DISSERTATION

VIABILITY AND INVASIVE POTENTIAL OF HYBRIDS BETWEEN YELLOW TOADFLAX *(LINARIA VULGARIS)* AND DALMATIAN TOADFLAX *(LINARIA DALMATICA)*

Submitted by

Marie F.S. Turner

Department of Soil and Crop Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 2012

Doctoral Committee:

Advisor: Sarah Ward

Christopher Richards
David Steingraeber
George Beck
Sharlene Sing

ABSTRACT

VIABILITY AND INVASIVE POTENTIAL OF HYBRIDS BETWEEN YELLOW TOADFLAX *(LINARIA VULGARIS)* AND DALMATIAN TOADFLAX *(LINARIA DALMATICA)*

Although outcomes of hybridization are highly variable, it is now considered to play an important role in evolution, speciation, and invasion. Hybridization has recently been confirmed between populations of yellow (or common) toadflax (*Linaria vulgaris*) and Dalmatian toadflax (*Linaria dalmatica*) in the Rocky Mountain region of the United States. The presence of hybrid toadflax populations on public lands is of concern, as both parents are aggressive invaders already listed as noxious weeds in multiple western states. A common garden experiment was designed to measure differences in quantitative (shoot length, biomass, flowering stems, seed capsule production) phenological (time of emergence, first flowering and seed maturity) and ecophysiological (photosynthesis, transpiration and water use efficiency (WUE)) traits for yellow and Dalmatian toadflax, F1 and BC1 hybrids, as well as natural field-collected hybrids from two sites. Genotypes were cloned to produce true replicates and the entire common garden was also replicated at two locations (Colorado and Montana); physiological data were collected only in Colorado. All genotypes grew larger and were more reproductively active in Colorado than in Montana, and hybrids outperformed parent taxa across vegetative and reproductive traits indicating heterosis. Hybrids also emerged earlier, but did not flower or set seed sooner than parent taxa, and all genotypes set seed more quickly in Montana than in Colorado indicating a strong environmental influence on this trait. There were indications that for some traits, yellow toadflax alleles conferred a relative advantage in Montana and Dalmatian toadflax alleles conferred a similar advantage in Colorado. Natural hybrids collected from Montana had higher rates of overall germination than any other class, suggesting selection for transgressive germination; they also emerged earlier in the Montana common garden suggesting possible local adaptation. Aside from these indications of GxE , general patterns of genotypic class performance remained relatively consistent across sites. Ecophysiological patterns were intermediate: yellow toadflax had the highest rates of photosynthesis and WUE; Dalmatian toadflax had the lowest rates of these two metrics and hybrid rates were distributed between them. Plants with higher rates of photosynthesis and transpiration reached phenological stages earlier than those with lower rates. For natural hybrids, the direction of the correlation between ecophysiological traits and quantitative and phenological traits changed depending on when the physiological data was collected.

Overall, results indicate most toadflax hybrids may have increased fitness relative to their parents as well as multiple phenotypic attributes which may enable them to expand and invade. However, which of these specific genotypes become invasive will also depend on the direction of crossing and location at which hybridization occurs. Given the observed heterosis, transgressive trait expression, a lack of other apparent phenotypic shortcomings and the potential impacts of hybridization on current mechanisms of control, known hybridization sites, as well as other locations where yellow and Dalmatian toadflax are co-invading should be prioritized for management.

TABLE OF CONTENTS

Chapter One: Literature Review

Ecology and Genetics of Invasive Species:

Whether he knew it or not, C.S. Elton's fundamental 1958 book, "The Ecology of Invasions by Animals and Plants" would help to trigger a scientific landslide. This prophetic work, coupled with a globalized network capable of moving species (deliberately or not) around the world has initiated a deluge of research which has sought to address the problem of invasion. Economic and ecological impacts of invasions are staggering (Pejchar and Mooney, 2009) and so have sparked to life an entire field of study which is ultimately a multi-disciplinary integration of diverse fields of natural science. We now study invasions as real-time experiments in community and species ecology and evolution, we examine them from the sky with geographic information systems, we model them statistically, mathematically and spatially, and we trace them historically through shipping logs and herbarium specimens and genealogies. We measure invaded environments, and we measure invaders, in every way we can think of- and this, in and of itself is becoming a discipline within the field of invasion biology.

In particular, there is extensive and ongoing discussion in the literature about which traits make species prone to become invasive. Much of this discourse goes back to the work of H.G. Baker (1974), who made one of the earliest explicit lists of "weedy" traits, and although modern inventories of such characters are certainly expanded and more nuanced, the spirit of much invasion biology research remains linked to this list. A seminal 1965 symposium volume edited by Baker and Stebbins was entitled "Genetics of Colonizing Species". This tome offered a first summary of how genetics and evolution might impact movement, establishment and expansion of species around the globe. This recognition of the importance of process has helped invasion biology transition away from treating invasive organisms and invaded environments as fixed entities to be examined at single points in time. Researchers are increasingly aware that invasion is not a singular pattern or event, but that post-introduction, both species and colonized habitats continue to change. This, in turn, has resulted in the realization that rapid evolution of introduced species impairs ability to directly compare benign-native and introduced-invasive behavior and that it may be rapid evolution itself which confers invasive properties. The patterning of traits across invasive and non-invasive species does not necessarily become any less important as a component of analysis, but such patterns must be considered dynamic and as existing in equally dynamic spatial and temporal networks. The goal of this section is to provide an overview of plant qualities related to invasion and also a description of how they change across circumstance. Aspects of species' phenotypes and biology which may contribute to their ability to colonize and subsequently invade are discussed (pattern), and linked to population and evolutionary scales (process).

Pattern: What does an invasive species look like?

Baker's list of "weedy" traits certainly contained some discrete characters such as "not easily drawn from the ground" or "pollinated [by] unspecialized visitors or wind" (Baker, 1974). However, his list was quite farsighted in so far as it also addressed environmental contingencies for many traits, for instance "produces some seed in a wide range of environmental conditions" or "continuous seed production for as long as growing conditions permit". Conditional formulations are perhaps the best way to look at any trait if we truly want to understand its relevance to invasions because phenotypes are never independent of the environments in which they originate, travel and establish. However, any model seeking to predict a mosaic of characters resulting in species invasion must begin by defining its parameters, and so the following section attempts to do. The focus here is less upon qualities which make species subject to initial translocation, and rather upon which qualities encourage colonization and expansion, although many of these are intimately linked.

Reproduction:

No single or suite of traits has been found to be consistent across invasive taxa, but certain characters do appear in many case studies and are often related to growth and reproduction by both sexual and asexual means. However, to reach the levels of reproduction which facilitate invasion, the qualities of an organism's reproductive biology which enable it to gain purchase where it lands must be considered. A key trait on Baker's 1974 list is a capacity to self-fertilize. While the link between colonization and self-fertilization is intuitive, numerous invasive species are obligately outcrossing, so additional mechanisms are required. Often, no such limitation exists because more than a single propagule is introduced, and some colonists are able to hybridize with native congeners (this will be discussed further in the "process" section). Asexual reproduction, either by fragmentation or apomixis, can also alleviate initial needs of colonists for demographic increase. Expansion or persistence by rhizome, bulbil or other vegetative propagule coupled with sexual reproduction is particularly powerful as seen in *Butontus*, an aquatic plant native to Europe. In its home range, the species is largely triploid, and limited sexual reproduction takes place; however, due to an apparent founder effect in the invaded American range, populations are mostly diploid and fully capable of both vegetative and sexual reproduction which has perhaps facilitated invasion (Kliber and Eckert, 2005).

With either mode of reproduction, both fecundity and dispersal must be taken into consideration, rather, how many, how far and in what manner propagules can travel. Short-distance dispersal may be important to establish a population, but modeling by Neubert and Caswell (2000) suggests it may be long-distance dispersal, even infrequent, which governs the rate of invasion. Therefore, it is likely a combination of dispersal distances (long and short) which ultimately govern in-site invasion and expansion off-site; this is supported by Levin et al. (2003) who showed that even though mean dispersal distances are important, it is the distribution of possible distances which influences how fast ranges expand. As the quantity of viable seed or asexual fragments a plant makes increases, the propagule pressure within and perhaps across locations is expected to increase. Propagule pressure has been demonstrated to be one of the consistent drivers of invasions (Daehler, 2006; Maron, 2006; Pauchard et al., 2009; Pysek et al., 2009; Richardson and Pysek, 2006; Von Holle and Simberloff, 2005; Williamson and Fitter, 1996) and total seed production and viability as measured by germination have both been shown to be significantly correlated with invasiveness (Colautti et al., 2006).

In addition to influencing asexual reproduction, vegetative growth may also be an important indicator of competitive ability. The size and functional traits of a plant's vegetative architecture can be integral to its ability to capture resources in a heterogeneous and competitive community environment. Abundant biomass is often associated with increased invasive abilities e.g. (Flory et al., 2011; Zheng et al., 2009) as it not only provides a spatial advantage when vying for light and other resources, but is also implicated in physical displacement of other habitat occupants e.g. (Molina-Montenegro et al., 2012). On the other hand, maintenance of large plant structure is likely to require substantial resources, and if no plastic mechanism is in place during times of stress, greater size may actually be a maladaptive phenotype as suggested by He et al. (2010). These authors examined invasive *Centaurea*, and found that the largest plants under good circumstances grew most slowly when times were hard. Similar tradeoffs between reproductive capacity and other traits are quite likely given genotypic and resource limitations and are discussed more extensively in the following sections.

Phenology and Growth:

In addition to the idea that biggest is not necessarily best, the previously cited study (He et al., 2010) also suggests extensive tradeoffs among quantity, duration, and speed of plant productivity as well as with the resources necessary to meet minimal versus optimal requirements for growth. For instance, there is speculation that some invaders use a strategy of early germination and/or emergence followed by rapid growth; these plants have been termed “gap grabbers” (Newsome and Noble, 1986). Such a species is thought to have an advantage of preemptive resource capture and avoidance of late season stress via early life cycle completion. However, just as large is not always adaptive, early emergence and completion may not be beneficial in every environment. Furthermore, the rate at which a plant is able to grow and reach phenological landmarks will depend strongly on availability of resources as well as ability to capitalize under differing conditions.

Environmental variability makes universal optimum phenological strategies impossible, but so does an often distinct negative association (tradeoff) between plant size and time to reproductive maturity as observed in weedy *Raphanus* by Campbell et al. (2009) or in *Lythrum* by Colautti and Barrett (2010). The implication is that even if earlier reproductive completion might seem adaptive, stronger selection for fecundity or plant size may constrain evolution of phenological shifts. In contrast, if harsh environments promote adaptive quick completion of reproduction, selection for smaller plants may be concurrent with a shorter life cycle; though a meta-analysis by Kingsolver et al. (2001) suggests morphology tends to be under stronger selection than phenology. That said, it is difficult to generalize across environments, and since phenology often demonstrates strong association with latitude, it may be this selection which exerts more consistent influence across species. Additionally, matching of latitude between home and introduced ranges is a reiterated characteristic of invasions (Lonsdale, 1999; Maron, 2006), and there are documented cases of rapid evolution of phenological clines in novel ranges e.g. (Colautti et al., 2010; Dlugosch and Parker, 2008b). However, it also seems possible that phenological differences of natives and colonists might in some cases enable the latter to capitalize on underexploited abiotic (i.e., nutrients, light, space, water etc.) and biotic (i.e., pollinators, facilitators) resources.

Physiology:

Plasticity of physiological traits is often suggested as a mechanism by which introduced plants may establish (Nicotra and Davidson, 2010; Schlichting and Levin, 1986). However, a recent meta-analysis by Dawson et al. (2012) suggests it is not so much a multi-directional plasticity facilitating widespread distribution and abundance of species, but rather an ability to make use of resources under favorable conditions. These authors forge the connection between resource use and species abundance using phenotypic characters related to resource capture (i.e. specific leaf area (SLA) and root-to-shoot ratio). Another way to examine the relevance of resource capture to distribution and abundance (or invasion) is by measuring physiological processes which underlie invasive abilities; a tactic becoming increasingly common in the invasive-trait literature. Since photosynthesis is the primary mechanism of carbon assimilation in plants, it is one such ecophysiological trait receiving increasing scrutiny. Although in some cases it appears invaders demonstrate superior photosynthetic performance relative to natives, for example, *Phragmites* (Mozdzer and Zieman, 2010) and *Eupatorium* (Zheng et al., 2009), other research finds no difference in ability to capture carbon e.g. (Brodersen et al., 2008; Smith and Knapp, 2001). It may not be surprising that the relationship between invasion and photosynthetic rate is inconsistent; data are often noisy and also prone to change across environment, season, developmental stage and leaf architecture and form. There is also evidence to support extensive genetic variation of photosynthesis even within species (Arntz and Delph, 2001; Flood et al., 2011). A lack of distinct ecophysiology for invasive species may also not be remarkable: Dawson et al. (2012) noted the importance of the ability to capitalize in the context of plentiful resources, but also noted that under this scenario, observed magnitude of the physiological process will depend strongly on the environment in which it is measured. A study by McAlpine et al. (2008) exemplifies this principle. This research showed that under high light, invasive *Berberis* was twice as photosynthetically active than its non-invasive congener, but under lower light, there was no difference between them. The observation that a very specific type of plastic response may further widespread distribution and abundance of species, makes sense in the context of recent theory proposing that beneficial plasticity of traits under selection can be genetically incorporated into populations (Pigliucci et al., 2006; West-Eberhard, 2005). There may also be specific directional selection on plastic traits rather than selection for a general magnitude of plasticity, highlighting just how important real-time acquisition responses to resources may be for future evolution of plant populations.

It is quite possible similar selection pressures will act upon other physiological characteristics such as drought tolerance. However, a problem with measurement of broad phenotypic characters is that often multiple mechanisms may create the same phenotype and may co-vary in different ways with plant architecture and environment. Additionally, there are tradeoffs among mechanisms, such as between photosynthesis and transpiration which are physically coupled via the stomata. This structural association makes it difficult for plants to acquire carbon without subjecting themselves to water loss. Such a coupling is likely to result in reciprocal evolutionary constraints on opposing physiological processes unless other mechanisms of carbon capture or wilting avoidance are at play (such as the 'turgor loss point' suggested by Bartlett et al. (2012). An evolutionary tradeoff between carbon acquisition and water loss is described in a recent study by Hodgins and Rieseberg (2011). These authors demonstrated that while introduced *Ambrosia* increased growth and reproduction in most benign environments and even under certain stressors (light, herbivory and nutrient), they also wilted and died more quickly under water stress. Likewise, Matzek (2011) found non-invasive *Pinus* had higher water use efficiency (WUE) than a faster growing invasive congener. These data seem to suggest plants must choose between strategies of conservation and growth. However, as touched upon earlier, measured ecophysiological processes will co-vary not only with functional type and leaf construction (Reich et al., 1999) but also across environmental conditions and developmental stages and should therefore be only cautiously and contextually interpreted as adaptive traits.

Chemistry:

Despite the fact that plants are constantly in contact with other organisms including plentiful microbial life, only a few associations ever become severely antagonistic or pathogenic (Bednarek et al., 2010). One reason is that plants produce a dazzling array of secondary metabolites which mediate diverse biotic interactions including resistance to herbivores and pathogens, attraction of pollinating organisms and both beneficial and antagonistic interactions with other plant and animal life. Cappuccino and Arnason (2006) compared invasive and non-invasive exotic plant chemistry and found invasives were far more likely to contain novel (relative to native flora) secondary compounds important in regulating relationships with herbivores, fungi, microbes or other plants. The inference is that these chemical differences may be a reason why some exotics become invasive and others do not. For instance, resistance to detrimental organisms in its new environment may be one result of novel chemistry: if colonists are immune to organisms which affect other community members, they may gain a window to establish and/or expand. Chemistry can also serve to attract rather than repel, and facilitation of mutualistic host shifts is another way novel secondary chemistry may play out; that is, if a beneficial organism prefers the chemical profile of an invader, it may alter its behavior. This may affect pollination dynamics, fruit/seed consumption and subsequent dispersal, or symbioses such as with soil mycorrhizae. In contrast to advantageous soil relationships, certain plants produce chemicals (typically root exudates) which are antagonistic to other plants and organisms and therefore further invasion, though they may have primary roles within the plant as well (Inderjit et al., 2011). This production of chemicals which suppress growth in competitors is known as allelopathy and has been suggested for some *Fallopia* (Murrell et al., 2011) and *Centaurea* (Pollock et al., 2009) invasions. However, allelopathy is methodologically difficult to confirm, and a summary study by Lind and Parker (2010) finds that while “deterrent chemistry” does occur in some invasive species, it is not a particularly broad motif.

A final note in this brief discussion of secondary chemistry is that there is much discussion in the literature of the “EICA” (evolution of increased competitive ability) hypothesis, proposed by Blossey and Notzhold, (1995). This theory states that there exist tradeoffs within organisms between defense compound production and other traits such as growth rate, and that introduced species are able to reallocate resources to growth which would otherwise be devoted to defense in the native range. As discussed above, evolutionary constraint among traits is likely to cause tradeoffs (such as that proposed by EICA) and so it seems reasonable to expect that in some cases, plants have been able to invade because they are not as busy defending themselves. However, there is no reason to expect this particular tradeoff with any more consistency than others, and in fact, as with many so-called unifying theories of invasion, it appears EICA is plausible in some cases, but not proven empirically.

Process: How do Genetics Affect Invasion?

It is widely accepted that genetic variation enables populations to adapt to changing environments. It has also been suggested that rather than any one particular trait, it is this variation which has enabled some colonizing species to rapidly evolve in response to natural selection and become invasive in novel environments (Lee, 2002). It is less intuitive how ample variation can be repeatedly gained across invasions in which migrants often have both demographic disadvantage and reduced gene pools due to a transit bottleneck (commonly referred to as a “founder effect”). On a geologic time scale, mutation makes the most significant contribution to genetic variation, but it is unlikely it will do so on the shorter time scale of colonization (Prentis et al., 2008). Tolerance of variable conditions and/or plasticity are other ways which species persist and increase in abundance despite low initial numbers and absence of variation; although there is not necessarily a clear distinction between heritable variation and plasticity (Pigliucci et al., 2006; West-Eberhard, 2005). So, if genetic variation is often greatly reduced in the initial phase of establishment, how do colonists overcome this obstacle?

As previously mentioned, self-fertilization is a mechanism by which population amplification is possible in absence of large numbers of colonists, but will do little to increase genetic variation and alleviate risk of future inbreeding. Similarly, some invasive species achieve vast populations via vegetative reproduction, as in the case of *Eichornia* (Ren and Zhang, 2007). However, this may be an extreme case and colonist abundance is often not quite so limiting. In fact, as has been previously mentioned, the intensity of incoming propagule pressure is often linked with the severity of invasion (Von Holle and Simberloff, 2005; Colautti et al., 2006). Some argue (e.g. Prentis et al., 2008) that even with moderate propagule pressure and limited variation, novel exposure to variable or disturbed environments may result in enough diversifying selection to increase additive variance and enable rapid evolution of an aggressive population. Propagule pressure is often referred to as the number of reproductive entities (i.e. seeds or vegetative fragments) entering a location over time, but may also be considered on a larger scale where multiple introductions from divergent populations in the native range are the components of pressure. Multiple introductions may set the stage for larger potential increases in collective additive (and non-additive) genetic variation as well assemblage of novel genotypic and phenotypic variation (Duglosch and Parker, 2008; Ellstrand and Schierenbeck, 2000; Lee, 2002). Intraspecific hybridization between previously allopatric members of a species has been implicated in invasions including *Schinus* (Geiger et al., 2011; Williams et al., 2005) and *Pyrus* (Culley and Hardiman, 2009). Similarly, interspecific hybridization between introduced species and native congeners can facilitate increased variation and may also lead to acquisition of alleles important for local adaptation. In some cases there has even been hybridization between multiple invasive non-native species such as *Tamarix* (Gaskin and Schaal, 2002) and *Linaria* (Ward et al., 2009). In the former case, recombination of two invasive genomes appears to have coincided with a worsening problem; in the latter, whether hybridization will prove to worsen an already significant problem is not yet known. However, the frequency of observation of sites where these two *Linaria* species naturally hybridize is increasing, and in one recently discovered site, it appears both pure parent species have been displaced (S. Ward, personal observation). Hybridization between invasive *Linaria* species is the focus of research described in the following chapters. In general, hybridization is increasingly recognized as a potential stimulus for rapid evolution in colonizing species leading to subsequent invasion (Ellstrand and Schierenbeck, 2000; Schierenbeck and Ellstrand, 2009).

The Significance of Hybridization in Evolution and Invasion:

"In 1742, seven years after writing '*nullae dantur species novae*' (there are no new species) Carolus Linnaeus was brought a fertile floral mutant of *Linaria* that he called '*Peloria*'. The unusual floral structure convinced Linnaeus that the plant was of hybrid origin, and the fertility of *Peloria* and other hybrids led Linnaeus to abandon his earlier certainty in the fixed nature of species. Instead, he proposed the radical evolutionary hypothesis that new species could arise via hybridization" (Baack and Rieseberg, 2007).

History of Research and Theory:

Hybridization among plants is of crucial significance to our agricultural history as well as a major evolutionary force governing diversification and speciation. However, it took humans a long while to recognize the capacity for plants to hybridize. This was likely because plant sex was less obvious than in analogous animal counterparts (Zirkle, 1935). The first generally recognized example of deliberate cross-pollination of plants by humans is that of date palms, memorialized in Assyrian reliefs from the 800s B.C (Roberts, 1929), but it was much later when the idea of sex between plants was assimilated into common scientific knowledge. Although the Greek and Roman Philosophers were aware of plant sex, the first person to actually confirm hybridization via pollen transfer was the German botanist Rudolph Jakob Camerer, and not until the late 1600s. A decade or two later, the first known artificial hybrid was made between *Dianthus* spp. by Thomas Fairchild, an English gardener and nurseryman (Rieseberg and Carney, 1998).

As the study of hybridization began in earnest, the general perception, fostered by botanists Carl Linnaeus and Joseph Kohlreuter, was that though plant hybridization did occur in nature, it was rare in most taxonomic groups; and rarer still that hybrids were fertile, fully functional organisms themselves (although Linnaeus would later recant this position - Baack and Rieseberg, 2007). This perception delayed research into the role of hybridization in evolution and speciation, until after the turn of the 20th century when the interaction of hybridization events and subsequent offspring with the environment began to be investigated (Anderson, 1948) and shortly thereafter, hybridization-induced allopolyploidy as an isolating mechanism (Stebbins, 1958). A slow paradigm shift began as realization came that not only does hybridization in many cases produce fertile, viable offspring, but that it can also be a fountain of novel variation upon which selection can act (Anderson and Stebbins, 1954; Lewontin and Birch, 1966).   A parallel body of work on hybridization also developed surrounding its potential as a route to speciation and many, still relevant models of reproductive isolation and hybrid incompatibility were developed from this original work (e.g. Dobzhansky, 1940; Mayr, 1942; 1963). While this research was extremely valuable in its own right, the study of enforcement of reproductive isolation and maintenance of the biological species concept did little to dispel the idea that hybridization faces strong opposing forces (see for example Mayr, 1963). It is only within the last few decades that an increasing number of researchers have convincingly argued that while the outcomes of hybridization are extraordinarily variable (Burke and Arnold, 2001; Rieseberg and Carney, 1998), it has nevertheless played a significant role in the evolution and diversification of organisms, including the introgression of important phenotypic traits across taxa, particularly in plants (Twyford and Ennos, 2012). It is now thought that while the incidence of hybridization varies across taxonomic groups (Whitney et al., 2010), at least 25% of plant species can cross with one or more other species (Mallet, 2005). Ultimately the number is likely to be much higher than this estimate because of the difficulty of detecting cryptic introgression (Currat et al., 2008).

Invasions are costly, but also provide us with real time ecological and evolutionary laboratories. A good portion of this research has shifted towards the idea that in many cases, an invasive organism is not necessarily pre-adapted in its native range to become invasive elsewhere, but rather the colonization process and demographic and/or genetic disruption of transit coupled with selection in a novel environment may facilitate rapid evolution of aggressively invasive species. One increasingly recognized mechanism facilitating rapid evolution is hybridization. In an updated review, Schierenbeck and Ellstrand (2009) listed 35 known examples in which hybridization predated invasiveness in plants, in addition to a handful of examples in other organisms. Some of this increase is likely due to human facilitation. As our globe has become increasingly connected, we have substantially increased the frequency of opportunity for hybridization via the creation of novel species sympatries. Previously geographically separated congeners now often end up in proximity by chance. Similarly, intraspecific hybridization among differentiated populations derived from multiple introductions of a single species may cause recombination into novel genetic and phenotypic variation, sometimes restoring a bottleneck-induced deficit (e.g. Dlugosch and Parker, 2008a). Additionally, disturbed, modern environments may present good opportunities for rapid evolution (Reznick and Ghalambor, 2001) and colonization and invasion in particular (Allan and

Pannell, 2009; Elton, 2000). A corresponding link is also established between hybridization and disturbance where diverse genotypes generated in hybrid events may be especially well-equipped to adapt to similarly diverse microtopographies of disturbed environments (Anderson, 1948; Lamont et al., 2003). Overall, reshuffling gene pools and exposing them to new ecosystems presents the opportunity to understand ecological and evolutionary unfolding of invasion (or transient event) in real time.

Stabilizing the Effects of Hybridization:

The outcomes of hybridization are variable, and there are many scales at which to consider these outcomes. In some species pairings, hybridization may be so infrequent or unsuccessful that it has little or no impact on the populations. However, if successful hybridization occurs, it may increase genetic variation upon which selection may act in populations and serve as fuel for evolution. As previously mentioned, hybridization may be particularly important in invasions because it can give demographically challenged colonists increased numbers, variation or locally adapted traits needed to survive and become established. Hybridization may also result in heterosis (increased growth and reproduction in early generations) with transgressive segregation where trait values fall outside the phenotypic realm of both parents. For early generation novelty or heterosis to be of adaptive or invasive significance, however, such genotypes must be fixed or stabilized in the population. One route to stabilization is allopolyploidy (where a full complement of each species' genome is retained), which is sometimes an immediate consequence of hybridization, but may also evolve subsequently and restore fertility to sterile hybrids or isolate new heterotic lineages (Rieseberg, 1997). Likewise, fixation by asexual reproduction may provide a route to demographic increase of heterotic ramets in the absence of sexual reproductive capacity as has apparently been the case in invasions such as *Myriophyllum* (Moody and Les, 2002). Additionally, fertile yet rare hybrid segregates may become established because of pre or post-mating barriers which prevent backcrossing into either original species (Rieseberg, 1997). When hybrids are transient, novel combinations may still persist in populations through introgression, so even if early generation hybrids do not gain a permanent demographic foothold they may be conduits for transfer of adaptive genes between parents (Rieseberg, 1997, Ellstrand and Schierenbeck, 2000). The occurrence of any of the above-discussed mechanisms is highly contingent on many factors which influence the relative fitness of a hybrid including the degree of divergence and genomic architecture of parent taxa, the relationship of this architecture with phenotype, the abundance of parents and hybrids and other sources of inherent asymmetry (such as genome permeability), and the interaction of all these factors with a temporally and spatially variable environment.

Hybridization and Responses of the Genome:

It is likely there are many hybridization “events” which are so strongly selected against by pre and post zygotic barriers that it is impossible to observe at least under natural conditions, whether they even occurred. However, we are increasingly aware of instances in which hybridization is successful, either transiently as in introgression, or more permanently as in speciation or hybrid complex stabilization. For hybridization to be of long-term significance in a population, an organism must first be able to overcome the genomic disruption of having two more or less differentiated sets of instructions attempt to operate in a single entity. McClintock (1984) suggested hybridization could yield a sort of “genomic shock” which might cripple a hybrid organism, yet alternatively provide fuel for rapid evolution. The meiotic pairing of structurally divergent chromosomes often results in a higher rate of both large scale mutations (e.g. rearrangements, inversions, indels) as well as in smaller changes such as single nucleotide and microsatellite mutations (Zou et al., 2011); variation can be so great as to result in differing karyotypes even among siblings (Lim et al., 2008). This disruptive phenomenon has also been called “hybrid dysgenesis” (Barton and Hewitt, 1985) and it appears such early generation genomic confusion is now considered “the rule rather than the exception” (Rieseberg, 1997).

It has also been shown that the same interspecific crosses can repeatedly generate similar genomic architecture and that even the same multi-locus epistatic interactions can be achieved across cross repetitions (Rieseberg et al., 1996). Kovarik et al. (2005) revealed that the same parental sequences were often quickly and repeatedly eliminated from the genomes of hybrid offspring in subsequent generations. In addition to major architectural changes, it appears additional mutation may be induced by the post-hybridization activation of transposons in both plants (Baker, 1974; Zou et al., 2011) and animals (Labrador et al., 1999). Zou et al. (2011) demonstrated that this activity is not always detrimental and was in some cases associated with improvements in traits related to crop yield. Similarly, some of this transposon activity has been shown to result in epigenetic changes to expression and heritable reorganization of hybrid genomes (Parisod et al., 2010) in addition to other epigenetic mechanisms such as “buffering of genomic shock via siRNAs” in *Arabidopsis* (Ha et al., 2009). Methylation and other modes of epigenetic regulation are now thought to have a significant impact on the phenotypic evolution of plant populations (Kalisz and Purugganan, 2004) and also appear to have a role in mitigating the more damaging effects of interspecific hybridization as in rice hybrids studied by Jin and Hu (2008), and *Arabidopsis* hybrids (Mosher and Melnyk, 2010). It is thought that the differential expression regimes of individual parent species or “heteromes”, so dubbed by Comai et al. (2003) are part of what causes genomic shock in hybrids, but may also increase beneficial processes as seen with metabolic activity in *Arabidopsis* hybrids (Meyer et al., 2012). However the majority of this work has been conducted in model and allopolyploid systems, therefore it should be interpreted with caution in the context of natural homoploid hybrids (although see Lai et al. (2006) and Karrenberg et al. (2007).

Silencing of gene expression via methylation appears to have played an important role in the rapid evolution of the genus *Spartina* (Ainouche et al., 2004; Ainouche et al., 2009) in what is now perhaps one of the most famous (and environmentally costly) examples of a hybridization-facilitated invasion. This aggressive invader is just one indication that “genomic shock” does not always result in disarray and that moreover, an assortment of regulatory mechanisms, such as those discussed above, can facilitate phenotypic responses of heterosis and transgressive segregation (Chen, 2010; Schlichting and Levin, 1986). However, selection will have a significant impact on the progression of hybrid phenotypes across time and either stabilization of initial benefits of genomic shock or a decrease in fitness via hybrid breakdown may be observed.

Finally, hybridization is bound to result in substantial interactions between nuclear and organelle genomes of plants and hybrid performance may often depend on the direction of the initial cross. This is because mitochondrial and chloroplast DNA are uniparentally inherited and so often have a large influence on genotypic and phenotypic expression (the latter is discussed in the next section and is often particularly noticeable in the F1 generation). In some cases, it has been demonstrated that the possession of local versus exotic cytoplasm was associated with higher fitness as shown in *Arabidopsis* by Leonin and Feng (2011) or in *Penstemon* by Kimball et al. (2008). Likewise, Campbell et al. (2008) found that in *Ipomopsis* hybrids, cytoplasmic and genotype by environment interactions had a greater influence on performance than did epistatic nuclear incompatibilities. In general, while phenotypic patterns of hybrids are often classified into categories such as “heterotic” or “transgressive”, the genomic mechanisms which create them are often very different; yet another reason the outcomes of hybridization can be so difficult to predict.

Hybridization and Responses of the Phenotype:

While there is likely a great deal of cryptic hybridization with impacts to the phenotype so subtle they go unnoticed in the absence of extensive study and molecular tools (Currat, 2008), there are also major phenotypic changes of all kinds which can result from the combination of two species. Heterosis is only one possible outcome of hybridization. The magnitude of heterosis in a hybrid is very often proportional to the divergence between its parents, however, this is not always true (Nosrati et al., 2011) and it has also been demonstrated that closely related lines can generate disproportionate amounts of heterosis when crossed (Chen, 2010). While this ephemeral vigor is often a very poorly understood genetic phenomenon, it may also be the one most straightforwardly related to increased fitness or invasive ability because it typically affects traits which either directly (e.g. flower and seed) or indirectly (e.g. vegetative structure and biomass) contribute to fecundity.

Number of seeds produced, and the rates at which they accumulate, germinate or remain dormant in the soil will ultimately govern a sexually reproducing plant’s lifetime reproductive fitness. From an evolutionary standpoint, individuals with the highest fitness are those that have the best chance to successfully pass on their genetic material. In invasion, this fitness, and its interaction with population size and migration rates will contribute to propagule pressure, a key factor in successful initiation, persistence and amplification of colonizing species (Von Holle and Simberloff, 2005; Colautti et al., 2006). However, not just sheer numbers govern fitness and/or invasive potential; seed must also germinate, develop normally and survive. Particularly with hybrids, there may be increased chances of low or abnormal germination if not enough parental compatibility exists between genomes to produce a viable embryo. Bleeker (2004) saw such a trend where hybrids of *Rorippa spp.* had higher rates of seed set than parents, but substantially lower germination rates. However, as hybrid seed fitness is quite variable, fitness may not always be compromised, as seen in Johnston et al. (2003) who observed that germination as well as survival and growth of seedlings was greater in hybrid offspring than at least one and sometimes both *Iris* parents. Likewise, *Schinus* hybrids demonstrated increased germination relative to parent species and also had greater survivorship (Geiger et al., 2011). Nevertheless, as with many hybrid traits, variability within even a single generation can be high and direction of hybridization can affect the magnitude of trait expression. This is seen with differential rates of seed set in *Narcissus* (Marques et al., 2011) and also in differential F1 germination rates in *Geum* (Ruhsam et al., 2011).

In addition to seed production, heterotic flowering and biomass accumulation may also result from hybridization. The effect of more floral or vegetative structure production at the level of the individual phenotype is often to make it a more effective competitor for resources; for instance, a more intense or constant floral display may more effectively attract or otherwise mediate pollinator selection (Levin and Anderson, 1970; Sandring and Ågren, 2009). Likewise, larger plants may compete more effectively for light and other resources (Guesewell et al., 2006), though bigger is not always better under stress (He et al., 2010). Hybridization resulting in phenotypic increases is often but not necessarily linked to increased fitness and rapid evolution of populations and the same may be said of its impact on invasiveness. In the notorious *Spartina* complex, increased vegetative vigor in the form of taller tillers and an overall increased tillering rate were demonstrated in hybrids and thought to be associated with aggressiveness (Castillo et al., 2010). Often plant size and reproductive capacity are correlated, but there may also be tradeoffs between them when there are genomic limitations or only finite resources to invest in either one. Increased levels of reproductive or vegetative mass are not always reflected by a parallel increase in physiological process (e.g. photosynthesis) as physiological traits are often expressed intermediately in hybrids as in Kimball and Campbell (2009). However, both higher rates of physiological processes such as photosynthesis (Mozdzer and Zieman, 2010)as well as higher plasticity of these processes (Molina-Montenegro et al., 2012) may be associated with capacity to adapt as well as invade.

Tradeoffs exist among quantitative aspects of a plant phenotype (e.g. total floral production, display intensity), and phenological aspects such as initiation and duration of flowering. For instance, a plant which flowers earlier (such as transgressive *Helianthus* hybrids observed by Rosenthal et al. (2002) may be smaller and have a less intense floral display, while a plant which flowers later may produce more inflorescences, but over a shorter period of time. The phenology, or timing of organisms' major biological events, is another way which hybridization can alter species' existing patterns. The timing of important life cycle landmarks (i.e., emergence, first flowering, first seed set, and senescence) will have implications for the stresses a plant is exposed to, as well which resources it is able to capture. A plant which emerges from the ground early and completes its reproductive cycle quickly may have access to spring resources in advance of other species and may also avoid late season drought or frost. In hybrids between wild and cultivated *Raphanus*, earlier emergence appeared to be associated with higher seed outputs and contributed to the ability to colonize new places (Davidson et al., 2011). However, plants which initiate flowering later in the season may produce larger displays on bigger vegetative structures and have access to entirely different sets of resources, including pollinators. For instance, increased floral display is correlated with pollinator visitation and relative performance in invasive hybrids of *Carpobrotus* (Suehs et al., 2006). In two slightly different examples, later flowering and seed set increased late season herbivory in populations of hybrid *Lupinus* (Bishop and Schemske, 1998) and in *Populus*, hybridization-induced changes in leaf phenology have helped to promote a hybrid zone as a sink for herbivores (Floate et al., 1993; Floate and Whitham, 1993). In a later publication, the same authors even suggest that the genotypic association between tree genotype and herbivore is so strong that it can be used as a supplementary hybrid diagnostic tool (Floate and Whitham, 1995). Clearly, complexity of potential tradeoffs between resources, stress, biota and reproductive timing requires examination on a case-by-case basis.

Alteration of morphological characteristics is yet another way which hybridization can affect a plant phenotype. In a summary of 41 different morphological traits of *Helianthus* hybrids, Schwarzbach et al. (2001) found hybrids were either intermediate or transgressive for nearly half these characters. Novel or intermediate expression of floral traits can substantially alter existing biotic interactions of all kinds. Indeed, it has been shown that small genetic changes resulting in discrete morphological alterations can have large impacts on assortative mating (Schemske and Bradshaw, 1999). A study of *Ipomopsis* found hybrids could be at either selective advantage or disadvantage depending upon which parental pollinator was most dominant in the system (Campbell et al., 1997). In this study as well as others, maternal phenotype has exerted strong influence over the morphology of early generation hybrids (Favre and Karrenberg, 2011).   In Louisiana *Iris* species Martin et al. (2008) discovered that hybridization caused changes to flower structure which changed preferences of pollinators.

In addition to visually obvious changes in morphology, hybridization can facilitate host shifts through novelties of secondary chemistry in both floral and vegetative plant components (Orians, 2000) and ultimately, such secondary metabolites can be important factors in the evolutionary success of populations (Nicotra et al., 2010). As with changes in morphology, alterations in the way a flower smells to a pollinator can compromise existing relationships as well as create new ones, as seen in the system studied by Vereecken et al. (2010) where a pollinator preference shift in *Ophrys* orchids was driven by novel, hybridization-induced odor compounds. Besides an obvious link with pollinators, transformations of both floral and vegetative chemistry may impact additional insect, microbe and other mutualisms, both beneficial and antagonistic. Such distortions of chemistry may have particular significance for invasion, especially where they affect biological control systems. Attempts are often made to control aggressive species via biocontrol mechanisms, which are typically highly specific interactions so that they avoid impacts on non-target organisms. Muller-Scharer et al. (2004) suggest that rapid evolution during invasion could have strong implications for the future efficacy of this management tactic. The potential transgressive or novel chemistry which can be caused by hybridization-induced alterations to metabolic pathways may be of particular concern. Although not specifically related to biocontrol (but an example of hybridization-altered secondary chemistry), Cheng et al. (2011) showed pyrrolizidine alkaloid composition (compounds known to be important in the mediation of microbe and herbivore relationships) was altered in *Jacobea* hybrids relative to their parents. In another instance, *Artemisia* hybrids produced most terpenes common to parent species in intermediate amounts, but some of the compounds produced by both parents were absent in hybrids which themselves possessed some completely novel terpenes (Nicotra and Davidson, 2010). If hybridization occurs between one or more species which are typically managed with biocontrol, a disruption of secondary chemistry could in turn disrupt the relationship between host plant(s) and the biological control agent. In addition, it is thought that plants with compounds novel to their location (such as those generated by hybridization or those accompanying an introduction), may help to facilitate future invasive behavior (Cappucino et al. 2006).

Hybridization may also alter abiotic tolerances such as abilities to withstand drought, salinity or extreme temperatures. In hybrids, this may increase ecological amplitude enabling a plant to withstand a wider range of environmental conditions (Milne and Abbott, 2000), or facilitate niche shift, where a new phenotype is suited to a different set of conditions than its parents (Rieseberg et al., 1999). Increased amplitude may be intermediate or transgressive, that is, a hybrid may have an expanded tolerance relative to a single parent, or may actually exceed what is tolerated by both parents.

All potential responses of hybrid phenotypes have implications for both initial and continued hybridization in a population as well as for phenotypic evolution because individual fitness will vary over environments and generations. In addition, individual surrogate measures of fitness can change over a lifetime in perennial plants as has been demonstrated in a long-term study of fitness of *Artemisia* hybrids (Miglia et al., 2005). Furthermore, plasticity of any novel phenotypic trait induced by hybridization may enable it to persist and evolve in a population longer than an inflexible trait, and can cause this adaptive novelty to be selected for and genetically integrated over time (West-Eberhard, 2005; Pigliucci et al., 2006).

Hybridization and Responses of the Population:

For the most part, evolution happens at the scale of the population, so while an individual hybridization event may have substantial phenotypic implications for a these offspring, it may not have any long-term impact on the population as a whole. However, depending on hybrid phenotypes, demography and gene flow, the same phenomena observed in individuals may occur at population scales: 1) increase in variation, 2) heterosis 3) transgressive segregation, or 4) purging of genetic load. Some potentially beneficial effects of hybridization, such as an overall increase in genetic variation due to the recombination of two or more previously isolated lineages, only have significant impact at the population level. With invasion, interspecific or intraspecific exchange may alleviate genetic limitations of any given introduction. An additional hypothesis of how hybridization may benefit fitness and/or invasive capabilities of a population is that hybridization is especially good at generating populations which carry high "genetic load" (Ellstrand and Schierenbeck, 2000) and subsequent strong selection against such epistatic load may purge otherwise hidden deleterious alleles (Rieseberg et al., 1997; Ellstrand and Schierenbeck, 2000). As yet, there is little empirical evidence to support this concept. For hybrid-induced heterosis and transgressive segregation to be of evolutionary significance at a population level, they must be fixed, or stable introgression of novel or pre-existing variation must take place across species. As discussed earlier, unless early generation heterosis can be fixed via asexual reproduction, polyploidy or immediate isolation of heterotic segregates, it will likely be transient (Prentis et al., 2008), although such transience will depend strongly upon underlying genetic architecture of the trait(s).

If heterosis does become established, however, the consequences for the population could be demographic increase (via augmented fecundity) or competitive advantage over and subsequent displacement of other community members. Hybrids with higher fitness can also help to change the adaptive landscape (relative fitness peaks) within a species complex. This can result in parent-hybrid competition for the same niche as in *Populus* (Smulders et al., 2008) or in one or more of the parents being displaced as has been the case in *Narcissus* hybrid populations (Marques et al., 2010). In the case of invasion, it may be that more than just the parent species are evicted if relative performance is shifted, especially if heterosis is accompanied by increased ecological amplitude. Increased tolerances for both biotic and abiotic conditions acquired through transgressive segregation or introgression may enable hybrids to occupy more diverse niches than either parent and may facilitate within-community spread or range expansion given appropriate geography and dispersal capabilities. Therefore, heterosis is not the only way in hybridization may facilitate rapid evolution and lead to species displacement, spread and expansion. Transgressive segregation, when intransient and adaptive, may facilitate hybrid speciation when it alters or initiates a new stable niche preference relative to the parent species and thereby isolates the segregates as in Rieseberg et al. (2007) where an increased tolerance for salinity and drought apparently helped to isolate new *Helianthus* hybrid lineages. Likewise, if a population of alien colonists expresses a beneficial transgressive trait, it may provide means for avoidance of direct competition with natives, persistence in the novel environment and even the ability to expand from it.

In most cases, hybridization between two species increases size and diversity of the gene pool, a merger which enhances the raw material available for evolution. For instance, increased variation resulting in novel genotypic combinations in the invasive hybrid species *Senecio squalidus* apparently facilitated its spread (James and Abbott, 2005) and likewise it appears that repeated introductions of seed and subsequent hybridization between two *Onopordum* thistles triggered their invasion (O'Hanlon et al., 1999). Spread facilitated by increased variation also occurred in weedy *Silene vulgaris* where at least three differentiated groups underwent intraspecific recombination resulting in increased fitness (Keller and Taylor, 2010).

In some cases, hybridization events may be relatively transient, while in other cases hybrids may be more persistent, but still not result in major short-term demographic shifts (such as partial or total displacement) for parental populations. In these enduring ‘hybrid zones’ as well as in populations which have sporadic and/or transient hybridization, hybrids may act as conduits for genetic transfer between parent taxa, that is, bridges for introgression. Unlike bigger heterotic plants or novel transgressive phenotypes, the potential subtleties of the effects of introgression are reflected in what Edgar Andersen wrote in 1949: “The more imperceptible introgression becomes, the greater its biological importance.” Similarly, Rieseberg et al. (2007) suggested introgression may be the simplest and therefore most common adaptive outcome of hybridization. While hybridization may be infrequent, or produce only a handful of less fit individuals, the contribution it ultimately makes to advanced generations may still result in increased fitness (Arnold and Hodges, 1995). In other words, introgression is important because it serves to transfer alleles which are adaptive from one species to another. This can alter environmental tolerance and amplitude of either parent, or introgression of locally adapted alleles into a genetically depauperate migrant population, may alleviate a founder effect and be the difference between ephemeral existence versus establishment and expansion. For instance, in *Raphanus* hybridization between wild and cultivated species apparently resulted in changes to several life history characters and conferred an ability to expand to new areas (Hovick et al., 2012). Likewise, in the case of *Rhododendron*, introgression between two species resulted in improved cold tolerance and perhaps facilitated northern expansion (Milne and Abbott, 2000).

Rusham et al. (2011) speculate that adaptive tolerances for both waterlogging and manganese tolerance have introgressed across *Geum* species boundaries. Hybridization has even resulted in the introgression of herbicide resistance across species (Flood et al., 2011; Gaines et al., 2012) a trait which has serious implications for management.

Despite the apparent variety of traits which may introgress across species, previous research has observed repetition of how hybrid genomes are constructed across the same interspecific crosses (Rieseberg and Sinervo, 1996). This means limitations may exist even on adaptive trait introgression due to architectural constraints which make certain portions of the genome more permeable than others, or because certain trait structures are more able to introgress (Gross et al., 2004). One such example is seen with *Iris* where Taylor et al. (2009) demonstrated that while some regions of the genome showed strong signals of introgression, other regions were prevented from recombining entirely. Similar patterns have been shown in other organisms, although the degree of permeability varies substantially across species (Baack and Rieseberg, 2007). However, even strong reproductive barriers may not prevent introgression of particularly adaptive alleles (Barton and Hewitt, 1985; Yatabe et al., 2007) although low rates of gene flow can compromise this progression (Pialek and Barton, 1997).

The possible outcomes of hybridization at a population scale will be highly contingent on phenotypic and demographic interactions with the environment (Pigliucci et al., 2006). Lexer et al. (2003) confirmed the strong influence of environment on hybrid genotype fitness across study systems and also found that strong GxE interactions help maintain structure in persistent hybrid zones. Similarly, Gaskin and Kazmer (2009) found differential introgression was strongly correlated with latitude in invasive *Tamarix* hybrids. As previously mentioned, hybridization has potential to reshape relative fitness of community members, however, this a function of not only the fitness of genotypes in a population, but also of how such fitness changes over space and time. Nolte and Tautz (2010) propose biotic and abiotic processes directly following initial hybridization events may have the strongest impact on evolutionary trajectories, and Currat et al. (2008) suggest that in the case of invasion, introgression will almost always take place in the direction of the colonist due to demography and local adaptation. Gene flow will also determine how hybridization affects the capacity of a population for rapid evolution and speciation (Nolte, 2011) and/or subsequent invasion (Neubert and Caswell, 2000). Additionally, asymmetric gene flow between parents, whether governed by N_e (effective population size) or genomic architecture, will also be important in the long term outcomes of hybridization (Lepais et al., 2009).

In summary, in some ways the outcomes of introduction are akin to the outcomes of hybridization, that is, variable and reliant on many external factors. However, just like introductions, given the right combination of genomic architecture, phenotypic expression and environmental and demographic circumstance, hybrids are capable of heterosis, transgressive segregation or mediation of interspecies introgression, all of which may prove adaptive and lead to ecological shifts, population and range expansion, or invasion.

Toadflax (*Linaria)*:

Taxonomy:

The Linaria, collectively refered to as the “spurred snapdragons” comprise the largest genus belonging to the tribe Antirrhinae (Sutton, 1980). Although molecular phylogenetic analyses recently necessitated a substantial reclassification of many genera, including a reassignment of *Linaria* from Scrophulariaceae (Figwort) (Sutton, 1980) to the Plantaginaceae

(Plantain), the monophyly of the tribe Antirrhinae allowed it to remain largely intact (Albach et al., 2005; Olmstead et al., 2001). The *Linaria* are widely distributed with a proposed center of diversity in the Mediterranean basin and are almost entirely a group of short-lived, perennial diploids (Segarra-Moragues and Mateu-Andres, 2007). The native range of Dalmatian toadflax directly overlaps the center of origin of *Linaria* and this variation as well as ongoing geneflow is reflected in the complexity and uncertainty of *L. dalmatica-genistifolia* taxonomy (Tosevski et al., 2011). In general, two main morphotypes of Dalmatian toadflax, narrow-leaved and broad-leaved (De Clerck-Floate and Harris, 2002) are recognized in the introduced range although the scientific names are as yet used inconsistently.

Yellow Toadflax (Linaria vulgaris (Mill.):

Yellow toadflax is a diploid, herbaceous perennial first introduced to the east coast of North America sometime prior to 1672 as a medicinal and ornamental plant (Alex, 1962; Arnold, 1982) and is thought to be one of the first recorded US invasions (Mack, 2003) Lajeunesse (1999), holds that by the middle of the 20th century it had become widely distributed in the United States as well as a major problem in Canadian rangelands and agricultural fields (Saner et al., 1995), becoming especially invasive in no-till systems (McClay, 1992). The USDA Plants Database currently reports yellow toadflax as present in all contiguous states and Alaska, and listed as a noxious weed in seven; it is also present throughout Canada, except in Nunavut and Labrador (USDA NRCS, 2012). Yellow toadflax tends to establish in more disturbed environments (Arnold, 1982; Matzek, 2011), although there is evidence this species is appearing in more isolated and pristine habitats such as wilderness areas (Sutton et al., 2007) national parks (Pauchard et al., 2003) and remote national forest sites (M. Turner, personal observation). In its native range, which encompasses much of the British Isles, northern and central Europe and

Eurasia (Tutin et al., 1972), yellow toadflax was originally an inhabitant of rocky shores, creek and river banks, hillsides and clearings (Saner et al., 1995), but has expanded from coastal to inland Scandinavia, and now more commonly found along roads and other areas of man-made disturbance (Hellstrom et al., 2006).

In its introduced range, yellow toadflax is found in a wide variety of environments, soils and elevations, growing both at sea level as well as high-elevation mountain locations (Sutton et al., 2007). While it will tolerate drier soils, it tends to prefer a slightly more moist and nutrient rich environment than does Dalmatian toadflax (Wilson et al., 2005). Depending on location, yellow toadflax may bloom as early as mid-May, but typically enters its reproductive cycle later than Dalmatian toadflax, often not until the end of August into September. Yellow toadflax has a habit of multiple upright stems with alternate, narrow leaves and a raceme of lemon-yellow zygomorphic flowers with bearded orange palates and long nectar-containing spurs. The inflorescences bloom from bottom to top in a staggered sequence, which Arnold (1982) suggests may be a tradeoff between showiness and flowering duration. Newman and Thompson (2005) note that even post-reproductive flowers are retained on the plant long after they are able to be pollinated, thereby extending the duration of the floral display. The flowers are very attractive to insects and in addition to pollination services, the blooms often suffer from nectar-robbing, although this does not appear to result in pollen limitation (Stout et al., 2000). *Linaria* species belong to a functional form having ample lateral storage organs (roots) with adventitious buds capable of extensive lateral spread and resource exploitation (Hellstrom et al., 2006). Such clonal expansions are part of what makes yellow toadflax such a potent invader; in an extensive characterization of the root morphology of this species, Charlton (1966) describes single year seedlings capable of producing patches nearly a meter wide. In addition to such rapid lateral growth, *L. vulgaris* is also capable of producing a substantial taproot (Saner et al., 1995) which provides competitive access to moisture and nutrients (Wilson et al. 2005), perhaps conferring a competitive advantage under water-limited conditions (Sing and Peterson, 2011). The vegetative reproduction capabilities of yellow toadflax are formidable: in addition to basic patch expansion, adventitious shoots can also develop from roots at considerable distance from the original plant and form new independent physiological ramets. Furthermore, even very small pieces of root fragment are capable of generating a new clone, although they do not tend to generate shoot biomass as quickly as do sexually produced genets (Nadeau et al., 1992). However, a molecular study by Ward et al. (2008) indicates patch expansion, at least in the intermountain west, is mostly driven by sexual rather than vegetative reproduction, although Nadeau and King (1992) argue that once established, populations do much of their growth by asexual means.

Although it is a self-incompatible species, yellow toadflax is capable of abundant seed production (estimated at 15,000-30,000 per plant, (McClay, 1992) and is typically cross-pollinated by large, strong-flying generalist species (often *Apis* or *Bombus)* (Wilson et al., 2005). Despite its great fecundity, much of the seed produced by yellow toadflax is dormant or non-viable and germination rates are low (Clements and Cavers, 1990) but also quite variable (M. Turner, personal observation). Although wind dispersal was once thought to be a significant mechanism of seed movement of both yellow and Dalmatian toadflax (Robocker, 1974), it now appears it is not regularly dispersed over long distances, usually falling only a short distance from the parent plant (Nadeau and King, 1991; Ward et al., 2008). In the introduced range, yellow toadflax has high levels of genetic diversity both within and among populations (Ward et al. 2008), which is not surprising for an outcrossing generalist-pollinated species (Hamrick and Godt, 1996). High genetic diversity in yellow toadflax reflects not only its life history, but also a pattern of repeated introduction into North America for ornamental and medicinal purposes (Ward et al. 2008) followed by extensive additional anthropogenic dispersal (Lajeunesse, 1999). High amounts of genetic variation may also explain observations that the efficacy of yellow toadflax biological and chemical controls is fairly inconsistent because of extensive differentiation of responses among ecotypes (Lajeunesse, 1999). This, combined with wide environmental tolerance, strong vegetative and sexual reproductive capabilities (including maintenance of a persistent soil seed bank), makes yellow toadflax both an imposing competitor and a difficult challenge for management.

Dalmatian Toadflax (Linaria dalmatica (L.) Miller):

Dalmatian toadflax is also a gregarious plant, tending to grow in clusters and colonies in recently or chronically disturbed areas. It is also a diploid, herbaceous perennial, and shares the self-incompatibility system which appears to be consistent across *Linaria* spp. (Docherty, 1982). In North America, plants identified as Dalmatian toadflax and *L. dalmatica* may be subspecies of *L.dalmatica (*e.g. *ssp. dalmatica; ssp. macedonica)* (Vujnovic and Wein, 1997) or in some cases the “narrow-leaved Dalmatian toadflax” species, *L. genistifolia*. At the end of the 19th century, somewhat later than yellow toadflax, it is believed that one or more of the Dalmatian toadflax taxa were introduced into North America as ornamentals for rock gardens (Alex, 1962). As in the case of yellow toadflax, Dalmatian toadflax has been introduced multiple times and is now widely distributed across much of the US (excluding the Southeast) and Canada; it is listed as a noxious weed in 12 states and three Canadian provinces (USDA NRCS, 2012). The native range of Dalmatian toadflax overlaps the heart of *Linaria’s* center of distribution in the Mediterranean basin and extends northward into the countries of the former Yugoslavia and eastward to northern Iran (Alex, 1962). Like yellow toadflax, Dalmatian toadflax is an opportunistic species most often found after both man-made and natural disturbances such as fire, which has even been demonstrated to increase seed production and germination in some cases (Jacobs and Sheley, 2003). In its native range, Dalmatian toadflax shows a strong preference for sunny, hilly country and occurs on both natural lands and in agricultural settings from sea level up to slightly less than 3000 meters (somewhat lower than altitudes reached by yellow toadflax).

Like yellow toadflax, Dalmatian toadflax persists under broad environmental conditions in its introduced range. However, it has slightly more tolerance for drier, coarser and more alkaline soils than yellow toadflax and is often found in more open and gravelly locales with a particular preference for the direct sunlight of south-facing slopes (Blumenthal et al., 2012) though canopy cover does not necessarily exclude it (Allen and Hansen, 1999). Established Dalmatian toadflax typically overwinters as a (usually evergreen) rosette (Robocker, 1974) from which the next season's upright flowering shoots will emerge in early spring or even late winter (Wilson et al., 2005). Similar to yellow toadflax, Dalmatian toadflax produces a tap root as well as extensive lateral root development and establishment of independent ramets via adventitious root buds (Wilson et al., 2005). This root system makes Dalmatian toadflax quite competitive, even in poor and extremely water-limited environments, and substantial leaf plasticity (S. Ward, personal observation) may also contribute to its drought-tolerating capabilities.

Dalmatian toadflax is early to emerge and bloom, and often initiates the reproductive cycle earlier than yellow toadflax, although due to substantial climate-induced variability and the long duration of bloom for both species, the opportunity for flowering overlap is great. The growth habit of Dalmatian toadflax also consists of multiple upright stems, though it often forms more of a crown-type plant than a yellow toadflax-style mat. The leaves of Dalmatian toadflax are larger than yellow toadflax, although plastic and with a thicker waxy cuticle. It forms a raceme of bright yellow zygomorphic flowers often with orange palates (Lajeunesse, 1999), but relative to yellow, individual flowers are less densely packed along the stem. Blooming is staggered along the stem, although post-reproductive flowers tend to fall off the plant more quickly than those of yellow toadflax (M. Turner, personal observation). Dalmatian toadflax shares many of the same large, strong-flying bees that pollinate yellow toadflax. In addition to its capacity for asexual reproduction, Dalmatian toadflax is capable of producing even more seed than yellow toadflax, and can generate as many as half a million seeds per mature, well-established plant (Robocker, 1970). Dalmatian toadflax seed also has a lower proportion of dormancy and higher rates of germination than yellow toadflax, although dormant seed can remain so for up to a decade and create a problematic seed bank for managers. Newly germinated Dalmatian seedlings are quite drought susceptible (Robocker, 1974). Patch expansion in Dalmatian toadflax takes place by both sexual and asexual reproduction, possibly because seedlings fail to be competitive in the absence of precipitation (Lajeunesse, 1999).

A study evaluating genetic diversity of Dalmatian toadflax in its introduced range found high variation within and among populations and similar to yellow toadflax (Brown, 2008). Additionally, life history is a consistent determinant of diversity (Hamrick and Godt, 1996) and (Segarra-Moragues and Mateu-Andres, 2007) specifically note “breeding system is a reliable predictor of diversity in *Linaria*”. This is consistent with the difficulty managers often have with the use of biological and chemical controls, finding them effective on some toadflax biotypes but not in others (Lajenuesse, 1999). This diversity in combination with the ability to survive and reproduce across a wide range of conditions by both vegetative and reproductive means make Dalmatian toadflax, like its congener yellow toadflax, an aggressive colonist and substantial management challenge.

Impact and Control:

Toadflax has effects not only on community members such as other native and non-native plants, domestic and wild animals, insects (including biocontrol), pathogens, but also on human perceptions and expenses. In addition, these species may impact their abiotic surroundings in ways such as changes in soil structure, succession and fire cycles, erosion potential and water availability. There is some uncertainty as to whether toadflax is a threat to animals which graze on it. On the one hand, it appears that some ungulates, especially those deliberately employed as weed grazers, are not negatively affected by its consumption, and cattle, horses and deer have been known to willingly graze the stalks as well (Lajeunesse, 1999). However, toadflax does contain glycoside compounds thought to be distasteful and poisonous to livestock (Mitich, 1993), although no formal cases of toxicity have been recorded (Wilson et al. 2005). In general, toadflax species are not a major component of any bird or mammal diet, although some small animals may use the plants as structural protection (Lajeunesse, 1999). More indirectly, infestation of Dalmatian toadflax on rangelands may reduce the availability of other forage plants and compromise the number of animals which can be supported on a piece of land. It does not appear that many native herbivores predominantly utilize toadflax species as a new source of food, and this may be because of the deterrent secondary chemistry these plants produce (Sing and Peterson, 2011). It is possible yellow and Dalmatian toadflax are capable of displacing native plant species in the sites where they form dense infestations, however, there is not a great amount of empirical evidence to support this claim (although see Robocker (1974), Sutton (2003), and Sing and Peterson (2011) for examples of competitive effects of toadflax). Some invasive species have been implicated not only in damage and displacement of natives, but also in changes to surrounding ecosystems. The record of toadflax in this regard is empirically thin, although it has been noted that when a shift occurs from erosion controlling grass species to an ecosystem more spatially dominated by a forb such as toadflax, soil loss and surface run off can increase (Lajeunesse, 1999). However, in extreme cases where vegetative cover is limited, any plants, including toadflax, can be better than none at controlling erosion (Sing and Peterson, 2011).

In early or small invasions, repeated removal of toadflax by hand over a period of years can help stave off further infestation (Lajeunesse, 1999). In agricultural systems, regular tilling does not eradicate yellow toadflax, but keeps it at bay, allowing continued cultivation (McClay, 1992). On natural lands, species composition can be managed in order to attempt to provide a more competitive environment in which invasive species will find it more difficult to thrive. This can be done in conjunction with other management techniques, such as prescribed fire and/or herbicide treatments. Prescribed fire alone is a poor idea as it can actually stimulate the toadflax to become more aggressive (Jacobs and Sheley, 2003). Likewise, grazing at the wrong time of year (i.e. spring) can actually facilitate seedling competitive ability and worsen an infestation, but if performed later in the season can effectively limit seed production (Lajeunesse, 1999). Herbicides can be a part of an effective anti-toadflax strategy but the results vary quite widely (Sebastian and Beck, 1998; Sebastian and Beck, 1999) across species, genotype and the kind of herbicide used. Even when herbicide is an effective killer of aboveground vegetative growth, the deep roots and persistent seed bank may require repeated treatment for up to a dozen years. Recently, Krick (2011) showed that timing of herbicide application might be an especially important factor in control.

Another method used to manage invasive toadflax species is biological control (biocontrol), which typically refers to the deliberate introduction of an antagonistic organism (co-evolved natural enemy) to control a problem species. Most biocontrol programs seek to maximize control over the invasive organism at a population level, but also emphasize the specificity of the relationship between biocontrol agent and host plant because agents with too broad a host range may also damage non-target organisms. Species which are deliberately introduced for biocontrol are derived from the native range of the target organism and then extensively tested to ensure host specificity. Managers must also be able to successfully establish populations of the biocontrol agent in the field if long-term limitation of the invader is to occur. In the case of the two toadflaxes, a total of eight biocontrol insects which feed on flowers, seeds, stems, leaves and roots, are considered as biocontrol organisms; these are either moths (*Lepidoptera*) or beetles (*Coleoptera*) (Wilson et al., 2005). Some of these groups were introduced specifically for *Linaria* control, and others are accidentally imported exotic species which proved to feed on one or more parts on at least one of the two toadflax species. While all of these eight insects will, to some degree, feed upon both yellow and Dalmatian toadflax, their effectiveness varies depending on their host, and there is not a particularly optimal biological control which has a powerful impact on both *Linaria* species. The seed-feeding (*Rhinusa antirrhini* and *Rhinusa neta*) and flower-feeding (*Brachypterolus pulicarius)* beetles tend to be the most abundant on yellow toadflax, often occurring in concert, (Wilson, 2005), but overall exerting only moderate control over the species. Tosevski et al. (2011) established that in the native range, the stem mining weevil *Mecinus janthinus*, an agent widely released in North America, is actually two cryptic species. Within the original *M. janthinus* species designation, these two closely related weevils exhibited a strong host preference on native range plants, *M. janithinus* for *L. vulgaris* and *M. janthiniformis* for the *L.dalmatica – L. genistifolia* complex. In Montana, adult and larval feeding by putative well-established populations of both stem-mining weevil species significantly restricted flowering, seed production and above-ground growth of their preferred host (S.Sing, personal communication; I. Tosevski, D. Weaver, S. Sing, unpublished data).

Hybridization of Toadflax:

Narrow leaved toadflax plants are sometimes referred to as *Linaria genistifolia or L. dalmatica* ssp. *macedonica*, but it is also possible that some of these narrow-leaved types are hybrids (Wilson et al. 2005) or represent plastic phenotypes. In general *Linaria* is referred to as a “promiscuous” genus (Dillemann, 1950) and there are several documentations of hybrid crosses made among its members in the literature (Blaringhem, 1919; Brink, 1927; Bruun, 1936; Dillemann, 1950; East, 1933; Olsson, 1975; Viano, 1978; Ward et al., 2009). While some of these authors reported partial or full sterility, others found hybrid offspring fully fertile and apparently fit. In addition, Docherty (1982) found that the self-incompatibility system he elicited in yellow toadflax is not disrupted by hybridization. All of this evidence certainly suggests that while there is a reasonable amount of morphological diversity within the genus *Linaria*, including between yellow and Dalmatian toadflax, there do not appear to have been major evolutionary steps towards strong reproductive isolating barriers.

Interestingly, while *L. vulgaris* and *L. dalmatica* are largely allopatric in their native ranges and may not have had substantial opportunities (other than in laboratories and possibly gardens) to hybridize, they are now doing so in sympatric encounters in their introduced North American range (Ward et al., 2009), apparently with increasing frequency (S. Ward, S. Sing, A. Norton, personal observations). The fact that they are both capable of occupying diverse, albeit somewhat differentiated habitats, coupled with their sharing of the same generalist and strong-flying pollinators suggests that these opportunities may not be transient and may only increase as do the ranges of the two invasive parents. Given the diverse effects which hybridization may have on colonizing, expanding and invasive species and on rapid evolution, the outcomes of hybridization between yellow and Dalmatian toadflax are difficult to predict. However, since these two species already have many characteristics which make them problematic invaders (wide ecological amplitudes, strong sexual and asexual reproductive capacities, competitive ability, and variable responses to control mechanisms), the potential exists for augmentation of aggressive traits. Hybridization between two already problematic invasive plants could result in heterosis, transgressive segregation, and introgression of adaptive traits between the parent species, which may increase growth and reproduction and expand populations and ranges. Knowledge regarding the performance of toadflax species and hybrids is required so that if necessary, land managers may prioritize control at sites where these species co-occur.

In the following chapters, I studied the effects of hybridization on the phenotypes of early generation (BC1 and F1) hybrids and naturally occurring hybrids of unknown parentage. I examined quantitative measures of growth and reproduction (Chapter 2), the timing of major phenological events (Chapter 3), and ecophysiological traits and their relationship to growth and reproduction (Chapter 4). Because environments are expected to have significant impacts on the performance of hybrids, I investigated all traits in both parent taxa and a diverse array of hybrids at two common garden sites, one in Colorado and one in Montana. My primary research questions were:

(1) Are hybrids significantly phenotypically different than one or both parent species?
(2) Are there differences among hybrid genotypic classes in performance?

(3) Do performances of parent taxa and hybrids change across common garden sites i.e. (are there genetic by environmental interactions (GxE)?

(4) Are there tradeoffs between different phenotypic traits?

(5) Do answers to the above questions have implications for potential invasiveness of hybrids and management of toadflax?

Chapter Two: Vegetative and Reproductive Characters of Yellow and Dalmatian Toadflax and Hybrids

Introduction:

Exponential growth in the field of invasion ecology has been driven by substantial negative impacts to the environment (Callaway and Maron, 2006) and their associated financial and biological costs. Invasion by non-native plants is considered a serious global threat, and there is now no protected natural area in the world (apart from the Antarctic) which remains untouched. Recently, hybridization between species has also been implicated in the context of invasion, and in an updated review, Schierenbeck and Ellstrand (2009) cited 35 instances where hybridization has apparently facilitated rapid evolution and invasion of both plant and animal species, including the severe cases of *Spartina* (Daehler and Strong, 1997) and *Tamarix* (Gaskin and Schaal, 2002).

The potential outcomes of hybridization events between plant species are highly variable (Burke and Arnold, 2001) and this has generated controversy over the role and significance of hybridization in evolution and speciation. In many cases, diverged species generate hybrid offspring with reduced fitness (Barton and Hewitt, 1985), or fail to produce functional offspring at all. It has therefore been argued in the past that hybridization represents only transient phenomena in the field, with no significant impact upon evolutionary dynamics (Mayr, 1992). It is now believed hybridization plays an important role in generating diversity and in speciation (Lewontin and Birch, 1966); (Rieseberg and Carney, 1998). The links between hybridization and invasion are multi-faceted. Increasingly, global traffic creates novel sympatries between previously isolated congeners and provides new opportunities for hybridization. Human-disturbed environments are more common and the inherent variability of these disrupted habitats may provide particularly appropriate conditions for the variety of phenotypes produced during hybridization events (Anderson, 1948; Anderson and Stebbins, 1954). In addition, colonists often undergo severe genetic bottlenecks (founder effects) and hybridization with local species or other colonists may restore diversity and allow a response to selection (Ellstrand and Schierenbeck, 2000). Hybrids are also known to exhibit heterosis or fitness-related traits greater than those of parent species (Castillo et al. 2009). Another possible outcome of hybridization is transgressive segregation (Rieseberg et al., 1999) where phenotypic characters of hybrid offspring fall outside the range of either parent. Transgressive segregation may take the form of more vigorous vegetative and/or reproductive growth, but also novel ecological tolerances (Rieseberg, 2003), phenological shifts (Rieseberg, 1997), or changes to vegetative or floral volatile chemical profiles (Fritz et al., 1999; Orians, 2000). These phenomena are not well understood, but increasing evidence points to substantial epigenetic change during hybridization (Hegarty et al., 2011).

It is easy to see how heterosis and transgressive segregation may impact invasions. Larger or earlier emerging heterotic plants could more effectively compete for space, light and nutrients; more intense floral displays could serve as pollination sinks and changes to phenology and chemistry could alter mutualism dynamics. Increases in fecundity or germination rates could affect propagule pressure which has direct links to invasion (Von Holle and Simberloff, 2005). Novel resistance or tolerance can aid persistence or facilitate range expansion (Rieseberg et al., 2003). Additionally, even if hybrids are only transient they may still serve as bridges for gene flow between species (Rieseberg et al. 1999; Ellstrand and Schierenbeck, 2000).

Yellow toadflax (*Linaria vulgaris* Miller) is a diploid herbaceous perennial which is native to northern and central Europe. It was introduced into the northeast US just prior to 1700

(Alex 1962) and has subsequently spread throughout North America (Lajeunesse 1999). Yellow toadflax is often found in disturbed places (Arnold, 1982), but invasion of native plant communities in national parks (Pauchard et al. 2003) and at high elevations in the Rocky Mountains has also been reported (Sutton et al., 2007). Yellow toadflax is pollinated by strong-flying insects and is a self-incompatible outcrosser; it reproduces via ample seed but also asexually by adventitious shoots from rhizomes. Yellow toadflax has been shown to have high genetic diversity both within and among populations consistent with its self-incompatibility (Hamrick and Godt, 1996) short-distance seed dispersal and probable multiple introductions (Ward et al., 2008). Yellow toadflax is present in all contiguous states and a noxious weed in seven (USDA, 2012); it is also present in all provinces and territories of Canada (Sing and Peterson, 2011).

Dalmatian toadflax (*Linaria dalmatica* (L.) Miller) is also a diploid perennial herb but originates near the center of origin for *Linaria* in the Mediterranean, and its native range extends from former Yugoslavia to northern Iran (Alex, 1962). Dalmatian toadflax was first imported to North America in the late 1800s (Vujnovic and Wein, 1997) and has since become widely distributed in the U.S. and Canada. Dalmatian toadflax is also pollinated mostly by large, strong-flying bees and is a self-incompatible outcrosser (Bruun, 1936). Mature plants may produce up to 500,000 seeds which can germinate or remain dormant in the seed bank for a decade (Wilson et al., 2005). Dalmatian toadflax also has the capacity to reproduce asexually via adventitious buds on lateral roots. Dalmatian toadflax has been introduced multiple times as an ornamental and is now widely distributed across much of the US (excluding the Southeast), and is listed as a noxious weed in 12 states (USDA, 2012) as well as three Canadian provinces (Sing and Peterson, 2011).

Two invasive *Linaria* species (yellow toadflax and Dalmatian toadflax) are hybridizing at multiple sites of co-occurrence in the intermountain West (Ward et al. 2009). Yellow toadflax and Dalmatian toadflax tolerate diverse habitats but maintain slightly different habitat preferences. Dalmatian toadflax is often found in open hillsides and thrives even in poor soil; yellow toadflax prefers slightly richer ground with more moisture, often at higher altitudes. Dalmatian toadflax blooms in late spring onward, while yellow toadflax often does not flower until late summer. Despite this difference, blooming periods are long and there is often overlap. In addition, the species share a suite of large, long-distance flying pollinators, so even dispersed populations may be within range of gene flow. Molecular confirmation of hybridization has occurred and controlled crosses have proved hybrids are viable (Ward et al. 2009).

This study examined invasive potential of these toadflax hybrids by examining several potential surrogates of fitness in replicated common gardens at two latitudes. Multiple characteristics of vegetative and reproductive capacity were measured across pure parent species, early generation hybrids derived from controlled crosses and field-collected hybrids of unknown pedigree. The goal of this research was to answer the following questions:

1) Are there differences in fitness-related traits among genotype classes, and in particular, are there differences between individual parent species and between parents and hybrids?
2) If so, are the genotypic differences consistent across traits and across latitudes?
3) Is there evidence of heterosis or transgressive segregation?
4) What are the implications of these findings for managing sites where Dalmatian and yellow toadflax co-invade?

Materials and Methods:

Site Descriptions and Experimental Design:

The common garden experiment was designed as a randomized complete block, replicated at two sites. The first garden site was located in Fort Collins, CO (40°34’12”N, 105°05’29”W, elev. 1530 m) at the Plant Environmental Research Center on the Colorado State University campus. The second site was situated in Bozeman, MT (45°39’43”N, 111°39’45”W, elev. 1503 m) at the USDA-Forest Service Forestry Sciences Lab adjacent to the Montana State University campus. The experiment was initiated in 2009 and was designed to represent both parent species as well as a wide range of potential hybrid variation. A total of 48 genotypes were included, six in each of eight genotypic categories. These categories were as follows: two parental species classes (yellow and Dalmatian toadflax), two first generation hybrid (F1) classes (one with yellow toadflax as the female parent, one with Dalmatian toadflax), two backcross classes (one backcrossed to yellow toadflax, one backcrossed to Dalmatian toadflax), and two classes of molecularly confirmed hybrids (Ward et al., 2009) collected from natural populations at two different field sites. F1 and backcross classes were made by hand in the greenhouse at Colorado State University. Dalmatian toadflax genotypes were collected from sites along the Front Range in Colorado as well as two genotypes collected from populations outside of Helena, Montana. Yellow toadflax was also collected from both Colorado the Colorado Front Range and Paradise Valley, Montana. In addition, a single Yellow Toadflax genotype was collected in Alaska. Collections of naturally occurring hybrids were made near Boulder, MT, along a forest service road and the second was made northeast of Radersburg, MT on the Townsend Ranger District of the Helena National Forest (See Fig.1).

Figure 1. Collection sites in Colorado and Montana of genotypes used in common garden experiments. Blue triangles = natural hybrids; green dots = yellow toadflax; yellow dots = Dalmatian toadflax.

Generation of plant material and garden establishment:

Three cloned replicates of each genotype were included at each site. The clones were generated via rooted cuttings: five centimeter cuttings were stripped of leaves on the lower 16 mm and dipped in rooting hormone powder (Schultz, 0.1% Indole-3-butyric acid) before placement in Fafard 2SV potting medium. Cuttings were kept under humidity domes and misted twice daily until root establishment. Once rooted, replicates were grown in the Colorado State University greenhouse for 3 weeks and then transferred to an outside area next to the common garden site and kept under shade cloth for 2 weeks outdoor acclimation. Planting took place in late June 2009. Plants were placed in five gallon plastic pots sunk so that the lip of the pot was flush with ground level. Plants were grown in ProMix BX media from Premier Horticulture and fertilized with Osmocote 14-14-14 at a rate of two tablespoons per plant once per growing season in early August. When precipitation was not adequate (when the top five centimeters of soil became dry), plants were watered using drip irrigation at a rate of two gallons per hour for 15 minutes at a time. Site differences in precipitation and mean temperatures were recorded by the Montana State University weather station and the Colorado State University weather station (#53005) and are reported in Fig. 2 and 3. Two Dalmatian toadflax genotypes failed to root and generate clones, so only four Dalmatian genotypes were represented in the final installation of the garden.

Data collection:

The following data were collected from each plant on a weekly basis during the growing seasons of 2009 and 2010: (1) longest shoot (surrogate for plant height) (2) number of flowering stems per plant (defined as a stem with at least one inflorescence open and available to pollinators). At the end of each growing season (after the first killing frost), total above ground plant biomass was collected for each individual plant, air dried at room temperature to constant weight and weighed; seed capsules were separated and counted. Relative growth rate (RGR) was calculated as per Hoffman and Poorter (2002) where $[\mathrm{RGR} = (\ln W2-\ln W1)/(t2-t1)]$ and W2 and W1 were the dry biomass from 2010 and 2009, respectively and t1 and t2 were the dates of harvest. Cumulative mortality was recorded as replicates which did not emerge in 2011. Seed was collected in 2010 from every reproductively active plant at both garden sites. Tetrazolium testing for seed viability was conducted on 25 seeds selected at random for each genotype at the Colorado State Seed Laboratory. Seed samples for germination and survival studies were wet stratified for two weeks at 4°C. Stratified seeds were sterilized on a stir plate in 10% Clorox for five minutes followed by three sterile water rinses. Sterile 25-seed lots were plated in petri dishes with 0.8% agar and placed in a growth chamber maintained at 20°C under continuous light and observed for cumulative germination percentages. After two weeks, germinated seedlings were counted and transferred to Fafard Seedling Medium, misted and kept moist under humidity domes. After a period of two weeks, the number of surviving seedlings was recorded.

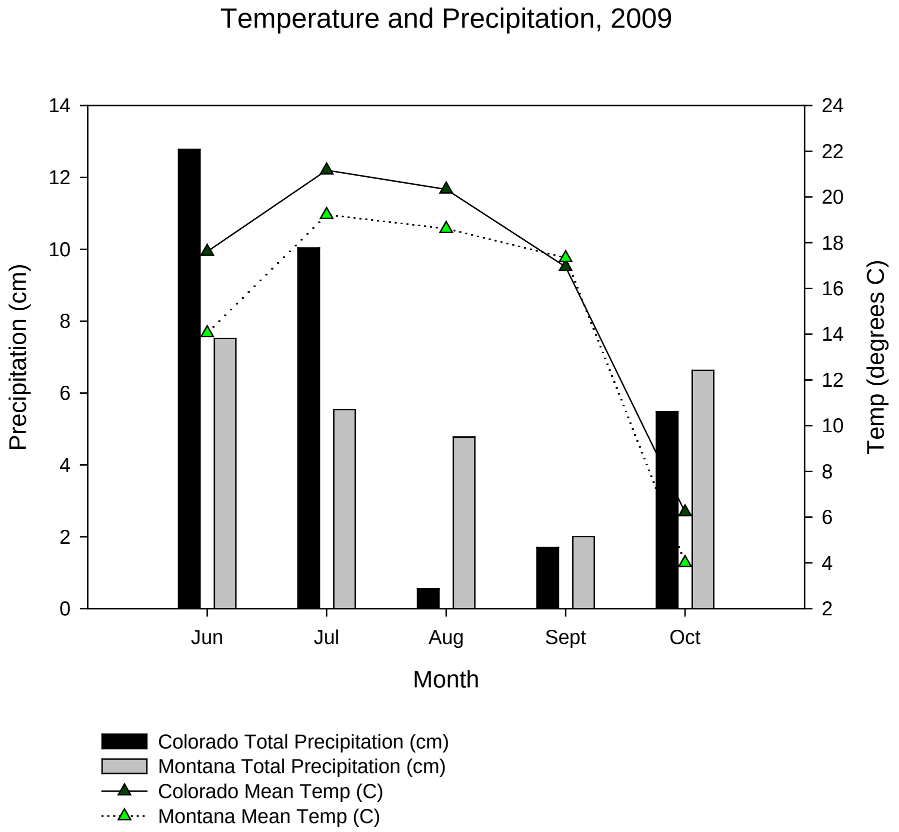


Fig 2. Mean monthly temperature (°C) and total precipitation (cm), 2009 growing season at Colorado and Montana common garden sites.

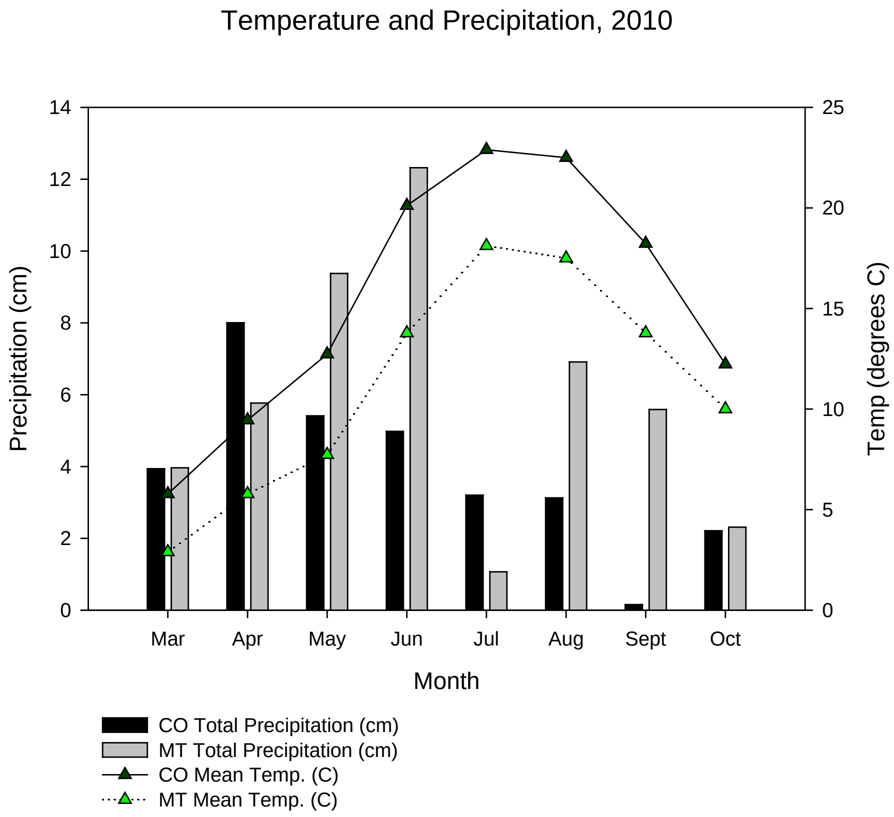


Fig. 3. Mean monthly temperature (°C) and total precipitation (cm), 2010 growing season at Colorado and Montana common garden sites.

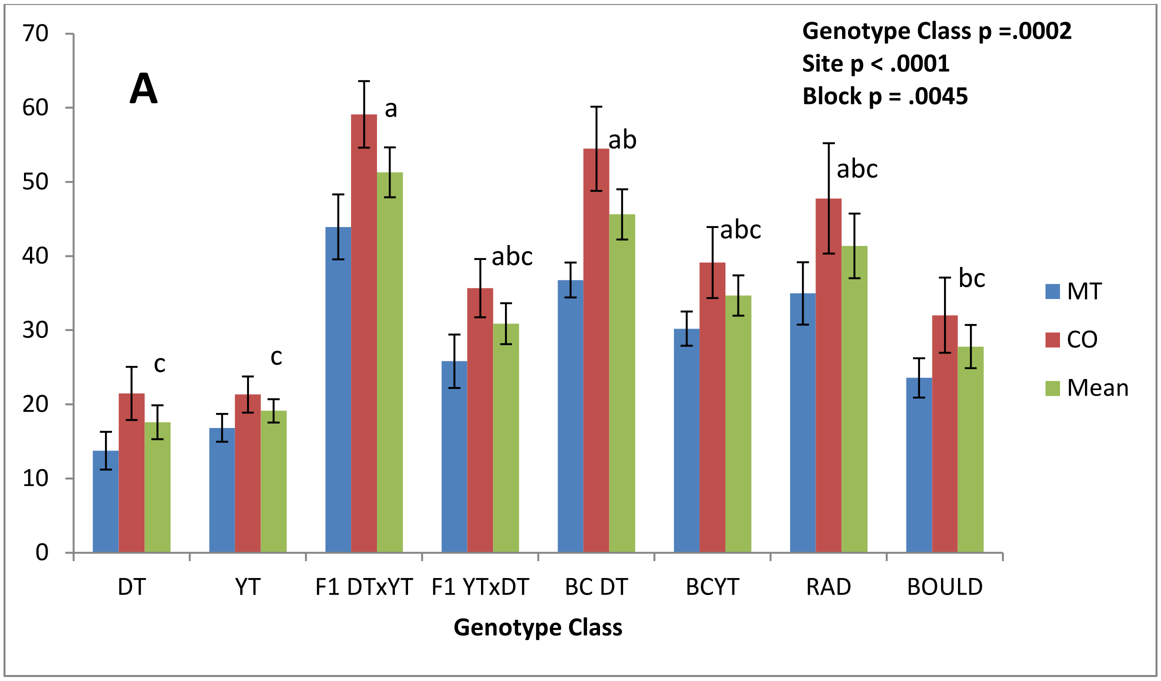
Data analysis:

Data analysis was carried out using JMP, Version 9.2 (SAS Institute Inc. Cary, NC). All trait data were Box-Cox transformed to meet assumptions of normality. Mixed model analysis was performed using Residual Maximum Likelihood (REML) for unbalanced data and we tested for fixed effects of genotype class, site, block (nested in site) and all interactions. Individual genotypes within classes were included as random effects. The distribution of final model residuals was examined to ensure assumptions of normality were met. Tukey's Honestly Significant Difference was used to compare overall differences among genotype classes and sites and multiple individual hypotheses. Logistic regression was performed on mortality data. All analyses were considered significant at a level of 0.05. In addition, Principal Component Analysis (PCA) was performed to examine variation across traits.

Results:

Vegetative traits, 2009:

The model for dry biomass had significant effects of genotype class, site, and block with all genotypes producing more biomass in Colorado than in Montana. A Tukey's HSD group with highest biomass included both F1 hybrid classes, both BC1s and one field-collected hybrid class. The HSD group with least biomass included both parent classes which also had the two lowest means overall (Table 1, Fig. 4B). Pooled and contrasted parent classes produced significantly less biomass than pooled hybrids (Table 2). F1 hybrids with a Dalmatian toadflax maternal parent combined with backcrosses to Dalmatian toadflax had significantly more biomass when compared with an analogous pool of yellow toadflax hybrids. In contrast to biomass, longest shoot had higher values at the Montana common garden site values than Colorado (Table 1, Fig. 4A); genotype class also significantly impacted this trait at both sites. The parent species produced shorter shoots than did hybrids in every individual class contrast with the exception of the comparison of yellow with a pool of hybrids with a yellow maternal parent or backcrossed to yellow.



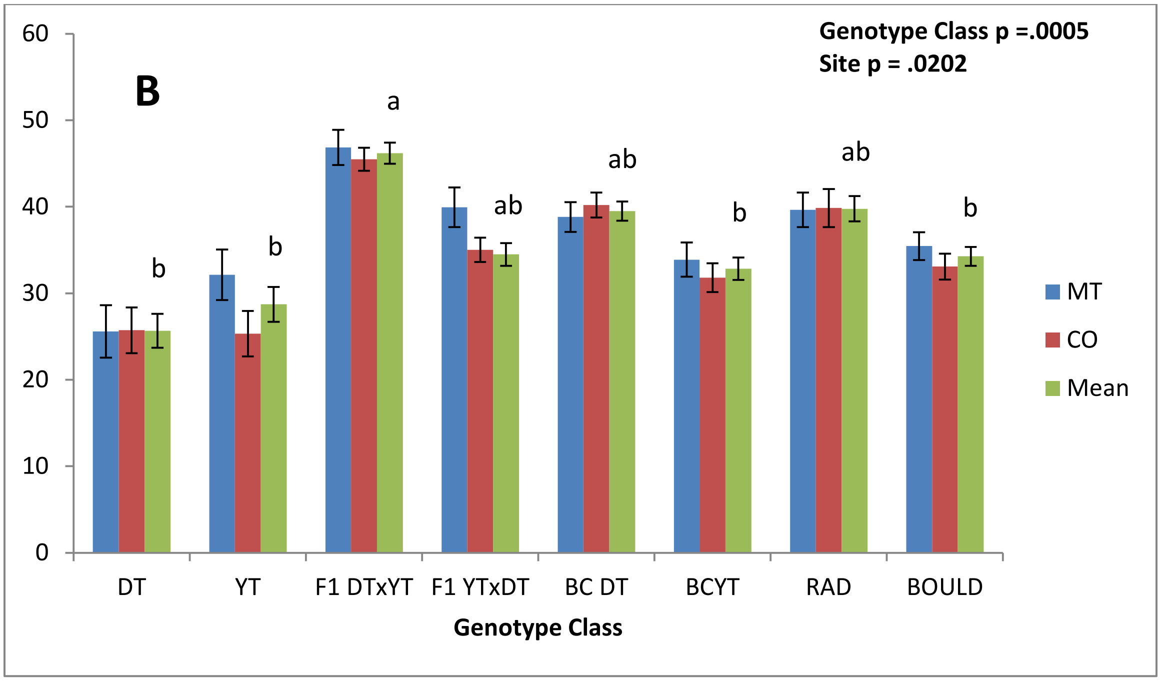
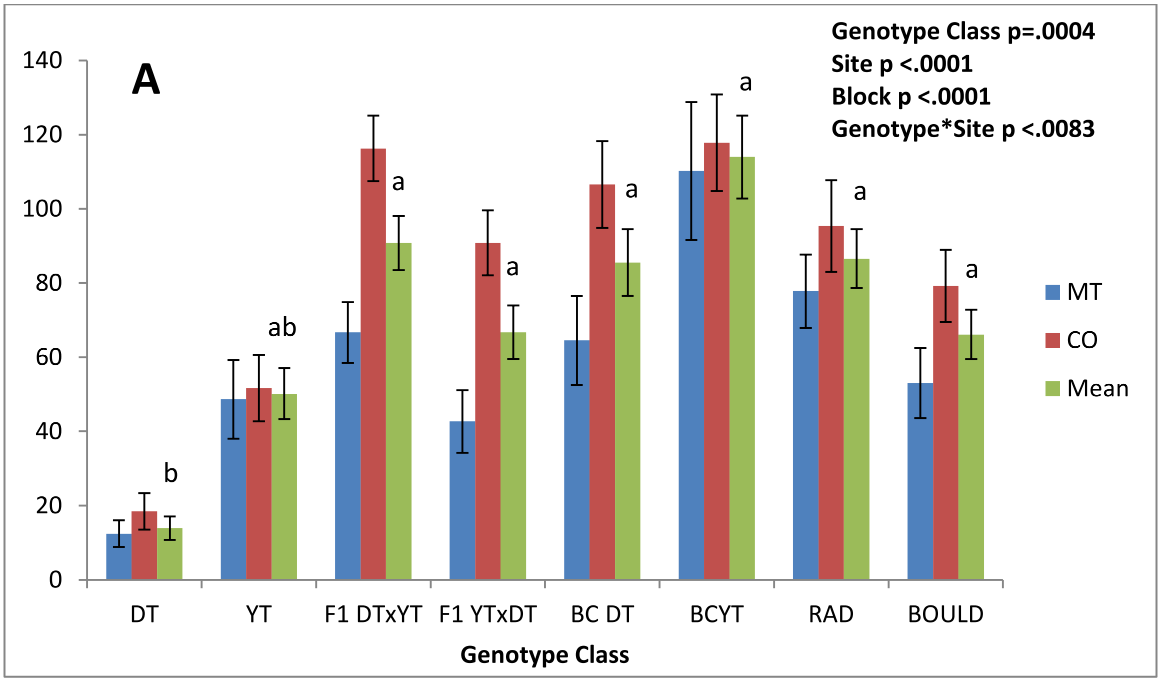


Figure 4. Vegetative trait means in 2009 and standard errors for genotypic classes for individual and combined sites. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.) Overall model fixed effects and parameter significance are shown in upper right hand corner. A. Dry biomass, season total (g). B. Longest shoot per plant (as proxy for height) in cm.

Reproductive traits, 2009:

The effects of genotype class, site, block and genotype class*site were significant in the flowering stem model; the mean number of flowering stems per plant was greater in Colorado than Montana and a significant HSD split occurred between parent and hybrid classes (Fig. 5A). The genotype class*site interaction was driven by natural hybrids and yellow toadflax improving rank at the Montana site. Contrast of hybrid vs. parents showed fewer floral stems in parental classes in individual and combined contrasts (Table 2). No contrasts between hybrid classes were significant. Seed capsule production (Fig. 5B) was correlated with floral production, so the same model effects were significant in the same directions, and rankings and contrast results were similar (Table 2).



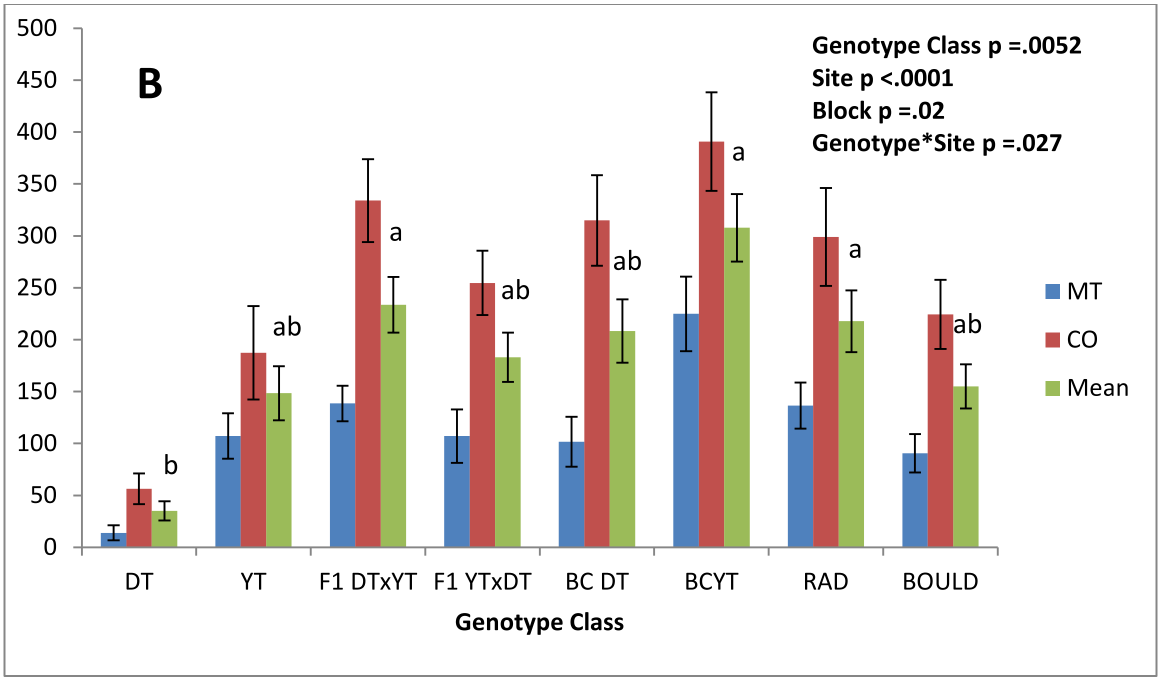


Figure 5. Reproductive trait means for 2009 and standard errors for genotypic classes for individual and combined sites. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.)Overall model fixed effects and parameter significance are shown in upper right hand corner. A. Total flowering stems (season). B. Total seed capsules (season).

Principal components, 2009:

When multiple traits are measured on individual plants, a high degree of correlation among them is possible. Potential correlations and multidimensional fitness variation were therefore explored in reproductive and vegetative traits in a principal component analysis. A large portion of variation (~73%) was associated with a single dominant eigenvalue which was loaded relatively evenly among the four quantitative traits; second and third principal components contained approximately 17% and 7% of variation, respectively (data not shown). We modeled this first principal component (“fitness”) with genotype class, site and block effects and found a trend similar to those of the individual traits emerged (Table 2). HSD groups of "fitness" revealed the highest performing group to be composed of all hybrid and no parent classes.

Mortality 2009-2010:

The original design intent was to analyze a year effect on quantitative data to examine impact of establishment and differential climate on the performance of genotypic classes. However high mortality in Dalmatian toadflax in during the 2009-2010 winter meant the statistical assumption of population equivalency across years was no longer met. Therefore the same traits were analyzed separately for 2010. Additionally, no formal statistical analysis (logistic regression) of survivorship was performed for 2009-2010 since some genotype classes had 100% survival resulting in unstable parameter estimates (i.e., mortality of these classes was impossible to estimate). Analysis of cumulative 2009-2011 mortality is presented following the next section (Fig. 8).

Vegetative traits, 2010:

The model for dry biomass in 2010 had effects of genotype class, site, and block; all genotypes produced more biomass in Colorado than in Montana. As in 2009, an HSD-separated group with highest biomass contained Dalmatian toadflax maternal F1 and backcross hybrids, *y*ellow toadflax maternal parent F1s, and one natural hybrid class (Fig. 6B). Parent species were in the bottom HSD group again along with hybrids backcrossed to yellow toadflax. Contrasts again showed hybrid classes produced significantly more biomass than either parent, although unlike 2009, F1s were now significantly larger than backcrosses. The longest shoot model had genotype class, site, block and genotype class*site terms. The direction of the site effect was reversed relative to 2009; Colorado shoots were longer than in Montana. The interaction was derived from changes in rankings of F1DTxYT and BCDT (longer shoots in CO than MT), and F1YTxDT and BCYT (longer shoots in MT than CO)(Fig. 6A).

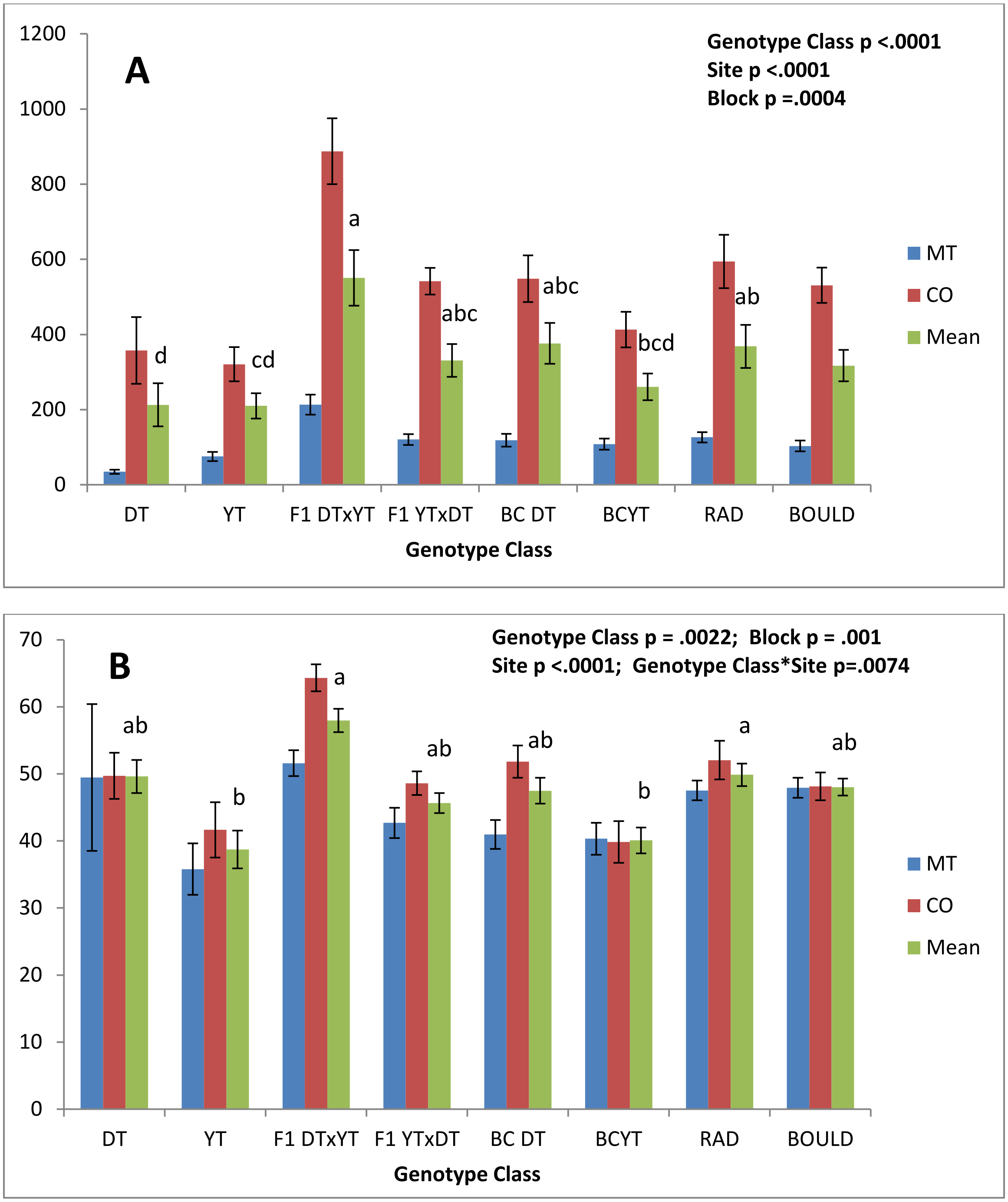
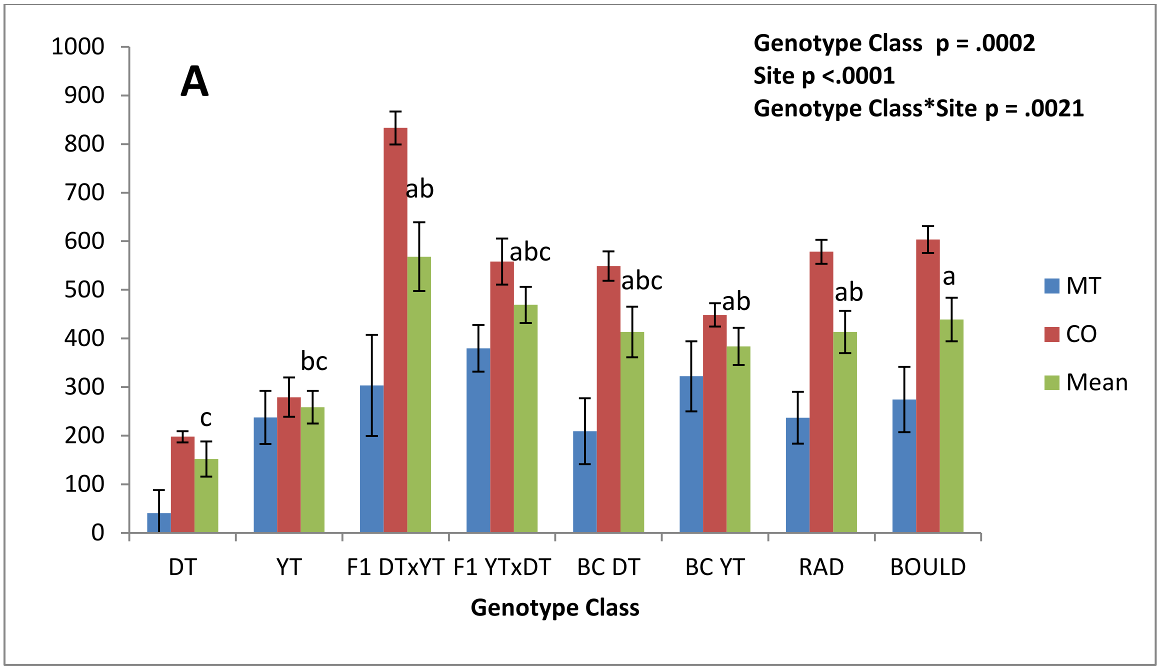


Figure 6. Vegetative trait means for 2010 and standard errors for genotypic classes for individual and combined sites. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.) Overall model fixed effects and parameter significance are shown in upper right hand corner. A. Dry biomass, season total (g). B. Longest shoot per plant (as proxy for height) in cm.

Reproductive traits, 2010:

The model for flowering stems had effects of genotype class, site, and genotype*site; the mean was higher in Colorado than in Montana. The interaction is derived from the tendency of F1DTxYT and BCDT to produce more in Colorado and likewise for F1YTxDT and BCYT in Montana. This trend also appeared for pure parents, but was non- significant. HSD for flowering stems placed all hybrids and no parents in the group with the highest means and the lowest performing group contained only the two parental classes (Fig. 7A). Contrast patterns revealed parents to be significantly less prolific in terms of floral production than hybrids across all class comparisons (Table 2). No significant interaction between site and genotype class was found in the seed capsule trait model. The HSD group of highest capsule producers again contained all hybrids and no parents, but now the lowest producing group contained the parents and both backcross classes (Fig. 7B). One of these (BC YT) had been the top seed producer overall in 2009. Natural hybrid classes increased rank in 2010. Contrast patterns of capsule production are consistent with those observed for flowering stems (Table 2).



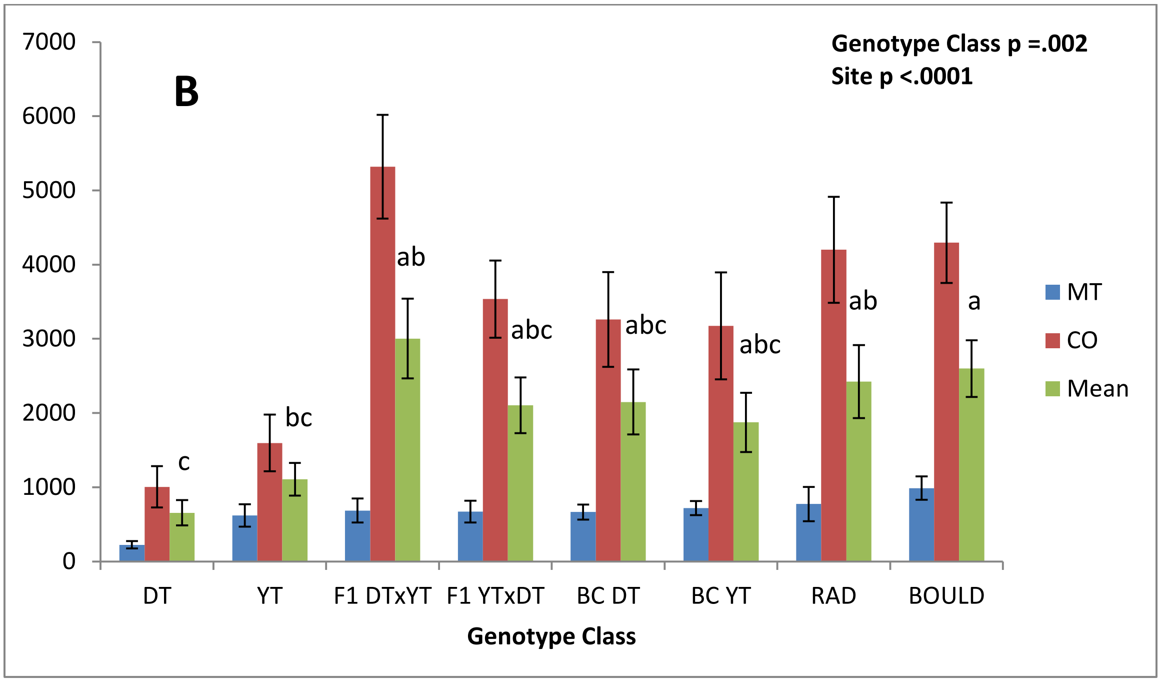


Figure 7. Reproductive trait means, 2010 and standard errors by genotypic class for individual and combined sites. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.)Overall model fixed effects and parameter significance are shown in upper right hand corner. A. Total flowering stems (season). B. Total seed capsules (season).

Principal components, 2010:

Principal component analysis was performed on four vegetative and reproductive traits. Again, much variation (~79.6%) was associated with a single dominant eigenvalue distributed loaded evenly among the four traits, second and third principal components contained approximately 12.5% and 4.8% of variation, respectively. The first principal component “fitness” was modeled with genotype class and site effects, and as in 2009, HSD separation of “fitness” included all hybrids but no parents in the group with the highest performance, and plants in Colorado outperformed those in Montana.

Table 1: Quantitative trait means±SE averaged across sites, by year for dry biomass (g), longest shoot (cm), flowering stems and seed capsules are season totals. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.)

Year	Genotype Class	Trait (Means±SE)			
		Dry Biomass	Longest Shoot	Flowering Stems	Seed Capsules
2009	DT	17.6±2.29	25.7±1.97	13.9±3.12	35.2±9.15
	YT	19.1±1.58	28.7±2.02	50.2±6.86	148.5±26.0
	F1 DTxYT	51.3±3.36	46.2±1.21	90.8±7.29	233.5±26.8
	F1 YTxDT	30.9±2.76	34.5±1.32	66.8±7.24	183.0±23.6
	BC xDT	45.6±3.38	39.5±1.11	85.5±8.96	208.3±30.5
	BC xYT	34.7±2.72	32.8±1.29	113.9±11.2	307.9±32.5
	RAD	41.4±4.37	39.8±1.46	86.6±7.93	217.8±29.8
	BOULD	27.8±2.90	34.3±1.10	66.1±6.70	155.0±21.4
2010	DT	212.7±57.2	49.6±2.46	152.0±36.4	654.9±170
	YT	209.9±33.7	38.7±2.82	258.3±33.6	1108±221
	F1 DTxYT	550.3±74.0	58.0±1.76	568.2±70.9	3003±537
	F1 YTxDT	331.0±43.4	45.7±1.51	469.0±37.2	2103±375
	BC xDT	376.2±54.2	47.5±1.92	413.1±52.1	2148±438
	BC xYT	260.7±35.6	40.1±1.93	383.5±38.1	1875±400
	RAD	368.4±57.5	49.9±1.68	413.4±43.7	2423±490
	BOULD	317.1±42.0	48.0±1.26	439.0±44.5	2599±382

Table 2: Contrasts of Traits and principal component 1 ("Fitness") derived from all four traits and averaged across sites. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.)a)All parents vs. all hybrids. b)Dalmatian toadflax vs. Dalmatian-influenced hybrids. c) Yellow toadflax vs. Yellow-influenced hybrids. d) All F1s vs. all backcrosses. e)Boulder vs. Radersburg hybrids. f)Field hybrids (Radersburg and Boulder) vs. controlled-cross-generated hybrids. g)Yellow-influenced hybrids vs. Dalmatian-influenced hybrids. F Ratios and p-values are given.

Year		Contrast	Trait				
			Longest Shoot	Dry Biomass	Flowering Stems	Seed Capsules	PC1 "Fitness"
2009	a	Parents v. Hybrids	F=18.25 p<.0001	F=23.97 p<.0001	F=27.32 p<.0001	F=16.95 p=.0002	F=30.63 p<.0001
	b	DT v. (F1DTxYT+BCDT)	F=18.93 p<.0001	F=21.82 p<.0001	F=23.42 p<.0001	F=14.36 p=.0005	F=27.66 p<.0001
	c	YT v. (F1YTxDT+BCYT)	F=2.226 p=.144	F=5.804 p=.0209	F=5.7 p=.022	F=4.041 p=.0515	F=6.249 p=.0168
	d	F1s v. BCs	F=2.996 p=.0916	F=.0022 p=.9631	F=.8452 p=.3637	F=.2819 p=.5986	F=0.001 p=.9993
	e	BOULD v. RAD	F=2.013 p=.1641	F=.3276 p=.0782	F=1.266 p=.2675	F=1.192 p=.2817	F=2.519 p=.1208
	f	(Field v. Controlled)	F=.4074 p=.5271	F=2.049 p=.1605	F=.624 p=.4344	F=.6999 p=.4081	F=1.201 p=.28
	g	(F1YTxDT + BCYT) v. (F1DTxYT + BCDT)	F=10.23 p=.0028	F=8.0275 p=.0073	F=.0003 p=.9872	F=.1538 p=.6972	F=2.39 p=.1304
2010	a	Parents v. Hybrids	F=3.892 p=.0556	F=40.78 p<.0001	F=33.87 p<.0001	F=25.49 p<.0001	F=26.63 p<.0001
	b	DT v. (F1DTxYT+BCDT)	F=.7454 p=.3929	F=36.18 p<.0001	F=22.13 p<.0001	F=12.99 p=.0008	F=16.21 p=.0002
	c	YT v. (F1YTxDT+BCYT)	F=2.255 p=.1417	F=8.148 p=.0069	F=9.943 p=.0033	F=6.816 p=.0129	F=7.614 p=.0091
	d	F1s v. BCs	F=8.087 p=.0073	F=8.914 p=.005	F=3.065 p=.0888	F=.2980 p=5885	F=4.46 p=.0424
	e	BOULD v. RAD	F=.2056 p=.6531	F=.7959 p=.3783	F=.0023 p=.9617	F=.0671 p=.7972	F=.047 p=.8298
	f	(Field v. Controlled)	F=.2894 p=.594	F=.4051 p=.5285	F=.0501 p=.8243	F=1.194 p=.2819	F=.0587 p=.81
	g	(F1YTxDT + BCYT) v. (F1DTxYT + BCDT)	F=8.04 p=.0075	F=5.67 p=.0225	F=.0381 p=.8464	F=.2201 p=.6417	F=2.446 p=.1274

Cumulative Mortality (2009-2011):

Over two winters, at least one replicate of each genotype class had perished, which allowed a logistic regression analysis of cumulative mortality. Only genotype class was significant in the model. The results revealed that Dalmatian toadflax suffered the highest mortality, but that both parental classes experienced significantly higher mortality than any of the hybrid classes (See Fig. 8).

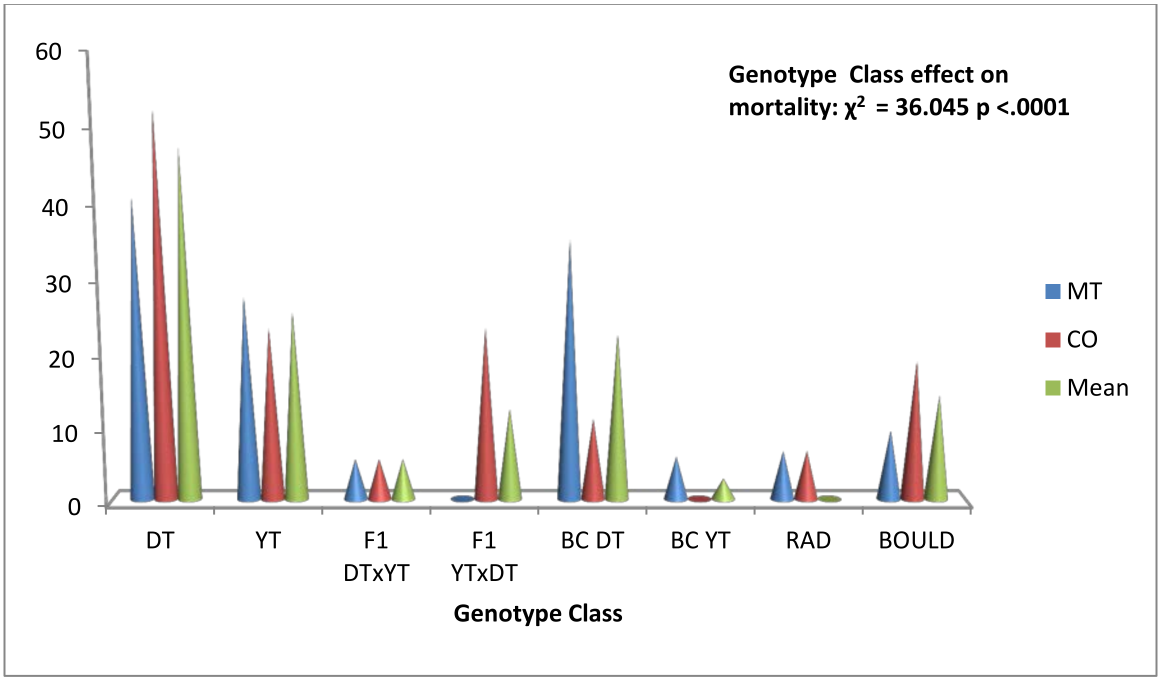


Figure 8. Mortality percentage for genotype classes by individual (Colorado and Montana) and combined sites. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.)

Germination, Viability and Seedling Survival:

Genotype class was the only significant effect on germination percentage, field-collected hybrids had the highest percentages followed by Dalmatian toadflax, F1DTxYT and BCDT.

Yellow toadflax, F1YTxDT and BCYT had the lowest rates of germination. (Fig. 9) Only the site effect was significant in the model for seedling survival; seed from plants in the Montana common garden had a higher rate of survivorship past initial germination. Rates of dormancy were no different between genotype classes or sites. Of the seeds which germinated, a limited number were classified as abnormal, but this was not significantly different between genotype classes or sites.

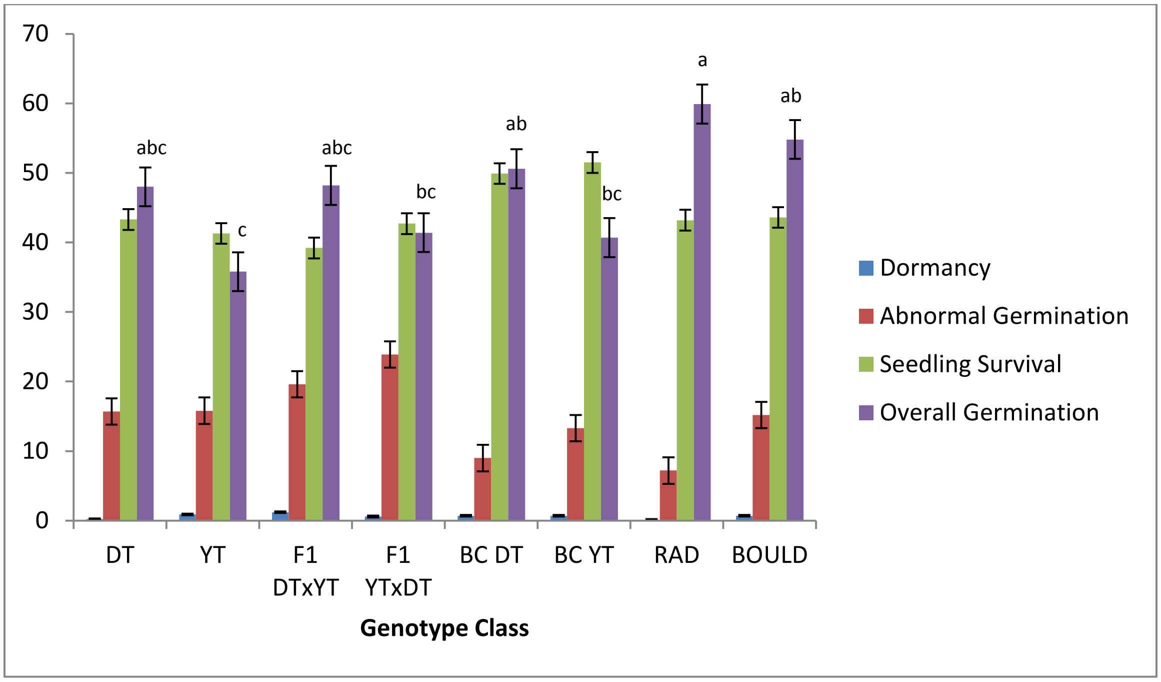


Figure 9. Seed germination and viability of open pollinated seed collected in 2010. Overall germination and dormancy are presented as a percentage of total seed; abnormal germination and seedling survival are presented as a percentage of germinated seed. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.)

Discussion:

There is concern that ongoing hybridization of two already invasive species (Dalmatian toadflax and yellow toadflax) may lead to an even more severe threat in areas where these exotics co-invade. However, outcomes of hybridization are extremely variable and depending on the specific case studied, may result in offspring which can be more or less fit than either parent, have transgressively segregating and/or novel phenotypes, or result in the introgression of adaptive genes from one species to another. In these common gardens, an attempt was made to measure diverse surrogates of fitness and invasiveness via quantitative vegetative and reproductive metrics. Increased size and biomass may represent a stronger ability to compete for space, light and other resources, while increased floral production can alter pollination dynamics and increased seed production can intensify propagule pressure which has been linked to invasive ability. Although results are genotype class dependent, hybrids demonstrate apparent heterosis and transgressive vegetative and seed production, which could increase competitive and/or invasive ability in single or across multiple traits (Fig. 4-6; Table 2). In contrast, germination appears more intermediate but still exhibits mid-parent heterosis.

Hybrids vs. Parent Species

Hybrids consistently outperformed parents across traits, sites and years. However, within this pattern there was variation in the magnitude and distribution of hybrid performance. Trait expression was dependent on differences in genomic composition (i.e., contributions of maternal or backcross parents) (Table 2). In 2010, differences between generations appeared, suggesting that over time, magnitude of heterosis and transgressive trait expression are contingent on generation as well as dominant genomic proportion and cross direction. In both years, F1s with a

Dalmatian maternal parent were top performers for all quantitative traits (Table 1). Dalmatian toadflax, given the appropriate environmental conditions tends to be the larger of the two parent species, so it appears the F1 DTxYT class has not only the size advantage of its maternal parent (perhaps augmented by heterosis) but also transgressive fecundity and high rates of germination (Fig. 9). Previous work by Ward et al. (2009) found substantially higher rates of seed set in interspecific crosses when yellow toadflax was the maternal parent: 49.1% compared to only 10.9% when Dalmatian was the female parent. There is further evidence of this asymmetry from the field, as the majority of genotyped natural hybrids have yellow toadflax cpDNA haplotypes (Boswell, 2012). So despite the apparent fitness advantage of this F1 class, initial compatibility may be a barrier to establishment. Such asymmetries are not rare and have implications for persistence and evolution of hybrids in nature. Viable seed also relies on compatible genomic combinations, so in spite of increased capsule production, it is possible hybrid seed might actually present a lesser sexual propagule threat if seed embryos are non-functional. All seed analyzed in this study was open-pollinated, so any effect of genotype class refers to the maternal parent. Certain hybrid classes demonstrated heterosis for germination percentages, while others did not, although none had rates lower than yellow toadflax (Fig. 9). This suggests that the increased capsule production observed in some hybrids is not subject to any greater limitation to viable embryo formation than are the parent species. Lower germination could be due to higher dormancy, but among hybrids dormancy was low and similar for all classes. Potentially higher rates of abnormal germinations might be expected in early generation hybrid seed with newly recombined genomes of two species not yet exposed to selection; but there were no significant differences among classes. Hybrids with yellow toadflax as a maternal or backcross parent, like yellow toadflax itself, germinated at a lower rate when compared to hybrids with Dalmatian toadflax as a maternal or backcross parent, indicating once again a strong maternal or cytoplasmic effect as well as influence of parental genomic proportions. Natural hybrid classes had highest rates of germination overall and when compared with each parent species individually have significantly higher rates of germination as well as higher rates compared to early generation hybrids (Fig. 9). This suggests there may have been selection for transgressive fecundity in the field. Alternatively, given the diversity of toadflax, it could be that an unknown parent, presumably Dalmatian toadflax (the species with higher germination rates), contributed high germination alleles to the Radersberg and Boulder hybrids.

Site Effect and GxE:

All genotype classes grew larger and produced more flowering stems and seed capsules in Colorado (Table 1; Fig 4-6), which is consistent with a longer growing season and warmer temperatures (Fig 2 and 3). In general, the size of the site effect on the longest shoot trait is not as great as for the biomass and reproductive traits. This may suggest that while environment has a substantial impact on the overall vegetative and reproductive output of a plant, the architecture of the plant appears under more direct genetic control. There was no site effect on germination but this did not translate to seedling survival; once germinated, genotype class was no longer a significant effect but the site effect was highly significant and seed collected from Montana was far more likely to survive from germination to seedling stage (data not shown). While there is no immediate explanation for this phenomenon, it appears that compromised seed performance in the early stages from germination to seedling is highly impacted by the environment in which it was originally grown.

No GxE was detected for vegetative traits in 2009, but the interaction was significant for both reproductive traits (Fig. 5a&b). The flowering stem rank of natural hybrid classes (both collected in Montana) improved from Colorado to Montana, indicating possible local adaptation to latitude or other site characteristics. Likewise, yellow toadflax improved rank for both flowering stems and total seed capsules in Montana, which could be related to its origin in northern latitudes. In 2010, GxE was only significant in models for shoot length and flowering stems (Fig. 6a&7a). No strong pattern caused the interaction for longest shoot, while for flowering stems the source of the interaction is that yellow toadflax, F1s hybrids with yellow toadflax maternal parents and backcrosses to yellow toadflax improve rank from Colorado to Montana. Like the pattern in 2009, this may mean yellow genomic background or cpDNA confers increased floral production at more northern latitudes. However, it does not necessarily translate into capsule production. Model analysis of first principal component (“fitness”) also had no significant genotype*site term, so while there might be subtler genotypic interactions with site for particular traits, multivariate performance of genotype classes for roughly holds across sites and years (Table 2).

Mortality and Study Caveats:

Patterns of survivorship were significantly non-random, and therefore worth interpreting in the context of fitness and invasive ability. Overall, both parent species, and especially Dalmatian toadflax experienced substantially higher rates of mortality than almost every hybrid class (Fig. 8). A straightforward interpretation of the high mortality might be that Dalmatian toadflax is far less robust than the other genotypic classes measured; however, there is some reason to question the overall performance of Dalmatian toadflax in the context of this experiment. The primary reasoning behind use of common garden designs is that environmental variability is controlled in order to identify strictly genotypic differences. Unfortunately, this control is also a major limitation of these experiments, because (1) it is not representative of competitive and resource limited conditions of the field and (2) if the specific condition(s) of the garden are an especially bad match for its subjects, the realistic comparison of performance may be skewed. High mortality leads us to believe Dalmatian toadflax may have underperformed in the common garden and that its growth and reproductive output may have been constrained. It was more difficult to generate rooted cuttings with Dalmatian toadflax and morphologically similar hybrids, which may have resulted in less vigorous plants. Or, it might be that our deliberately created benign environment (i.e., adequate nutrients and water) or the potted environment was suboptimal for a species such as Dalmatian toadflax. The possibility Dalmatian toadflax may not represent an accurate benchmark on which to compare fitness must be considered when interpreting the results of this study, i.e., estimates of transgressive segregation and heterosis may be high.

Conclusion:

In general, we found hybrids to be larger and more fecund regardless of the latitude at which they are grown. From the standpoint of invasive species management, this multi-trait heterosis is alarming, because it indicates not only may the hybrids have potential to be more invasive than the noxious parents, but they may have multiple mechanisms (both vegetative and sexual) of establishment and spread. There is much genotypic variation among hybrid classes, and their performance appears at least some degree contingent upon their environment; the genome(s) of yellow toadflax may confer an advantage at higher latitudes, the same may be true of Dalmatian at lower latitudes. While extreme size and reproductive capacity of the F1 generation might not persist in later generations, these plants can still become problematic by perennating in the field via rhizomatous growth. Natural site hybrids, whether they are the product of repeated introgressions or persistent early generation hybrids, appear to be nearly as large and fecund as the most heterotic hybrids from controlled classes.

Demography, asymmetrical compatibility, environment, species ecology and pedigree make outcomes of hybridization between yellow and Dalmatian toadflax complex. However, hybridization is a real phenomenon across the intermountain west, and hybrids can persist or even displace invasive parents. This study demonstrated that despite high genotypic variation and environmental contingency, in common gardens at two latitudes, most early generation and natural hybrids are capable of outperforming parents across multiple vegetative and reproductive traits. This suggests that areas of co-invasion should not only be prioritized for management, but should include populations within potential traveling distances of these species’ strong flying pollinators.

Chapter Three: Phenology of Toadflax Species and Hybrids and its Association with Fitness

Introduction:

Phenological timing is often a relatively stable character of a species, but the capacity to make opportunistic adjustments to the timing of various landmarks of a life cycle may prove to be highly adaptive under certain environments (Alpert and Simms, 2002). However, such a plastic ability to respond to real-time fluctuations in resources is thought to come at the cost of a tradeoff with other critical adaptive traits whose genetic architecture renders them less flexible (Bradshaw, 1965). For instance, the opportunity to mature may be limited by the duration of the growing season, so regardless of what resources become available at what time, plants need to begin their reproductive cycle early enough set viable seed. In this case, a plastic response enabling a plant to continue vegetative expansion when it needs to begin flowering could be maladaptive. Any strategy, whether based in fixed genetic variation or upon plastic response should be favored if it can reliably result in higher fitness (Bradshaw, 1965). However, it remains uncertain what strategy will be most effective if the environment is highly variable and its effect on phenological timing and fitness are more uncertain. Aside from the potential tradeoff between fixed and plastic phenology, there is often a similar compromise between plant size and time of first reproductive maturity (Campbell and Snow, 2009; Colautti and Barrett, 2010). Similarly, there may also be tradeoffs among floral characteristics such as total production, peak intensity, initiation and duration. This means that even if earlier seed set may appear adaptive, intense selection for fecundity may constrain phenological evolution. The reverse may be true as well, although Kingsolver and Hoekstra (2001) observe that quantitative plant traits generally appear to be under stronger selection than phenology.

It has been suggested that phenological mismatch can facilitate invasion of species which - due to timing- may have access to resources other community members do not. For instance, some invaders may use strategies of early germination and/or emergence followed by rapid growth and have been termed “gap grabbers” (Newsome and Noble, 1986). This scenario emphasizes preemptive resource capture and avoidance of late season stress via early life cycle completion. In contrast, similarity of latitude between native species and invasive ranges has been identified as a potential predictor of invasion (Lonsdale, 1999; Maron, 2006). In this instance it may be more likely that at least some aspects of a species’ phenology are pre-adapted to resemble that of its introduced community. In some cases, rapid evolution of phenological clines during establishment has also been shown (Dlugosch and Parker, 2008a; Colautti and Barett, 2010).

Hybridization among multiple introductions as well as between introductions and natives has been implicated as a factor in many invasions (Ellstrand and Schierenbeck, 2000; Schierenbeck and Ellstrand, 2009). Outcomes of hybridization are unpredictable and may have strong effects on the genome and transcriptome (Zou et al. 2011) as well as at phenotypic and population levels (Rieseberg et al. 2007). During establishment and invasion, hybridization may alleviate founder effects by restoring genetic variation, purge detrimental alleles from parental genomes, or result in heterosis and transgressive segregation (Ellstrand and Schierenbeck, 2000). Heterosis refers to the phenomenon by which a hybrid’s phenotypic performance exceeds both parents; similarly, transgressive segregation refers to all hybrid phenotypic expression outside the range of its parents (Rieseberg et al. 1999). It is possible altered phenology can result from hybridization and such changes may encourage evolutionary divergence or affect both the process of invasion and its subsequent impacts on the community. Such a pattern was observed in *Raphanus*, where an earlier emergence time drove higher seed output and facilitated novel colonization (Hovick et al. 2012). Alternatively, larger floral display in *Carpobrotus* increased pollinator visitation and fitness of invasive hybrids (Suehs et al., 2006.) Yet another way in which hybridization has altered phenology occurred in *Populus*, where changes in leaf timing promote hybrids as an herbivore sink (Floate et al. 1993).

This research focuses on two invasive *Linaria* species (yellow toadflax and Dalmatian toadflax) which are hybridizing at multiple sites of co-occurence (Ward et al. 2009). The biology of these two species is described in detail in Chapter 2. Both yellow and Dalmatian toadflax are tolerant of diverse conditions; however they maintain habitat preferences. In the mountain west, Dalmatian has a preference for somewhat lower elevations with open terrain and tolerance of poor soils while yellow toadflax prefers slightly richer and moister terrain, often at higher altitudes. Dalmatian toadflax initiates reproduction quite early in the season (late spring), while yellow toadflax often does not begin blooming until August into early September. Despite these staggered phenologies, both species have long blooming periods and there is often substantial overlap between the two. In addition, the same strong-flying pollinators (typically bumble and honey bees) visit both species at distances of up to two miles, and therefore the different habitat preferences of the toadflaxes are within potential gene flow distances. Hybrids in the field have been confirmed (Ward et al. 2009) and previous data collected from the same common garden as in this study suggested that most hybrids perform as well or better than either parent species (Chapter 2). Given these results, land managers confronting these two already problematic species have concerns about hybridization, which may be well founded. The outcomes of hybridization are extremely variable and can affect a broad range of phenotypic traits (Burke and Arnold, 2001) including phenological timing. Changes in phenology, especially when coupled with increased fitness or competitive ability can have impacts on ecological amplitude or facilitate further colonization and expansion of populations (Hovick et al. 2012). However, if there are tradeoffs between growth, reproduction, and phenological timing, any one of these traits may be evolutionarily constrained. If there is no compromise among traits, hybridization may stimulate rapid evolution of multiple phenotypic optimizations. Examination of the relationship of traits to one another in hybrid toadflax will add to the ability of managers to effectively prioritize co-invasion sites for control.

In this study, phenological landmarks of emergence from overwintering dormancy, first flowering and seed maturation were measured because they are key points in the initiation, duration and completion of a plant's reproductive cycle. These traits were compared across a range of early generation (F1 and BC1) and naturally occurring hybrids (collected two different sites) as well as parent species. The correlation of phenology with quantitative surrogate measures of fitness was then examined. The goal of this research was to answer the following questions:

1) Are there differences in phenology among genotype classes, and in particular, are there differences between individual parent species and between parents and hybrids?
2) Is phenology correlated with quantitative measures of fitness?
3) If so, what does this relationship look like: i.e., is there a tradeoff between phenological timing and magnitude of quantitative traits? Does this relationship change across sites or genotypes?

Materials and Methods:

Site descriptions:

This common garden experiment was designed as a randomized complete block, replicated at two sites. The first garden site was located in Fort Collins, CO (40°34’12”N, 105°05’29”W, elev. 1530 m) at the Plant Environmental Research Center on the Colorado State University campus. The second site was situated in Bozeman, MT (45°39’43”N, 111°39’45”W, elev. 1503 m) at the USDA-Forest Service Forestry Sciences Lab adjacent to the Montana State University campus.

Experimental design:

The experiment was initiated in 2009 and was designed to represent both parent species as well as a wide range of potential hybrid variation. A total of 48 genotypes were included, six in each of eight genotypic categories. These categories were: two parental species classes (yellow and Dalmatian toadflax), two first generation hybrid (F1) classes (one with yellow toadflax as the female parent, one with Dalmatian toadflax), two BC1 backcross classes (F1s backcrossed to yellow, F1s backcrossed to Dalmatian), and two classes of molecularly confirmed hybrids (Ward et al., 2009) collected from naturally occurring hybrid populations at two different field sites. F1 and BC1 classes were generated by controlled crosses in the greenhouse at Colorado State University. Dalmatian toadflax genotypes were collected from sites along the Front Range in Colorado as well as from populations outside of Helena, Montana. Yellow toadflax was collected from the Colorado Front Range and Paradise Valley, Montana (See Figure 1, Chapter two). In addition, a single yellow toadflax genotype was collected near Fairbanks, Alaska. The first collection of naturally occurring hybrids was made near Boulder, MT, along a forest service road and the second was made northeast of Radersburg, MT on the Townsend Ranger District of the Helena National Forest.

Generation of plant material and garden establishment:

Three cloned replicates of each genotype were included at each site. The clones were generated via rooted cuttings. Five centimeter shoot cuttings were stripped of leaves on the lower 16 mm and dipped in rooting hormone powder (Schultz, 0.1% Indole-3-butyric acid) before placement in Fafard 2SV potting medium. Cuttings were kept under humidity domes and misted twice daily until root establishment. Once rooted, replicates were grown in the Colorado State University greenhouse for 3 weeks and then transferred to an outside area next to the common garden site and kept under shade cloth for 2 weeks outdoor acclimation. Planting took place in late June 2009. Plants were placed in five gallon plastic pots sunk so that the lip of the pot was flush with ground level. Plants were grown in ProMix BX media from Premier Horticulture and fertilized with Osmocote 14-14-14 at a rate of two tablespoons per plant once per growing season in early August. When precipitation was not adequate (when the top five centimeters of soil became dry), plants were watered using drip irrigation at a rate of two gallons per hour for 15 minutes at a time. Site differences in both natural precipitation and supplemental irrigation were recorded. Two Dalmatian toadflax genotypes failed to root and generate clones, so only four Dalmatian genotypes were represented in the final installation of the garden.

Data collection:

Phenological data were collected beginning in 2010 one full year after establishment, as timing of phenological landmarks in the first year were disrupted by the cloning, acclimation and planting processes. In the spring and summer of 2010 the plots were inspected on a weekly basis for first signs of emergence. The day upon which the first emerged plant was observed was defined as day one and all other phenological landmarks were counted from this point. For each of three clonal replicates at both sites the following data were collected: 1) Days to first emergence (defined as the day of first observed new season growth). 2) Days of first flowering (defined as the date when a raceme had at least one open flower capable of being pollinated) 3) Days to first maturation of seed (defined as the first appearance of an open capsule). The latter two time points were measured from the time of emergence. The intervals between emergence and flowering and between flowering and set of mature seed were also recorded, however each of the two intervals was strongly correlated with its endpoint in absolute days, so for statistical analysis, only the three non-interval traits were used (Table 3).

Data analysis:

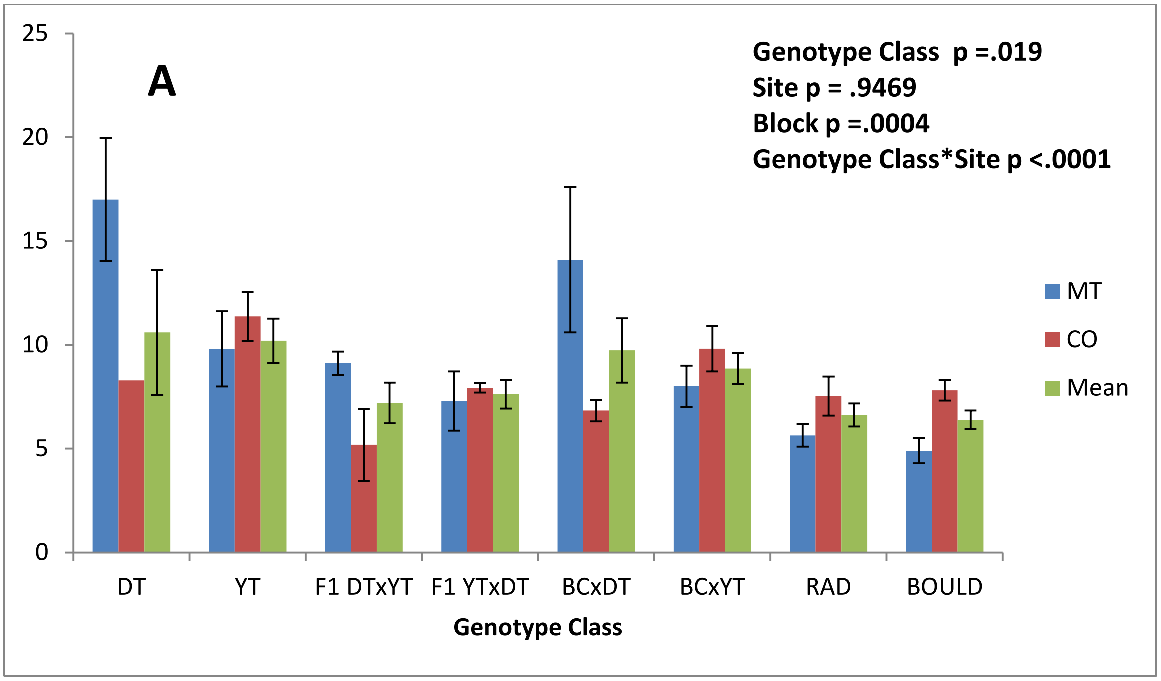
Data analysis was carried out using JMP, Version 9.2 (SAS Institute Inc., Cary, NC). Phenological data were examined and all data were Box-Cox transformed to meet assumptions of normality. Mixed model analysis was performed using Residual Maximum Likelihood (REML) to test for fixed effects of genotype class, site, block and all interactions. Individual genotypes within classes were included as random effects. The distribution of final model residuals was examined to ensure assumptions of normality were met. Tukey's Honestly Significant Difference (HSD) was used to compare overall differences among genotype classes and collection dates, and multiple individual contrast tests of interest were made between various pooled and individual genotypic classes to test more specific hypotheses. All analyses were considered significant at $\alpha=0.05$. Principal Components on combined phenological data and quantitative data from the same common garden experiment (Chapter 2) were examined to look for tradeoffs between timing and magnitude of growth and reproduction.

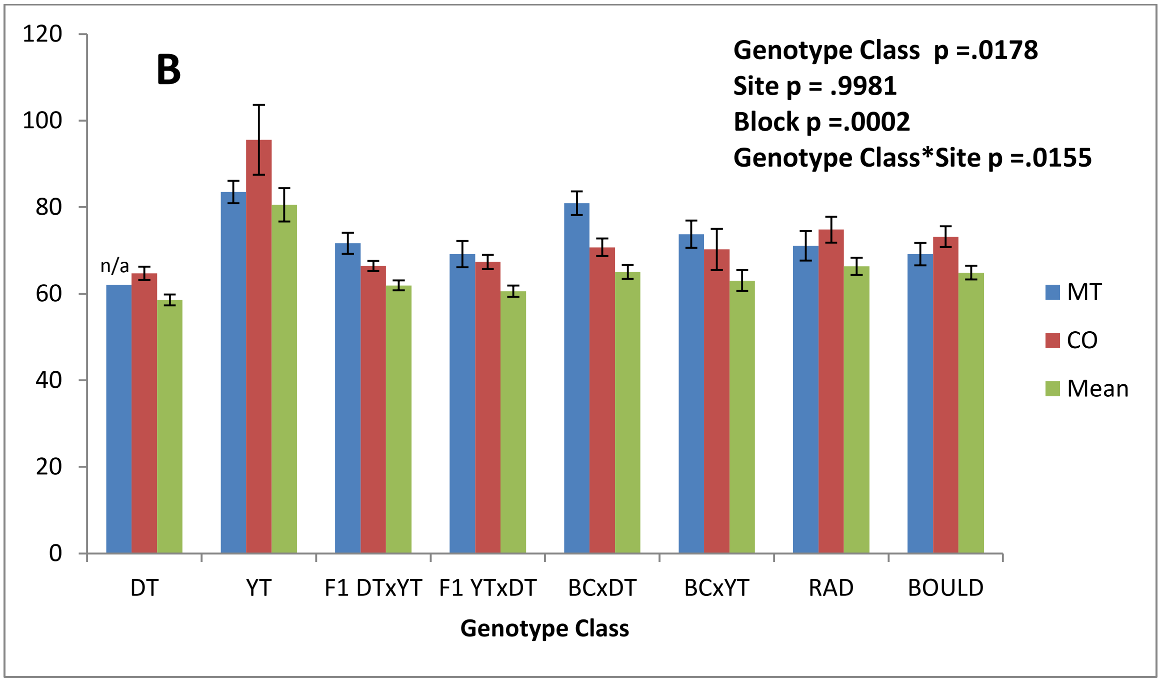
Results:

In the model for days until emergence, although the overall effect of site was not significant, its interaction with genotype class was, so the term remained in the model. Means are presented in Table 3. The conservative HSD was unable to separate groups, but individual contrasts and parameter estimates shed light on differences between individual and pooled classes (Table 4). Overall, hybrid classes emerged significantly earlier than either parental class and F1s and field-collected hybrids emerged earlier than backcrosses. When backcross classes are compared to parents without pooling with other hybrids, they did not emerge any earlier. Table 4 shows the complete list of contrasts. Though site means were not different from each other, closer examination of genotype class means within each site reveals that parent species influence appears to be a source of the strongly significant class by site interactions. That is, yellow toadflax, as well as the hybrids upon which it exerts the most genetic influence (i.e., F1YTxDT and BCYT) emerged earlier in Montana than in Colorado, while the reverse is true of Dalmatian toadflax and its F1DTxYT and BCDT hybrids. In addition, both field-collected hybrid classes emerged earlier at the Montana site than in Colorado (Fig. 10A).

For days to first flowering, the same effects remained significant (Fig. 10B). Overall, the contrast between parents and hybrids was no longer significant, nor were there significant differences between individual hybrid classes, but the contrast between parents was significant. The same general trend governed the genotype class*site interaction in that genomic composition appeared to influence timing differentially by site, but the trends were not as strongly supported. Yellow toadflax alleles still appeared to enable earlier flowering in Montana, but Dalmatian alleles no longer significantly affected timing of flowering by site although the direction of the trend remained consistent. However, Dalmatian suffered substantial cumulative mortality (see Fig. 8, Chapter 2), so only one Dalmatian toadflax plant in Montana actually flowered and yielded an emergence to flowering time point, limiting insight. As with emergence, both natural hybrid classes flowered earlier in Montana.

No significant interaction between genotype class and site remained in the model for days to mature seed set; rather, variation was governed by differences between genotype classes and sites (Fig. 10C). Plants in Montana achieved mature seed set in a significantly shorter time than in Colorado. Accordingly, both field-collected hybrid classes set seed earlier in Montana, as did hybrids with a yellow toadflax influenced pedigree, consistent with the pattern observed in the other two phenological time points. However, absence of an interaction implies that all genotypes set seed earlier in Montana, or substantial environmental impact on this character; however, as surviving Dalmatian toadflax in Montana had waned to a meager N=1, this assertion is compromised for this genotypic class.


A
Genotype Class p =.019
Site p = .9469
Block p =.0004
Genotype Class*Site p <.0001
25
20
15
10
5
0
DT
YT
F1 DTxYT
F1 YTxDT
BCxDT
BCxYT
RAD
BOULD
Genotype Class
MT
CO
Mean


B
Genotype Class p =.0178
Site p = .9981
Block p =.0002
Genotype Class*Site p =.0155
120
100
80
60
40
20
0
n/a
DT
YT
F1 DTxYT
F1 YTxDT
BCxDT
BCxYT
RAD
BOULD
Genotype Class
MT
CO
Mean

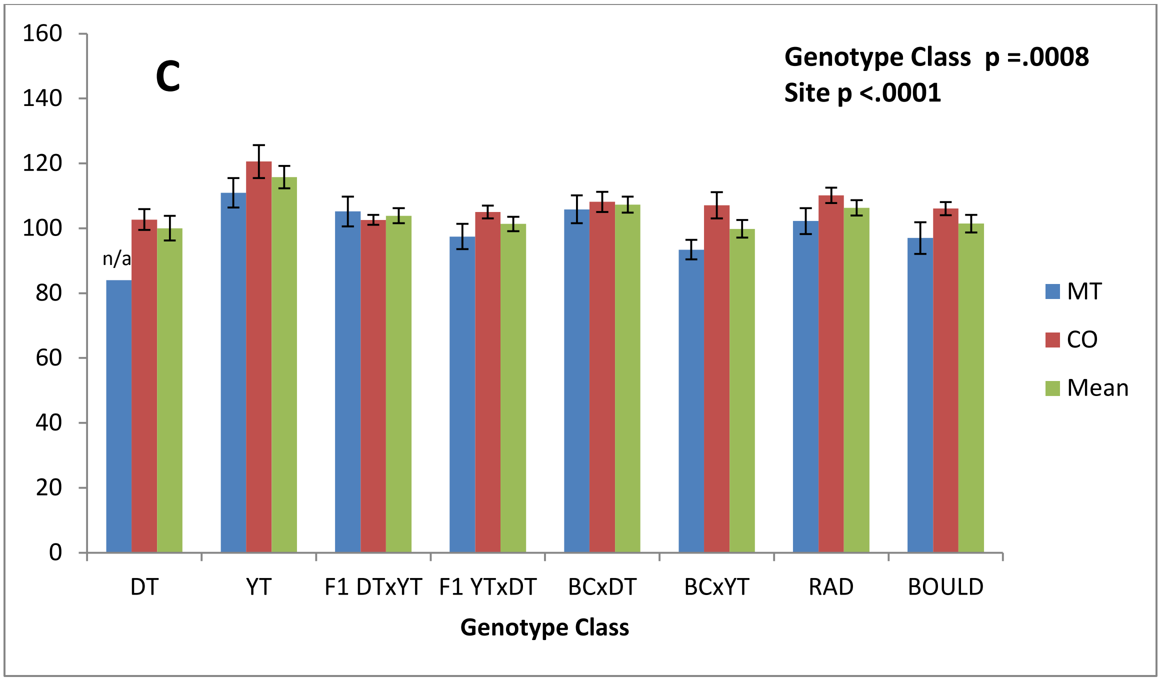


Figure 10. Phenological trait means and standard errors by genotypic class for individual and combined sites in 2010. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.) Overall model fixed effects and parameter significance are shown in upper right hand corner. A. Day of first emergence (first plant emerged defines day one). B. Days from emergence to first flowering. C. Days from emergence to first mature seed.

Table 3: Phenological Means ±SE with HSD groupings by genotype class in 2010. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.) For emergence, the first plants to emerge are defined as day one, all subsequent emergence dates are counted from that point. First flowering and first mature seed are counted as days after an individual plant's first emergence.

Year	Genotype Class	Trait Mean ±SE)/ HSD		
		Emergence	First Flowering	First Mature Seed
2010	YT	10.6±1.06/ a	89.76±4.47/ a	115.8±3.49/ a
	DT	10.2±3.01/ a	64.29±1.38/ ab	100.0±3.79/ ab
	F1 YTxDT	7.62±.687/ a	68.21±1.67/ b	101.4±2.22/ b
	F1 DTxYT	7.20±.977/ a	69.1±1.43/ b	103.9±2.33/ ab
	BC xYT	8.85±.741/ a	71.9±2.78/ b	99.82±2.71/ b
	BC xDT	9.73±1.55/ a	74.6±1.86/ ab	107.3±2.47/ ab
	RAD	6.62±.554/ a	73.0±2.25/ ab	106.3±2.37/ ab
	BOULD	6.39±.447/ a	71.2±1.77/ b	101.4±2.76/ b

Table 4: Contrasts of phenological traits in 2010. a) All parents vs. all hybrids. b) Dalmatian vs. Yellow toadflax. c) Dalmatian toadflax vs. Dalmatian-influenced hybrids. d) Yellow toadflax vs. Yellow-influenced hybrids. e) All F1s vs. all backcrosses. f) Boulder vs. Radersburg hybrids. g) Field hybrids (Radersburg and Boulder) vs. controlled-cross-generated hybrids. h) Yellow-influenced hybrids vs. Dalmatian-influenced hybrids. i) Dalmatian toadflax vs. natural hybrids j) Yellow toadflax v. natural hybrids. F Ratios and p-values are given in bold.

Year		Contrast	Trait		
			Emergence	Flowering	Mature Seed
2010	a	Parents v. Hybrids	F=7.121 p=.0097	F=1.845 p=.1785	F=.9568 p=.3291
	b	DT v. YT	F=1173 p=.733	F=6.355 p=.0137	F=8.636 p=.0037
	c	DT v. (F1DTxYT+BCDT)	F=2.424 p=.1232	F=.5684 p=.4528	F=2.058 p=.1529
	d	YT v. (F1YTxDT+BCYT)	F=2.18 p=.1502	F=15.31 p=.0004	F=19.26 p<.0001
	e	F1s v. BCs	F=4.683 p=.038	F=1.754 p=.1937	F=.0115 p=.9145
	f	BOULD v. RAD	F=.0587 p=.8102	F=.0786 p=.7809	F=3.204 p=.0749
	g	(Field v. Controlled)	F=4.173 p=.0498	F=.057 p=.8128	F=.1083 p=.7424
	h	(F1YTxDT + BCYT) v. (F1DTxYT + BCDT)	F=.297 p=.5895	F=.7924 p=.3793	F=3.974 p=.0475
	i	DT v. (RAD + BOULD)	F=5.2213 p=.0248	F=.4144 p=.5214	F=1.241 p=.2666
	j	YT v. (RAD + BOULD)	F=9.528 p=.0044	F=11.77 p=.0015	F=11.99 p=.0006

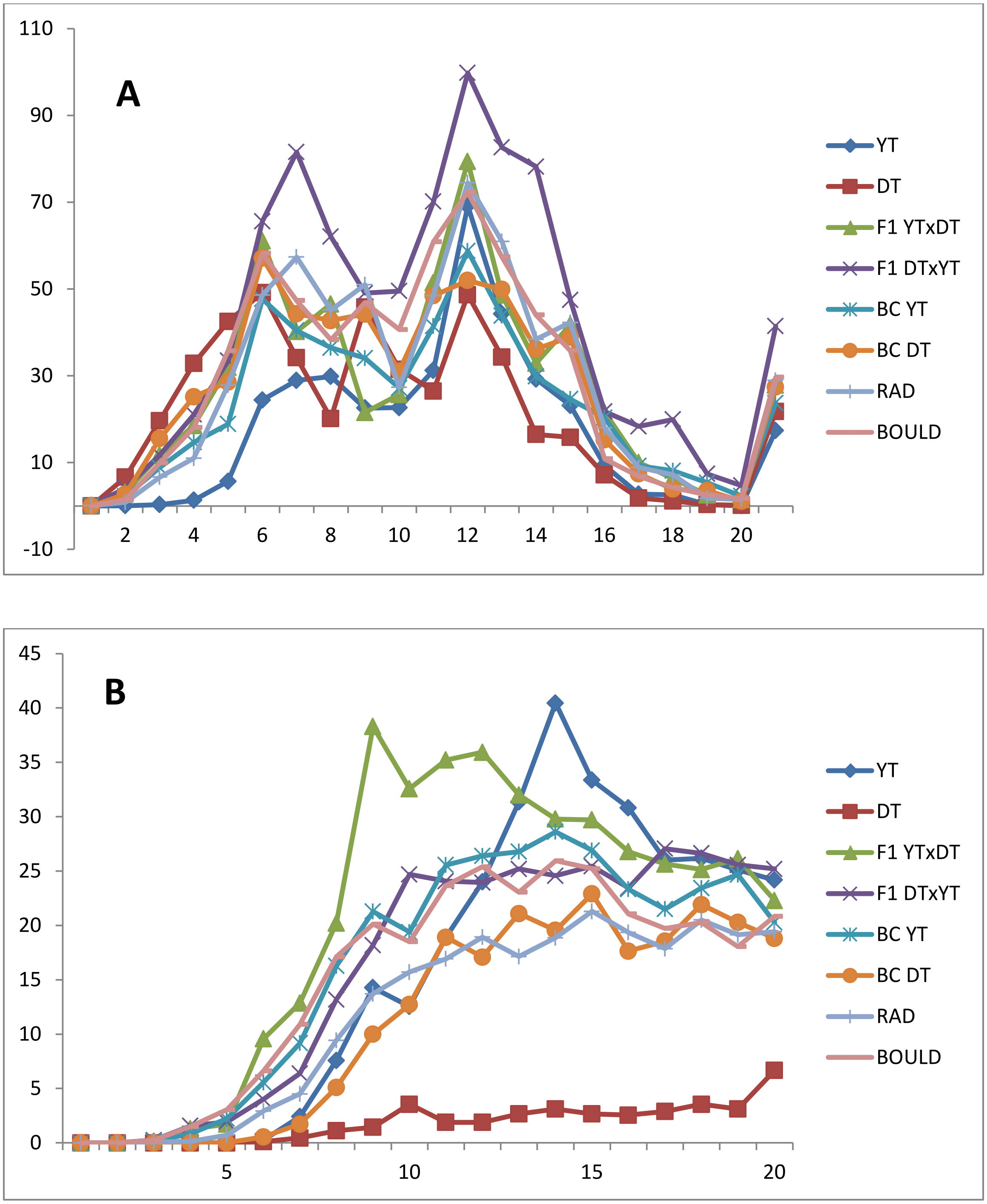


Figure 11: Flowering by Week in 2010: Average number of stems per genotypic class recorded each week with at least one fully open flower. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.) A. In Colorado B. In Montana.

Principal Components:

Previous data describing the biomass and reproductive capacity of parents and hybrids were collected from the common gardens (Chapter 2). These data and the phenological data from this study were used for Principal Components Analysis (PCA) (Table 5). The first axis of variation (~52.4%) was composed largely of correlated quantitative characters and correlated emergence characters. This implies that within this first axis, an increase in plant size was associated with earlier emergence. Within the second axis of variation (~24.1%), plants with increased biomass took a longer time to reach phenological landmarks (Table 5).

Table 5. Loadings of phenological and quantitative traits on the first three principal components.

Trait	PC1	PC2	PC3
Emergence	-0.32771	0.40162	-0.50761
First Flowering	-0.39743	0.38376	0.16714
First Mature Seed	-0.27283	0.54222	0.42102
Shoot Length	0.40128	0.05458	0.59600
Aboveground Dry Biomass	0.35874	0.49459	0.08027
Total Flower Production	0.44027	0.23496	-0.47265
Total Seed Capsule Production	0.42031	0.30761	-0.31582
Percentage Variation Explained	52.4%	24.1%	10.1%

Discussion:

Phenological surrogates of fitness were assessed by measuring phenological time points and intervals, but the latter were strongly correlated with two of the former, thus, analysis was performed using only time points. Hybridization has the potential to alter phenological components of life history, for example Hovick et al. (2012), where earlier emergence time in

Raphanus was related to increased fecundity and led to the ability to invade a new region. In theory, reaching phenological landmarks earlier could also confer a competitive edge when acquiring resources for space and light, and changes in flowering time have the potential to alter plant-pollinator interactions or other mutualisms in the field. Earlier maturation of seed could increase ecological amplitude as reproduction could be achieved in regions with shorter growing seasons. Generally, in these common gardens, hybrids emerged earlier than parents, although this varied somewhat between sites and did not directly translate to faster flowering or seed set. Although it varied by genotype class, early emergence appeared to be transgressive in many hybrids, while flowering time and seed set settled into an intermediate but also site dependent pattern. Therefore, it appears that hybridization in nature resulting in earlier emergence times is one way toadflax might increase competitive establishment or invasive ability. However, shifts in other more intermediately expressed phenological landmarks such as flowering or seed maturity might have more centralizing or transient effects on the population. Such impacts could be difficult to discern and may also be highly environmentally influenced.

Hybrids vs. Parent Species:

A contrast of all hybrids versus both parent species showed hybrids mostly emerged from dormancy earlier than parents, but this did not result in earlier flowering or seed set. This trend may be partially explained by the biology of the two *Linaria* species and intermediate hybrid trait expression. While closely related, Dalmatian and yellow toadflax do have somewhat distinct ecological and particularly, floral phenological patterns. Dalmatian toadflax is an early-flowering species, often blooming in May and even earlier if temperatures are warm; yellow toadflax typically does not bloom until well into the late summer or early fall months. While a contrast of parent species showed no difference between emergence dates for these classes, when first flowering and seed set were compared, their differing life histories resulted in increasingly significant differences. Similarly, when hybrids with a Dalmatian maternal or backcross parent were compared to Dalmatian toadflax itself, no substantial differences in any phenological characters were found. However, an analogous comparison made between yellow toadflax and its maternal F1s and backcrosses demonstrated that while they were no different in emergence time, hybrids did flower and set seed substantially earlier than yellow itself. This suggests that under the right demographic and environmental circumstances, introgression of Dalmatian flowering-time alleles into a yellow toadflax background could confer an advantage in situations where earlier flowering-cycle onset and completion would be adaptive. For instance, earlier flowering might be beneficial in environments with a high degree of late season drought or other stressor. A similar trend was observed when individual parent species were compared to pooled natural hybrid classes (hybrids from the Radersburg and Boulder sites were not, themselves, different from each other for any phenological trait). When natural classes were compared to Dalmatian toadflax, they emerged earlier, but did not flower or set seed any earlier. When the same comparison was made with yellow toadflax, natural hybrids reached every phenological landmark significantly earlier. Again, this is consistent with delayed reproductive biology of yellow toadflax compared to Dalmatian toadflax, but it also indicates natural hybrids may have acquired and retained the earlier-flowering trait of Dalmatian toadflax in addition to transgressive emergence via hybridization.

Site Effect and GxE:

The site effect was not significant in the models for emergence and first flowering, but its interaction with genotype class was. In some cases, missing data prevented the explicit statistical comparison of traits within sites, but class rankings were examined to deduce the source of the interaction. In Colorado, hybrids with Dalmatian maternal and backcross parents and Dalmatian toadflax emerged first, followed by natural hybrids, then hybrids with yellow toadflax maternal and backcross parents, and finally yellow toadflax. By contrast, in Montana early emergence was dominated by the natural hybrid classes, followed by the F1s, and then the remaining backcrosses and parents. The early emergence of the natural hybrid classes (both collected from Montana field sites) is interesting because it suggests potential local adaptation to more northern latitudes. The Colorado trend of early emergence by Dalmatian toadflax and hybrids with Dalmatian maternal and backcross parents, the middling performance of the MT natural hybrids and late emergence of yellow toadflax and its maternal and backcross hybrids, suggests a slightly different pattern where emergence appears more likely to be governed by genomic composition at least in early (F1 and BC1) generations. Without comparable natural hybrids from Colorado and explicit knowledge of the natural hybrid pedigrees it is difficult to say what long-term changes in emergence would look like. Flowering patterns within sites are comparable to the trends observed across sites: in both Colorado and Montana, Dalmatian toadflax flowers first, yellow toadflax flowers last and the source of GxE appears to be reordering of intermediate hybrid ranks. Within this intermediate range there is no readily apparent pattern across classes and sites. For first seed set, a similar pattern is observed, although in Colorado, F1 hybrid plants with a Dalmatian maternal parent set seed earliest and grouped separately (data not shown). In Montana, Dalmatian was first to mature seed, yellow toadflax last, but no differences were significant among classes. All genotype classes set seed earlier in Montana than in Colorado, despite cooler temperatures. It may be that longer days at northern latitudes and a shorter season govern quicker times to maturity regardless of genotype.

As discussed earlier, plants may have to compromise between being large and being early. To look at tradeoffs between size, flowering and fecundity with phenological timing, Principal Components Analysis (PCA) was performed on data from this study and data collected on the same common garden and comprised of plant height, dry biomass, floral and seed capsule production (Chapter 2). The dominant axis of variation (~52.4%) consisted of relatively equal contributions of reproductive and vegetative characters, and phenological characters (emergence, first flowering and mature seed) had opposing signs. This first axis describes bigger plants reaching phenological landmarks earlier than smaller plants, and vice versa and suggests that in large part there is no tradeoff between plant size and completion of life cycle. However, there may be a substantial influence of species biology at play in the distribution of variation in this system. In Chapter 2 it was noted that plants more genomically influenced by Dalmatian toadflax are often larger in both reproductive and vegetative metrics than hybrids related to yellow toadflax; the reproductive cycle of Dalmatian toadflax tends to begin and end at earlier times during the growing season than yellow toadflax. This suggests that the pattern observed in this PCA may be partly governed by a pre-existing genomic association of phenology and plant architecture, rather than indicative of a lack of tradeoff between the two. In contrast to the first axis of variation, the second (~24.1%) revealed a pattern where plants with increased biomass took longer to reach phenological landmarks, suggesting that at least some of the time a bigger growth habit means a longer time to complete reproduction. PCs were also examined across genotypes (data not shown) to check whether dominant axes of variation were different for parents and hybrids, but there were no notable differences.

Conclusion:

In general, hybrids emerged earlier than parent species, which suggests that if they are established in an environment where early emergence confers higher fitness (and an apparent association with increased size and fecundity suggests that it may) then some hybrids may have a further advantage over invasive parents. As the reproductive cycle progressed, an increasing environmental impact was observed in the form of a slope sign change from early Colorado emergence and flowering to earlier Montana seed set suggesting that despite earlier emergences, seed maturity date is largely constrained by growing season, regardless of genotype.

A lack of transgressive expression of traits does not necessarily mean intermediate shifts of phenology will have no impact on the population or community at large. However, strong tendencies towards parental phenological expression have been demonstrated in hybrids in previous research (Oberprieler et al., 2010), and Barton and Hewitt (1985) suggest that over time selection is more likely to diversify a phenological intermediate distribution to a bimodal one with strong parental peaks, or a single strong parental mode. In this case, it seems likely that whichever strategy is most consistently beneficial in a particular environment will increase in frequency. In drought prone habitats, like those often occupied by Dalmatian toadflax, it has been demonstrated that an early reproductive cycle is advantageous because it escapes the increasingly water-limited late season environment (Geber and Dawson, 1997; Ludlow, 1989; McKay et al., 2003). At higher elevation sites often occupied by yellow toadflax the reverse may be true, and a strategy of delayed emergence and flowering to avoid inclement conditions in late winter and early spring may be of greater adaptive significance. It also appears natural hybrids have retained an earlier flowering and emergence time, which suggests the former strategy. These hybrids performed especially well at the Montana common garden site close to their latitude of origin also suggesting local adaptation.

Regarding whether hybridization between these two toadflax species will worsen invasion via stabilized hybrids and/or introgression of adaptive alleles, it appears that hybrids, particularly those with Dalmatian genomic influence have certain potential fitness advantages, specifically earliness, size and fecundity. However, previous research has shown that the success of controlled crosses between these toadflax species is asymmetrical in favor of yellow toadflax as a maternal parent (Ward et al. 2009) and that a majority of field-collected toadflax hybrids analyzed have yellow chloroplast haplotypes (Boswell, 2012). So, while some toadflax hybrids may outperform the parent species, the ultimate evolutionary trajectory depends on hybrid genotype, effective population size and species ratio, and environmental factors, specifically climate and latitude.

Chapter Four: Ecophysiology of Toadflax Species and Hybrids and Association with other Phenotypic Traits

Introduction:

Natural variation in ecophysiological processes and its role in acquisition of limiting resources is a potential mechanism plants may use to adapt to differing environments (Arntz and Delph, 2001; Donovan et al., 2009). Such adaptation may take place over generations as heritable variation, or in the short term, in the form of plasticity. Both magnitude and plasticity of ecophysiological traits are also often suggested as mechanisms which may help plants colonize novel environments (Schlichting and Levin, 1986; Nicotra and Davidson, 2010). A review by Dawson et al. (2012) proposes that it is not so much broad adaptation or multi-directional plasticity which supports species with a wide distribution and abundance, but rather an ability to make use of resource pulses (i.e., favorable conditions). If in fact, ecophysiological processes are differentiated among species according to the environments in which they have evolved (or those they have been exposed to), substantial variation may also exist even within species or between closely related ones (Arntz and Delph, 2001, Flood et al. 2011) and that an ability to capitalize on resources will also differ.

Expected differentiation is one reason ecophysiological processes such as photosynthesis have become a closely examined topic in the field of invasion biology and particularly in the sub-field of native-invasive comparison biology. However, so far the results are rather mixed. Some studies have found invaders have increased photosynthetic rates relative to natives, such as in *Phragmites* (Mozdzer and Zieman, 2010) and *Eupatorium* (Zheng et al. 2009). Other research shows a lack of ecophysiological differentiation between invader and native for resource capture (e.g. Smith and Knapp, 2001; Brodersen et al. 2008). This inconsistency is perhaps unsurprising given that ecophysiological data are often noisy and subject to methodological, environmental and seasonal variation as well as to change according to developmental stage and functional type. Nonetheless, it does appear that at least in certain instances, measured differences in ecophysiology are at least correlated with more invasive plants.

Hybridization, both within and among species, has also been suggested as a facilitator of invasion (Ellstrand and Schierenbeck, 2000); and linked with some very problematic species complexes such as *Spartina* (Ayres et al., 2004; Baumel et al., 2003; Daehler and Strong, 1997) or *Tamarix* (Gaskin and Schaal, 2002). The outcomes of hybridization are extremely variable and have complex hierarchical effects on everything from genomic architecture and regulatory mechanisms (Zou et al. 2011) to population divergence and speciation (Rieseberg et al. 2007). During the colonization of new habitats, hybridization may restore genetic variation lost to bottlenecks, purge a parental load of hidden detrimental alleles, or cause heterosis and/or transgressively segregating phenotypes (Ellstrand and Schierenbeck, 2000). Heterosis refers to expression of reproductive or growth characteristics which exceed phenotypic performance of one or both parents in a positive direction; likewise, transgressive segregation describes all phenotypes outside the realm of parental expression (Rieseberg et al. 1999).

In this study system, two introduced and already invasive *Linaria* species (yellow toadflax and Dalmatian toadflax) are hybridizing in multiple locations where they co-occur (Ward et al. 2009). Both have broad environmental tolerances in their introduced range, but still distinct habitat preferences. These species also share a suite of strong-flying pollinators capable of visiting populations separated by as much as two miles, and therefore the microtopographies preferred by each are often well within distances subject to potential gene flow. Observation of apparently viable toadflax hybrids in the field (Ward et al. 2009) with increasing frequency in addition to experimentally observed heterosis (Chapter 2) has raised concerns that hybridization between these two invasive species may expand their already considerable ecological amplitude or increase vegetative growth or fecundity. An expectation of pre-existing natural variation in ecophysiological rates between species coupled with the often disruptive nature of hybridization suggests hybridization may give rise to increased or novel variation in these traits which may be significant in both evolution and invasion. Furthermore, investigating correlations or tradeoffs between ecophysiological and heterotic traits may help us understand the mechanisms by which some species are more successful than others. In the case of hybrid toadflax, these results could help managers who decide whether they should prioritize control at sites of species co-occurrence.

This study measured ecophysiological rates and leaf characters of parental and hybrid genotypes in a common garden early and late in the growing season. Photosynthesis was analyzed because it is the primary mechanism by which plants assimilate carbon, and transpiration because it is a driving force of water and limiting nutrient uptake as well as water loss. The ratio of these two traits (carbon uptake to water loss) was used to estimate water use efficiency (WUE). This metric is often used as a proxy for drought tolerance because it signifies carbon acquisition per the unit water loss. In addition, specific leaf area (SLA) was measured together with leaf succulence, which are believed to be associated with differences in physiology. Ecophysiological traits were correlated with leaf, quantitative, phenological, and relative growth rate (RGR) as measured in two previous studies (Chapters 2&3). This research addressed the following questions:

1) Are there differences in ecophysiology among genotype classes, and in particular, are there differences between individual parent species and between parents and hybrids?

2) Are there differences among genotype classes for leaf traits thought to be associated with ecophysiological traits?
3) Is ecophysiology correlated with leaf traits or other quantitative or phenological measures of fitness? If so, do the associations of physiology and other trait change between genotypes and across collection dates?

Materials and Methods:

Study Species:

Yellow toadflax (*Linaria vulgaris*) is a diploid herbaceous perennial native to northern and central Europe. It was introduced into the northeast US just prior to 1700 (Alex 1962) and has subsequently spread throughout North America (Lajeunesse 1999). Yellow toadflax is often found in disturbed places (Arnold, 1982), but invasion of native plant communities in national parks (Pauchard et al. 2003) and at high elevations in the Rocky Mountains has also been reported (Sutton et al., 2007). Yellow toadflax is pollinated by strong-flying insects and is a self-incompatible outcrosser; it reproduces via ample seed but also asexually by adventitious shoots from rhizomes. Yellow toadflax has been shown to have high genetic diversity both within and among populations consistent with its self-incompatibility, (Hamrick and Godt, 1996) short-distance seed dispersal and probable multiple introductions (Ward et al., 2008). At last estimate, the USDA has yellow toadflax listed as present in all contiguous states and listed as a noxious weed in seven (The Plants Database, USDA, 2012); it is also present in all provinces and territories of Canada (Sing and Peterson, 2011).

Dalmatian toadflax (*Linaria dalmatica*) is also a diploid perennial herb but originates near *Linaria's* center of origin in the Mediterranean, and its native range extends from former

Yugoslavia to northern Iran (Alex, 1962). *L. dalmatica* was first imported to North America in the late 1800s (Vujnovic and Wein, 1997) and has since become widely distributed in the U.S. and Canada. Dalmatian toadflax is also pollinated mostly by large, strong-flying bees and is a self-incompatible outcrosser (Bruun, 1936). Mature plants may produce up to 500,000 seeds which can germinate or remain dormant in the seed bank for a decade (Wilson et al., 2005). Dalmatian toadflax also has the capacity to reproduce asexually via adventitious buds on lateral roots. Dalmatian toadflax has been introduced multiple times as an ornamental and is now widely distributed across much of the US (excluding the Southeast), and is listed as a noxious weed in 12 states (The Plants Database, USDA, 2012) as well as three Canadian provinces (Sing and Peterson, 2011).

Site Description and Source Material:

Ecophysiological data were collected from plants in an established common garden. The experiment was designed as a randomized complete block and was located in Fort Collins, CO (40°34’12”N, 105°05’29”W, elev. 1530 m) at the Plant Environmental Research Center on the Colorado State University campus. The garden was initiated in 2009 and designed to measure characters of two parent toadflax species as well as multiple hybrid classes. F1 and backcross classes were made by hand in the greenhouse at Colorado State University. Dalmatian toadflax was collected along the Front Range in Colorado as well as from populations near Helena, Montana. Yellow toadflax was collected from the Colorado Front Range, the Colorado Flat Tops Wilderness, and Paradise Valley, Montana and a population near Fairbanks, Alaska. Natural hybrids were collected near Boulder, MT, along a forest service road and northeast of Radersburg, MT on the Townsend Ranger District of the Helena National Forest. (See Figure 1, Chapter 2).

Generation of plant material and garden establishment:

Cloned replicates of each genotype were generated by rooted cuttings. Five centimeter shoot cuttings were stripped of leaves on the lower 16 mm and dipped in rooting hormone powder (Schultz, 0.1% Indole-3-butyric acid) before placement in Fafard 2SV potting medium. Cuttings were kept under humidity domes and misted twice daily until root establishment. Rooted replicates were grown in the Colorado State University greenhouse for 3 weeks and then transferred outside to the common garden site for two weeks acclimation under shade cloth. Planting took place in June 2009 in five gallon plastic pots sunk so that the lip of the pot was flush with ground level. Plants were grown in ProMix BX media from Premier Horticulture and fertilized with Osmocote 14-14-14 at a rate of two tablespoons per plant once per growing season in early August. Plants were watered twice weekly using drip irrigation at a rate of two gallons per hour for 15 minutes at a time.

Data collection:

Ecophysiology and leaf trait data were collected two years post-establishment in the summer of 2011. Photosynthetic rate, transpiration and WUE (water use efficiency) were measure on three cloned replicates of each of three genotypes in each of eight genotypic categories. These categories were as follows: two parental species classes (yellow and Dalmatian toadflax), two first generation hybrid (F1) classes (one with yellow toadflax as the maternal parent, one with Dalmatian), two backcross classes (one backcrossed to yellow toadflax, one backcrossed to Dalmatian), and two classes of molecularly confirmed hybrids (Ward et al., 2009) collected from natural occurring populations at two different field sites. Three healthy leaf replicates from the lower part of the top third of each plant were collected and physiological data were obtained using a LI-COR 6400XT (LI-COR Biosciences, Inc., Lincoln, NE). Individual leaves were collected at spaced intervals over the data collection period (i.e., the first leaf from each plant was collected, then the second, then the third.) Data collection periods occurred on three consecutive days and a different replicate of each plant was sampled each day. Each leaf was analyzed immediately after collection for carbon assimilation rate (µmol CO_2 $m^{-2}s^{-1}$), transpiration (mmol H_2O $m^{-2}s^{-1}$). System settings were reference CO_2 at 400 ppm; flow rate at 400 µmol s^{-1}; photosynthetically active radiation (PAR) 1400 and 10% blue. Once stabilized, five readings per leaf were taken at three second intervals). Measured leaves were scanned and leaf area was calculated using Adobe Photoshop. Leaves were oven-dried at 80 C and SLA was calculated as the ratio of leaf area to dry mass (Garnier et al. 2001) and leaf succulence was calculated as [Wet Biomass – Dry Biomass]/Leaf Area (Jennings, 1976). Instantaneous WUE was calculated as the ratio of photosynthetic rate to transpiration rate (µmol CO_2 $m^{-2}s^{-1}$/ mmol H_2O $m^{-2}s^{-1}$). photosynthetically active radiation (PAR) 1400 and 10% blue. Default leaf areas were replaced with individually calculated leaf areas and used in the calculations of photosynthesis and transpiration. To examine seasonal variation in ecophysiology, the entire analysis was replicated both in early summer (June 23-25), and in late summer (August 24-26).

Data analysis:

Data analysis was carried out using JMP, Version 9.2 (SAS Institute Inc., Cary, NC). Multivariate analysis was conducted on system variables of LI-COR 6400XT to remove data collected when these variables were anomalous (i.e., disruptions in flow rate or PAR). Outliers which did not make biological sense (i.e., negative photosynthesis) were also removed and data were Box-Cox transformed to meet assumptions of normality. Mixed model analysis was performed using Residual Maximum Likelihood (REML) to test for fixed effects of genotype class, month, collection date (block) and interactions. Leaf replicate and individual genotypes within classes were included as random effects. The distribution of final model residuals was examined to ensure distribution assumptions were met. Tukey's Honestly Significant Difference was used to compare overall differences among genotype classes and collection dates, and individual contrasts were made between pooled and individual genotypic classes to test more specific hypotheses. Spearman's correlations of physiological data with previously collected phenological and quantitative metrics (Chapters 2&3) were performed to determine whether particular physiological processes are associated with these phenotypes. All correlations, model effects and contrasts were considered significant at an alpha level of 0.05.

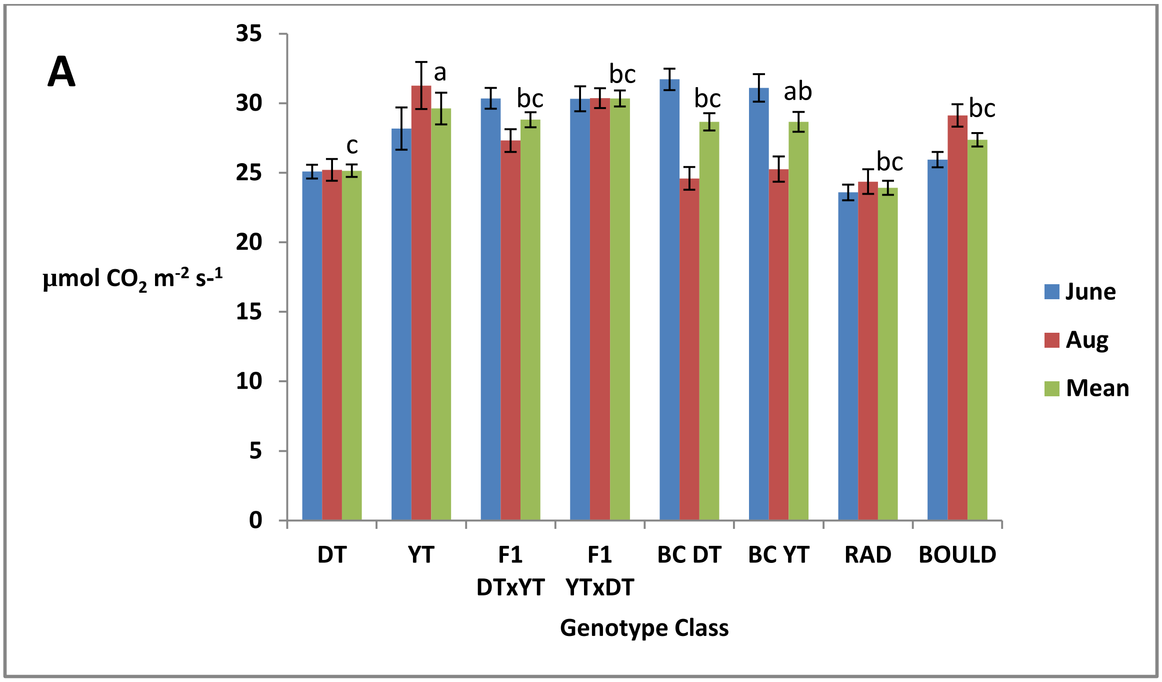
Results:

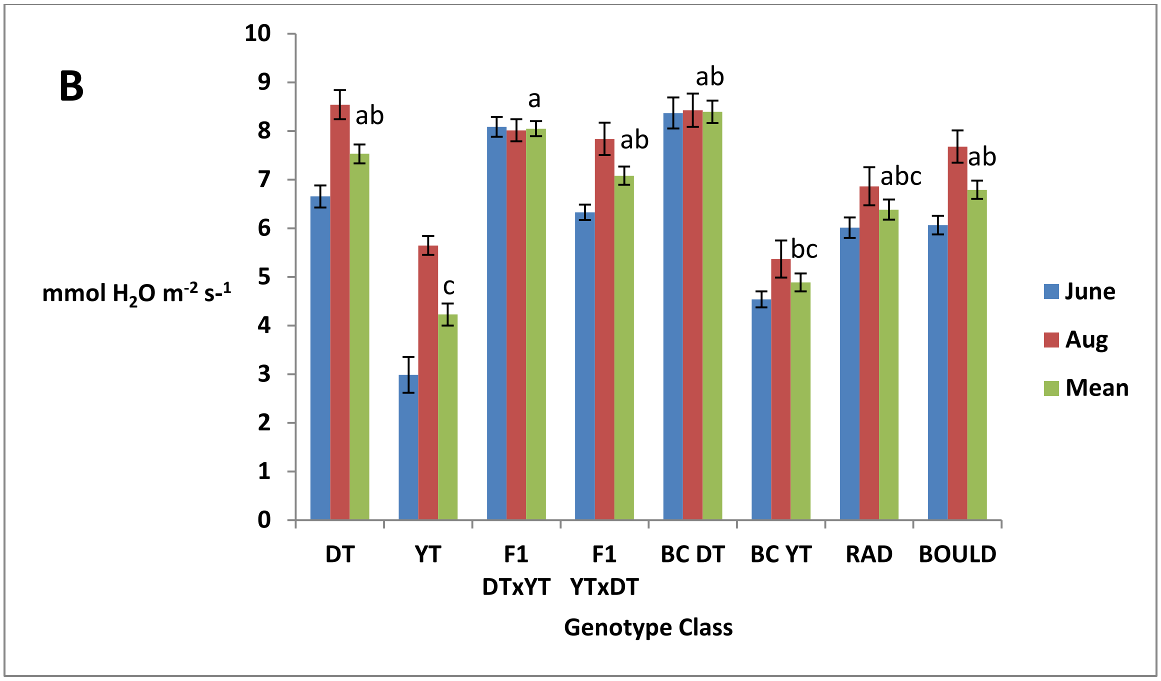
Photosynthesis:

Ecophysiological data were collected on a subset of the original Colorado common garden so the full model was slightly different than for the quantitative and phenological traits (Chapters 1&2). In the model for photosynthesis, genotype class, block, month, genotype class*month were significant. As expected, carbon assimilation was impacted by environmental variation, as evidenced by the effects of month and date of collection. Examination of HSD separation of genotype class means of revealed an intermediate trend in which yellow toadflax was significantly more photosynthetically active than Dalmatian toadflax and hybrids were distributed in between (Fig 12A). Both F1 classes, both backcross classes and one natural hybrid class (Boulder) were in the HSD group with the highest photosynthetic rates. In general, rates of photosynthesis were higher in June than August, but the month effect was not consistent across all genotype classes: Yellow toadflax, both F1 classes and Boulder natural hybrids were top performers in both months, while backcross classes were less photosynthetically active in August than June.

Transpiration, Water Use Efficiency and Leaf Traits:

The model for transpiration had the same significant effects as photosynthesis. Genotype classes with the highest rates of transpiration were F1s with a Dalmatian toadflax maternal parent, backcrosses to Dalmatian and Dalmatian toadflax itself (Fig. 12B). In contrast, yellow toadflax, backcrosses to yellow toadflax and one natural hybrid class were grouped by HSD with the lowest rates of transpiration. Overall, transpiration was higher in August than in June, and while genotype class*month interaction was significant, it was driven by only a weak tendency of Dalmatian-type hybrids (those with Dalmatian toadflax maternal and backcross parents) to transpire more in June. The model for WUE had the same significant effects as the photosynthesis and transpiration models. HSD separated a group of the two most water use efficient genotype classes: yellow toadflax and backcrosses to yellow toadflax (Fig. 12C). This is consistent with WUE as a ratio of photosynthesis to transpiration since previous analyses revealed yellow toadflax to have high photosynthesis relative to Dalmatian toadflax, yet also relatively low transpiration. Overall, WUE was higher in June, and this trend was nearly universal, though not always significant within individual genotype classes. Models for leaf characteristics (SLA and leaf succulence) had no significant model effects, nor were there any correlations of these traits with physiological (photosynthesis and transpiration) rates (data not shown).


A
35
30
25
20
15
10
5
0
μmol CO2 m-2 s-1
a
c
bc
bc
bc
ab
bc
bc
DT
YT
F1 DTxYT
F1 YTxDT
BC DT
BC YT
RAD
BOULD
Genotype Class
June
Aug
Mean


B
10
9
8
7
6
5
4
3
2
1
0
mmol H_2O m^{-2} s^{-1}
ab
c
a
ab
ab
bc
abc
ab
DT
YT
F1 DTxYT
F1 YTxDT
BC DT
BC YT
RAD
BOULD
Genotype Class
June
Aug
Mean

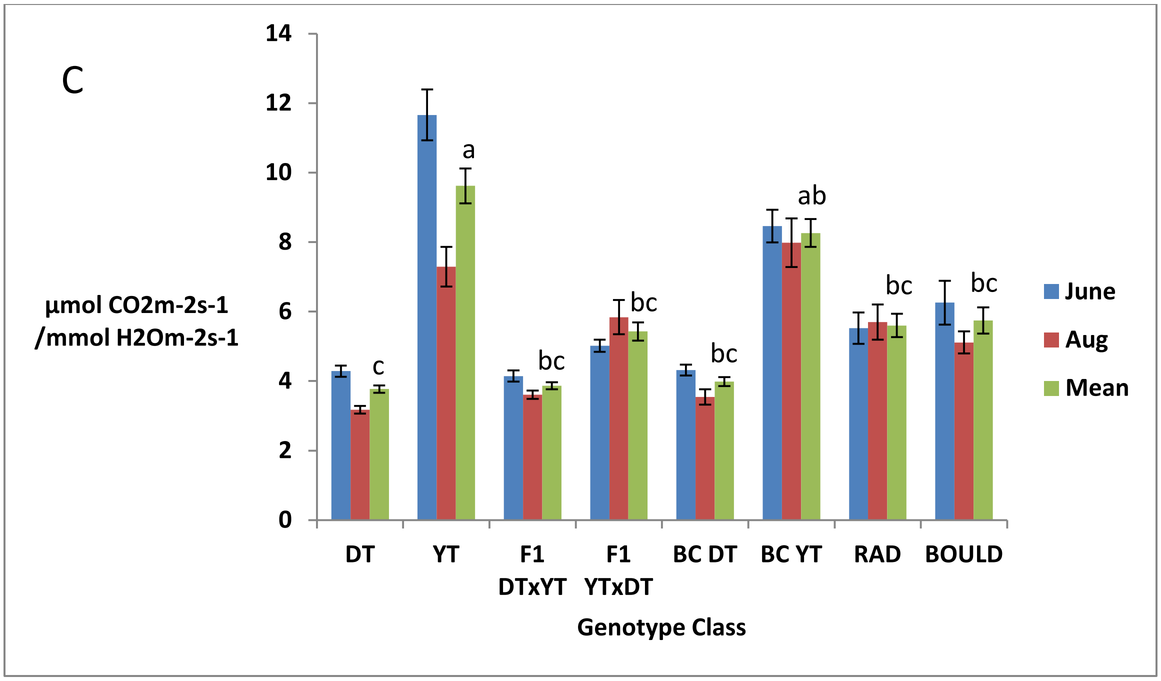


Figure 12. Physiological data by month and by genotype class; combined-month mean separation also shown. (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.) A. Photosynthetic rate as measured in CO_2 assimilation (µmol CO_2 $m^{-2}s^{-1}$). B. Transpiration rate in (mmol H_2O $m^{-2}s^{-1}$). C. Instantaneous water use efficiency (WUE) as a ratio of photosynthesis to transpiration (µmol CO_2 $m^{-2}s^{-1}$/ mmol H_2O $m^{-2}s^{-1}$).

Table 6: Contrasts of physiological data. a) All parents vs. all hybrids. b) Dalmatian vs. Yellow toadflax. c) Dalmatian toadflax vs. Dalmatian-influenced hybrids. d) Yellow toadflax vs. Yellow-influenced hybrids. e) All F1s vs. all backcrosses. f) Boulder vs. Radersburg hybrids. g) Field hybrids (Radersburg and Boulder) vs. controlled-cross-generated hybrids. h) Yellow-influenced hybrids vs. Dalmatian-influenced hybrids. i) Dalmatian toadflax vs. natural hybrids j) Yellow toadflax v. natural hybrids. F Ratios and p-values are given.

	Contrast	Trait		
		Photo	Transp	WUE
a	Parents v. Hybrids	F=.048 p=.8288	F=7.89 p=.01	F=10.2 p=.0039
b	DT v. YT	F=15.6 p=.0007	F=19.24 p=.0002	F=41.5 p<.0001
c	DT v. (F1DTxYT+BCDT)	F=10.24 p=.0043	F=1.65 p=.2122	F=.0002 p=.9882
d	YT v. (F1YTxDT+BCYT)	F=1.54 p=.2299	F=7.32 p=.0135	F=14.19 p<.0011
e	F1s v. BCs	F=2.22 p=.1552	F=2.00 p=.1724	F=1.25 p=.2765
f	BOULD v. RAD	F=7.20 p=.0187	F=.167 p=.6876	F=.669 p=.4238
g	(Field v. Controlled)	F=8.88 p=.0098	F=.924 p=.3487	F=.0073 p=.9326
h	(F1YTxDT+BCYT)v. (F1DTxYT+BCDT)	F=.051 p=.8241	F=.13.5 p=.0015	F=13.6 p=.0013
i	DT v. (RAD + BOULD)	F=1.30 p=.2673	F=1.56 p=.2251	F=2.89 p=.1018
j	YT v. (RAD + BOULD)	F=11.12 p=.004	F=12.66 p=.0021	F=20.3 p<.0001

Correlations of Ecophysiologcial with Quantitative and Phenological traits:

To simplify the correlation of ecophysiological traits with growth rate, quantitative, and phenological data, the first principal component of the quantitative traits “PC1Q” (called “fitness” in chapter 2) and the first principal component derived from phenological traits from Chapter 3 (“PC1P”), were used in addition to RGR (relative growth rate). No combined month ecophysiological trait was correlated with RGR; transpiration was correlated with PC1Q; photosynthesis, transpiration and WUE were all correlated with PC1P. Correlation of PC1Q and transpiration was positive: i.e., collectively bigger and more fecund plants transpired at higher rates. Correlations of photosynthesis and transpiration with phenology were negative: plants with shorter times to phenological landmarks had higher rates. WUE was positively correlated to phenology: more water use efficient plants reached phenological stages later than those with lower WUE. When physiological data were divided by sampling month, patterns changed slightly; where no trait correlations with photosynthesis were previously significant, RGR was now associated with photosynthesis, but in opposing directions (RGR was negatively correlated with photosynthesis in June and positively in August). In other words, plants which grew faster overall had higher photosynthetic rates in August and but lower rates in June. RGR demonstrates an analogous relationship with WUE, that is, faster growing plants are more water use efficient in June than August. Similarly, PC1Q has a negative association with WUE in June only meaning bigger and more reproductively active plants are less water use efficient at that time (Table 7b).

Since there appeared to be an impact of genomic composition upon ecophysiological expression in parents and hybrids, differences between genotypic classes in the way ecophysiological processes were associated with quantitative and phenological traits were examined. However, when broken down into smaller groups of data, much power to detect significant relationships was lost, so strong patterns were generally not observed. However, the same positive/negative split relationship of ecophysiology with phenotype when the data were divided by month was seen, but only for natural hybrid classes (Table 7a.)

Table 7a. Correlation of physiological rates with phenotypic traits by genotype. (Photosynthesis (A), Transpiration (E), and Water Use Efficiency (A/E)) with Relative Growth Rate (RGR), and the first principal component of combined quantitative traits (PC1Q) and phenological traits (PC1P). (YT is Yellow toadflax; DT is Dalmatian toadflax; F1s are listed with maternal parent first, Backcrosses are listed by the pure parent; RAD=Radersburg and BOULD=Boulder are natural hybrid site classes.) Data are given for individual genotypic classes for each of two collection time points (June and August) as well as the average. Spearman's P is presented with significance level.

Genotype	Trait	A (Photo)			E (Transp)			A/E (WUE)		
		Jun	Aug	Avg.	Jun	Aug	Avg.	June	Aug	Avg.
YT	RGR	NS	NS	NS	NS	NS	NS	NS	NS	NS
	PC1Q	NS	NS	NS	NS	NS	NS	NS	NS	NS
	PC1P	NS	-.82*	NS	NS	NS	NS	NS	NS	NS
BCxYT	RGR	-.52**	NS	NS	NS	NS	NS	NS	NS	NS
	PC1Q	NS	NS	NS	NS	NS	NS	NS	-.52*	NS
	PC1P	NS	NS	NS	NS	NS	NS	NS	NS	NS
FYTxDT	RGR	NS	-.48*	NS	.48*	NS	NS	NS	NS	NS
	PC1Q	NS	NS	NS	NS	NS	NS	NS	NS	NS
	PC1P	NS	NS	NS	NS	NS	NS	NS	NS	NS
RAD	RGR	-.41*	.51*	NS	.36*	NS	NS	-.58***	NS	NS
	PC1Q	NS	NS	NS	.66***	-.60**	NS	-.74***	.70***	NS
	PC1P	NS	NS	NS	NS	-.52*	NS	NS	NS	NS
BOULD	RGR	-.37*	.48*	NS	NS	-.63**	NS	-.48**	.53*	NS
	PC1Q	-.35*	.48*	NS	NS	NS	NS	NS	NS	NS
	PC1P	NS	NS	NS	NS	NS	-.40**	NS	NS	NS
FDTxYT	RGR	NS	-.61***	NS	NS	-.53**	NS	NS	NS	NS
	PC1Q	NS	NS	NS	NS	-.43*	NS	NS	NS	NS
	PC1P	NS	NS	NS	NS	NS	NS	NS	NS	NS
BCxDT	RGR	NS	NS	NS	NS	NS	NS	NS	NS	NS
	PC1Q	NS	NS	NS	NS	NS	NS	NS	NS	NS
	PC1P	NS	NS	NS	-.47*	NS	-.38*	NS	NS	NS
DT	RGR	-.63**	NS	-.37*	.88*	NS	.88*	NS	NS	NS
	PC1Q	NS	NS	NS	NS	-.57*	NS	-.47*	NS	NS
	PC1P	NS	NS	NS	NS	NS	NS	-.88*	NS	-.88*

Table 7b. Correlation of physiological rates with phenotype, by month. (Photosynthesis (A), Transpiration (E), and Water Use Efficiency (A/E)) with Relative Growth Rate (RGR), and the first principal component of combined quantitative traits (PC1Q) and phenological traits (PC1P). Data are averaged across genotype classes for each of two collection time points (June and August) as well as the average. Spearman's P is presented with significance level.

Trait	A(Photo)			E(Transp)			A/E (WUE)		
	Jun	Aug	Avg.	Jun	Aug	Avg.	Jun	Aug	Avg.
RGR	-.22**	.22**	NS	NS	-.18*	NS	-.20**	.30***	NS
PC1Q	NS	NS	NS	.32***	NS	.14**	-.28***	NS	NS
PC1P	-.16*	-.20*	-.17**	-.19*	-.34***	-.8***	NS	.18*	.14*

Discussion:

Photosynthesis is the primary mechanisms by which plants acquire carbon to build biomass. Therefore, variation in photosynthetic capacity and other related processes such as transpiration may result in differential growth rates and productivity which can affect fitness and competitive ability (Flood et al., 2011). Extensive heritable genetic variation in photosynthetic rate and other associated physiological traits has been demonstrated both within and among populations of a single species (Arntz and Delph, 2001) although the magnitude of heritability estimates differs substantially across studies and organisms. Ecophysiological variation is often associated with an environmental factor or gradient to which the genetic differences are thought to be adaptive (e.g., Teramura and Strain, 1979) although no published study explicitly rules out drift as the cause rather than selection. Therefore, it seems reasonable to expect ecophysiological variation between the two hybridizing species in this study (each occupying a somewhat different niche), and perhaps between their offspring as well. However, physiological differences may not translate to selective differences in fitness or ability to invade, and they may also interact with other functional plant traits in the effects they do have on fitness, as seen in (Dudley, 1996). Additionally, traits which initially appear advantageous such as a higher photosynthetic rate may actually prove maladaptive in certain environments where high growth rates or consistently open stomata are risky. Ecophysiological measures are also expected to fluctuate over time with developmental stage and growing season, space and available resources, limiting inference about their influence on fitness without extensive data (Arntz and Delph, 2001).

Results from this study suggest ecophysiological trait expression in toadflax hybrids follows a largely intermediate pattern distributed between a high and a low parent although, which parent was high or low depended on the trait. Intermediacy of ecophysiological traits in hybrids has been reported in the literature (e.g. Brock and Galen, 2005; Kimball and Campbell, 2009) and indicates physiological expression in hybrids is likely to be via an at least partially dominant mechanism derived from one parent rather than overdominance derived from both. Combined data from June and August in this study demonstrate substantial differences in both photosynthesis and transpiration between species: yellow toadflax had the highest photosynthetic rates, Dalmatian toadflax the lowest (Fig. 1A&B). This was initially unexpected, given that higher rates of photosynthetic activity have been shown to be likely adaptations in populations with higher irradiance (Sims and Kelley, 1998) and that Dalmatian toadflax prefers sunny, open ground, versus more fertile and moist habitat preference of yellow toadflax. However, higher rates of photosynthesis have also been shown to be higher in plants from greater altitude (Gurevitch, 1992), and yellow toadflax often establishes in such locales. More likely, it may be different life history characters cause the species differential. Campbell et al., (2005) showed that photosynthesis was greater in vegetative plants versus those which had reached reproductive maturity and while the June sampling in this study was intended to be at a purely vegetative stage, extremely warm weather in the days prior to data collection meant some Dalmatian plants as well as some Dalmatian-like hybrids had just begun to flower. Therefore, the true photosynthetic peak may have been missed for Dalmatian toadflax and the relatively later yellow toadflax may have been caught just as it was ramping up.

Hybrid transpiration was also intermediate, but re rates reversed for the parent species i.e., yellow toadflax had very low rates of transpiration, Dalmatian, significantly higher (1B). It was surprising that a drought tolerant species such as Dalmatian toadflax had such rapid rates of water loss. However, it may be that transpiration rates in Dalmatian toadflax are plastic, and under benign common garden conditions, lack of drought stress results in no conservation. Yellow toadflax had the highest WUE (CO_2 uptake to H_2O lost), as it assimilated more carbon than Dalmatian toadflax but also consistently gave up less water doing it (Fig. 1C). This result is striking given the perceived drought tolerance of Dalmatian toadflax, but perhaps not surprising because although WUE is often interpreted as an indicator of drought tolerance, it is also (like the metrics from which it is derived) a flexible species trait which changes across environments (Blum, 2005) and plant architectures (Dudley, 1996). Therefore, it is likely were full reaction norms not visualized for this trait with only two sampling periods and unstressed conditions, but also that WUE is a more a measure of differential species architecture rather drought tolerance.

Hybrids and Parents:

There was no significant difference in photosynthetic rate between parents and hybrids, which is not surprising given intermediate expression of the trait (Table 6). On the other hand, there were marginally significant differences between parents and hybrids for transpiration and WUE indicating mid-parent heterosis for these traits. Transpiration differences between parents and hybrids were driven by the high rates of early generation hybrids with a Dalmatian maternal or backcross parent (Fig 12A). Overall, WUE of hybrids was also greater than parents, but varied across genotype classes and was derived in multiple ways, that is, a high WUE could be generated by either high relative photosynthetic rates or low relative transpiration rates and might have variable adaptive significance in different environments. Wu and Campbell (2006), found different routes toward similar WUEs to be associated with different cytotypes in an F2 generation of *Ipomopsis* and data in this study show a similar, albeit non-significant pattern in the two F1 classes. Such a pattern may reflect interaction between cytoplasmic (highly photosynthetically relevant) and nuclear genomes. This indicates any fitness advantages of hybridization-altered ecophysiology might be especially contingent upon the direction(s) of the initial cross. Compared with early generation hybrids for which it was a maternal or backcross parent, yellow toadflax was not photosynthetically different, yet transpired less and had higher WUE (Table 6). This suggests hybridization with Dalmatian toadflax, at least in early generations, does not substantially compromise photosynthetic capacity, but may increase potential water loss. In the reciprocal case, early generation Dalmatian hybrids appeared to gain photosynthetic capacity via hybridization with yellow, but did not see significantly reduced transpiration or increased WUE.

As differences between hybrid generations are often observed (e.g. Arnold and Hodges, 1995; Ruhsam, 2011), early generations (F1s and BCs) were compared with each other and with natural hybrids (Table 6). No significant differences were observed between F1s and BCs or between natural and early generation hybrids for transpiration or WUE. However, early generations did have a higher rate of photosynthesis relative to field-collected hybrids and there was also a photosynthetic difference between field sites. Whether such differences are a result of local adaptation or lineage is unknown, but comparison of natural hybrids and each parent shows that while natural hybrids are no different from Dalmatian toadflax for any physiological trait, they differ from yellow toadflax for *every* physiological trait. Again, this pattern could develop by selection for a Dalmatian-like suite of traits or a demographic ratio (i.e., more Dalmatian than yellow toadflax in a population).

Month Effect and Genotype by Month Interaction:

In general, photosynthesis and WUE were higher in June and transpiration was higher in August; all traits had a significant month by genotype class interaction. The interaction of genotype class and month for photosynthesis was driven by the increased (and temporarily transgressive) rates of F1 DTxYT and backcross classes in June followed by a steep drop off in August. It is possible this trend was partially driven by differences in species phenology, as all these genotypes are at least marginally influenced by earlier flowering in Dalmatian toadflax and therefore its earlier photosynthetic peak. Transpiration and WUE remained mostly consistent across months with only minor changes to genotype class ranks. In combination with intermediate expression of these traits across classes, these results suggest that at least within the common garden, WUE may be more closely associated with species differences and genomic composition than with environmental conditions.

Correlations of Ecophysiological with Quantitative and Phenological traits:

Ecophysiological processes are often found to have associations or tradeoffs with plant architecture and phenology (Kimball and Campbell, 2009; Wright et al., 2004) or vegetative and reproductive output (Meyer and Hull-Sanders, 2008; Mozdzer and Zieman, 2010). In previous work, quantitative and phenological traits were measured in a single common garden environment and hybrids demonstrated both transgressive and intermediate expression depending on the class and character measured (Chapters 2&3). To examine how ecophysiological processes relate to expression of phenological and quantitative traits pairwise correlations were performed, revealing that the relationship is not only contingent on the time of physiological sampling, but also varies by trait and genotype class.

Correlation between PC1Q and transpiration was positive: bigger, more fecund plants had higher transpiration rates and also reached key phenological stages earlier (Table 7b). Transpiration is a major mechanism by which plants draw up minerals, nutrients and additional water from the soil, many of which cannot be produced by any metabolic process within the plant itself. Therefore, one explanation for association of increased rates of transpiration with bigger, earlier plants may be that higher transpiration is advantageous by allowing increased uptake of otherwise limiting resources. Not only might transpiration be physiologically related to increased proximate measures of fitness, it may also suggest a mechanism by which certain genotypic classes compete for nutrients in poor environments (Table 7a), although it may come at an increased water cost. However, correlations across genotypic classes may also be due to genomic composition. For instance, the Dalmatian toadflax genome appears to confer not only an increased rate of transpiration, but also increased size (Chapter 2), so size and transpiration traits may related by genetics, but not physiology. The same may be true of the correlation between phenology and transpiration because it is unclear whether the observed physiological process actually drives maturation or just coexists in an early flowering genome. Likewise, any correlation may be fueled by covariance with something unmeasured or unobserved, so these results should be interpreted with caution.

Data were also examined within months to identify whether ecophysiology contributes to phenotypes at different developmental stages. Relative growth rate was significantly associated with photosynthesis across dates but with opposing effects (negative in June and positive in August) (Table 7b). At first glance, this is surprising because photosynthetic rate would be expected to positively impact the rate of biomass accumulation (RGR). However, RGR estimates in this study were derived across seasons rather than within them (which plants grew fastest on a year to year basis) so this means plants which gained biomass fastest over time were those which maintained higher rates of photosynthesis late in the season. A similar observation for WUE suggests faster biomass accumulators were also more water use efficient late in the season, so even though rates of photosynthesis in these plants remain relatively high in August, some mechanism was mitigating stomatal or other water loss. Decoupling of these processes could prove adaptive in water-limited environments.

Genomic composition impacted ecophysiological expression; therefore, genotype class differences were tested for within patterns of correlation (Table 7a). As above, when the data are split between months, opposing correlations were still observed between June and August. However, these relationships appeared only in the two natural hybrid classes, but not in other parents or hybrids. For both Radersberg and Boulder hybrids, faster accumulators of biomass were those with higher late but lower early season photosynthesis, and the same was true for PC1Q of Boulder hybrids. Likewise, natural hybrid classes with increased late season WUE were likely to grow faster and be more fecund. These patterns occurred exclusively in hybrid classes which have been exposed to some degree of selection in the field. An absence of this trend in the parent species suggests such plasticity might be a capability unique to hybrids (although, not one which is obvious in early hybrid generations). It could be that such a trait might not be explicitly derived in early generations, but rather increased genomic amplitude may be created during this process, but only stabilized under subsequent introgression and natural selection.

Conclusion:

There is ample evidence to support extensive natural genetic variation of physiological traits within species (Arntz and Delph, 2001; Flood et al. 2011), but results comparing invasives and natives and hybrids and parent species are inconsistent. In this study, distinct differences in physiological rates between parent taxa and consistent physiological differences intermediate to parents among hybrid classes were observed, although no differences in SLA or leaf succulence were found. In general, physiological patterns showed stronger correlations with phenology than with quantitative growth and reproduction. When individual genotypic classes were examined within months, natural hybrids had strong relationships between ecophysiology and other phenotypic traits but the direction of the correlation changed across sampling times indicating that timing of ecological processes may be as relevant as their magnitude. This agrees with Dawson et al. (2012) who noted the importance of plant ability to capitalize under favorable conditions and that it may be this very specific type of response to resource availability which furthers widespread distribution and abundance of species. This emphasizes the importance of an organism’s environment in determining its fitness, and how short-term acquisition responses to fluctuating resources may be crucial for both immediate success and future evolution of novel hybrid and/or invasive populations. If stabilized toadflax hybrids have an improved ability to exploit dynamic resource ability, this may contribute to their persistence or facilitate invasive spread.

Summary:

Hybridization is occurring between two invasive toadflax species at multiple sites across the Rocky Mountain region. Because such hybridization can lead to adaptive novel phenotypes or heterosis, land managers who are already dealing with these two problematic species are concerned that hybrids may present an even more aggressive threat. Therefore, this study addressed the comparative performance of yellow and Dalmatian toadflax and their hybrids at two latitudes and from multiple angles via collection of reproductive, vegetative, phenological and physiological data.

Generally speaking, hybrids had higher fecundity and greater biomass at both the Colorado and Montana common garden sites. This apparent multiple trait heterosis is worrisome, because it suggests that not only may the hybrids have potential to be more invasive than the noxious parents, but that they may also have multiple mechanisms (both vegetative and sexual) to achieve this increased invasiveness. Despite the overall increased performance of hybrid classes, there is substantial variability as well, and it does appear their performance is at least somewhat contingent upon where they are grown. For example, it seems that yellow toadflax alleles may confer potential advantages as latitudes increase, while when they decrease, it may be a Dalmatian genomic composition which is most beneficial. It is difficult to say to what extent the extreme size and reproductive capacity of the F1 generation might persist in later generations, but they may still perennate and expand via asexual growth. Additionally, as both backcross and natural hybrid classes in this study were nearly as large and fecund as our most heterotic F1 hybrids, some potential for the retention of heterosis certainly exists.

Phenological observation of toadflax parent species and hybrids revealed that in general, hybrid genotypic classes emerge from dormancy earlier. This suggests that if hybridization occurs and offspring become established in environments where early emergence is beneficial they may have an adaptive advantage relative to one or both parent species. As the season progressed beyond emergence, a strong environmental impact was seen as early Colorado flowering changed into earlier Montana seed set which suggests that in spite of earlier hybrid emergence, time to maturity is dictated by the site and latitude at which the plant grows, regardless of genotype.

Over time, selection on phenological traits is more likely to change an intermediate hybrid distribution to one with strong parental peaks, or a single parental mode. In this case, it seems likely that whichever strategy is most consistently beneficial in a particular environment will increase in frequency, but that this could potentially vary quite a bit across the Rock Mountain region. In areas prone to drought, such as those often occupied by Dalmatian toadflax, early reproductive completion may be advantageous because it escapes an often water-limited late season environment. At higher elevation sites often occupied by yellow toadflax the reverse may be true, and a strategy of delayed emergence and flowering to avoid frost or other stress in late winter and early spring may be of greater adaptive significance. It appears natural hybrids have earlier flowering and emergence times, which suggests the former strategy (or a stronger demographic influence of Dalmatian toadflax) and the fact that these hybrids performed especially well at the Montana common garden site (close to where they were sampled) also suggests local adaptation.

There is often extensive natural genetic variation in physiological traits within species, but results comparing the ecophysiology of invasive vs. native and hybrid vs. parent species are inconsistent. Distinct differences in physiological rates were observed between parent taxa and consistent and intermediate physiological differences among hybrid classes, though these differences were not found for SLA or leaf succulence. Physiological patterns showed stronger correlations with phenological timing than with quantitative growth and reproduction. When individual genotypic classes were examined within months, natural hybrids had strong relationships between ecophysiology and other phenotypic traits but the direction of the correlation changed across sampling times indicating that the timing of changes in ecophysiological processes may be as relevant to plant performance as their magnitude. It is this ability of plants to capitalize under favorable conditions and respond to resource availability which may ultimately govern the distribution and abundance of species. Thus, an organism's environment and its response to resource pulses is a key determinant of its fitness and will be crucial for both colonization and future evolution of novel hybrid and/or invasive populations.

Whether hybridization between toadflax species will further aggravate invasion via stabilized hybrids or introgression of adaptive genetic material remains unknown, but it does appear that hybrids, particularly those with Dalmatian genomic influence have certain potentially adaptive traits such as early reproduction, size and fecundity. However, an inherent asymmetry in viable crosses in favor of yellow toadflax as a maternal parent and a predominance of yellow chloroplast haplotypes in natural hybrids indicates hybrid phenotypes may be only one of many considerations.

In addition to asymmetrical compatibility, demography, environmental heterogeneity, species ecology and pedigree make the potential outcomes of hybridization quite complex. However, there is no doubt that hybridization is happening in natural populations in multiple states, that hybrids persist in multiple years and have in some cases displaced invasive parents.

Furthermore, results from these common garden studies at two latitudes confirm that most early generation and natural hybrids are capable of outperforming parents across multiple vegetative and reproductive traits. This suggests that not only should areas where yellow and Dalmatian toadflax are co-invading be prioritized for future management, but also that managers should also prioritize yellow toadflax and Dalmatian toadflax populations which fall within the potential traveling distances of these species' strong flying pollinators.

Literature Cited

Ainouche M.L., Baumel A., Salmon A., Yannic G. (2004) Hybridization, polyploidy and speciation in *Spartina* (Poaceae). New Phytologist 161:165-172.

Ainouche M.L., Fortune P.M., Salmon A., Parisod C., Grandbastien M.A., Fukunaga K., Ricou M., Misset M.T. (2009) Hybridization, polyploidy and invasion: lessons from *Spartina* (Poaceae). Biological Invasions 11:1159-1173.

Alex J.F. (1962) The taxonomy, history, and distribution of *Linaria dalmatica*. Canadian Journal of Botany 40:295-307.

Allan E., Pannell J.R. (2009) Rapid divergence in physiological and life-history traits between northern and southern populations of the British introduced neo-species, *Senecio squalidus*. Oikos 118:1053-1061.

Allen K., Hansen K. (1999) Geography of exotic plants adjacent to campgrounds, Yellowstone National Park, USA. Great Basin Naturalist 59:315-322.

Alpert P., Simms E.L. (2002) The relative advantages of plasticity and fixity in different environments: when is it good for a plant to adjust? Evolutionary Ecology 16:285-297.

Anderson E. (1948) Hybridization of the habitat. Evolution 2:1-9.

Anderson E. (1949) Introgressive hybridization J. Wiley, New York.

Anderson E., Stebbins G.L. (1954) Hybridization as an evolutionary stimulus. Evolution 8:378-388.

Arnold M.L., Hodges S.A. (1995) Are natural hybrids fit or unfit relative to their parents. Trends in Ecology & Evolution 10:67-71.

Arnold R.M. (1982) Pollination, predation and seed set in *Linaria vulgaris* (SCROPHULARIACEAE). American Midland Naturalist 107:360-369.

Arntz A.M., Delph L.F. (2001) Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. Oecologia 127:455-467.

Ayres D.R., Smith D.L., Zaremba K., Klohr S., Strong D.R. (2004) Spread of exotic cordgrasses and hybrids (*Spartina sp.*) in the tidal marshes of San Francisco Bay, California, USA. Biological Invasions 6:221-231.

Baack E.J., Rieseberg L.H. (2007) A genomic view of introgression and hybrid speciation. Current Opinion in Genetics & Development 17:513-518.

Baker H.G. (1974) The Evolution of Weeds. Annual Review of Ecology and Systematics 5:1-24.

Bartlett M.K., Scoffoni C., Sack L. (2012) The determinants of leaf turgor loss point and prediction of drought tolerance of species and biomes: a global meta-analysis. Ecology Letters 15:393-405.

Barton N.H., Hewitt G.M. (1985) Analysis of hybrid zones. Annual Review of Ecology and Systematics 16:113-148.

Baumel A., Ainouche M.L., Misset M.T., Gourret J.P., Bayer R.J. (2003) Genetic evidence for hybridization between the native Spartina maritima and the introduced *Spartina alterniflora* (Poaceae) in South-West France: Spartina X neyrautii re-examined. Plant Systematics and Evolution 237:87-97.

Bednarek P., Kwon C., Schulze-Lefert P. (2010) Not a peripheral issue: secretion in plant-microbe interactions. Current Opinion in Plant Biology 13:378-387.

Bishop J.G., Schemske D.W. (1998) Variation in flowering phenology and its consequences for lupines colonizing Mount St. Helens. Ecology 79:534-546.

Blaringhem L. (1919) Floral anomalies observed in the issue of the hybrid of the *Linaria vulgaris X L striata*. Comptes Rendus Hebdomadaires Des Seances De L Academie Des Sciences 169:1103-1105.

Bleeker W. (2004) Genetic variation and self-incompatibility within and outside a *Rorippa* hybrid zone (Brassicaceae). Plant Systematics and Evolution 246:35-44.

Blossey B., Notzold R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants - a hypothesis. Journal of Ecology 83:887-889

Blum A. (2005) Drought resistance, water-use efficiency, and yield potential - are they compatible, dissonant, or mutually exclusive? Australian Journal of Agricultural Research 56:1159-1168.

Blumenthal D.M., Norton A.P., Cox S.E., Hardy E.M., Liston G.E., Kennaway L., Booth D.T., Derner J.D. (2012) *Linaria dalmatica* invades south-facing slopes and less grazed areas in grazing-tolerant mixed-grass prairie. Biological Invasions 14:395-404.

Bradshaw A.D. (1965) Crop adaptation and distribution - WILSIE,CP. Journal of Ecology 53:234-235.

Brink R.A. (1927) Studies on pollen tube development in a partially sterile hybrid between *Linaria vulgaris* & *L. purpurea* Molecular and General Genetics MGG 44:129-148.

Boswell, A. 2012 Development of PCR-RFLP and DNA Barcoding Chloroplast Markers for Yellow Toadflax and Dalmatian Toadflax, Thesis, Colorado State University

Brodersen C., Lavergne S., Molofsky J. (2008) Genetic variation in photosynthetic characteristics among invasive and native populations of reed canarygrass (*Phalaris arundinacea*). Biological Invasions 10:1317-1325.

Brown, Lorena Sue. 2008. Genetic variation of the invasive *Linaria dalmatica* in its introduced range in western North America and the impact of its predominant biological control agent, *Mecinus janthinus*. Thesis (M.S., Environmental Science)--University of Idaho, December 2008.

Bruun H.G. (1936) Genetical notes on *Linaria*, I-II. Hereditas 22:395-400.

Buerkle C.A. (2009) Ecological context shapes hybridization dynamics. Molecular Ecology 18:2077-2079.

Burke J.M., Arnold M.L. (2001) Genetics and the fitness of hybrids. Annual Review of Genetics 35:31-52.

Burow M., Halkier B.A., Kliebenstein D.J. (2010) Regulatory networks of glucosinolates shape *Arabidopsis thaliana* fitness. Current Opinion in Plant Biology 13:348-353.

Byrd D.W., McArthur E.D., Wang H., Graham J.H., Freeman D.C. (1999) Narrow hybrid zone between two subspecies of big sagebrush, *Artemisia tridentata* (Asteraceae). VIII. Spatial and temporal pattern of terpenes. Biochemical Systematics and Ecology 27:11-25.

Callaway R.M., Maron J.L. (2006) What have exotic plant invasions taught us over the past 20 years? Trends in Ecology & Evolution 21:369-374.

Campbell D.R., Waser N.M., MelendezAckerman E.J. (1997) Analyzing pollinator-mediated selection in a plant hybrid zone: Hummingbird visitation patterns on three spatial scales. American Naturalist 149:295-315.

Campbell D.R., Galen C., Wu C.A. (2005) Ecophysiology of first and second generation hybrids in a natural plant hybrid zone.

Campbell D.R., Waser N.M., Aldridge G., Wu C.A. (2008) Lifetime fitness in two generations of *Ipomopsis* hybrids. Evolution 62:2616-2627.

Campbell L.G., Snow A.A., Sweeney P.M. (2009) When divergent life histories hybridize: insights into adaptive life-history traits in an annual weed. New Phytologist 184:806-818.

Cappuccino N., Arnason J.T. (2006) Novel chemistry of invasive exotic plants. Biology Letters 2:189-193.

Castillo J.M., Ayres D.R., Leira-Doce P., Bailey J., Blum M., Strong D.R., Luque T., Figueroa E. (2010) The production of hybrids with high ecological amplitude between exotic *Spartina densiflora* and native *S. maritima* in the Iberian Peninsula. Diversity and Distributions 16:547-558.

Charlton W.A. (1966) Root system of *Linaria vulgaris mill* .1. Morphology and anatomy. Canadian Journal of Botany 44:1111.

Chen Z.J. (2010) Molecular mechanisms of polyploidy and hybrid vigor. Trends in Plant Science 15:57-71.

Cheng D., Kirk H., Mulder P.P.J., Vrieling K., Klinkhamer P.G.L. (2011) Pyrrolizidine alkaloid variation in shoots and roots of segregating hybrids between *Jacobaea vulgaris* and *Jacobaea aquatica*. New Phytologist 192:1010-1023.

Clapham A.R. (1957) Flora of the British Isles: illustrations University Press, Cambridge [Eng.].

Colautti R.I., Barrett S.C.H. (2010) Natural selection and genetic constraints on flowering phenology in an invasive plant. International Journal of Plant Sciences 171:960-971.

Colautti R.I., Grigorovich I.A., MacIsaac H.J. (2006) Propagule pressure: A null model for biological invasions. Biological Invasions 8:1023-1037.

Colautti R.I., Eckert C.G., Barrett S.C.H. (2010) Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. Proceedings of the Royal Society B-Biological Sciences 277:1799-1806.

Comai L., Madlung A., Josefsson C., Tyagi A. (2003) Do the different parental 'heteromes' cause genomic shock in newly formed allopolyploids? Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 358:1149-1155.

Culley T.M., Hardiman N.A. (2009) The role of intraspecific hybridization in the evolution of invasiveness: a case study of the ornamental pear tree *Pyrus calleryana.* Biological Invasions 11:1107-1119.

Currat M., Ruedi M., Petit R.J., Excoffier L. (2008) The hidden side of invasions: Massive introgression by local genes. Evolution 62:1908-1920.

Daehler C.C. (2006) Invasibility of tropical islands by introduced plants: partitioning the influence of isolation and propagule pressure. Preslia 78:389-404.

Daehler C.C., Strong D.R. (1997) Hybridization between introduced smooth cordgrass (*Spartina alterniflora*; Poaceae) and native California cordgrass (*S. foliosa*) in San Francisco Bay, California, USA. American Journal of Botany 84:607-611.

Dawson W., Rohr R.P., van Kleunen M., Fischer M. (2012) Alien plant species with a wider global distribution are better able to capitalize on increased resource availability. New Phytologist 194:859-867.

De Clerck-Floate R.A., Harris, P. (2002). *Linaria dalmatica* (L.) Miller, Dalmatian toadflax (Scrophulariaceae). pp. 368-374. In: Biological control programmes in Canada, 1981-2000. Chapter 72. (P.G. Mason and J.T. Huber, eds.), CABI Publishing, Wallingford, Oxon, UK.

Dillemann G. (1950) Heredite du principe cyanhydrique chez les hybrides de 2nd generation et les hybrides en retour dans les croisements interspecifiques de *Linaria*. Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales 144:1472-1475.

Dlugosch K.M., Parker I.M. (2008a) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Molecular Ecology 17:431-449.

Dlugosch K.M., Parker I.M. (2008b) Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. Ecology Letters 11:701-709.

Dobzhansky T. (1940) Speciation as a stage in evolutionary divergence. American Naturalist 74:312-321.

Docherty Z. (1982) Self-incompatibility in *Linaria*. Heredity 49:349-352.

Donovan L.A., Ludwig F., Rosenthal D.M., Rieseberg L.H., Dudley S.A. (2009) Phenotypic selection on leaf ecophysiological traits in *Helianthus*. New Phytologist 183:868-879.

Dudley S.A. (1996) The response to differing selection on plant physiological traits: Evidence for local adaptation. Evolution 50:103-110.

East E.M. (1933) Genetic observations on the genus *Linaria*. Genetics 18:324-328.

Ellstrand N.C., Schierenbeck K.A. (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences of the United States of America 97:7043-7050.

Elton C.S. (2000) The ecology of invasions by animals and plants University of Chicago Press, Chicago.

Favre A., Karrenberg S. (2011) Stress tolerance in closely related species and their first-generation hybrids: a case study of *Silene*. Journal of Ecology 99:1415-1423.

Floate K.D., Whitham T.G. (1993) The hybrid bridge hypothesis - host shifting via plant hybrid swarms. American Naturalist 141:651-662.

Floate K.D., Whitham T.G. (1995) Insects as traits in plant systematics - their use in discriminating between hybrid cottonwoods. Canadian Journal of Botany-Revue Canadienne De Botanique 73:1-13.

Floate K.D., Kearsley M.J.C., Whitham T.G. (1993) Elevated herbivory in plant hybrid zones - *chrysomela confluens, populus* - and phenological sinks. Ecology 74:2056-2065.

Flood P.J., Harbinson J., Aarts M.G.M. (2011) Natural genetic variation in plant photosynthesis. Trends in Plant Science 16:327-335.

Flory S.L., Long F.R., Clay K. (2011) Greater performance of introduced vs. native range populations of *Microstegium vimineum* across different light environments. Basic and Applied Ecology 12:350-359.

Fritz R.S., Moulia C., Newcombe G. (1999) Resistance of hybrid plants and animals to herbivores, pathogens, and parasites. Annual Review of Ecology and Systematics 30:565-591.

Gaines T.A., Ward S.M., Bukun B., Preston C., Leach J.E., Westra P. (2012) Interspecific hybridization transfers a previously unknown glyphosate resistance mechanism in *Amaranthus species.* Evolutionary Applications 5:29-38.

Gaskin J.F., Schaal B.A. (2002) Hybrid *Tamarix* widespread in US invasion and undetected in native Asian range. Proceedings of the National Academy of Sciences of the United States of America 99:11256-11259.

Gaskin J.F., Kazmer D.J. (2009) Introgression between invasive saltcedars (*Tamarix chinensis and T-ramosissima*) in the USA. Biological Invasions 11:1121-1130.

Geber M.A., Dawson T.E. (1997) Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. Oecologia 109:535-546.

Geiger J.H., Pratt P.D., Wheeler G.S., Williams D.A. (2011) Hybrid vigor for the invasive exotic brazilian peppertree (*Schinus terebinthifolius* raddi., anacardiaceae) in Florida. International Journal of Plant Sciences 172:655-663.

Greaves I.K., Groszmann M., Ying H., Taylor J.M., Peacock W.J., Dennis E.S. (2012) Trans Chromosomal Methylation in *Arabidopsis* hybrids. Proceedings of the National Academy of Sciences of the United States of America 109:3570-3575.

Grime J.P. (1977) Evidence for existence of 3 primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111:1169-1194.

Gross B.L., Kane N.C., Lexer C., Ludwig F., Rosenthal D.M., Donovan L.A., Rieseberg L.H. (2004) Reconstructing the origin of *Helianthus deserticola*: Survival and selection on the desert floor. American Naturalist 164:145-156.

Guesewell S., Jakobs G., Weber E. (2006) Native and introduced populations of *Solidago gigantea* differ in shoot production but not in leaf traits or litter decomposition. Functional Ecology 20:575-584.

Gurevitch J. (1992) Differences in photosynthetic rate in populations of *Achillea lanulosa* from 2 altitudes. Functional Ecology 6:568-574.

Ha M., Lu J., Tian L., Ramachandran V., Kasschau K.D., Chapman E.J., Carrington J.C., Chen X.M., Wang X.J., Chen Z.J. (2009) Small RNAs serve as a genetic buffer against genomic shock in *Arabidopsis* interspecific hybrids and allopolyploids. Proceedings of the National Academy of Sciences of the United States of America 106:17835-17840.

Hamrick J.L., Godt M.J.W. (1996) Effects of life history traits on genetic diversity in plant species. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 351:1291-1298.

He W.M., Thelen G.C., Ridenour W.M., Callaway R.M. (2010) Is there a risk to living large? Large size correlates with reduced growth when stressed for knapweed populations. Biological Invasions 12:3591-3598.

Hegarty M.J., Batstone T., Barker G.L., Edwards K.J., Abbott R.J., Hiscock S.J. (2011) Nonadditive changes to cytosine methylation as a consequence of hybridization and genome duplication in *Senecio* (Asteraceae). Molecular Ecology 20:105-113.

Hellstrom K., Kytoviita M.M., Tuomi J., Rautio P. (2006) Plasticity of clonal integration in the perennial herb *Linaria vulgaris* after damage. Functional Ecology 20:413-420.

Hodgins K.A., Rieseberg L. (2011) Genetic differentiation in life-history traits of introduced and native common ragweed (*Ambrosia artemisiifolia*) populations. Journal of Evolutionary Biology 24:2731-2749.

Hoffman, W.A. and Poorter, H. (2002) Avoiding Bias in Calculations of Relative Growth Rate. Annals of Botany 90:37-42.

Hovick S.M., Campbell L.G., Snow A.A., Whitney K.D. (2012) Hybridization Alters Early Life-History Traits and Increases Plant Colonization Success in a Novel Region. American Naturalist 179:192-203.

Inderjit, Wardle D.A., Karban R., Callaway R.M. (2011) The ecosystem and evolutionary contexts of allelopathy. Trends in Ecology & Evolution 26:655-662.

Jacobs J.S., Sheley R.L. (2003) Prescribed fire effects on Dalmation toadflax. Journal of Range Management 56:193-197.

James J.K., Abbott R.J. (2005) Recent, allopatric, homoploid hybrid speciation: The origin of *Senecio squalidus* (Asteraceae) in the British Isles from a hybrid zone on Mount Etna, Sicily. Evolution 59:2533-2547.

Jin H.J., Hu W., Wei Z., Wan L.L., Li G., Tan G.X., Zhu L.L., He G.C. (2008) Alterations in cytosine methylation and species-specific transcription induced by interspecific hybridization between *Oryza sativa* and *O. officinalis*. Theoretical and Applied Genetics 117:1271-1279.

Johnston J.A., Arnold M.L., Donovan L.A. (2003) High hybrid fitness at seed and seedling life history stages in Louisiana irises. Journal of Ecology 91:438-446.

Kalisz S., Purugganan M.D. (2004) Epialleles via DNA methylation: consequences for plant evolution. Trends in Ecology & Evolution 19:309-314.

Karrenberg S., Lexer C., Rieseberg L.H. (2007) Reconstructing the history of selection during homoploid hybrid speciation. American Naturalist 169:725-737.

Kashkush K., Feldman M., Levy A.A. (2003) Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nature Genetics 33:102-106.

Keller S.R., Taylor D.R. (2010) Genomic admixture increases fitness during a biological invasion. Journal of Evolutionary Biology 23:1720-1731.

Kimball S., Campbell D. (2009) Physiological differences among two *Penstemon* species and their hybrids in field and common garden environments. New Phytologist 181:478-488.

Kimball S., Campbell D.R., Lessin C. (2008) Differential performance of reciprocal hybrids in multiple environments. Journal of Ecology 96:1306-1318.

Kingsolver J.G., Hoekstra H.E., Hoekstra J.M., Berrigan D., Vignieri S.N., Hill C.E., Hoang A., Gibert P., Beerli P. (2001) The strength of phenotypic selection in natural populations. American Naturalist 157:245-261.

Kliber A., Eckert C.G. (2005) Interaction between founder effect and selection during biological invasion in an aquatic plant. Evolution 59:1900-1913.

Kovarik A., Pires J.C., Leitch A.R., Lim K.Y., Sherwood A.M., Matyasek R., Rocca J., Soltis D.E., Soltis P.S. (2005) Rapid concerted evolution of nuclear ribosomal DNA in two *tragopogon* allopolyploids of recent and recurrent origin. Genetics 169:931-944.

Krick N.J. (2011) Examining the unpredictable nature of yellow toadflax, Colorado State University. Libraries.

Labrador M., Farre M., Utzet F., Fontdevila A. (1999) Interspecific hybridization increases transposition rates of *Osvaldo*. Molecular Biology and Evolution 16:931-937.

Lai Z., Nakazato T., Salmaso M., Burke J.M., Tang S.X., Knapp S.J., Rieseberg L.H. (2005) Extensive chromosomal repatterning and the evolution of sterility barriers in hybrid sunflower species. Genetics 171:291-303.

Lajeunesse, S. 1999. Dalmatian and Yellow Toadflax. Pages 202-216 In R.L. Sheley and J.K. Petroff, eds. Biology and Management of Noxious Rangeland Weeds. Corvallis, OR: Oregon State University Press.

Lamont B.B., He T., Enright N.J., Krauss S.L., Miller B.P. (2003) Anthropogenic disturbance promotes hybridization between *Banksia* species by altering their biology. Journal of Evolutionary Biology 16:551-557.

Lee C.E. (2002) Evolutionary genetics of invasive species. Trends in Ecology & Evolution 17:386-391.

Leinonen P.H., Remington D.L., Savolainen O. (2011) Local adaptation, phenotypic differentiation, and hybrid fitness in diverged natural populations of *Arabidopsis lyrata*. Evolution 65:90-107.

Lepais O., Petit R.J., Guichoux E., Lavabre J.E., Alberto F., Kremer A., Gerber S. (2009) Species relative abundance and direction of introgression in oaks. Molecular Ecology 18:2228-2242.

Levin D.A., Anderson W.W. (1970) Competition for pollinators between simultaneously flowering species. American Naturalist 104:455

Levin S.A., Muller-Landau H.C., Nathan R., Chave J. (2003) The ecology and evolution of seed dispersal: A theoretical perspective. Annual Review of Ecology Evolution and Systematics 34:575-604.

Lewontin R.C., Birch L.C. (1966) Hybridization as a source of variation for adaptation to new environments. Evolution 20:315

Lexer C., Randell R.A., Rieseberg L.H. (2003) Experimental hybridization as a tool for studying selection in the wild. Ecology 84:1688-1699.

Lim K.Y., Soltis D.E., Soltis P.S., Tate J., Matyasek R., Srubarova H., Kovarik A., Pires J.C., Xiong Z.Y., Leitch A.R. (2008) Rapid Chromosome Evolution in Recently Formed Polyploids in *Tragopogon* (Asteraceae). Plos One 3.

Lind E.M., Parker J.D. (2010) Novel Weapons Testing: Are Invasive Plants More Chemically Defended than Native Plants? Plos One 5.

Liu B., Wendel J.F. (2000) Retrotransposon activation followed by rapid repression in introgressed rice plants. Genome 43:874-880.

Lonsdale W.M. (1999) Global patterns of plant invasions and the concept of invasibility. Ecology 80:1522-1536.

Ludlow M.M. (1989) Strategies of response to water-stress.

Mack R.N. (2003) Plant naturalizations and invasions in the eastern United States: 1634-1860. Annals of the Missouri Botanical Garden 90:77-90.

Mallet J. (2005) Hybridization as an invasion of the genorne. Trends in Ecology & Evolution 20:229-237.

Maron J.L. (2006) The relative importance of latitude matching and propagule pressure in the colonization success of an invasive forb. Ecography 29:819-826.

Marques I., Nieto Feliner G., Martins-Loução M.A., Fuertes Aguilar J. (2011) Fitness in *Narcissus* hybrids: low fertility is overcome by early hybrid vigour, absence of exogenous selection and high bulb propagation. Journal of Ecology 99:1508-1519.

Marques I., Feliner G.N., Munt D.D., Martins-Loucao M.A., Aguilar J.F. (2010) Unraveling cryptic reticulate relationships and the origin of orphan hybrid disjunct populations in *Narcissus*. Evolution 64:2353-2368.

Martin N.H., Sapir Y., Arnold M.L. (2008) The genetic architecture of reproductive isolation in louisiana irises: Pollination syndromes and pollinator preferences. Evolution 62:740-752.

Matzek V. (2011) Superior performance and nutrient-use efficiency of invasive plants over non-invasive congeners in a resource-limited environment. Biological Invasions 13:3005-3014.

Mayr E. (1942) Systematics and the origin of species from the viewpoint of a zoologist Columbia University Press, New York.

Mayr E. (1963) Animal species and evolution Belknap Press of Harvard University Press, Cambridge.

Mayr E. (1992) A local flora and the biological species concept. American Journal of Botany 79:222-238.

McAlpine K.G., Jesson L.K., Kubien D.S. (2008) Photosynthesis and water-use efficiency: A comparison between invasive (exotic) and non-invasive (native) species. Austral Ecology 33:10-19.

McClay A.S. (1992) Effects of *Brachypterolus-pulicarius* (l) (coleoptera, nitidulidae) on flowering and seed production of common toadflax. Canadian Entomologist 124:631-636.

McClay A.S., Hughes R.B. (2007) Temperature and host-plant effects on development and population growth of *Mecinus janthinus* (Coleoptera : Curculionidae), a biological control agent for invasive Linaria spp. Biological Control 40:405-410.

McClintock B. (1984) The significance of responses of the genome to challenge. Science 226:792-801.

McKay J.K., Richards J.H., Mitchell-Olds T. (2003) Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. Molecular Ecology 12:1137-1151.

Meyer G.A., Hull-Sanders H.M. (2008) Altered patterns of growth, physiology and reproduction in invasive genotypes of *Solidago gigantea* (Asteraceae). Biological Invasions 10:303-317.

Meyer R.C., Witucka-Wall H., Becher M., Blacha A., Boudichevskaia A., Dörmann P., Fiehn O., Friedel S., von Korff M., Lisec J., Melzer M., Repsilber D., Schmidt R., Scholz M., Selbig J., Willmitzer L., Altmann T. (2012) Heterosis manifestation during early *Arabidopsis* seedling development is characterized by intermediate gene expression and enhanced metabolic activity in the hybrids. The Plant Journal

Miglia K.J., McArthur E.D., Moore W.S., Wang H., Graham J.H., Freeman D.C. (2005) Nine-year reciprocal transplant experiment in the gardens of the basin and mountain big sagebrush (*Artemisia tridentata*: Asteraceae) hybrid zone of Salt Creek Canyon: the importance of multiple-year tracking of fitness. Biological Journal of the Linnean Society 86:213-225.

Milne R.I., Abbott R.J. (2000) Origin and evolution of invasive naturalized material of *Rhododendron ponticum* L. in the British Isles. Molecular Ecology 9:541-556.

Mitich L.W. (1993) Intriguing world of weeds .41. Yellow toadflax. Weed Technology 7:791-793.

Molina-Montenegro M.A., Penuelas J., Munne-Bosch S., Sardans J. (2012) Higher plasticity in ecophysiological traits enhances the performance and invasion success of *Taraxacum officinale* (dandelion) in alpine environments. Biological Invasions 14:21-33.

Moody M.L., Les D.H. (2002) Evidence of hybridity in invasive watermilfoil (*Myriophyllum*) populations. Proceedings of the National Academy of Sciences of the United States of America 99:14867-14871.

Mosher R.A., Melnyk C.W. (2010) siRNAs and DNA methylation: seedy epigenetics. Trends in Plant Science 15:204-210.

Mozdzer T.J., Zieman J.C. (2010) Ecophysiological differences between genetic lineages facilitate the invasion of non-native *Phragmites australis* in North American Atlantic coast wetlands. Journal of Ecology 98:451-458.

Muller-Scharer H., Schaffner U., Steinger T. (2004) Evolution in invasive plants: implications for biological control. Trends in Ecology & Evolution 19:417-422.

Murrell C., Gerber E., Krebs C., Parepa M., Schaffner U., Bossdorf O. (2011) Invasive knotweed affects native plants through allelopathy. American Journal of Botany 98:38-43.

Nadeau L.B., King J.R. (1991) Seed dispersal and seedling establishment of *Linaria vulgaris* mill. Canadian Journal of Plant Science 71:771-782.

Nadeau L.B., King J.R., Harker K.N. (1992) Comparison of growth of seedlings and plants grown from root pieces of yellow toadflax (*Linaria vulgaris*). Weed Science 40:43-47.

Neubert M.G., Caswell H. (2000) Demography and dispersal: Calculation and sensitivity analysis of invasion speed for structured populations. Ecology 81:1613-1628.

Newman D.A., Thomson J.D. (2005) Interactions among nectar robbing, floral herbivory, and ant protection in *Linaria vulgaris*. Oikos 110:497-506.

Nicotra A.B., Davidson A. (2010) Adaptive phenotypic plasticity and plant water use. Functional Plant Biology 37:117-127.

Nolte A.W. (2011) Dispersal in the course of an invasion. Molecular Ecology 20:1803-1804.

Nolte A.W., Tautz D. (2010) Understanding the onset of hybrid speciation. Trends in Genetics 26:54-58.

Nosrati H., Price A.H., Wilcock C.C. (2011) Relationship between genetic distances and postzygotic reproductive isolation in diploid *Fragaria* (Rosaceae). Biological Journal of the Linnean Society 104:510-526.

O'Hanlon P.C., Peakall R., Briese D.T. (1999) Amplified fragment length polymorphism (AFLP) reveals introgression in weedy *Onopordum* thistles: hybridization and invasion. Molecular Ecology 8:1239-1246.

Oberprieler C., Barth A., Schwarz S., Heilmann J. (2010) Morphological and phytochemical variation, genetic structure and phenology in an introgressive hybrid swarm of *Senecio* *hercynicus* and *S. ovatus* (Compositae, Senecioneae). Plant Systematics and Evolution 286:153-166.

Olsson U. (1975) Hereditary transmission of microstructures of testa in reciprocal crosses between *Linaria-vulgaris* and *Linaria-repens*. Hereditas 79:251-254.

Orians C.M. (2000) The effects of hybridization in plants on secondary chemistry: Implications for the ecology and evolution of plant-herbivore interactions. American Journal of Botany 87:1749-1756.

Parisod C., Alix K., Just J., Petit M., Sarilar V., Mhiri C., Ainouche M., Chalhoub B., Grandbastien M.A. (2010) Impact of transposable elements on the organization and function of allopolyploid genomes. New Phytologist 186:37-45.

Pauchard A., Alaback P.B., Edlund E.G. (2003) Plant invasions in protected areas at multiple scales: *Linaria vulgaris* (Scrophulariaceae) in the west Yellowstone area. Western North American Naturalist 63:416-428.

Pauchard A., Kueffer C., Dietz H., Daehler C.C., Alexander J., Edwards P.J., Arevalo J.R., Cavieres L.A., Guisan A., Haider S., Jakobs G., McDougall K., Millar C.I., Naylor B.J., Parks C.G., Rew L.J., Seipel T. (2009) Ain't no mountain high enough: plant invasions reaching new elevations. Frontiers in Ecology and the Environment 7:479-486.

Pejchar L., Mooney H.A. (2009) Invasive species, ecosystem services and human well-being. Trends in Ecology & Evolution 24:497-504.

Peterson R.K.D., Sing S.E., Weaver D.K. (2005) Differential physiological responses of Dalmatian toadflax, *Linaria dalmatica* L. Miller, to injury from two insect biological control agents: Implications for decision-making in biological control. Environmental Entomology 34:899-905.

Pialek J., Barton N.H. (1997) The spread of an advantageous allele across a barrier: The effects of random drift and selection against heterozygotes. Genetics 145:493-504.

Pigliucci M., Murren C.J., Schlichting C.D. (2006) Phenotypic plasticity and evolution by genetic assimilation. Journal of Experimental Biology 209:2362-2367.

Pollock J.L., Callaway R.M., Thelen G.C., Holben W.E. (2009) Catechin-metal interactions as a mechanism for conditional allelopathy by the invasive plant *Centaurea maculosa*. Journal of Ecology 97:1234-1242.

Prentis P.J., Wilson J.R.U., Dormontt E.E., Richardson D.M., Lowe A.J. (2008) Adaptive evolution in invasive species. Trends in Plant Science 13:288-294.

Pysek P., Krivanek M., Jarosik V. (2009) Planting intensity, residence time, and species traits determine invasion success of alien woody species. Ecology 90:2734-2744.

Reich P.B., Ellsworth D.S., Walters M.B., Vose J.M., Gresham C., Volin J.C., Bowman W.D. (1999) Generality of leaf trait relationships: A test across six biomes. Ecology 80:1955-1969.

Ren M.X., Zhang Q.G. (2007) Clonal diversity and structure of the invasive aquatic plant *Eichhornia crassipes* in China. Aquatic Botany 87:242-246.

Reznick D.N., Ghalambor C.K. (2001) The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. Genetica 112:183-198.

Richardson D.M., Pysek P. (2006) Plant invasions: merging the concepts of species invasiveness and community invasibility. Progress in Physical Geography 30:409-431.

Rieseberg L.H. (1997) Hybrid origins of plant species. Annual Review of Ecology and Systematics 28:359-389.

Rieseberg L.H., Carney S.E. (1998) Plant hybridization. New Phytologist 140:599-624.

Rieseberg L.H., Archer M.A., Wayne R.K. (1999) Transgressive segregation, adaptation and speciation. Heredity 83:363-372.

Rieseberg L.H., Sinervo B., Linder C.R., Ungerer M.C., Arias D.M. (1996) Role of gene interactions in hybrid speciation: Evidence from ancient and experimental hybrids. Science 272:741-745.

Rieseberg L.H., Kim S.C., Randell R.A., Whitney K.D., Gross B.L., Lexer C., Clay K. (2007) Hybridization and the colonization of novel habitats by annual sunflowers. Genetica 129:149-165.

Rieseberg L.H., Raymond O., Rosenthal D.M., Lai Z., Livingstone K., Nakazato T., Durphy J.L., Schwarzbach A.E., Donovan L.A., Lexer C. (2003) Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301:1211-1216.

Roberts H.F. (1965) Plant hybridization before Mendel Hafner Pub. Co., New York.

Robocker W.C. (1974) Life history, ecology, and control of Dalmatian toadflax. Technical Bulletin, Washington Agricultural Experiment Station., Washington State University. 20 p

Robocker W.C. (1970) Seed characteristics and seedling emergence of Dalmatian toadflax. Weed Science 18:720-725.

Rosenthal D.M., Schwarzbach A.E., Donovan L.A., Raymond O., Rieseberg L.H. (2002) Phenotypic differentiation between three ancient hybrid taxa and their parental species. International Journal of Plant Sciences 163:387-398.

Ruhsam M., Hollingsworth P.M., Ennos R.A. (2011) Early evolution in a hybrid swarm between outcrossing and selfing lineages in *Geum*. Heredity 107:246-255.

Sandring S., Ågren J. (2009) Pollinator-mediated selection on floral display and flowering time in the perennial herb *Arabidopsis lyrata*. Evolution 63:1292-1300.

Saner M.A., Clements D.R., Hall M.R., Doohan D.J., Crompton C.W. (1995) The biology of Canadian weeds. 105. *Linaria vulgaris* Mill. Canadian Journal of Plant Science 75:525-537.

Schemske D.W., Bradshaw H.D. (1999) Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proceedings of the National Academy of Sciences of the United States of America 96:11910-11915.

Schierenbeck K.A., Ellstrand N.C. (2009) Hybridization and the evolution of invasiveness in plants and other organisms. Biological Invasions 11:1093-1105.

Schlichting C.D., Levin D.A. (1986) Phenotypic plasticity: an evolving plant character. Biological Journal of the Linnean Society 29:37-47.

Schwarzbach A.E., Donovan L.A., Rieseberg L.H. (2001) Transgressive character expression in a hybrid sunflower species. American Journal of Botany 88:270-277.

Segarra-Moragues J.G., Mateu-Andres I. (2007) Levels of allozyme diversity in closely related toadflaxes (*Linaria*, Plantaginaceae) and their correspondence with the breeding systems of the species. Conservation Genetics 8:373-383.

Sims D.A., Kelley S. (1998) Somatic and genetic factors in sun and shade population differentiation in *Plantago lanceolata* and *Anthoxanthum odoratum*. New Phytologist 140:75-84.

Sing S.E., Peterson R.K.D. (2011) Assessing Environmental Risks for Established Invasive Weeds: Dalmatian (*Linaria dalmatica*) and Yellow (*L. vulgaris*) Toadflax in North

America. International Journal of Environmental Research and Public Health 8:2828-2853.

Smith M.D., Knapp A.K. (2001) Physiological and morphological traits of exotic, invasive exotic, and native plant species in tallgrass prairie. International Journal of Plant Sciences 162:785-792.

Smulders M.J.M., Beringen R., Volosyanchuk R., Broeck A.V., van der Schoot J., Arens P., Vosman B. (2008) Natural hybridisation between *Populus nigra* L. and *P. x canadensis* Moench. Hybrid offspring competes for niches along the Rhine river in the Netherlands. Tree Genetics & Genomes 4:663-675.

Snow A.A., Andersen B., Jorgensen R.B. (1999) Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. Molecular Ecology 8:605-615.

Stebbins G.L. (1958) On the hybrid origin of the angiosperms. Evolution 12:267-270.

Stout J.C., Allen J.A., Goulson D. (2000) Nectar robbing, forager efficiency and seed set: Bumblebees foraging on the self incompatible plant *Linaria vulgaris* (Scrophulariaceae). Acta Oecologica-International Journal of Ecology 21:277-283.

Suehs C.M., Charpentier S., Affre L., Medail F. (2006) The evolutionary potential of invasive *Carpobrotus* (Aizoaceae) taxa: are pollen-mediated gene flow potential and hybrid vigor levels connected? Evolutionary Ecology 20:447-463.

Sutton D.A. (1988) A Revision of the Tribe Antirrhineae. British Museum of Natural History, Oxford Univ. Press, London. 575 p.

Sutton D.A. (1980) A new section of *Linaria* (scrophulariaceae, antirrhineae). Botanical Journal of the Linnean Society 81:169-184.

Sutton, J.R. 2003 Prediction and Characterization of Yellow Toadflax (Linaria vulgaris Mill.) Infestations at two Scales in the Flat Tops Wilderness of Colorado, Thesis, Colorado State University

Sutton J.R., Stohlgren T.J., Beck K.G. (2007) Predicting yellow toadflax infestations in the flat tops wilderness of colorado. Biological Invasions 9:783-793.

Taylor S.J., Arnold M., Martin N.H. (2009) The genetic architecture of reproductive isolation in louisiana irises: hybrid fitness in nature. Evolution 63:2581-2594.

Tosevski I., Caldara R., Jovic J., Hernandez-Vera G., Baviera C., Gassmann A., Emerson B.C. (2011) Morphological, molecular and biological evidence reveal two cryptic species in *Mecinus janthinus* Germar (Coleoptera, Curculionidae), a successful biological control agent of Dalmatian toadflax, *Linaria dalmatica* (Lamiales, Plantaginaceae). Systematic Entomology 36:741-753.

Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A. (1972) Flora Europaea. Cambridge University Press, Cambridge, UK.

Twyford A.D., Ennos R.A. (2012) Next-generation hybridization and introgression. Heredity 108:179-189.

USDA, NRCS. 2012. The PLANTS Database (http://plants.usda.gov, 13 October 2012). National Plant Data Team, Greensboro, NC 27401-4901 USA.

Vereecken N.J., Cozzolino S., Schiestl F.P. (2010) Hybrid floral scent novelty drives pollinator shift in sexually deceptive orchids. Bmc Evolutionary Biology 10.

Viano J. (1978) Interspecific experimental cross-breedings within the genus Linaria. Caryologia 31:383-425.

Von Holle B., Simberloff D. (2005) Ecological resistance to biological invasion overwhelmed by propagule pressure. Ecology 86:3212-3218.

Vujnovic K., Wein R.W. (1997) The biology of Canadian weeds .106. *Linaria dalmatica* (L.) Mill. Canadian Journal of Plant Science 77:483-491.

Ward S.M., Sing S.E., Turner M.F., Fleischmann C.E. (2009) Hybridization between Invasive Populations of Dalmatian Toadflax (*Linaria dalmatica*) and Yellow Toadflax (*Linaria vulgaris*) Invasive plant science and management 2:369-378.

Ward S.M., Reid S.D., Harrington J., Sutton J., Beck K.G. (2008) Genetic variation in invasive populations of yellow toadflax (*Linaria vulgaris*) in the western United States. Weed Science 56:394-399.

West-Eberhard M.J. (2005) Developmental plasticity and the origin of species differences. Proceedings of the National Academy of Sciences of the United States of America 102:6543-6549.

Whitney K.D., Ahern J.R., Campbell L.G., Albert L.P., King M.S. (2010) Patterns of hybridization in plants. Perspectives in Plant Ecology Evolution and Systematics 12:175-182.

Williams D.A., Overholt W.A., Cuda J.P., Hughes C.R. (2005) Chloroplast and microsatellite DNA diversities reveal the introduction history of Brazilian peppertree (*Schinus terebinthifolius*) in Florida. Molecular Ecology 14:3643-3656.

Williamson M., Fitter A. (1996) The varying success of invaders. Ecology 77:1661-1666.

Wright I.J., Reich P.B., Westoby M., Ackerly D.D., Baruch Z., Bongers F., Cavender-Bares J., Chapin T., Cornelissen J.H.C., Diemer M., Flexas J., Garnier E., Groom P.K., Gulias J., Hikosaka K., Lamont B.B., Lee T., Lee W., Lusk C., Midgley J.J., Navas M.L., Niinemets U., Oleksyn J., Osada N., Poorter H., Poot P., Prior L., Pyankov V.I., Roumet C., Thomas S.C., Tjoelker M.G., Veneklaas E.J., Villar R. (2004) The worldwide leaf economics spectrum. Nature 428:821-827.

Wu C.A., Campbell D.R. (2006) Environmental stressors differentially affect leaf ecophysiological responses in two *Ipomopsis* species and their hybrids. Oecologia 148:202-212.

Yatabe Y., Kane N.C., Scotti-Saintagne C., Rieseberg L.H. (2007) Rampant gene exchange across a strong reproductive barrier between the annual sunflowers, *Helianthus annuus* and *H. petiolaris*. Genetics 175:1883-1893.

Zheng Y.L., Feng Y.L., Liu W.X., Liao Z.Y. (2009) Growth, biomass allocation, morphology, and photosynthesis of invasive *Eupatorium adenophorum* and its native congeners grown at four irradiances. Plant Ecology 203:263-271.

Zirkle C. (1935) The beginnings of plant hybridization. University of Pennsylvania Press, Philadelphia.

Zou J., Fu D.H., Gong H.H., Qian W., Xia W., Pires J.C., Li R.Y., Long Y., Mason A.S., Yang T.J., Lim Y.P., Park B.S., Meng J.L. (2011) De novo genetic variation associated with retrotransposon activation, genomic rearrangements and trait variation in a recombinant inbred line population of Brassica napus derived from interspecific hybridization with *Brassica rapa*. Plant Journal 68:212-224.