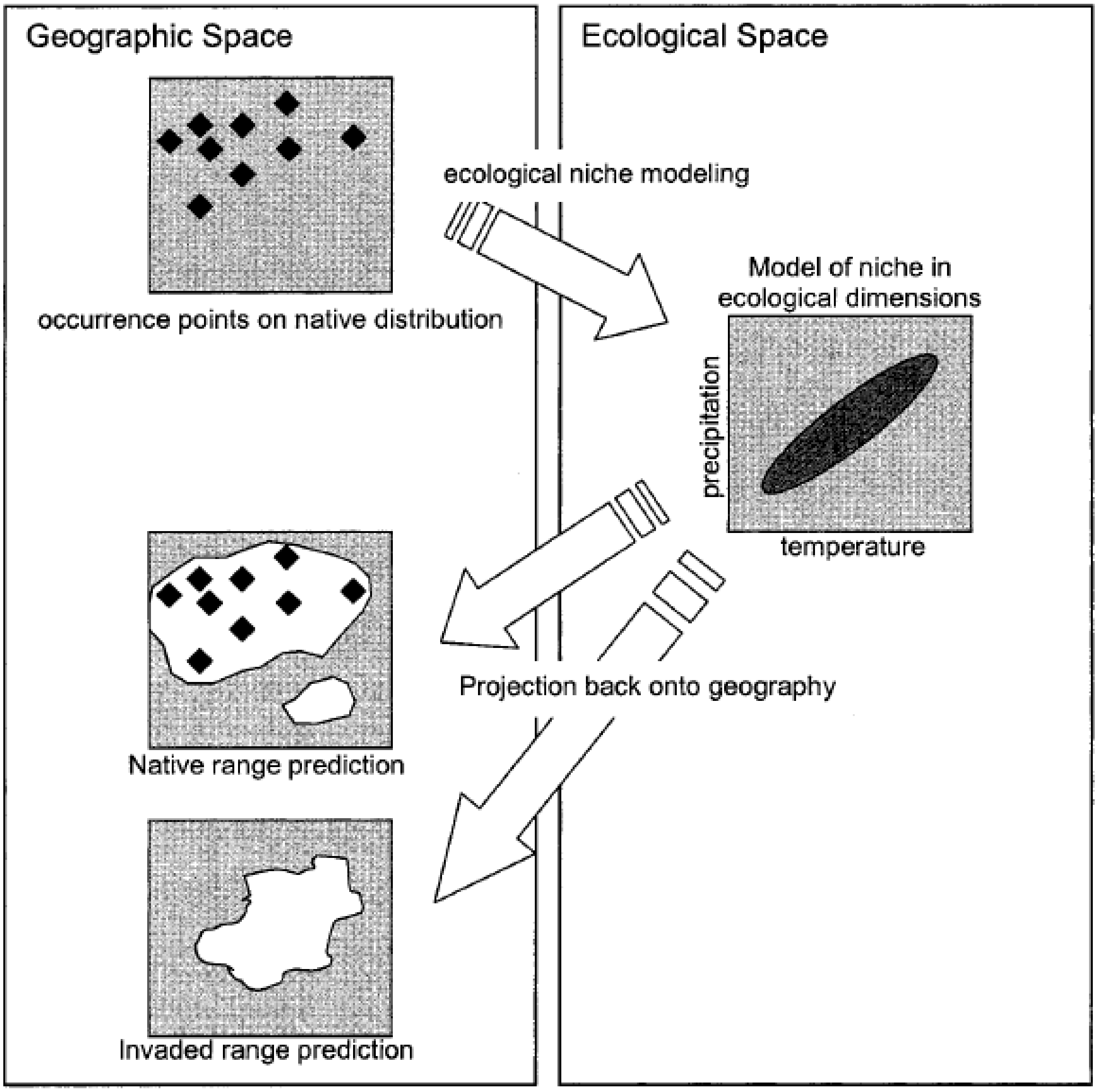

Figure 5. This diagram shows the technique of niche modeling to predict the range of species' invasion (Peterson 2003)

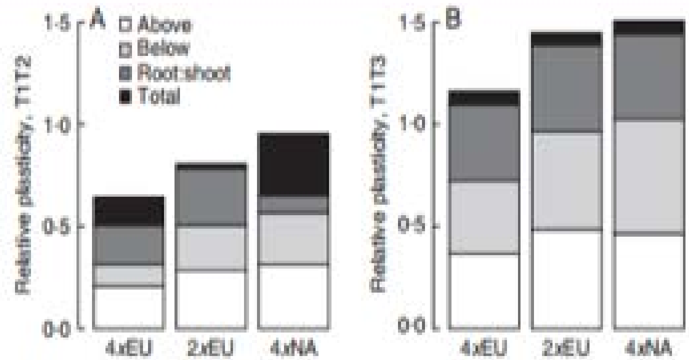

Figure 6. Relative plasticity indices for biomass of *Centaurea stoebe* below ground, above ground, in the roots and shoots, and total. (A) Growth under control versus moderate water stress (B) Growth under control versus severe water stress. (Mraz, Tarbush, & Muller-Scharer, 2014).

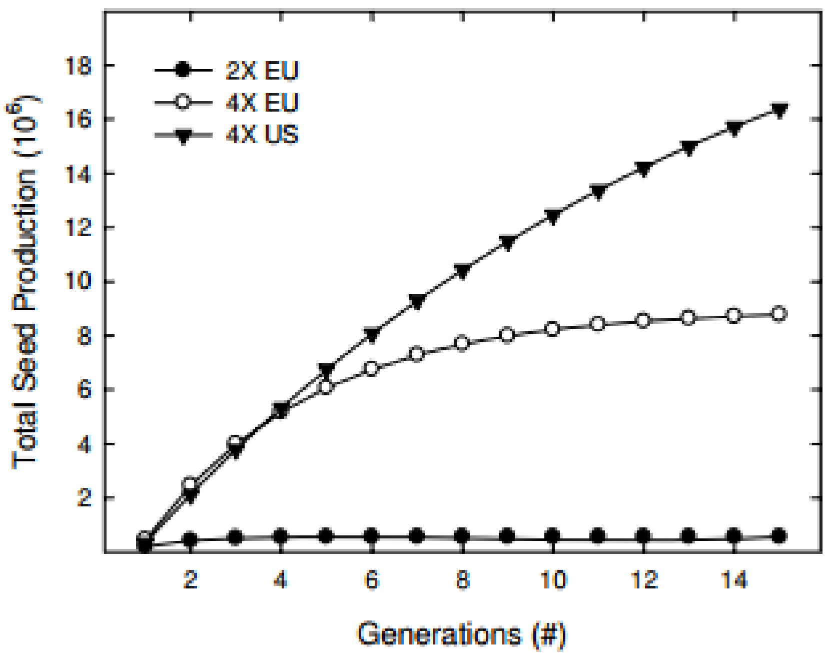

Figure 7. Seed production characteristics were measured in a common garden experiment. This model represents a prediction for 15 generations at measured production rates. (Broz et al., 2009a).

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