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Title: Hybridization Between Yellow Starthistle (*Centaurea solstitialis*) and Meadow Knapweed (*Centaurea* × *moncktonii*).

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Andrew G. Hulting

Centaurea solstitialis L. (yellow starthistle) and *C.* × *moncktonii* Britt. (meadow knapweed) are members of the genus *Centaurea* in the Asteraceae family. Both species have become serious management concerns as invasive species in North America, often displacing native vegetation and costing land managers millions of dollars to eradicate. Seven plants were found in southwestern Oregon that appeared to be hybrids between *C. solstitialis* and *C.* × *moncktonii*. These plants were identified as hybrids based on bract shape, flower color, and the presence of the putative parent species at the same sites. Hybridization between these two species may present potential management problems, such as the hybrids developing into a viable species or gene flow between the parent species. Meadow knapweed originated through hybridization and colonized a larger range than either of its parent species. If hybrids produce viable pollen or fertile seed, backcrossing with one of the parent species could transfer alleles from one parent species to the other. Hybrids have the potential to transgress parent species for some traits and show increased fitness relative to the parent species. The putative *C. solstitialis* × *C. moncktonii* hybrids were identified based primarily on intermediate morphological traits. To test the hypothesis that these species can produce hybrids, controlled crosses between yellow starthistle and meadow knapweed were attempted. These crosses produced thirty hybrids that fit the morphological description used by Roché and Susanna (2010) to identify plants as *C. solstitialis* × *C. moncktonii* hybrids. The hybrids generated from the controlled crosses germinated from seeds that came from yellow starthistle plants.

Genome size, measured using flow cytometry, and four quantifiable morphological characters were measured on the putative hybrids, hybrids generated through controlled crossing, and the parent species. When the group means were compared, there was no significant difference between the putative hybrids and the hybrids generated through controlled crossing for any of the characters. Both putative and artificial hybrids were backcrossed with the parent species to determine the likelihood of backcrossing. Backcrossing did occur, with the hybrids serving as both maternal parent and pollen parent at very low rates (<1%). Management of the hybrid should focus on prevention of pollination to prevent introgression between the parent species.

Hybridization Between Yellow Starthistle (*Centaurea solstitialis*) and Meadow Knapweed (*Centaurea* × *moncktonii*)

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APPROVED:

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

John Miskella, Author

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1. INTRODUCTION

1.1 General Introduction

Yellow starthistle (*C. solstitialis* L.) and meadow knapweed (*C.* × *moncktonii* Britt.) are invasive in North America and are classified as noxious weeds in several states (USDA Plants). Yellow starthistle is characterized as one of the worst weeds of western United States rangelands (DiTomaso and Healy 2007). In the western United States, both species colonize disturbed areas and can form monotypic stands. Yellow starthistle is an annual herbaceous plant and meadow knapweed is typically a perennial herbaceous plant. Both species are insect-pollinated, primarily outcrossing species.

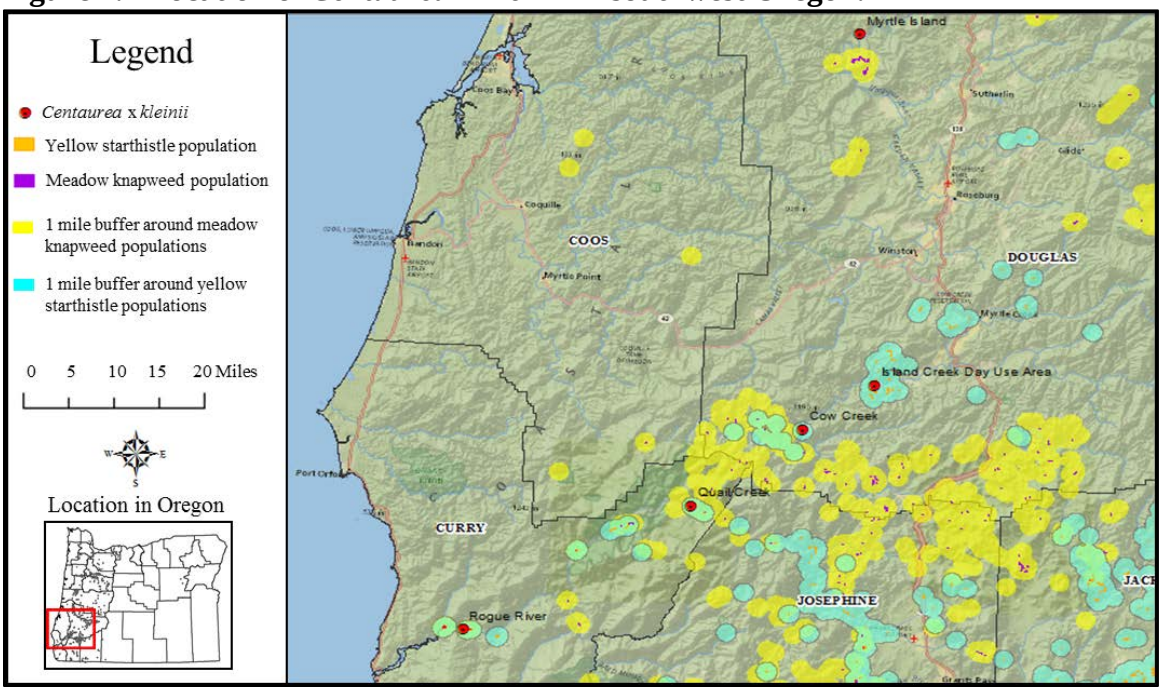
The species have different ecological requirements, and generally occur in different habitats, as well as different geographic areas. Yellow starthistle inhabits dry sites, while meadow knapweed generally becomes established at sites near water such as riverbanks and irrigation ditches. In Eurasia, yellow starthistle is native to the Mediterranean region, while meadow knapweed occurs in the United Kingdom and northern Europe. In Oregon, yellow starthistle is established in eastern and southern Oregon. Meadow knapweed occurs in the Willamette Valley and western Oregon. The two species do occur together in southwestern Oregon, near the Rogue River.

In this region, an unidentified plant in the Asteraceae family was located during a vegetation survey for the Bureau of Land Management in 1998 (Jeanne Klein). Four additional plants with the same flower color and bract shape were located from 2000-2006 (Figure 1.1). Roché and Susanna (2010) identified these plants as *C. solstitialis* × *C. moncktonii* hybrids, based on floret color, bract shape, and the presence of the parent species. They named the putative hybrid *Centaurea* × *kleinii*. Two additional putative hybrids were located in 2012 (personal collection).

1.2 Taxonomy

Yellow starthistle and meadow knapweed are members of the Asteraceae family and the genus *Centaurea*. This genus contains approximately 300 species (Garcia-Jacas et al. 2006). The genus contains species that have become invasive in North America, including fourteen that are listed as noxious weeds in at least one state (USDA Plants).

Figure 1.1 Location of *Centaurea* × *kleinii* in southwest Oregon.



There were a number of attempts to classify the taxonomic relationships among the species within *Centaurea* during the nineteenth and twentieth centuries. These classifications were based on morphology, geographic distribution, and karyology. None of these attempts were accepted as definitive (Garcia-Jacas et al. 2006).

More recent analysis used molecular methods to delineate the taxonomy of the genus. These methods have delineated taxonomic relationships that are significantly different from those described by the earlier taxonomies. There are currently areas of uncertainty and disagreement over the taxonomic organization of *Centaurea*. Garcia-Jacas et al. (2006) hypothesize that hybridization led to some of the inconsistencies between taxonomic organization based on morphology and taxonomic organization based on molecular data.

Within *Centaurea*, species are grouped in sub-generic clades called groups. These groups are further divided into sections, based on morphology. One of the most important characters used to differentiate species into groups and sections within *Centaurea* is pollen type. Yellow starthistle and meadow knapweed both have *Jacea*

pollen type and are therefore included in the Jacea group (Roché and Susanna 2010). The *Jacea* pollen type has a scabrate surface, with rough circular spots less than 1 µm across. Within the *Jacea* group, yellow starthistle and meadow knapweed were placed in separate sections by Garcia-Jacas et al. (2006).

Determining whether the hybridization between the two species can be replicated under controlled conditions is critical due to the phylogenetic distance between the two species. There are species that are much more closely related than yellow starthistle and meadow knapweed. If yellow starthistle and meadow knapweed are related closely enough to hybridize, it potentially means that the other species considered more closely related to these two may also be able to hybridize or that the phylogenetic tree is not accurate. Gene flow may have been so extensive that distantly related species share enough of their genomes that they are able to produce hybrid offspring, despite there being dozens of species between these two species on phylogenetic trees. Alternatively, current phylogenies may not accurately describe the relationship between these species and need revision.

At the species level, the taxonomic status of yellow starthistle is well-defined. The taxonomy of meadow knapweed, however, is more complicated. Meadow knapweed is considered to have originated as a fully fertile hybrid between *C. nigra* L. (black knapweed) and *C. jacea* L. (brown knapweed) (Roché and Roché 1991a). It often occurs in hybrid swarms containing plants whose morphological characters range from black knapweed through meadow knapweed to brown knapweed. Because the plants occur in hybrid swarms with a range of morphological characteristics, they are collectively referred to as the *C. nigra/jacea* complex. Briquet (1931) proposed characterizing the complex as one polymorphic species, though this recommendation has not received support. Currently, they are treated as three species (Roché and Roché 1991a).

Three different scientific names have been used for meadow knapweed in the scientific literature. The confusion in nomenclature is a result of disagreement over whether historical botanical descriptions are describing the species that resulted from *C. nigra* × *C. jacea* hybridization. The Weed Science Society of America lists *Centaurea*

debeauxii Gren. & Godr. as the legitimate name and *Centaurea pratensis* Thuill. as illegitimate. An authority on the genus, Wagenitz (1987) lists *C.* × *moncktonii* Britton as the correct name. Roché and Susanna (2010), when publishing the initial identification of plants as putative hybrids, follow Wagenitz in using *C.* × *moncktonii* as the legitimate name for meadow knapweed. *C.* × *moncktonii* is used here for meadow knapweed.

1.3 Hybridization

The genus *Centaurea* contains species which experienced frequent hybridizations. Wagenitz (1983) documented 232 reports of hybrids between members of *Centaurea*. Hybridizations and backcrossing facilitated gene flow between *Centaurea* species. Phylogenies based on nuclear DNA have yielded differing phylogenetic trees from phylogenies based on plastid DNA, indicating that gene flow has continued to occur during and after speciation within the genus (Font et al. 2009).

Ellstrand and Schierenbeck (2000) documented cases where hybridization has led to the evolution of invasiveness. They propose the following mechanisms that may lead hybrids to develop increased invasiveness potential compared to the parent species: evolutionary novelty, increased genetic variation, decreased genetic load and heterosis. The exact mechanism for increased invasiveness is not understood for each case, and multiple mechanisms may be affecting each case where increased invasiveness has been documented.

Hybridization between *C. jacea* (brown knapweed) and *C. nigra* (black knapweed), and the development of fertile meadow knapweed, were documented in Europe prior to documentation in North America. Both of the parent species of meadow knapweed were reported in North America in the nineteenth century, before any report of meadow knapweed in North America. It is unclear if these two species hybridized independently in Europe and North America, or if meadow knapweed was transported to North America after hybridization in Europe.

If a *C. solstitialis* × *C. moncktonii* hybrid can produce viable offspring, it could follow the pattern of meadow knapweed and become a management concern as a noxious

weed. Both yellow starthistle and meadow knapweed have characteristics that allow them to invade disturbed ecosystems. A hybrid between these two species will begin with two successful genotypes, adapt to its environment, and develop a suite of traits that best suits the environment. Transgressive segregation could lead to hybrid plants with traits that are more extreme than those found in the parent species.

C. solstitialis × *C. moncktonii* hybrids could develop greater ecological amplitude than the parent species. This occurred with meadow knapweed, which eventually invaded more territory than either of its parent species, decades after being introduced in the Pacific Northwest. In the evolution of competitive ability (EICA) hypothesis, a lag period is often observed between the time a species is introduced to a new area and when that species becomes widespread (Blossey and Nötzold 1995).

Compared to parent species, hybrids have a greater range of alleles. As alleles are selected for during natural selection, the greater range of available alleles may allow hybrids to be more successful than the parent species in a wider range of habitats. Within a habitat, hybrids may be able to out-compete other species due to the range of alleles from the parent species.

1.4 Distribution

Yellow starthistle is native to the eastern Mediterranean region and has expanded its range across southern Europe. It has become invasive in North America, South America, and Australia (Roché and Susanna 2010). It first became established in North America during the nineteenth century. It was introduced either in alfalfa (*Medicago sativa* L.) seed or in soil dumped after being used as ship ballast. Yellow starthistle was recorded in ballast dumping areas in Oakland, CA, in 1869 (Roché and Roché 1991a). In Washington State, farmers reported that yellow starthistle was introduced in alfalfa seed (Roché 1965). Gerlach (1997) analyzed the locations of herbarium samples of yellow starthistle collected before 1900 and reported that all were located near alfalfa fields or areas where alfalfa was consumed. There were likely multiple introductions of yellow starthistle into North America.

Yellow starthistle now occurs extensively in pastures, grasslands, rangelands, and disturbed areas. It is a noxious weed that grows over large areas in California, Oregon, Idaho, and Washington. It has also been reported in Colorado, Wyoming, Montana, Utah, Arizona, and New Mexico. It occurs on over four million hectares in the United States, including 3.2 million ha in California (Susanna and Roché 2011, Roché and Roché 1991a).

In its native range in southern Europe, yellow starthistle thrives in dry grasslands. In North America, it is found most frequently in regions with a Mediterranean climate. In the northern portion of its North American range, yellow starthistle is generally found on south-facing slopes. Some populations have become monocultures, excluding some native species (Susanna and Roché 2011).

Black knapweed and brown knapweed, the parent species of meadow knapweed were reported in North America in the nineteenth century, before any report of meadow knapweed. Brown knapweed was first reported in Victoria on Vancouver Island in British Columbia in 1887. The first report of black knapweed in the United States was in Pullman, WA, in 1895 (Roché and Roché 1991b). It is not known whether meadow knapweed was introduced after having first hybridized in Europe or whether it independently hybridized in North America. In many areas, brown knapweed and black knapweed have declined in abundance in North America during the twentieth century, while meadow knapweed has expanded its range.

The first report of meadow knapweed in the Pacific Northwest is from Eugene, OR, in 1918. Roché and Roché (1991b) hypothesized that meadow knapweed was introduced as seed in ship ballast. It mainly occurs as a weed species, but it was also intentionally grown as winter forage near Roseburg, OR, in 1952. It is now found in Oregon, Washington, Idaho, Montana, and British Columbia. Prior to 1960, it was principally found west of the Cascade Mountain Range. In 1991, it was estimated to cover 1900 hectares in these four states and province (Roché and Roché 1991a). Meadow knapweed is listed as a Class B noxious weed in Oregon and as a noxious weed in Colorado and Idaho (DiTomaso and Healy 2007).

Yellow starthistle and meadow knapweed occur in many of the same states, but they normally occur in different habitats. Yellow starthistle occurs on dry sites, and requires light on the soil surface for the development of its rosette. Meadow knapweed occurs in moist meadows, pastures, river banks, open woodlands and disturbed sites with sufficient moisture (Roché and Roché 1991a). In their native European ranges, the two species do not occur close enough for a pollinating insect to carry viable pollen from the flower of one species to the flower of the other species. This is also true throughout most of their introduced range in the US. However, southwestern Oregon has habitats that support both species within the flight range of a single pollinator (Osborne et al. 2008). If hybridizations between these two species occurred in this region, there is the possibility of future hybridizations occurring at other locations where yellow starthistle and meadow knapweed may spread to within the range of an insect pollinator.

1.5 Morphology

Yellow starthistle and meadow knapweed are both herbaceous species. Both species can vary in height and generally reach 1 meter. Yellow starthistle plants of up to 2 meters have been reported (DiTomaso and Healy 2007). Yellow starthistle has branched stems with multiple heads per stem. Meadow knapweed heads occur singly on stems. Yellow starthistle stems are often winged, while meadow knapweed stems typically are not winged. Yellow starthistle generally has a thicker covering of hairs than meadow knapweed, giving yellow starthistle leaves and stems a grayish-green coloration. Meadow knapweed often has deep red color at the base of the stems and on rosette leaves.

Yellow starthistle basal leaves are deeply lobed, with six to sixteen lobes per rosette leaf and a triangular terminal lobe. The upper leaves on yellow starthistle stems are entire and lanceolate. Meadow knapweed leaves range from entire to lobed, with up to eight lobes per rosette leaf. Meadow knapweed leaves, when lobed, are not as deeply lobed as yellow starthistle. Yellow starthistle rosette leaves are crisped, or wavy, while meadow knapweed leaves are flat. Meadow knapweed lower leaves are borne on stalks, while upper leaves are not stalked.

The flowers of both species occur in clusters on capitula (heads). Each head is subtended by involucral bracts. Bract shape provides one of the most important morphological characteristic for identification of *Centaurea* species (Figure 1.2). The appendages along the margin of yellow starthistle bracts are called spines and the appendages along the margin of meadow knapweed bracts are called phyllaries. Each bract has 3 or 5 spines, with the long, sharp central spine reaching 25 mm. The central spine recurves away from the head, which likely discourages herbivory. The bracts of meadow knapweed range from papery to pectinate. The phyllaries are not sharp and are appressed to the head. Unlike yellow starthistle, where the terminal spine is much longer than the other spines on the bract, the papery to pectinate phyllaries on meadow knapweed bracts have a relatively uniform length. The phyllaries range from less than 1 mm to 4 mm and number from 14 to 28 per bract. Meadow knapweed phyllaries are brown to black, while the spines of yellow starthistle are tan. The lower portion of the bracts of both species are green.

Figure 1.2. Capitula of yellow starthistle, putative *C.* × *kleinii* hybrids, and meadow knapweed.

Yellow starthistle florets are dark yellow, with little variation in color (Figure 1.2). Both ray and disk flowers are present. There is considerable variation in flower coloration among meadow knapweed plants, from purple to white. On individual plants, ray and disk flowers may differ in color. Meadow knapweed ray flowers are often sterile. Both species have perfect flowers. Fused anthers form an anther cone through which the stigma emerges. Maturation of male and female reproductive structures happens at slightly different times on the same flower.

1.6 Ecology

Yellow starthistle is an annual, insect-pollinated plant. In California, Roché and Thill (2001) reported that approximately 50% of pollination was by introduced European honeybees. It reproduces primarily through outcrossing, but self-pollination can occur (Maddux et al. 1996). Sun and Ritland (1998) found a 97.5% outcrossing rate.

Yellow starthistle generally flowers in late summer or early autumn (Roché 1965). In Oregon, it typically flowers in July and develops seeds by August. The seeds are achenes and occur in two different forms. The tan, plumed form has a pappus on the end opposite the hilum, while the black, plumeless form does not have a pappus. The seeds are 2-3 mm in length and the pappus bristles are 2-5 mm in length (DiTomaso and Healy 2007). A single head may produce both types of achenes. The plumed achene is produced in the center of the capitula and falls to the ground at maturity. The plumeless achenes do not fall from the head until the head disintegrates through the fall and winter months (Roché and Roché 1991a).

The achenes typically germinate with first precipitation of autumn. The plants overwinter in the rosette stage and bolt in the spring. Vernalization does not appear to increase the rate or decrease the timing of flowering (Roché and Thill 2001). The plumeless achenes require a higher temperature to germinate (Roché and Roché 1991a). As the heads develop, involucral bracts develop into sharp spines as a possible defense against herbivory. Yellow starthistle displays ruderal traits that allow it to colonize disturbed areas. The achenes are small and produced in large quantities. The two different forms of achenes allow the species both concentrated germination in the environment of the mother plant and dispersal over greater distances resulting from the wind-mediated movement of plumed achenes.

Meadow knapweed is a perennial. It spreads primarily by seed, but can regrow vegetatively. It becomes established in moist sites such as pastures, river banks, and irrigation ditches and is an aggressive invader in disturbed areas (Roché and Johnson,

2003). Like yellow starthistle, vernalization does not appear to induce flowering. It is reported to be self-incompatible by Roché and Roché (1991a).

Meadow knapweed achenes typically remain in the capitula until the capitula disintegrates throughout the autumn and winter. The achenes may be spread by water, vehicles, or humans (Roché and Roché 1991a). Meadow knapweed achenes do not exhibit the two forms that yellow starthistle achenes, but some meadow knapweed achenes have short pappus bristles.

1.7 Genetics

The potential hybridization between yellow starthistle and meadow knapweed would be relatively rare because they have different ploidy levels and different base numbers of chromosomes. Yellow starthistle is diploid with $2n = 16$ chromosomes. In contrast, meadow knapweed occurs as a tetraploid in North America, with $2n = 4x = 44$ chromosomes (Susanna and Roché 2011). In North America, black knapweed and brown knapweed, the parent species of meadow knapweed are also tetraploid with $2n = 4x = 44$ chromosomes (Roché and Roché 1991).

Hardy et al. (2000) characterized a Belgian population of meadow knapweed and its parent species as a hybrid complex with a continuum of morphological features rather than clear differences in morphology among the constituent species. They reported that there are both diploid ($2n = 22$) and tetraploid ($2n = 44$) meadow knapweed individuals in the populations they characterized.

Holoploid (2C) genome size is the "DNA content of the whole complement of chromosomes characteristic for the organism" (Greilhuber et al. 2005), measured in picograms (pg) of DNA. The 2C genome size of yellow starthistle is 1.74 pg (Bancheva and Greilhuber 2006, Kew Angiosperm Database). For the knapweed species complex, the 2C-values are 3.60 pg for black knapweed, 4.00 pg for brown knapweed, and 4.30 pg for meadow knapweed (Grime et al. 1985, Bancheva and Greilhuber 2006, Kew Angiosperm Database).

1.8 Objectives

The primary objective of this research was to determine if yellow starthistle and meadow knapweed can produce hybrids. To investigate this, two methods of controlled crossing were used to transfer pollen from meadow knapweed to yellow starthistle, and vice versa. The descriptive morphological criteria (flower color and bract shape) described in Roché and Susanna (2010) were used to determine if any of the F_1 progeny from either parent species were hybrids. Genome size and four quantitative morphological characters were measured for both parent species, the putative hybrids collected in the field, F_1 hybrids produced through controlled crossing, self-pollinated yellow starthistle progeny, and self-pollinated meadow knapweed progeny. Each of the characters was analyzed using a generalized linear model in SAS 9.3. Roché and Susanna's (2010) hypothesis that the putative hybrids found in soutwestern OR were hybrids between yellow starthistle and meadow knapweed was tested by analyzing whether there were differences between the putative hybrids and the hybrids generated through controlled crossing for genome size or any of the four quantifiable morphological traits.

Roché and Susanna (2010) hypothesized that meadow knapweed was likely the maternal parent of hybrids. This hypothesis was based on the fact that the putative hybrids physiologically and morphologically resemble meadow knapweed more than yellow starthistle. This hypothesis was tested as seed from the controlled crosses was germinated and F_1 hybrids were separated from self-pollinated progeny.

The putative hybrids and the F_1 hybrids generated through controlled crossing were both crossed with the both parent species to test if backcrossing was possible.

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2. HYBRIDIZATION BETWEEN YELLOW STARTHISTLE (*CENTAUREA SOLSTITIALIS*) AND MEADOW KNAPWEED (*CENTAUREA* × *MONCKTONII*)

2.1 Abstract

Seven plants were identified in southwestern Oregon that appeared to be hybrids between yellow starthistle (*Centaurea solstitialis* L.) and meadow knapweed (*Centaurea* × *moncktonii* Britt.). Roché and Susanna (2010) characterized these plants as hybrids based on morphological characteristics that are intermediate between the putative parent species. To test the hypothesis that these plants are hybrids, controlled crosses were made between yellow starthistle and meadow knapweed. These crosses produced 30 hybrids that fit the morphological description used by Roché and Susanna (2010) to identify plants as *C. solstitialis* × *C. moncktonii* hybrids. All the hybrids generated from the controlled crosses germinated from yellow starthistle maternal parents. Five quantifiable characters were measured on the putative hybrids, hybrids generated through controlled crossing, and the parent species. For all five characters, there was no difference between the putative hybrids and the hybrids generated through controlled crossing. The putative hybrids and the hybrids generated by controlled crossing were different from yellow starthistle for all five characters. For two of the characters, the putative hybrids were not different from meadow knapweed. The hybrids generated from controlled crosses were not different from meadow knapweed when comparing length of the apical bract appendages, but showed differences for the other four characters. The analysis of these characters provided strong evidence that the putative hybrids are hybrids between yellow starthistle and meadow knapweed.

2.2 Introduction

Bureau of Land Management (BLM) employee Jeanne Klein (now Standley) located an unidentified member of the Asteraceae family as part of a vegetation survey in 1998. Cindy Roché, an expert on the genus *Centaurea*, identified the plants as putative hybrids between yellow starthistle and meadow knapweed. Four additional plants were located by BLM and U.S. Forest Service employees and identified as putative hybrids. In 2010, Roché coauthored an article identifying and naming the putative hybrids *Centaurea* × *kleinii* (Roché and Susanna 2010).

The hypothesis of hybrid origin was based on the morphological characteristics of flower color and bract shape, as well the presence of the yellow starthistle and meadow knapweed at the same sites as the putative hybrids. The putative hybrids have recurved bracts with distinct, comb-like appendages. The flowers have light yellow disk flowers and lavender or light yellow ray flowers. Due to the invasive nature of the two parent species and the fact that meadow knapweed originated through hybridization, additional evidence is critical in confirming or refuting the *C. solstitialis* × *C. moncktonii* hybridization hypothesis.

To determine whether the two species hybridize, controlled crosses were done between the two putative parent species. Both parent species are pollinated by insects and are primarily out-crossers. Yellow starthistle will self-pollinate at low rates. Maddox et al. (1996) counted the number of viable achenes in self-pollinated yellow starthistle compared to outcrossed yellow starthistle plants. There were 0.02 viable achenes per head per plant for self-pollinated plants compared to 9.1 viable achenes per head per plant for out-crossed plants. Meadow knapweed was reported to be self-incompatible (Roché and Roché 1991b), but this has not been tested. Tetraploid brown knapweed (*C. jacea* L.) produced 0.8 achenes per capitulum when self-pollinated (Koutecký et al. 2011).

In addition to the characteristics used by Roché and Susanna (2010), two additional methods were used to determine whether the seedlings generated in controlled crosses were hybrids or the products of self-pollination of the parent species. The first method was to use flow cytometry to determine the genome size of the parent species, the F_1 seedlings, and the putative hybrids. The second method was to measure quantifiable morphological characteristics to determine if these traits can be used to differentiate hybrids from the parent species.

Yellow starthistle is diploid with a base number of 8 chromosomes ($2n = 2x = 16$) and meadow knapweed is tetraploid with a base number of 11 chromosomes ($2n = 4x = 44$). Meadow knapweed has been reported to occur as a diploid in Europe, but only tetraploids have been reported in North America. Brown knapweed (*C. jacea*), black

knapweed (*C. nigra* L.), and meadow knapweed generally occur in hybrid swarms containing various backcrossed combinations of the three species. Brown knapweed and black knapweed are both tetraploid with $2n = 4x = 44$ (Hardy et al. 2000). These differences in ploidy level and base number of chromosomes between yellow starthistle and the knapweed species complex make confirming the reported hybridization through controlled crossing necessary.

Holoploid (2C) genome size, measured in picograms (pg) of DNA, is the "DNA content of the whole complement of chromosomes characteristic for the organism" (Greilhuber et al. 2005). The 2C-value is "the unreplicated non-reduced chromosome content" (Greilhuber et al. 2005). The 2C genome size of yellow starthistle is 1.74 pg (Bancheva and Greilhuber 2006, Kew Angiosperm Database). For the knapweed species complex, the 2C-values are 3.60 pg for black knapweed, 4.00 pg for brown knapweed, and 4.30 pg for meadow knapweed (Grime et al. 1985, Bancheva and Greilhuber 2006, Kew Angiosperm Database).

Both yellow starthistle and meadow knapweed flowers are arranged in heads, or capitula. The flowers are closely grouped and the head is subtended by involucral bracts. Bract shape is one of the morphological characters that are used to delineate species in the genus *Centaurea*. The appendages along the margin of yellow starthistle bracts are spines and the appendages along the margin of meadow knapweed bracts are phyllaries. Yellow starthistle bracts have one long apical spine, with one or two much smaller appendages on either side of the long spine. Meadow knapweed bracts have a papery or comb-like margin with 12-24 phyllaries (Marsden-Jones and Turrill 1954). The number and length of the bract appendages of the species are two characters that can be used to distinguish the species.

2.3 Materials and Methods

Hybrids: Carol Mallory-Smith (OSU), Jeanne Standley, and Susan Carter, scouted the reported locations of the putative hybrids and collected three putative hybrids in 2004. Mallory-Smith potted these plants and maintained them in greenhouses at

Oregon State University (OSU) in Corvallis, OR. All three survived for more than five years. The plants periodically produced stems and flowered. This is characteristic of perennial meadow knapweed, but not of annual yellow starthistle.

The three putative hybrids collected in the field were catalogued with consecutive numbers. These putative hybrids were vegetatively propagated to create clones. The clones were placed in different pots. The clones were catalogued using the putative hybrid number, followed by a lower case letter. This propagation was done to generate more plant material to characterize morphological characters and to maintain the genomes of each putative hybrid.

In the summer of 2012, the GPS coordinates of the five putative hybrids described by Susanna and Roché (2011) were mapped using ArcGIS 10.0 (ESRI 2010). At one location (42°29’59”N 124°15’33”W), a putative hybrid was identified and collected. Both putative parent species were located within 1 meter of the putative hybrid. In this region, the flowering periods of yellow starthistle and meadow knapweed overlap.

Areas in southwest Oregon with populations of both of the parent species were also scouted. In most of these locations, there are large populations of meadow knapweed, along with isolated yellow starthistle plants. One additional putative hybrid was identified and collected. The location (42°49’01”N 123°36’31”W, along Cow Creek Rd. in Douglas County, OR) was recorded and photographs were taken of the putative hybrid in the field (Figure 2.7). The two putative hybrids collected in 2012 were cloned. Insect pollinators were observed visiting both parent species and the putative hybrid in late July and August 2012 (Figure 2.8).

Seeds: Yellow starthistle and meadow knapweed seeds were collected from populations found in southwest OR and the Willamette Valley. Yellow starthistle seed was collected from populations in southwest OR by Cindy Roché. Meadow knapweed seeds were collected from Oregon State University property located on Tampico Rd., 18 km north of Corvallis. Additional seeds from both species were collected within five meters of the locations of putative hybrids when these locations were scouted in 2012.

The seed was stored at room temperature, and then stratified at 4°C for 600 hours to break dormancy. To germinate the seed, seed was placed on germination paper in Petri dishes. The Petri dishes were placed in a germination chamber with a photoperiod of 14 hours of light and 10 hours of dark. Two temperature regimes were tested (at 20°C/10°C light/dark temperature and 25°C/10°C light/dark temperature) initially (Table 2.1).

Based on the germination test, the 25°C/10°C light/dark temperature was used for additional germination tests. One hundred twenty seeds of each parent species were placed in the germination chamber. One hundred fourteen of the 120 meadow knapweed seeds (95.00%) and 110 of the 120 yellow starthistle seeds (91.67%) germinated (Table 2.2).

Seedlings were potted in 342 cm^3 pots containing Sunshine Mix No.1 potting soil (Sungrow Horticulture, Seba Beach, AB, Canada) and placed in a greenhouse with a temperature of 21°C. The seedlings were later transferred to 2470 cm^3 pots.

Table 2.1. Germination test results of parent species at two temperature regimes.

Species	Seeds	Seeds Germinated	Percent germination (%)
Meadow knapweed			
20°C/10°C	128	94	73.44
25°C/10°C	128	109	85.16
Yellow starthistle			
20°C/10°C	128	55	42.97
25°C/10°C	128	103	80.47

Table 2.2. Combined germination of parent species at two temperature regimes.

Species	Seeds	Seeds Germinated	Percent germination (%)
Meadow knapweed			
20°C/10°C	128	94	73.44
25°C/10°C	248	228	91.94
Yellow starthistle			
20°C/10°C	128	55	42.97
25°C/10°C	248	213	85.89

2.4 Pollination

To test whether yellow starthistle and meadow knapweed can produce hybrids, two controlled crossing methods were used to cross-pollinate the species. The first method used an insect pollinator. The second method was hand-pollination. Reciprocal crosses were performed to determine which parent species served as the maternal parent. Precautions, described below, were taken to reduce the possibility of self-pollination for both pollination methods.

Insect Pollination: Isolation pollination cages containing *Calliphora vomitoria* L. (blue bottle flies, Forked Tree Ranch, Porthill, ID), were used for insect pollination. The flies were maintained in the pupae stage at 4°C until they were needed for pollination.

Three pollination cages measuring 1.08 m by 1.28 m by 1.52 m were constructed and placed in different rooms within the greenhouse at Oregon State University. The yellow starthistle and meadow knapweed parent populations were each isolated in separate rooms from the pollination cages to prevent the transfer of pollen outside the controlled crosses.

For each cross, a meadow knapweed plant in flower and a yellow starthistle plant in flower were placed in the same pollination cage. The blue bottle fly pupae were moved from 4°C storage to the pollination cage in the 21°C greenhouse room, where they metamorphosed into adult flies, used for pollination. As controls, two plants of the same species were crossed following the same protocol used with the two different species.

Each head used in a cross was tagged with a unique identifying number and the date. Each head was only involved in one cross. When a plant was used in a cross, all the flowering heads except the tagged head were bagged to prevent the movement of pollen.

Hand Pollination: For hand pollination, a flowering meadow knapweed head was chosen and a flowering yellow starthistle head were selected. A tag was tied to each

head with a unique identifying number and the date. The plant number on which each head was located was recorded. The flowers on each head develop centripedally over the course of 24-72 hours. Disc flowers for both species contain both male and female reproductive parts. Heads were chosen when there were both flowers with mature pollen and flowers with receptive stigmas to perform reciprocal crosses.

Pollen from meadow knapweed flowers was brushed against receptive yellow starthistle stigmas. Using the same heads, pollen from yellow starthistle flowers was also brushed against receptive meadow knapweed stigmas. The flowers on each head are small and close together. None of the flowers were emasculated. Though precautions were taken to minimize pollen from one flower being brushed onto stigmas on the same head, the potential for a low rate of self-pollination remained.

The heads were collected eight weeks after pollination. Each head was placed in an envelope marked with the head number, date pollinated, and date collected. The seed was cleaned and stratified at 4°C for 600 hours.

The seed from each head was placed on germination paper in Petri dishes marked with the unique identifying number. The Petri dishes were placed in a germination chamber with a photoperiod of 14 hours of light (25°C) and 10 hours of dark (10°C).

When the seed germinated, the number of seedlings was recorded. Seedlings were potted in 342 cm^3 pots containing Sunshine Mix No.1 potting soil (Sungrow Horticulture, Seba Beach, AB, Canada) and placed in a greenhouse with a temperature of 21°C. The seedlings were later transferred to 2470 cm^3 pots.

The putative hybrids morphologically appear to be intermediate to the parent species. However, meadow knapweed has variability in many morphological characters so it is possible that the putative hybrid may be meadow knapweed plants with unusual flower color and bract shape. To test this possibility, two methods with quantifiable variables were used in addition to the criteria used by Roché and Susanna (2010) to distinguish hybrids from the parent species. These methods were quantification of the genome size and of four morphological traits.

2.5 Genome Size

Flow cytometry was used to measure the holoploid genome size (2C-value) of the two parent species populations, the five putative hybrid plants collected in the field, and the seedlings generated when the two parent species were crossed under controlled conditions. *Raphanus sativus* L. 'Saxa' (2C-value = 1.11 pg) was used as a standard in this study (Doležel 1998).

For each sample plant, leaf tissue was collected. Approximately 0.5 cm^2 of the sample plant was placed in a Petri dish, along with 0.5 cm^2 of leaf tissue from a *R. sativus* 'Saxa' plant and 500 µL of nuclei extraction buffer (CyStain ultraviolet Precise P Nuclei Extraction Buffer; Partec, Münster, Germany). The leaf tissue of both plants was finely chopped and this solution was filtered through Partec CellTrics filters with a pore size of 50 µm to separate nuclei from the rest of the leaf tissue. The nuclei were then stained with 4',6-diamidino-2-phenylindole (CyStain Precise P DAPI Staining Buffer; Partec, Münster, Germany) and the suspension was analyzed using a Partec CyFlow Ploidy Analyzer to determine the relative fluorescence of the sample plant and the standard plant.

The fluorescence values of each molecule are plotted on a histogram. When the sample plant and standard plant are run together, two peaks are generated on the histogram. The 2C-value of the sample can be found by multiplying the 2C-value of the reference peak (*R. sativus* 'Saxa' = 1.11 pg) by the mean fluorescence value of the sample divided by the mean fluorescence value of the standard.

Table 2.3. Genome Size (2C-value) of the parent species (from Kew Royal Botanical Garden DNA C-values).

Species	Chromosome Number	2C-value
Yellow starthistle (*Centaurea solstitialis*)	$2n = 2x = 16$	1.74 pg
Black knapweed (*Centaurea nigra*)	$2n = 4x = 44$	3.60 pg
Brown knapweed (*Centaurea jacea*)	$2n = 4x = 44$	4.00 pg
Meadow knapweed (*Centaurea* × *moncktonii*)	$2n = 4x = 44$	4.30 pg

The 2C-values obtained using flow cytometry were compared to the values for yellow starthistle and meadow knapweed obtained by Bancheva and Greilhuber (2006) and compiled in the Kew Royal Botanical Garden Angiosperm DNA C-values database (Kew) (Table 2.3). Because meadow knapweed generally occurs as hybrid swarm containing various backcross combinations with black knapweed and brown knapweed, the values were compared to the range of accepted values for the three knapweed species.

2.6 Morphology

Quantifiable morphological characters were measured to determine whether the hybrids produced by controlled crosses were different from the five putative hybrids from the field. The putative hybrids were compared to populations of both parent species and to seedlings generated when the parent species were crossed.

The first morphological character measured was the mean number of involucral bracts per head. For each plant measured, the number of involucral bracts was counted on each of the four fully developed heads. These values were averaged to calculate a mean for each plant.

The second character measured was the mean number of appendages per bract. Three fully developed heads were randomly chosen for each plant. On each of these heads, three bracts were randomly chosen. The number of appendages was counted on each of these nine bracts. The values were averaged to calculate a mean for each plant.

The third morphological character measured was the mean length of apical bract appendages. For each plant, three fully developed heads were chosen at random. On each of these heads, three bracts were chosen and the apical appendages were measured. The appendages were measured from the point where the apical appendage separated from the surrounding appendages. The appendage lengths measured on each plant were averaged to calculate a mean apical appendage length for each plant.

The fourth morphological character measured was the mean number of lobes per basal leaf. For each plant, five basal leaves were chosen at random and the number of

lobes was counted on each. The terminal lobe was not included in this count. For each plant, the number of lobes per basal leaf for the five leaves was averaged to calculate the mean. The mean number of lobes per rosette leaf was analyzed to determine if hybrids could be identified prior to the development of flower heads, or capitula. The criteria Roché and Susanna (2010) used to identify hybrids were flower color and bract shape, which are visible after the plant has bolted and produced capitula. Among the quantifiable morphological characters used in this study, bracts per capitula, appendages per bract, and length of apical appendage all are measured on the capitula. Genome size cannot be analyzed in the field. Because the parent species are invasive and it is a management goal to limit the spread of hybrids, it would be advantageous to identify hybrids prior to the plants flowering and anthesis. There are 12-20 days from when the bracts are visible on the capitula to when to the plant produces pollen. If the number of lobes per rosette leaf could be used to differentiate hybrids from the parent species, management of the hybrids could be done throughout the year, rather than a short period of time in July and August.

Each of the five variables described was analyzed using generalized linear model (PROC GLM) in SAS 9.3. A generalized linear model was used because there were an unequal number of samples in each group. The groups tested were the meadow knapweed and yellow starthistle parent plants, the meadow knapweed and yellow starthistle self-pollinated plants, the F_1 hybrid plants generated from controlled crossing, and the putative hybrids collected in the field. For each variable, the H_0 tested was that there was no significant difference between any of the means of each group.

Two way comparisons were used between each of the groups. These comparisons used the differences in least square means to determine if there was a significant difference between means of the two groups. For each two-way comparison, the H_0 was that there was no significant difference between the means of the two groups. For each comparison, the p-value was the probability that randomization would lead to a greater difference in group means than the observed difference. P-values below 0.05 were

considered significant and p-values above 0.05 were considered not significantly different.

2.7 Results

Eight seeds that originated from meadow knapweed heads germinated and six of these seedlings survived (Table 2.3). The plants had rosette leaves which were flat, dark green and entire to lobed. Both meadow knapweed and hybrids have flat, dark green leaves and entire to shallowly lobed leaves. Three of the meadow knapweed progeny matured and flowered, while a fourth produced a head with visible bracts. The flowers had the papery or partially united comb-like bracts (Figure 2.3) and the purple and white flowers that are characteristic of meadow knapweed. None of the F_1 progeny from seed from meadow knapweed capitula had morphology intermediate between meadow knapweed and yellow starthistle. Intermediate morphology would be indicative of yellow starthistle serving as the pollen parent. The progeny also did not meet the criteria Roché and Susanna (2010) used to describe the yellow starthistle × meadow knapweed putative hybrid, *C.* × *kleinii*. The putative hybrids have recurved bracts with distinct, comb-like phyllaries. The putative hybrid flowers have light yellow disk flowers and lavender or light yellow ray flowers. The meadow knapweed progeny appear to be the result of self-pollination.

Table 2.4. Percentage of seeds germinated from parent species after cross-pollination using an insect pollinator.

Species	Pollination Method	Seeds	Seeds Germinated	Percent germination	Established
Meadow knapweed	Hand	678	7	1.03%	6
Meadow knapweed	Insect	1018	1	0.10%	0
Yellow starthistle	Hand	908	9	0.99%	8
Yellow starthistle	Insect	1292	88	6.81%	71

Ninety-seven seeds that came from yellow starthistle heads germinated and 79 seedlings became established and survived. Forty-nine of these yellow starthistle F_1 progeny had grayish-green crisped, or wavy, leaves with deeply cleft lobes. They did not have entire leaves and most rosette leaves had more than eight lobes per leaf ($\mu = 11.00$,

$\sigma = 2.74$). Thirty-four of these plants bolted and flowered. The plants had stem wings, bracts with sharp spines, and three or five spines per bract (Figure 2.3). Both ray and disk flowers were dark yellow. These characteristics indicate that these were self-pollinated yellow starthistle plants.

Thirty of the seedlings that germinated from yellow starthistle seeds pollinated by meadow knapweed had flat, dark green leaves and entire to shallowly lobed leaves which are not deeply cleft. Twenty-three of these plants bolted and produced capitula. They lacked stem wings, had recurved bracts with distinct comb-like phyllaries (Figure 2.3), light yellow disk flowers, and lavender or light yellow ray flowers. These plants had characteristics which make them appear intermediate between meadow knapweed and yellow starthistle. These 23 plants share these characteristics with the five putative hybrids and match the description used by Roché and Susanna (2010) to identify the putative hybrids.

Table 2.5. Results of hypothesis tests (see Appendices for complete results).

Character	degrees of freedom	F-value	p-value
Genome Size	148	1935.69	$p < 0.0001$
Bracts per Head	139	50.30	$p < 0.0001$
Appendages per Bract	139	202.02	$p < 0.0001$
Length of Appendage	139	211.43	$p < 0.0001$
Lobes per Rosette Leaf	138	62.83	$p < 0.0001$

The H_0 of no difference between the means of any groups was rejected for each variable tested (Table 2.5).

Genome Size: The yellow starthistle population used in the controlled crosses had a 2C-value range from 1.50 - 1.76 pg, with a mean of 1.70 pg ($\sigma = 0.07$). The

Table 2.6. Genome size (2C-value) of yellow starthistle and knapweed species complex.

	Accepted 2C-value Genome Size (Bancheva and Greilhuber 2006)	Range of 2C-value of parent populations
Yellow starthistle	1.74 pg	1.50 - 1.76 pg ($\mu = 1.66$, $\sigma = 0.07$)
Knapweed species complex	3.60 - 4.30 pg	3.61 - 4.47 pg ($\mu = 3.85$, $\sigma = 0.16$)

meadow knapweed population used in the controlled crosses had a 2C-value range from 3.61 - 4.47 pg, with a mean of 3.85 pg ($\sigma = 0.16$) (Table 2.6).

Four of the five putative hybrids collected from the field had 2C-values ranging from 2.55 - 2.71 pg (Table 2.7). The fifth putative hybrid had a 2C-value of 4.67 pg. This plant appears to be a different ploidy level than the other putative hybrids. The monoploid values (1x) were calculated, based on the ploidy levels and 2C genome sizes of the parent species and the putative hybrids, to determine the likely ploidy level of this plant (Table 2.8). A ploidy level of 5x, with a monoploid value of 1x = 0.93, was more consistent with the monoploid values for the other groups than a ploidy of 4x (1x = 1.16) or 6x (1x = 0.78).

Table 2.7. Genome size (2C-value) of the putative hybrids.

Putative hybrid	Genome Size (2C-value) (pg)
h1	2.55
h2	2.65
h3	2.66
h4	2.71
h5	4.67

Table 2.8. Ploidy levels of the putative hybrids.

Group	2C genome size	Ploidy	Monoploid value (1x)
Yellow starthistle parent pop.	1.66	2x	0.83
Yellow starthistle progeny	1.70	2x	0.85
Meadow knapweed parent pop.	3.85	4x	0.96
Meadow knapweed progeny	3.73	4x	0.93
Putative hybrids (h1-h4)	2.64	3x	0.88
F_1 hybrids from yellow starthistle maternal parent	2.70	3x	0.90
5th putative hybrid (h5)	4.67	5x	0.93

The six self-pollinated meadow knapweed progeny had 2C-values that ranged from 3.66 - 3.81 pg (Table 2.9).

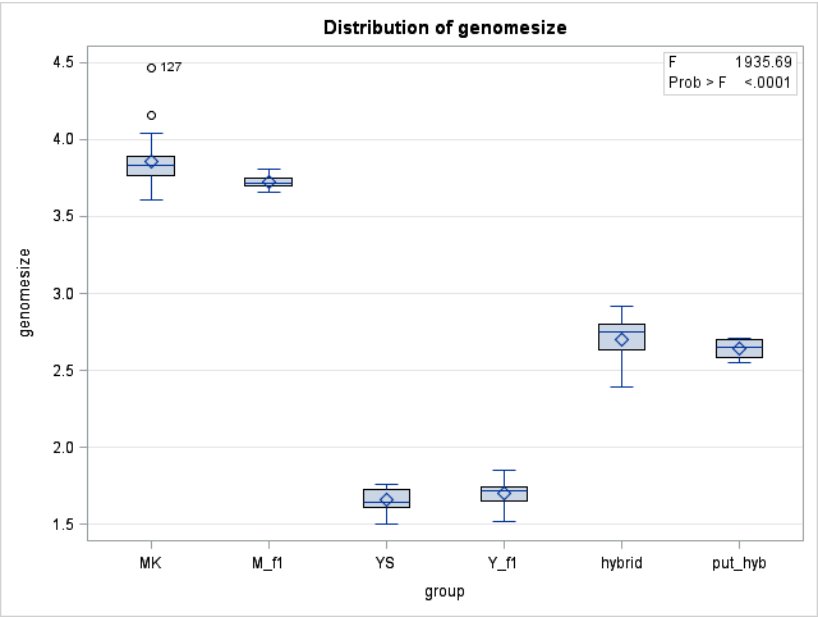
Table 2.9. Genome size (2C-value) of the putative hybrids from the field and the F_1 generation from controlled crosses.

Group	n	Range of 2C-value of sample populations (pg)
Putative hybrids	5	2.55 – 2.71, 4.67
Meadow knapweed progeny	6	3.66 – 3.81
Yellow starthistle progeny	49	1.52 – 1.85
F_1 hybrids from yellow starthistle maternal parent	30	2.39 – 2.92

The 2C-values of the yellow starthistle F_1 progeny fell into two groups. One group, which was comprised of the F_1 plants with grayish-green, crisped, deeply-lobed leaves; sharp spines; three or five spines per bract; and dark yellow flowers had 2C-values of 1.52 – 1.85 pg. The second group, comprised of the F_1 plants with hybrid morphology (flat, dark green leaves, shallow lobes, distinct comb-like phyllaries, light yellow disk flowers, and lavender or light yellow ray flowers) had a 2C-value of 2.39 - 2.92 pg (Table 2.9).

The groups analyzed were the yellow starthistle parent population (n = 30), the meadow knapweed parent population (n = 30), the putative hybrids collected from the field with the 2C-value range of 2.55 - 2.71 pg (n = 4), the progeny from meadow knapweed maternal parents (n = 6), the self-pollinated yellow starthistle progeny (n = 49), and hybrids from yellow starthistle maternal parents (n = 30).

Figure 2.1. Genome size (2C-value) of parent species, putative hybrids, and progeny generated in controlled crosses.



The self-pollinated progeny of both parent species had genome sizes which were close to their respective parent species (Figure 2.1). The mean 2C-value of the yellow starthistle parent population ($\mu = 1.66$, $\sigma = 0.07$) was not different from the mean 2C-

value of the self-pollinated yellow starthistle progeny ($\mu = 1.70$, $\sigma = 0.07$, $p = 0.1090$). Genome size analysis supports the hypothesis that these plants were self-pollinated yellow starthistle plants. Though the meadow knapweed parent population was different compared to the F_1 progeny ($\mu = 3.73$, $\sigma = 0.05$) from this population ($p = 0.0101$), the ranges of the parent population (3.61 - 4.47 pg) and F_1 plants (3.66 - 3.81 pg) overlap. The meadow knapweed progeny did not share intermediate morphological traits with the putative hybrids or the hybrids germinated from yellow starthistle, but had meadow knapweed morphology.

The hybrids germinated from yellow starthistle seed had similar 2C-values to the four putative hybrids from the field, intermediate to the parent species ('hybrid' and 'put_hyb,' respectively, in Figure 2.1). When the mean 2C-value of four putative hybrids collected in the field ($\mu = 2.64$, $\sigma = 0.07$) was compared to the mean 2C-value of the hybrids from the controlled crosses ($\mu = 2.70$, $\sigma = 0.14$), there was no significant difference ($p = 0.3132$). The putative hybrids and the F_1 hybrids created by controlled crosses have genome sizes that fall in a distinct range between the parent species (Figure 2.1). Because yellow starthistle is diploid and meadow knapweed is tetraploid, it appears that these hybrids are triploid. Both the putative hybrids and the F_1 hybrids showed significant differences with both parent species ($p < 0.0001$ for all four comparisons). Analysis of genome size provided strong evidence that the putative hybrids lack a difference in genome size with the hybrids from controlled crossing, but are different from parent species.

Bracts per head: When the mean number of bracts per head of the yellow starthistle parent population ($\mu = 23.24$, $\sigma = 2.61$) and the mean for the self-pollinated yellow starthistle progeny ($\mu = 21.47$, $\sigma = 2.55$) were compared, there was no difference ($p = 0.2749$), supporting the hypothesis that these plants were self-pollinated yellow starthistle. The meadow knapweed F_1 progeny ($\mu = 37.50$, $\sigma = 1.32$) showed no difference from the meadow knapweed parent population ($p = 0.4106$) and appear to be self-pollinated plants.

The putative hybrids from the field (μ = 29.65, σ = 7.28) and the hybrids generated through controlled crossing (μ = 31.39, σ = 6.26) have values intermediate to the parent species. When the putative hybrids were compared to the F_1 hybrids, there was no difference (p =0.4291). While the hybrids generated through controlled crossing were not significantly different from the putative hybrids, they were different from both the meadow knapweed parent population (μ = 40.43, σ = 8.75, p < 0.0001) and the yellow starthistle parent population (μ = 23.24, σ = 2.61, p < 0.0001). When the putative hybrids from the field (μ = 29.65, σ = 7.28) were compared the meadow knapweed parent population (μ = 40.43, σ = 8.75), there was a low probability (p = 0.0002) that the difference was due to chance. When the mean for putative hybrids was compared to the mean for the yellow starthistle parent population (μ = 23.24, σ = 2.61), they were significantly different (p = 0.0274). The hybrids generated through controlled crossing and the putative hybrids were not different, while both were different from the parent species. As with genome size, the number of bracts per head supports the hypothesis that the putative hybrids from the field are hybrids between meadow knapweed and yellow starthistle.

Figure 2.2. Number of bracts per head for the parent species, hybrids, and self-pollinated progeny.

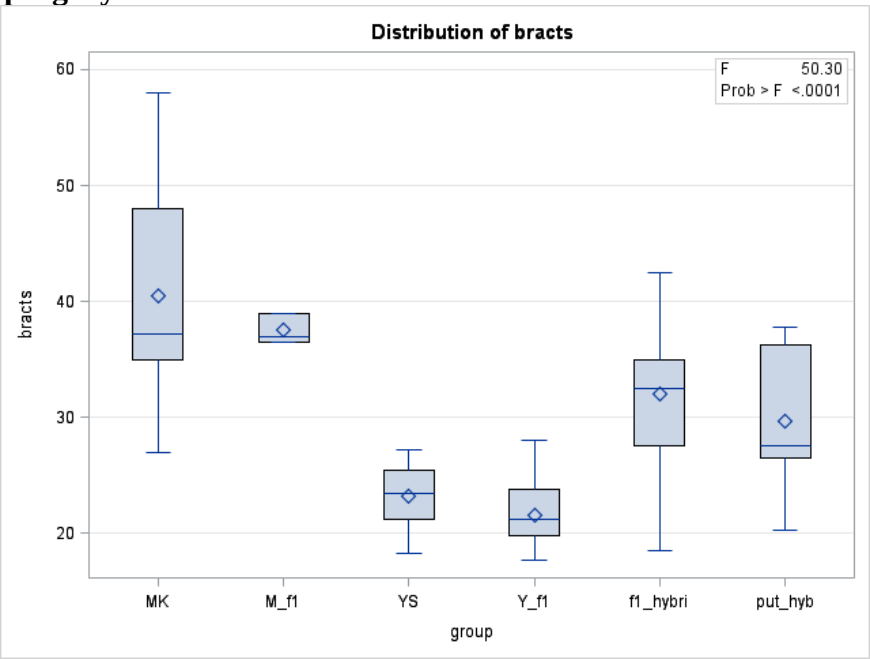


Table 2.10. Mean number of bracts per head for the parent species, hybrids, and self-pollinated progeny.

Group	n	Bracts per Head
Putative hybrids	5	29.65 (σ = 7.28)
F_1 hybrids	23	31.39 (σ = 6.26)
Yellow starthistle parent	30	23.24 (σ = 2.61)
Yellow starthistle self-pollinated	34	21.47 (σ = 2.55)
Meadow knapweed	45	40.43 (σ = 8.75)
Meadow knapweed self-pollinated	3	37.50 (σ = 1.32)

Appendages per Bract: The appendages on a yellow starthistle bract consist of one long, sharp apical spine with one or two short spines on either side of the long spine (Figure 2.3). All the yellow starthistle bracts measured in this study had either three or five total appendages per bract. The means for individual plants in the parent population ranged from 3.67 to 5.00. Meadow knapweed bracts have pectinate (comb-like) appendages. The mean number of appendages per bract for the meadow knapweed parent population ranged from 14.00 to 28.44. The number of appendages can clearly be used to differentiate the two species ($p < 0.0001$).

Figure 2.3. Bract appendages on yellow starthistle, hybrid, and meadow knapweed.

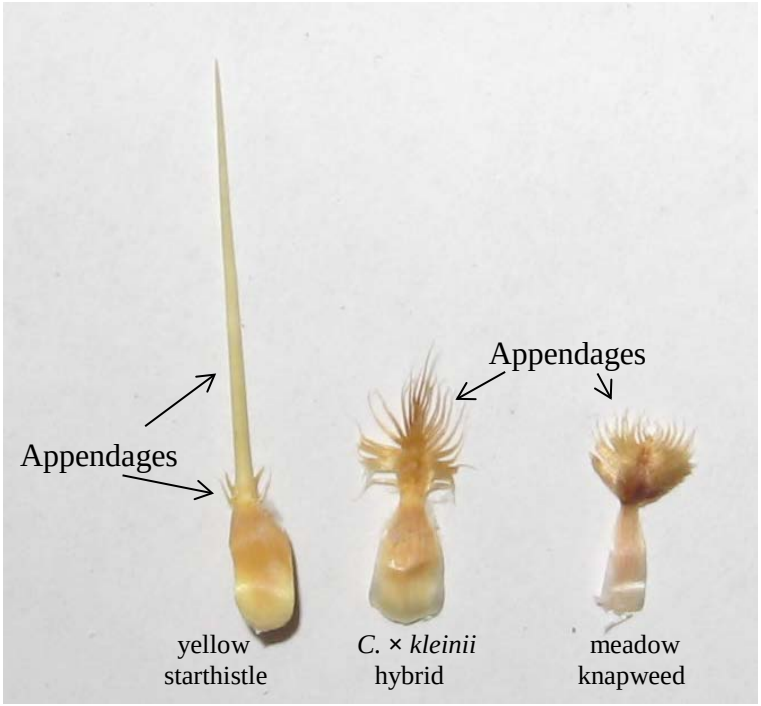
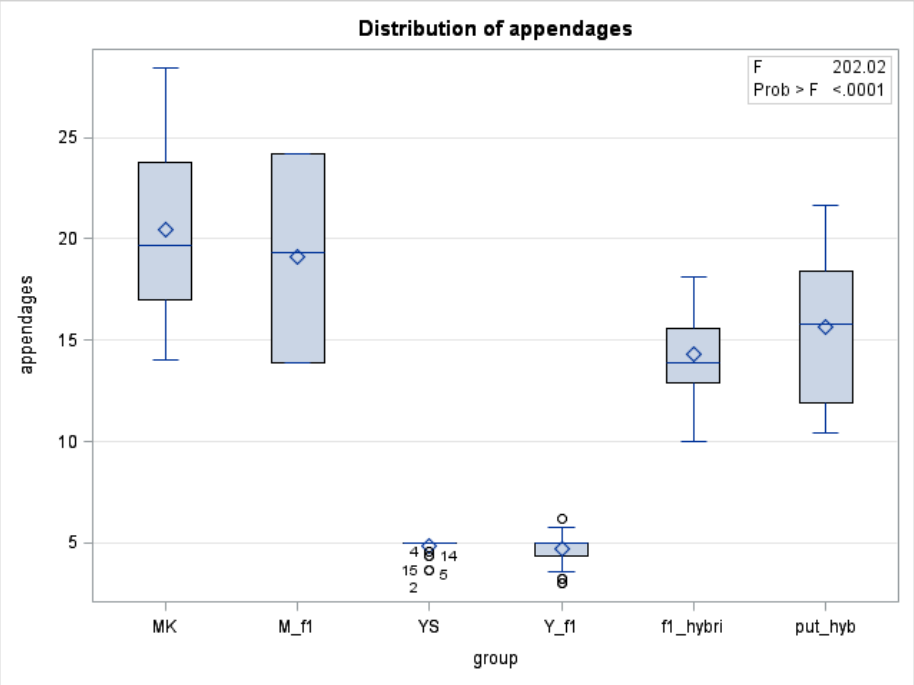


Table 2.11. Mean number of appendages per bract for the parent species, hybrids, and self-pollinated progeny.

Group	n	Appendages per Bract
Putative hybrids	5	15.64 (σ = 4.62)
F_1 hybrids	23	14.03 (σ = 2.05)
Yellow starthistle parent	30	4.85 (σ = 0.37)
Yellow starthistle self-pollinated	34	4.66 (σ = 0.78)
Meadow knapweed	45	20.45 (σ = 3.89)
Meadow knapweed self-pollinated	3	19.15 (σ = 5.17)

Figure 2.4. Number of appendages per bract for the parent species, self-pollinated progeny, and hybrids.



When the yellow starthistle parent population (μ = 4.85, σ = 0.37) was compared to the self-pollinated yellow starthistle progeny (μ = 4.66, σ = 0.78), there was no difference (p = 0.8272). When the meadow knapweed parent population (μ = 20.45, σ = 3.89) to the meadow knapweed self-pollinated progeny (μ = 19.15, σ = 5.17), there was no difference (p = 0.4043).

The putative hybrids from the field had between 10.44 and 21.67 appendages per bract. The F_1 hybrids had mean appendages per bract ranging from 10.00 to 18.11. Both groups can clearly be differentiated from yellow starthistle (p < 0.0001). Though the

ranges overlap with meadow knapweed (Figure 2.4), both groups also differ from meadow knapweed ($p = 0.0002$ for putative hybrids and $p < 0.0001$ for F_1 hybrids). When the putative hybrids were compared to the F_1 hybrids, there was no difference ($p = 0.3097$).

Though the F_1 hybrids germinated from yellow starthistle seeds, the number of appendages per bract is much closer to those of meadow knapweed. The hybrids have values intermediate to the parent species.

Length of Apical Bract Appendages: On yellow starthistle bracts, the apical appendage is a spine which is much longer than the other appendages (Figure 2.5). For the parent population in this study, the mean apical appendage lengths for the individual plants ranged from 9.11 to 23.56 mm. For meadow knapweed bracts, the apical appendage is of equal or shorter length than the other appendages. For the meadow knapweed parent population, the mean lengths ranged from 1.00 to 2.22 mm. Length of apical appendage clearly differentiated between the two species ($p < 0.0001$). Unlike the previous three characters analyzed, there was a difference between the yellow starthistle parent plants and self-pollinated progeny ($p < 0.0001$), though the reason for this was not clear. Both the yellow starthistle parent population and yellow starthistle self-pollinated progeny have much longer apical appendages (Figure 2.5) than the other groups of hybrids and meadow knapweed ($p < 0.0001$ for each of the comparisons).

Table 2.12. Mean length of apical appendage for the parent species, hybrids, and self-pollinated progeny.

Group	n	Mean Length (mm)
Putative hybrids	5	2.78 ($\sigma = 0.69$)
F_1 hybrids	23	2.30 ($\sigma = 0.53$)
Yellow starthistle parent	30	17.17 ($\sigma = 3.34$)
Yellow starthistle self-pollinated	34	12.21 ($\sigma = 3.80$)
Meadow knapweed	45	1.30 ($\sigma = 0.35$)
Meadow knapweed self-pollinated	3	0.41 ($\sigma = 0.53$)

Figure 2.5. Length of apical appendages on bracts of yellow starthistle, *C.* × *kleinii* hybrid, and meadow knapweed.

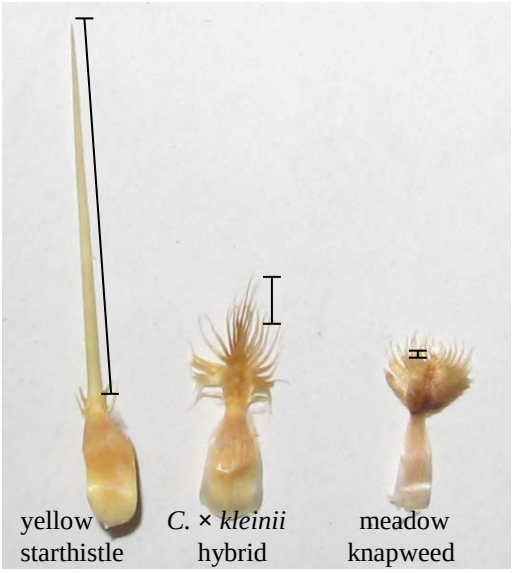
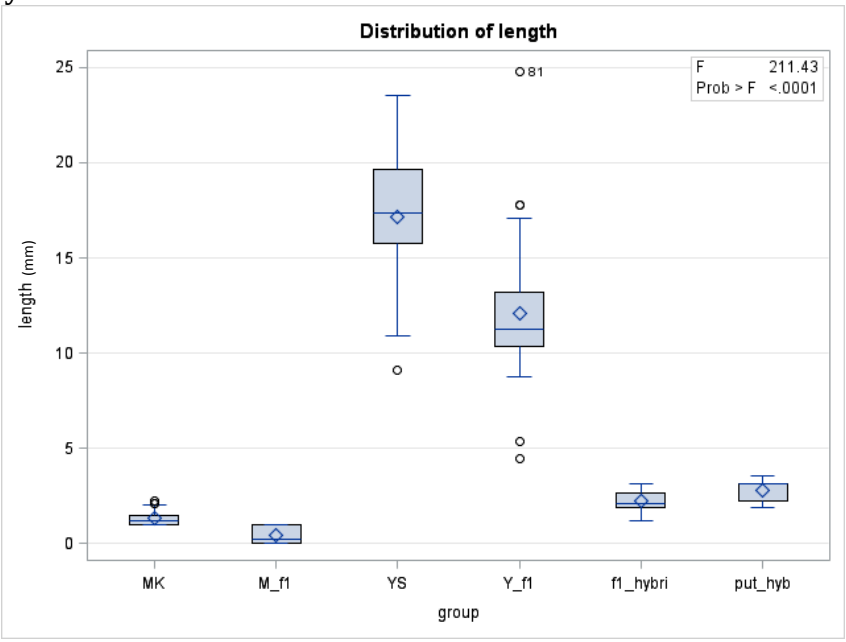


Figure 2.6. Length (mm) of apical appendages for the parent species, self-pollinated progeny, and hybrids.



Both the putative hybrids and the F_1 hybrids have apical appendage lengths which were intermediate to the parent species (Figure 2.6). The putative hybrids ranged from 1.89 to 3.56 mm and the F_1 hybrids ranged from 1.44 to 3.11mm. When compared, there

was no difference between the two groups ($p = 0.6501$). Both groups had means that were different from the yellow starthistle parent population ($p < 0.0001$). Unlike the other characters, neither hybrid group was different from the meadow knapweed parent population. For the putative hybrids, the p-value was 0.1947 and for the F_1 hybrids, the p-value was 0.1304. This character cannot be used by itself to distinguish hybrids from meadow knapweed. The lack of difference when compared to meadow knapweed supports the assertion that meadow knapweed is the pollen parent of these plants.

Lobes per Basal Leaf: For the yellow starthistle parent population, number of lobes ranged from 4.40 to 14.60 lobes ($\mu = 9.65$, $\sigma = 2.67$). Meadow knapweed leaves can range from entire to lobed. The lobes are shallower than yellow starthistle. The parent population had mean number of lobes per leaf which ranged from 0.20 to 6.80 ($\mu = 3.53$, $\sigma = 1.27$). Although there was a difference between the two ($p < 0.0001$), the number of lobes overlap. When combined with other leaf characteristics, such as color, the depth of the lobes, and being flat or wavy, it is possible to differentiate between the two species rosette leaves. However, the number of lobes per leaf cannot be used by itself to differentiate the species.

Figure 2.7. Lobes per rosette leaves for the parent species, hybrids, and self-pollinated progeny.

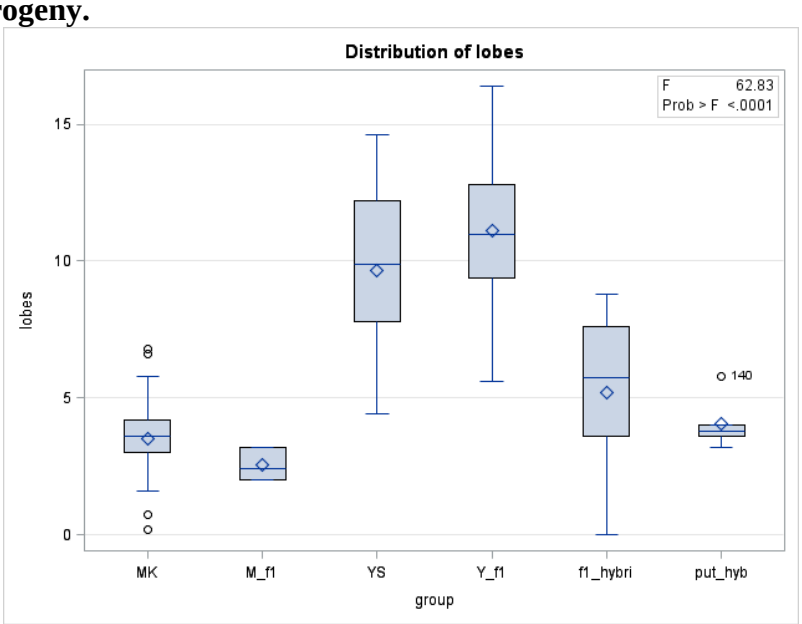


Table 2.13. Mean number of lobes per rosette leaves for the parent species, hybrids, and self-pollinated progeny.

Group	n	Lobes per Rosette Leaf
Putative hybrids	5	4.10 (σ = 1.01)
F_1 hybrids	23	5.04 (σ = 2.56)
Yellow starthistle parent	30	9.65 (σ = 2.67)
Yellow starthistle self-pollinated	33	11.00 (σ = 2.74)
Meadow knapweed	45	3.53 (σ = 1.27)
Meadow knapweed self-pollinated	3	2.53 (σ = 0.61)

Figure 2.8. Rosette leaves of yellow starthistle, *C.* × *kleinii* hybrid, and meadow knapweed.

The putative hybrids ranged from 3.20 to 5.80 lobes per rosette leaf. The F_1 hybrids ranged from entire (no lobes) to 8.80 lobes per rosette leaf. The means for both of these groups were intermediate to the parent species. When the putative hybrids (μ =

4.10, $\sigma = 1.01$) and F_1 hybrids ($\mu = 5.04$, $\sigma = 2.56$) were compared, there was no difference ($p = 0.2920$). This result adds to the evidence provided by the other characters that the putative hybrids are hybrids between yellow starthistle and meadow knapweed. The putative hybrids were not different when compared to meadow knapweed ($p = 0.5951$), but the F_1 hybrids were different ($p = 0.0031$). The range of both the putative hybrids and the F_1 hybrids overlap with yellow starthistle, but both show a difference when the means were compared ($p < 0.0001$).

2.8 Discussion

The F_1 hybrids generated through controlled crossing of yellow starthistle and meadow knapweed match the descriptive morphological evidence used by Roché and Susanna (2010). These *C. solstitialis* × *C. moncktonii* hybrids show no difference from the putative hybrids for genome size, bracts per head, appendages per bract, length of apical bract appendage, or number of lobes per basal leaf. Based on the evidence, we conclude that the putative hybrids are hybrids between yellow starthistle and meadow knapweed.

Neither hybrid group was different from the meadow knapweed parent population for mean length of apical bract appendage. The putative hybrids were not different when compared to meadow knapweed ($p = 0.5951$), but the F_1 hybrids were different ($p = 0.0031$). This result may be due to the fact that the putative hybrids had a sample size of $n = 5$.

The results of these comparisons provide quantitative support to Roché and Susanna's (2010) assertion that the putative hybrids are closer in morphology to meadow knapweed than yellow starthistle. Their hypothesis that meadow knapweed was the maternal parent was not proven, however. All the hybrids generated from the controlled crosses germinated from seeds that came from yellow starthistle plants.

For the number of lobes per rosette leaf, two-way comparisons showed that both groups of hybrids were different from yellow starthistle and were not different compared to each other, which helps confirm that these are both groups are hybrids. In the field,

when the maternal parent of a plant is unknown, counting the number of lobes in the field will not be an effective way of differentiating the hybrids from meadow knapweed in the field. Because the leaves of the hybrids share many leaf characteristics with meadow knapweed, it is difficult to differentiate them in the field.

Figure 2.9. Putative hybrid located at 42°29’59”N 124°15’33”W in Douglas County, OR.

Figure 2.10. Insect pollinator on a putative hybrid in Douglas County, OR (located at 42°29’59”N 124°15’33”).

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3. BACKCROSSING BETWEEN *CENTAUREA* × *KLEINII* HYBRIDS AND PARENT SPECIES

3.1 Abstract

Seven plants were found in southwestern Oregon that appeared to be hybrids between yellow starthistle (*Centaurea solstitialis* L.) and meadow knapweed (*Centaurea* × *moncktonii* Britt.). Roché and Susanna (2010) characterized these plants, named *C.* × *kleinii*, as hybrids based on morphological characteristics that are intermediate between the putative parent species. Five of the putative hybrids collected from the field were used in this study. Controlled crosses were made between yellow starthistle and meadow knapweed, producing 30 hybrids (Chapter 2). One of the management concerns that arise with hybridization between yellow starthistle and meadow knapweed is gene flow between the two species mediated by hybrids. Controlled crosses were made between hybrids and the parent species to determine if backcrosses were produced. One plant became established from hybrid seed pollinated by yellow starthistle. Nineteen plants became established from hybrid seed pollinated by meadow knapweed. Seventy-nine plants became established from yellow starthistle seed pollinated by hybrid pollen. Thirty-seven plants became established from meadow knapweed seed pollinated by hybrid pollen, all of which appear to be self-pollinated. Five quantifiable characters were measured on the hybrids, the parent species, and the plants produced in the controlled crosses. These characters were used to differentiate hybrids from the parent species (Ch. 2). Analysis of these characters provided evidence that hybrids are able to backcross with both parent species at very low levels. Eight of the plants germinated from yellow starthistle seed had a genome size indicating they were backcrosses. Measurement of the four morphological characters was only possible for three of the eight plants, because the others died or did not produce capitula. These characters indicate two of these mature plants are likely backcrosses, while it is not clear whether one of the plants was self-pollinated or a backcross. The small sample size limits the ability to statistically detect differences between plants. The plants produced from hybrid seeds are most likely backcrosses, but analysis of these characters did not eliminate the possibility that these plants may be self-pollinated hybrids. The *C.* × *kleinii* hybrids have a low level of fertility, but are able to produce viable offspring. The hybrid can likely act as a maternal parent and pollen parent for backcrosses with yellow starthistle and meadow knapweed,

but did not produce any progeny in crosses between *C.* × *kleinii* hybrids. These hybrids should be controlled prior to pollen release. Identification and control should continue to be a management priority.

3.2 Introduction

Management concerns related to the *C. solstitialis* × *C. moncktonii* hybridization depend on the hybrids producing viable pollen or seed. Neither parent species reproduces asexually and asexual reproduction has not been observed for the *C.* × *kleinii* hybrids. Fertile hybrids could lead to two undesirable outcomes. The first outcome is the hybrid continuing to evolve into a new species through sexual reproduction. Meadow knapweed, one of the parent species in this study, originated as a hybrid between black knapweed (*C. nigra* L.) and brown knapweed (*C. jacea* L.). Black knapweed and brown knapweed have the same base number of chromosomes and occur at the same ploidy level ($2n = 4x = 44$ chromosomes). Yellow starthistle ($x = 8$) and meadow knapweed ($x = 11$) have different base numbers of chromosomes and occur at different ploidy levels (Marsden-Jones and Turrill 1954). These two factors make speciation of *C.* × *kleinii* less likely than the meadow knapweed speciation.

Ploidy level difference acts as a reproductive barrier between *Centaurea* species (Koutecký et al. 2011). Yellow starthistle is diploid ($2n = 2x = 16$ chromosomes) with a 2C genome size of 1.66 pg and meadow knapweed is tetraploid ($2n = 4x = 44$ chromosomes) with a 2C genome size of 3.85 pg. Greilhuber et al. (2005) defined holoploid genome size as the "DNA content of the whole complement of chromosomes characteristic for the organism." The hybrids have a 2C genome size of 2.70 pg (Chapter 2).

C. × *kleinii* speciation would likely depend on the transfer of pollen from one hybrid to another hybrid. Self-pollination occurred at low rates for both yellow starthistle (2.2%) and meadow knapweed (0.4%) in this study (Chapter 2), so it is possible that *C.* × *kleinii* may self-pollinate. The majority of pollination for both parent species is facilitated by insect pollinators. Insects were observed visiting flowers on *C.* × *kleinii*

hybrids in the field. The hybrids found in the field occurred over three miles from each other. The distance makes hybrid cross-pollination by insects unlikely. Even if each of these hybrids was fully fertile, there is a very low probability that the pollen from one could reach the flowers of the other naturally-occurring hybrids.

Each of the hybrids in the field was located very close to plants of both parent species. There would be much more pollen transfer between these parent species plants and each hybrid than from one hybrid to another. The possibility of the hybrids becoming a species is small, but should be monitored and was tested in this study (Table 3.1).

A more pressing management concern with fertile hybrids is the potential for backcrossing between hybrids and the parent species. Backcrossing would facilitate gene flow between the two parent species. Gene flow is the change in allele frequency due to the movement of pollen or individuals between populations. This could occur between yellow starthistle and meadow knapweed if hybrids produced viable pollen that pollinates a plant of the parent species. Gene flow could also occur if pollen from one of the parent species pollinated a hybrid, and the backcrossed progeny produced seed or pollen which crossed with the parent species.

To determine whether hybrids are able to backcross with either parent species, controlled crosses were conducted between hybrids and both parent species. Flower color and bract shape, the characteristics used by Roché and Susanna (2010) to identify the putative hybrids, were used to determine whether the F_1 plants resulted from backcrossing or from self-pollination.

Two additional methods were used to determine whether the seedlings generated in the controlled backcrossing attempts were backcrosses or the result of self-pollination by the maternal parent. The first method was to use flow cytometry to determine the genome size of the yellow starthistle, meadow knapweed, and *C.* × *kleinii* hybrid plants used as parent material for the backcross attempts. The genome sizes of the seedlings

that were generated in the controlled backcrosses were measured and compared to the genome sizes of the parent material.

Greilhuber et al. (2005) defined holoploid genome size as the "DNA content of the whole complement of chromosomes characteristic for the organism." The 2C-value is "the unreplicated non-reduced chromosome content" (Greilhuber et al. 2005). The 2C-value is measured in picograms (pg) of DNA. When cytotypes occur at different ploidy levels 2C-value can be used to distinguish between the ploidy levels.

Yellow starthistle is diploid with a base number of 8 chromosomes ($2n = 2x = 16$) and meadow knapweed is tetraploid with a base number of 11 chromosomes ($2n = 4x = 44$). Meadow knapweed has been reported to occur as a diploid in Europe, but only tetraploids have been reported in North America. Brown knapweed, black knapweed, and meadow knapweed generally do not occur as distinct species, but rather as hybrid swarms containing various backcrossed combinations of the three species. Brown knapweed (*C. jacea*) and black knapweed (*C. nigra*), are both tetraploid with $2n = 4x = 44$ (Hardy et al. 2000). Despite the differences in ploidy level and chromosome base number, yellow starthistle and meadow knapweed can hybridize at a low rate.

The second method was to measure quantifiable morphological characteristics of the parent material and the F_1 plants produced through controlled crossing to determine if these traits can be used to differentiate backcrosses from the parent species and hybrids.

3.3 Materials and Methods

Bureau of Land Management employee Jeanne Klein located an unidentified member of the Asteraceae family as part of a vegetation survey in 1998. Cindy Roché, who had worked extensively with the genus *Centaurea*, helped to identify the plants as putative hybrids between yellow starthistle and meadow knapweed. In 2010, Roché coauthored an article identifying and naming the putative hybrids *Centaurea* × *kleinii* (Roché and Susanna 2010). Four additional plants were located and were identified as putative hybrids by BLM and U.S. Forest Service employees.

Carol Mallory-Smith (OSU) and Cindy Roché scouted the reported locations of the putative hybrids and collected three putative hybrids. Mallory-Smith potted these plants and maintained them in greenhouses at Oregon State University (OSU) in Corvallis, OR. Each putative hybrid collected in the field was catalogued with consecutive numbers.

In the summer of 2012, the GPS coordinates of the five putative hybrids described by Susanna and Roché (2011) were mapped using ArcGIS 10.0 (ESRI 2010). At one location (42°29'59"N 124°15'33"W), a putative hybrid was identified. This plant was collected and maintained at the OSU greenhouse. Areas in southwest Oregon with both of the parent species present were also scouted. In most of these locations, there are large populations of meadow knapweed, along with isolated yellow starthistle plants. One additional putative hybrid was identified and relocated to the OSU greenhouse. The location of this plant (42°49'01"N 123°36'31"W, along Cow Creek Rd. in Douglas County, OR) was recorded.

The five putative hybrids were vegetatively propagated to create clones. The clones were placed in different pots. The clones were catalogued using the unique putative hybrid numbers. Propagation was done to generate more plant material to characterize morphological characters, to maintain the genomes of each putative hybrid, and to generate more hybrid clones for backcross attempts.

In addition to the five putative hybrids collected from the field, hybrids were generated from the controlled crossing experiments described in Chapter 2. Thirty hybrids were generated through these crosses. These hybrids were produced from yellow starthistle seed which had been pollinated by meadow knapweed. After germinating, these hybrids were maintained in an OSU greenhouse and used as parent material for backcrossing.

Yellow starthistle seed was collected from populations in southwest OR by Cindy Roché. Meadow knapweed seeds were collected from Oregon State University property located on Tampico Rd., 18 km north of Corvallis, OR. Additional seed from both

species was collected within five meters of the locations of putative hybrids when these locations were scouted in 2012. Plants produced from these seeds were used in the controlled backcrossing.

3.4 Pollination

Both parent species are pollinated by insects and are primarily out-crossers. Yellow starthistle will self-pollinate at low rates. Maddox et al. (1996) reported 0.02 viable achenes per capitulum for self-pollinated plants yellow starthistle compared to 9.1 viable achenes per capitulum for out-crossed yellow starthistle plants.

Meadow knapweed was reported to be self-incompatible (Roché and Roché 1991b). Tetraploid brown knapweed (*C. jacea*) produced 0.8 achenes per capitulum when self-pollinated (Koutecký et al. 2011). The present study was not designed to test for self-compatibility in meadow knapweed, but in the course of attempting interspecific cross-pollination, 0.3% self-pollination was observed in meadow knapweed. There have been no previous studies testing the self-compatibility of the *C.* × *kleinii* hybrids. To reduce the possibility of self-pollination during hybrid cross-pollination and controlled backcrossing, flowers on the same head were not intentionally brushed again each other. Flowers are clustered in such close proximity on each head that it was not possible to prevent all pollen from contacting stigmas of flowers on that head.

Hybrid Cross-pollination: Hand pollination was used to test whether *C.* × *kleinii* hybrids were able to cross-pollinate. A flowering head from one of the hybrids was crossed with a flowering head from another hybrid. Each head was used for only one crossing attempt. Each head used in a cross was tagged and recorded. The heads involved in hybrid cross-pollination tests were collected eight weeks after pollination. Each head was placed in an envelope, which was marked with the head number, date pollinated, and date collected. The seed from each head was placed on germination paper in Petri dishes. The unique identifying number of each head was written on the Petri dish. The Petri dishes were placed in a germination chamber with a photoperiod of 14 hours of light (at 25°C) and 10 hours of dark (at 10°C). None of the seed from the heads

involved in these crosses germinated (Table 3.1). *C.* × *kleinii* hybrids did not produce viable offspring when pollinated by other *C.* × *kleinii* hybrids.

Table 3.1 Hybrid cross-pollination.

Number of crosses	Total Seeds	Germinated Seeds
42	1886	0

Each cross includes one maternal and one paternal parent.

Backcrossing: Hand pollination was used to test whether *C.* × *kleinii* hybrids were able to backcross with one or both parent species. Each backcross attempt involved one hybrid head crossed with either yellow starthistle or meadow knapweed. Each head was used for only one crossing attempt (HX_y1 was crossed with YX1, HX_y2 was crossed with YX2, etc.). Each head used in a cross was tagged and recorded (Table 3.2).

Table 3.2. Nomenclature used for backcrosses.

	Maternal parent	**Paternal parent**
HX_y	hybrid	yellow starthistle
HX_m	hybrid	meadow knapweed
YX	yellow starthistle	hybrid
MX	meadow knapweed	hybrid

The heads involved in backcross tests were collected eight weeks after pollination. Each head was placed in an envelope, which was marked with the head number, date pollinated, and date collected. The seed was cleaned and stratified at 4°C for 600 hours.

The seed from each head was placed on germination paper in Petri dishes marked with the unique identifying number (YX1, YX2, etc.) of each head. The Petri dishes were placed in a germination chamber with a photoperiod of 14 hours of light (25°C) and 10 hours of dark (10°C). The number of seedlings was recorded. Seedlings were potted in 342 cm^3 pots containing Sunshine Mix No.1 potting soil (Sungrow Horticulture, Seba Beach, AB, Canada) and placed in a greenhouse with a temperature of 21°C. The seedlings were later transferred to 2470 cm^3 pots.

Each of five putative hybrids collected from the field were crossed 15 times with yellow starthistle, generating 2902 seeds from yellow starthistle heads (YX) and 1714 seeds from hybrid heads (HX_y), and 15 times with meadow knapweed, generating 2677 seeds from meadow knapweed heads (MX) and 2118 seeds from hybrid heads (HX_m). Fifteen of the hybrids generated through controlled crossing produced flowers during the backcrossing study, and these were used for the controlled backcrossing tests. From these, 2624 yellow starthistle seeds pollinated by F_1 hybrids (YX) and 2259 seeds from F_1 hybrids pollinated by yellow starthistle (HX_y) were generated. Meadow knapweed pollinated by F_1 hybrids (MX) yielded 2281 seeds and F_1 hybrids pollinated by meadow knapweed (HX_m) yielded 2384 seeds.

Both of the parent species produced seedlings after being pollinated with pollen from hybrids (Table 3.3). Yellow starthistle produced 106 seedlings (YX), with 75 surviving, and meadow knapweed produced 42 seedlings (MX), with 37 surviving. Seeds from hybrid plants also germinated and produced seedlings after pollination with pollen from the parent species (Table 3.3). Two seeds from hybrid heads pollinated by yellow starthistle pollen (HX_y) germinated (0.05%), with one surviving and becoming established. Twenty-five seeds from hybrid heads pollinated by meadow knapweed pollen (HX_m) germinated (0.58%), with 19 surviving and becoming established (Table 3.3).

Flower color and bract shape were the criteria used by Roché and Susanna (2010) to distinguish hybrids from the parent species. Genome size and the four quantifiable morphological traits were chosen to test the differences in means between the parent species and the hybrids (Chapter 2). The same methods were used to compare yellow starthistle, meadow knapweed, *C.* × *kleinii* hybrids, and the progeny resulting from backcrossing.

Table 3.3. Cumulative totals for backcross attempts.

	Maternal Parent	Paternal Parent	Number of Crosses	Total Seeds	Germinated Seeds	Percent Germinated	Established Seedlings
YX	yellow starthistle	putative hybrids from the field	75	2902	70	2.41%	54
YX	yellow starthistle	hybrids from controlled crosses	53	2624	36	1.37%	21
MX	meadow knapweed	putative hybrids from the field	75	2677	27	1.01%	23
MX	meadow knapweed	hybrids from controlled crosses	50	2281	15	0.66%	14
HX-y	putative hybrids from the field	yellow starthistle	75	1714	1	0.06%	0
HX-y	hybrids from controlled crosses	yellow starthistle	53	2259	1	0.04%	1
HX-m	putative hybrids from the field	meadow knapweed	75	2118	23	1.09%	19
HX-m	hybrids from controlled crosses	meadow knapweed	50	2384	2	0.08%	0
Total				**18959**	**175**	**0.92%**	**132**

3.5 Genome Size

Flow cytometry was used to measure the holoploid genome size (2C-value) of the two parent species populations, the hybrid plants collected in the field, the hybrids produced through controlled crossing, and the seedlings generated after the seeds from each head involved in a backcross attempt were germinated. *Raphanus sativus* L. 'Saxa' (2C-value = 1.11 pg) was used as a standard in this study (Doležel 1998).

For each sample plant, leaf tissue was collected. Approximately 0.5 cm^2 of the sample plant was placed in a Petri dish, along with 0.5 cm^2 of leaf tissue from the *R. sativus* 'Saxa' plant used as a standard, and 500 µL of nuclei extraction buffer (CyStain ultraviolet Precise P Nuclei Extraction Buffer; Partec, Münster, Germany). The leaf tissue of both plants was finely chopped and this solution was filtered through Partec CellTrics filters with a pore size of 50 µm to separate nuclei from the rest of the leaf tissue. The nuclei were then stained with 4',6-diamidino-2-phenylindole (CyStain Precise P DAPI Staining Buffer; Partec, Münster, Germany) and the suspension was analyzed using a Partec CyFlow Ploidy Analyzer to determine the relative fluorescence of the sample plant and the standard plant.

The fluorescence values of each molecule were plotted on a histogram. When the sample plant and standard plant are run together, two peaks are generated on the histogram. The 2C-value of the sample can be calculated by multiplying the 2C-value of the reference peak (*R. sativus* 'Saxa'= 1.11 pg) by the mean fluorescence value of the sample divided by the mean fluorescence value of the standard.

Table 3.4 Genome size (2C-value) of yellow starthistle and knapweed species complex.

	Accepted 2C-value Genome Size (Bancheva and Greilhuber 2006)	Range of 2C-value of parent populations (pg)
Yellow starthistle	1.74 pg	1.50-1.76 (µ=1.66, σ=0.07)
Knapweed Species Complex	3.60 - 4.30 pg	3.67-4.47 (µ=3.85, σ=0.16)
Centaurea × *kleinii* hybrid	NA	2.55-2.71 (µ=2.64, σ=0.07), 4.67

3.6 Morphology

Quantifiable morphological characters were measured to determine whether the plants produced through controlled backcrossing were self-pollinated progeny of their maternal parent or were backcrosses between a hybrid plant and a parent species plant. Two way comparisons were used between the means for each of the groups for each variable. The parent population of meadow knapweed (MK), the parent population of yellow starthistle (YS), the five hybrids collected from the field, and the hybrids produced through controlled crossing were included in the analysis. The self-pollinated progeny from both meadow knapweed and yellow starthistle (Chapter 2) were included in the analysis. These progeny groups were included to determine if plants germinated in the backcross tests were self-pollinated progeny of the maternal parent.

Both yellow starthistle and meadow knapweed flowers are arranged in heads, or capitula. The flowers are closely grouped and the head is subtended by involucral bracts. Bract shape is one of the morphological characters that are used to delineate species in the genus *Centaurea*. The appendages along the margin of yellow starthistle are spines and the appendages along the margin of meadow knapweed are phyllaries. Yellow starthistle bracts have one long apical spine, with one or two much smaller appendages on either side of the long spine (see Figure 3.1). Meadow knapweed bracts have a papery or comb-like margin with 12-24 phyllaries (Marsden-Jones and Turrill 1954). The number and length of the bract appendages of the two species are two characters that can be used to distinguish the two species. The hybrids have appendages that are intermediate between the parent species. The mean number of bracts per head and mean number of lobes per basal leaf were also quantified and analyzed.

The first morphological character measured was the mean number of involucral bracts per head. For each plant measured, four fully-developed heads were randomly chosen and the number of involucral bracts was counted on each of these heads. These values were then averaged to calculate a mean for each plant.

The second character measured was the mean number of appendages per bract. Three fully developed heads were randomly chosen for each plant. On each of these heads, three bracts were randomly chosen. The number of appendages was counted on each of these nine bracts. The values were averaged to calculate a mean for each plant.

The third morphological character measured was the mean length of apical bract appendages. For yellow starthistle, the apical appendage is generally much longer than other appendages (see Figure 3.1). Meadow knapweed bract appendages are much more uniform in length. For each plant, three fully developed heads were randomly chosen. On each of these heads, three bracts were chosen and the apical appendages were measured. The appendages were measured from the point where the apical appendage separated from the surrounding appendages. The appendage lengths measured on each plant were averaged to calculate a mean apical appendage length for each plant.

The fourth morphological character measured was the mean number of lobes per basal leaf. For each plant, five basal leaves were randomly chosen and the number of lobes was counted on each. The terminal lobe was not included in this count. For each plant, the number of lobes per basal leaf for the five leaves was averaged to calculate the mean.

Each of the five variables described above was analyzed using a generalized linear model (PROC GLM) in SAS 9.3. A generalized linear model was used because there were an unequal number of samples in each group. Using two-way comparisons of means, the putative self-pollinated YX progeny, the putative backcross YX progeny, the MX progeny, and the HX_m progeny groups were each compared to the maternal parent group and the potential pollen parent group used in that backcross attempt. The means for each variable of the HX_y plant was visually compared to hybrid and yellow starthistle values for the five variables.

For each variable, the H_0 was that there was no significant difference between any of the means of the groups. Two way comparisons were conducted between each of the groups. These comparisons were done using the differences in least square means to

determine if there was a significant difference between means of each of the groups. For each two-way comparison, the H_0 was that there is no significant difference between the means of the two groups. For each comparison, the p-value was the probability that randomization would lead to a greater difference in group means than the observed difference. P-values below 0.05 were considered significant.

3.7 Results

Seed from Parent Species: Both yellow starthistle and meadow knapweed flowers produced seeds that germinated after being pollinated by the hybrid pollen. Thirty-seven of the 42 plants which germinated from seeds from meadow knapweed flowers pollinated by hybrid pollen (MX) survived and became established (Table 3.3). Twenty-three of these plants bolted and produced flowers. The 23 mature plants were analyzed using the criteria Roché and Susanna (2010) used to describe the yellow starthistle × meadow knapweed hybrid, *C.* × *kleinii*. The 23 mature plants had flower color, bract shape, leaf color, and leaf shape within the range of meadow knapweed (Marsden-Jones and Turrill 1954). They did not appear to have flower color or bract shape intermediate between meadow knapweed and hybrid morphology. Based on these descriptive morphological criteria, the 23 mature MX plants appeared to be self-pollinated meadow knapweed plants.

Seventy-five of the 106 plants which germinated from seeds of yellow starthistle flowers pollinated by hybrid pollen (YX) survived and became established. Eight of these YX progeny were grouped together as putative backcrosses based on a flat rosette leaf and dark green leaf color. Three of these plants bolted and produced capitula. The bract shape and flower color of these three plants appear to be intermediate to yellow starthistle and *C.* × *kleinii*. However, for bracts per head, appendages per bract, and length of apical spine, the three plants do not form a logical group. One of the plants, YX_bc1, has values which were similar to the yellow starthistle groups (Table 3.5). The other two putative backcrosses had values which were closer to the hybrids. YX_bc1

was excluded from this group when this group was analyzed. Therefore n = 2, which could cause potential Type II errors.

The remaining YX progeny appear to be self-pollinated, based on leaf shape, leaf color, and the presence of stem wings. Thirty of the putative self-pollinated YX progeny bolted and flowered. When these 30 were analyzed using Roché and Susanna's (2010) descriptive morphological criteria, they had dark yellow florets and bract shape characteristic of yellow starthistle.

Table 3.5. Putative backcrosses from yellow starthistle seed compared to yellow starthistle groups and hybrid groups.

	n	μ bract	σ bract	μ spine	σ spine	μ length	σ length	μ lobe	σ lobes
Yellow starthistle parent population	30	23.24	2.61	4.85	0.37	17.17	3.34	9.65	2.67
Yellow starthistle self-pollinated in previous experiment	34	21.47	2.55	4.66	0.78	12.21	3.80	11.00	2.74
Self-pollinated YX	30	23.17	3.50	4.54	0.64	10.69	3.44	11.97	2.42
Putative hybrids	5	29.65	7.28	15.64	4.62	2.78	0.69	4.10	1.01
F_1 hybrids	23	31.39	6.26	14.03	2.05	2.30	0.53	5.04	2.56
YX_bc1	1	22.75	-	6.78	-	16.22	-	6.00	-
YX_bc2	1	30.00	-	12.00	-	3.67	-	5.20	-
YX_bc3	1	33.75	-	16.11	-	3.89	-	3.60	-

Seed from Hybrids: Twenty-five seedlings germinated from hybrid seed after being pollinated by meadow knapweed pollen (HX_m). Nineteen seedlings survived and established. The morphological characters and 2C genome size means of these putative backcrossed plants were compared with the means of the other groups.

Two seedlings germinated from hybrid seed that had been pollinated by yellow starthistle (HX_y). One of these died as a seedling, before its genome size or mature morphological characters could be measured. The other established and produced capitula. The genome size (2C-value = 2.63 pg) and morphological characters were

measured. Because this is a group with n = 1, statistical tests cannot accurately test whether there are differences when compared to other groups.

Analysis: For each morphological variable, the groups tested included the yellow starthistle (YS) parent generation (n = 30), the meadow knapweed (MK) parent generation (n = 45), the meadow knapweed self-pollinated progeny (n = 3), the yellow starthistle self-pollinated progeny (n = 34), the F_1 hybrids generated from controlled crossing (n = 23), the putative hybrids collected in the field (n = 5), plants from yellow starthistle seeds (YX) that appear to be self-pollinated progeny (n = 30), the plants from a yellow starthistle seed (YX_bc) that appear to be backcrosses (n = 2), the plants from meadow knapweed seeds that appear to be self-pollinated progeny (MX) (n = 23), and the plants from hybrid seeds pollinated by meadow knapweed pollen (HX_m) (n = 19).

For the genome size analysis, the groups tested included the yellow starthistle (YS) parent generation (n = 30), the meadow knapweed (MK) parent generation (n = 30), the meadow knapweed self-pollinated progeny (n = 6), the yellow starthistle self-pollinated progeny (n = 49), the F_1 hybrids generated from controlled crossing (n = 30), the putative hybrids collected in the field (n = 4), plants from yellow starthistle seeds (YX) that appear to be self-pollinated progeny (n = 67), the plants from a yellow starthistle seed (YX_bc) that appear to be backcrosses (n = 8), the plants from meadow knapweed seeds that appear to be self-pollinated progeny (MX) (n = 37), and the plants from hybrid seeds pollinated by meadow knapweed pollen (HX_m) (n = 19).

There are groups in these analyses that have small sample sizes, which limited the ability to detect significance differences. There was only a single plant from hybrid seed pollinated by yellow starthistle (HX_y). This made a group with a sample size of one. For genome size analysis, the putative hybrid with a 2C genome size of 4.67 pg was analyzed as a separate group from the other four putative hybrids with 2C genome sizes ranging from 2.55 – 2.71 pg (μ = 2.64 pg, σ = 0.07 pg). For the morphological trait analysis, the putative YX backcrosses formed a group of two. Small sample sizes cause comparisons to have low statistical power. Comparisons with small sample sizes may

yield Type II errors, where no significant difference is detected in the analysis when one may be detected with larger samples sizes.

Table 3.6. Results of hypothesis tests (see Appendices for complete results).

Character	degrees of freedom	F-value	p-value
Genome Size	278	1888.01	$p < 0.0001$
Bracts per Head	213	67.96	$p < 0.0001$
Appendages per Bract	213	187.58	$p < 0.0001$
Length of Appendage	213	163.54	$p < 0.0001$
Lobes per Rosette Leaf	211	72.67	$p < 0.0001$

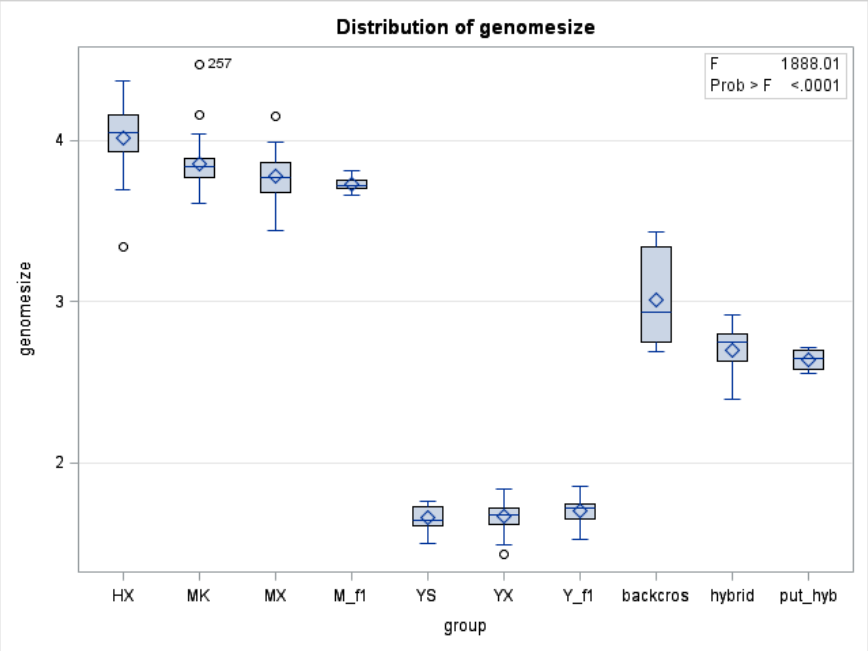
Genome Size: The putative self-pollinated YX ($\mu = 1.67$ pg, $\sigma = 0.08$ pg) has a mean genome size which is very similar to both the yellow starthistle parent population ($\mu = 1.66$ pg, $\sigma = 0.07$ pg) and the self-pollinated yellow starthistle progeny produced when testing whether yellow starthistle and meadow knapweed would produce hybrids (Chapter 2) ($\mu = 1.70$ pg, $\sigma = 0.07$ pg) (Figure 3.1). Neither showed a significant difference ($p = 0.7736$ and $p = 0.1597$, respectively). Comparing genome size supports the hypothesis that these are self-pollinated yellow starthistle plants, rather than backcrosses.

The putative self-pollinated YX progeny was also compared to the hybrids. Compared to the hybrids collected in the field ($\mu = 2.64$ pg, $\sigma = 0.07$ pg), the difference was significant ($p < 0.0001$). When compared to the hybrids produced through controlled crossing ($\mu = 2.70$ pg, $\sigma = 0.14$ pg), the difference was also significant ($p < 0.0001$). These comparisons support the hypothesis that these plants were not backcrosses, but are self-pollinated progeny.

The plants germinated from yellow starthistle seeds (YX) that appeared to be backcrosses had 2C genome sizes in the range of 2.54 – 3.34 pg ($\mu = 2.95$ pg, $\sigma = 0.31$). This group had a much larger genome size than yellow starthistle parent population and the YX plants that appeared to be self-pollinated. The other plants germinated from yellow starthistle seeds pollinated by hybrid pollen had 2C genome sizes in the range of 1.43 – 1.83 pg ($n = 67$, $\mu = 1.67$ pg, $\sigma = 0.08$ pg). The parent population of yellow starthistle (YS) had a 2C genome size range of 1.50 – 1.76 pg ($n = 30$, $\mu = 1.66$ pg, $\sigma =$

0.07 pg). The self-pollinated progeny produced when testing whether yellow starthistle and meadow knapweed would produce hybrids (Chapter 2) had a 2C genome size range of 1.52 – 1.85 pg (n = 49, μ = 1.70 pg, σ = 0.07 pg). The putative YX backcrosses were different from all the yellow starthistle groups ($p < 0.0001$). The putative YX backcrosses had a 2C genome size range closer to that of the hybrids (2.39 – 2.83 pg, n = 30, μ = 2.70 pg, σ =0.14) than any of the yellow starthistle groups. Though the ranges overlap, the means of these groups are different. It appeared likely that these plants were backcrosses, rather than self-pollinated yellow starthistle. Based on the genome size, it appears likely the putative YX backcrosses were the same ploidy level as the hybrids (Figure 3.1).

Figure 3.1. Genome size of the 10 groups analyzed.



The MX progeny have 2C-values (μ = 3.78 pg, σ = 0.14 pg) very similar to those of the meadow knapweed parent population 2C-values (μ = 3.85 pg, σ = 0.16 pg) and the self-pollinated meadow knapweed progeny 2C-values (μ = 3.73 pg, σ = 0.05 pg). When the means were compared, the MX progeny were different from the parent population ($p = 0.0109$) and not different from the meadow knapweed progeny generating during hybridization attempts ($p = 0.3776$). These comparisons provide evidence that supports

the hypothesis, based on the lack of intermediate phenotypical traits, that these are self-pollinated meadow knapweed plants.

The 2C genome size of the hybrid seeds pollinated by meadow knapweed (HX_m) ($\mu = 4.02$ pg, $\sigma = 0.23$ pg) appeared to be much closer to the meadow knapweed groups than the hybrid groups. This suggests that these plants were pollinated by meadow knapweed, rather than self-pollinated hybrids. The HX_m progeny group mean was different from all the meadow knapweed groups and the hybrid groups ($p < 0.0001$). This difference from the maternal parent group and similar range compared to the putative pollen parent was suggestive, but not definitive evidence, that these plants were backcrosses.

The 2C genome size of the plant germinated from a seed from a hybrid flower pollinated by yellow starthistle was 2.63 pg. This value was very close to the hybrids groups, which indicates it may be the result of self-pollination, but this is not clear based solely on genome size.

Based only on genome size analysis, it was not clear whether the plants which germinated from hybrid seeds (HX_y and HX_m) were self-pollinated *C.* × *kleinii* plants or backcrosses with the parent species. Because the 2C genome sizes of HX_y (2.63 pg) and HX_m ($\mu = 4.02$ pg, $\sigma = 0.23$ pg) are different, it appears that they have different ploidy levels. Because the two groups occur at sizes similar to *C.* × *kleinii* and meadow

Table 3.7. Mean 2C genome size of the parent species, hybrids, self-pollinated progeny, and backcross progeny.

Group	n	Mean 2C value (pg)
Putative hybrids	4	2.64 ($\sigma = 0.07$)
F_1 hybrids	30	2.70 ($\sigma = 0.14$)
Yellow starthistle parent	30	1.66 ($\sigma = 0.07$)
Yellow starthistle self-pollinated (Ch 2)	49	1.70 ($\sigma = 0.07$)
Putative YX self-pollinated	67	1.67 ($\sigma = 0.08$)
Putative YX backcrosses	8	2.95 ($\sigma = 0.31$)
Meadow knapweed parent	30	3.85 ($\sigma = 0.16$)
Meadow knapweed self-pollinated (Ch 2)	6	3.73 ($\sigma = 0.05$)
Meadow knapweed self-pollinated (MX)	37	3.78 ($\sigma = 0.14$)
HX_m (Hybrid seed pollinated by meadow knapweed)	19	4.02 ($\sigma = 0.23$)
HX_y (Hybrid seed pollinated by yellow starthistle)	1	2.63

knapweed, it is possible that one of these HX progeny groups was the result of cross-pollination, while the other group was the result of self-pollination. The difference in genome size makes it unlikely that both were the result of self-pollination of the hybrids. Whether these progeny were backcrosses or self-pollinated, the most important finding of this portion of the study was that the hybrids have a low level of fertility. The hybrids were able to produce seed which grew into viable offspring.

Bracts per Head: The YX progeny that appeared to be self-pollinated had a mean number of bracts per head much closer to yellow starthistle than to those of hybrids. These putative self-pollinated YX progeny were compared to the other groups of yellow starthistle and to the hybrid groups to investigate whether they were likely to have hybrids as pollen parents. The mean number of bracts per head of the putative self-pollinated YX ($\mu = 23.17$, $\sigma = 3.50$) was not different from the parent population of yellow starthistle ($\mu = 23.24$, $\sigma = 2.61$, $p = 0.9638$) or the self-pollinated yellow starthistle progeny produced when testing whether yellow starthistle and meadow knapweed would produce hybrids (Chapter 2) ($\mu = 21.47$, $\sigma = 2.55$, $p = 0.2912$). The lack of difference in the mean number of bracts per head supports the hypothesis that these were self-pollinated yellow starthistle plants, rather than backcrosses.

Table 3.8. Mean number of bracts per head for the parent species, hybrids, self-pollinated progeny, and backcross progeny.

Group	n	Mean Bracts per Head
Putative hybrids	5	29.65 ($\sigma = 7.28$)
F_1 hybrids	23	31.39 ($\sigma = 6.26$)
Yellow starthistle parent	30	23.24 ($\sigma = 2.61$)
Yellow starthistle self-pollinated (Ch 2)	34	21.47 ($\sigma = 2.55$)
Putative YX self-pollinated	30	23.17 ($\sigma = 3.50$)
Putative YX backcrosses	2	31.88 ($\sigma = 2.65$)
Meadow knapweed parent	45	40.43 ($\sigma = 8.75$)
Meadow knapweed self-pollinated (Ch 2)	3	37.50 ($\sigma = 1.32$)
Meadow knapweed self-pollinated (MX)	23	49.48 ($\sigma = 7.51$)
HX_m (Hybrid seed pollinated by meadow knapweed)	19	43.99 ($\sigma = 6.49$)
HX_y (Hybrid seed pollinated by yellow starthistle)	1	38.50

The mean number of bracts per head of the putative self-pollinated YX progeny was compared to the hybrids to determine the likelihood that hybrids were pollen parents of these plants. Both the hybrids collected in the field ($\mu = 29.65$, $\sigma = 7.28$, $p = 0.0241$)

and the hybrids generated through controlled crossing ($\mu = 31.39$, $\sigma = 6.26$, $p < 0.0001$) showed differences in mean number of bracts per head. The difference between the putative self-pollinated YX progeny and the hybrids supports the hypothesis that these are self-pollinated yellow starthistle.

The mean number of bracts per head of the two YX plants that appear to be backcrosses ($\mu = 31.88$, $\sigma = 2.65$) was compared to the yellow starthistle groups and the hybrid groups. The p-value when compared with the yellow starthistle parent population was 0.0465 and the p-value when compared with the self-pollinated YX plants was 0.0448. This is suggestive that these groups differ, though both p-values are very close to 0.05. The putative YX backcrosses was different from the self-pollinated yellow starthistle progeny produced when testing whether yellow starthistle and meadow knapweed would produce hybrids ($p = 0.0177$).

When the putative YX backcrosses were compared to the hybrid groups, they were not significantly different from either the hybrids collected in the field ($p = 0.6527$) or the hybrids generated through controlled crossing ($p = 0.9811$). This supports the hypothesis that these plants are backcrosses.

The meadow knapweed progeny produced after attempted backcrossing with hybrid pollen (MX) had a mean number of bracts per head ($\mu = 49.48$, $\sigma = 7.51$) that was higher than either of the meadow knapweed groups. The MX group was different from both the meadow knapweed parent population mean ($\mu = 40.43$, $\sigma = 8.75$, $p < 0.0001$) and the self-pollinated meadow knapweed progeny mean ($\mu = 37.50$, $\sigma = 1.32$, $p = 0.0011$). This group has more bracts per head than any other group and the mean was different from the mean of every other group. If the plants were backcrosses, this would be a transgressive, or more extreme, trait. Analyzing this character by itself does not provide clear evidence that these plants were self-pollinated meadow knapweed plants or backcrosses.

The mean number of bracts per head for the plants germinated from seed from hybrid flowers pollinated by meadow knapweed (HX_m) ($\mu = 43.99$, $\sigma = 6.49$) was

greater than the two hybrid groups and was closer to the meadow knapweed groups. Though HX_m plants germinated from hybrid seeds, they were different from both groups of hybrids ($p < 0.0001$). The HX_m plants have a mean number of bracts per head ($\mu = 43.99$) that was closer to the meadow knapweed groups (parent population $\mu = 40.43$ and self-pollinated $\mu = 37.50$) than to the maternal parent of HX_m, the hybrids (putative $\mu = 29.65$ and F_1 $\mu = 31.39$). This provides suggestive evidence that the HX_m plants were pollinated by meadow knapweed, rather than being self-pollinated progeny of hybrids.

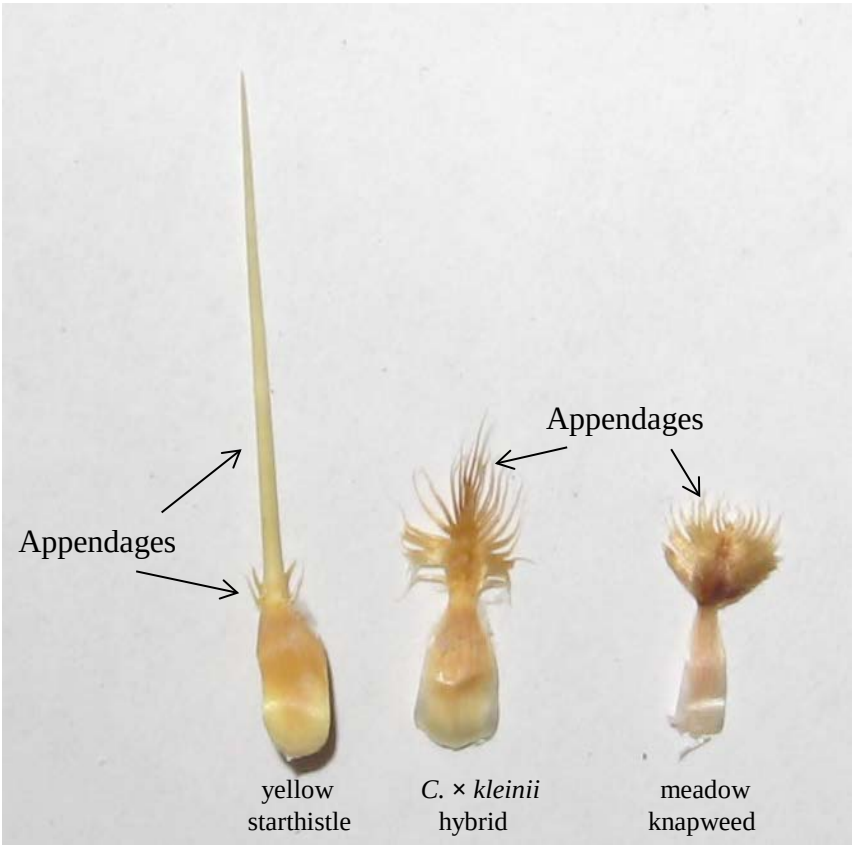
The mean number of bracts per head for the plant germinated from seeds from hybrids pollinated by yellow starthistle (HX_y) was 38.50, much greater than yellow starthistle (parent population $\mu = 23.24$ and self-pollinated $\mu = 21.47$). The mean number of bracts for the HX_y progeny (38.50) was closer to meadow knapweed (parent population $\mu = 40.43$ and self-pollinated $\mu = 37.50$) than it was to hybrids (putative $\mu = 29.65$ and F_1 $\mu = 31.39$). This character provides evidence that this plant was likely the result of self-pollination. The most important result for management is that hybrids can produce viable seed capable of either being pollinated by meadow knapweed or self-pollinating.

Appendages per Bract: The appendages on a yellow starthistle bract consist of one long, sharp apical spine with one or two short spines on either side of the long spine (Figure 3.2). The mean number of appendages per bract of the putative self-pollinated YX ($\mu = 4.54$, $\sigma = 0.64$) was not different from the parent population of yellow starthistle ($\mu = 4.85$, $\sigma = 0.37$, $p = 0.6402$) or the self-pollinated yellow starthistle progeny produced when testing whether yellow starthistle and meadow knapweed would produce hybrids (Chapter 2) ($\mu = 4.66$, $\sigma = 0.78$, $p = 0.7929$). Comparing the mean number of bracts per head supports the hypothesis that these were self-pollinated yellow starthistle plants, rather than backcrosses.

The putative self-pollinated YX progeny were different from both the hybrids collected in the field ($\mu = 15.64$, $\sigma = 4.62$, $p < 0.0001$ and the hybrids generated through

controlled crossing ($\mu = 14.03$, $\sigma = 2.05$, $p < 0.0001$). This also supports the hypothesis that these plants were self-pollinated yellow starthistle.

Figure 3.2. Bract appendages on yellow starthistle, hybrid, and meadow knapweed.



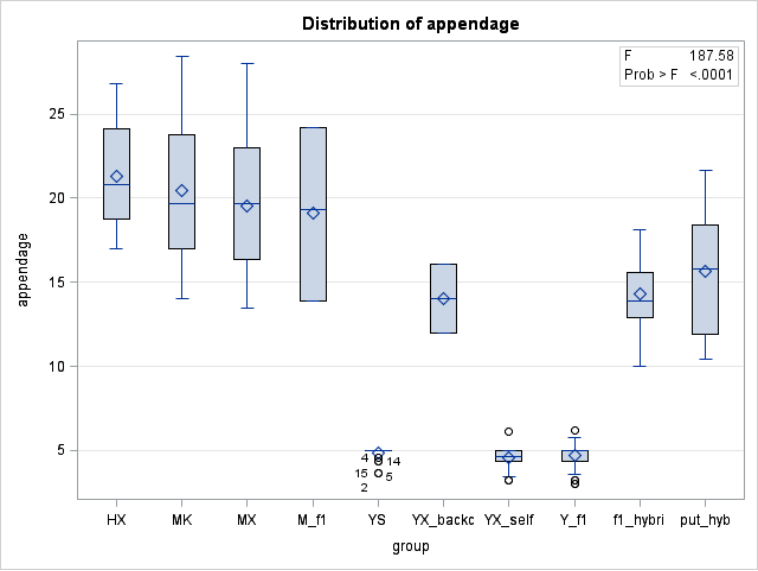
The appendages on the two mature YX plants which appeared to be backcrosses were more like hybrids than like yellow starthistle. They had 12.00 and 16.11 mean appendages per bract. Yellow starthistle has either three or five, and rarely seven appendages per bract. When the mean number of appendages per bract of the putative YX backcrosses ($\mu = 14.06$, $\sigma = 2.91$) was compared to the yellow starthistle parent population ($\mu = 4.85$), the self-pollinated yellow starthistle progeny from hybridization crosses ($\mu = 4.66$), and the self-pollinated yellow starthistle progeny from backcross tests ($\mu = 4.54$), there was a difference ($p < 0.0001$) for each comparison. The mean number of appendages per bract for the putative YX backcrosses was not different from the hybrids generated through controlled crossing ($\mu = 14.03$, $\sigma = 2.05$, $p = 0.8866$) or the putative hybrids from the field ($\mu = 15.64$, $\sigma = 4.62$, $p = 0.4666$). The difference with yellow

starthistle and lack of difference with hybrids supports the hypothesis that these plants were backcrosses between yellow starthistle and hybrids.

Table 3.9. Mean number of appendages per bract for the parent species, hybrids, self-pollinated progeny, and backcross progeny.

Group	n	Mean Appendages per Bract
Putative hybrids	5	15.64 (σ = 4.62)
F_1 hybrids	23	14.03 (σ = 2.05)
Yellow starthistle parent	30	4.85 (σ = 0.37)
Yellow starthistle self-pollinated (Ch 2)	34	4.66 (σ = 0.78)
Putative YX self-pollinated	30	4.54 (σ = 0.64)
Putative YX backcrosses	2	14.06 (σ = 2.91)
Meadow knapweed parent	45	20.45 (σ = 3.89)
Meadow knapweed self-pollinated (Ch 2)	3	19.15 (σ = 5.17)
Meadow knapweed self-pollinated (MX)	23	19.57 (σ = 3.71)
HX_m (Hybrid seed pollinated by meadow knapweed)	19	21.30 (σ = 2.83)
HX_y (Hybrid seed pollinated by yellow starthistle)	1	16.78

Figure 3.3. Number of appendages per bract for the 10 groups tested.



The mean number of appendages per bract for the MX progeny (μ = 19.57, σ = 3.71) was not different from the meadow knapweed parent population (μ = 20.45, σ = 3.89, p = 0.1861) or the self-pollinated meadow knapweed (μ = 19.15, σ = 5.17, p = 0.7939). The MX group showed no difference with the meadow knapweed groups or with the hybrids collected in the field (p = 0.0026). The MX progeny (μ = 19.57) does show a difference when compared to the hybrids generated through controlled crossing (μ

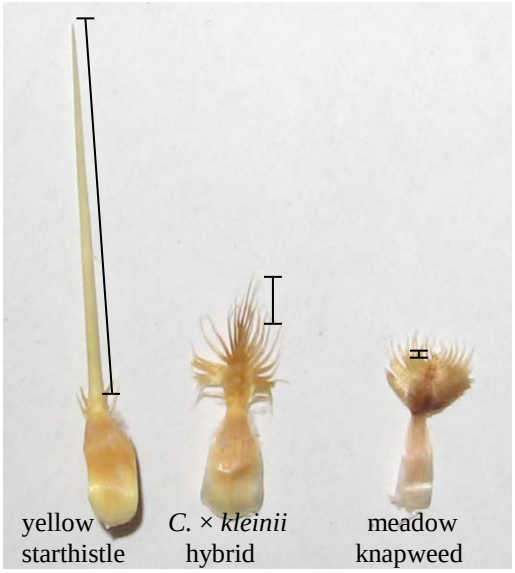
= 14.03, $\sigma = 2.05$, $p < 0.0001$). The hybrids from the field have a sample size of n = 5, so the lack of difference may be influenced by the small sample size. Based on the means for these groups, it appears the MX progeny were self-pollinated meadow knapweed, but because the two-way comparison with one of the hybrid groups was not significant, this character does not provide as strong evidence as the other characters.

The mean number of appendages per bract of plants which germinated from seeds from hybrid flowers pollinated by meadow knapweed (HX_m) ($\mu = 21.30$, $\sigma = 2.83$) was compared to the hybrid and meadow knapweed groups. When compared to the hybrids collected in the field ($\mu = 15.64$, $\sigma = 4.62$, $p < 0.0001$) and the hybrids generated through controlled crossing ($\mu = 14.03$, $\sigma = 2.05$, $p < 0.0001$), there was a difference. There was no difference in number of appendages per bract with the meadow knapweed parent population ($\mu = 20.45$, $\sigma = 3.89$, $p = 0.2317$) or the meadow knapweed progeny from hybridization attempts ($\mu = 19.15$, $\sigma = 5.17$, $p = 0.1836$). This character provides support to the hypothesis that these were backcrosses with meadow knapweed as the pollen parent.

The plant germinated from a seed from hybrid flowers pollinated by yellow starthistle (HX_y) had mean number of apical appendages of 16.78, which is much closer to the hybrid groups (putative $\mu = 15.64$ and F_1 $\mu = 14.03$) than the yellow starthistle groups (parent population $\mu = 4.85$ and self-pollinated $\mu = 4.66$), supporting the hypothesis that this plant was self-pollinated.

Length of Apical Appendages: On yellow starthistle bracts, the apical appendage is a spine which is much longer than the other appendages (Figure 3.4). For meadow knapweed bracts, the apical appendage is of equal or shorter length than the other appendages. The hybrids appear intermediate to the parent species. The apical appendage is longer than the other appendages, but is not a sharp spine. The bracts are somewhat recurved away from the capitula. In meadow knapweed, they are appressed and in yellow starthistle, the spines are very recurved away from the capitula.

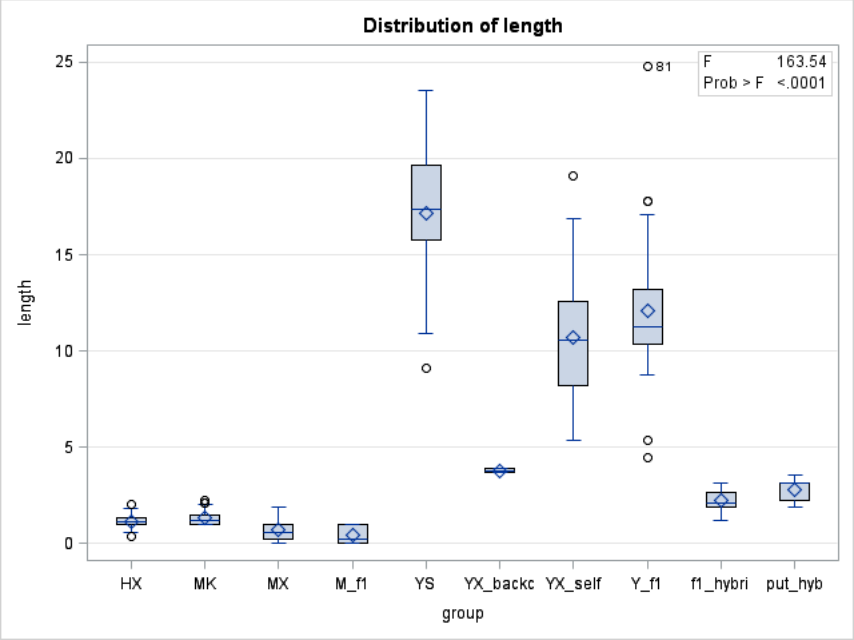
Figure 3.4. Length of apical appendages on bracts of yellow starthistle, *C.* × *kleinii* hybrid, and meadow knapweed.



In appearance, the putative self-pollinated YX progeny was similar to yellow starthistle. The self-pollinated YX progeny had mean apical appendage lengths in the range of the yellow starthistle parent population and was grouped closest to yellow starthistle self-pollinated progeny. However, when the means were compared, the self-pollinated YX plants (μ = 10.69 mm, σ = 3.44 mm) were different from the parent population of yellow starthistle (μ = 17.17 mm, σ = 3.34 mm, $p < 0.0001$) and the self-pollinated yellow starthistle progeny (Chapter 2) (μ = 12.21 mm, σ = 3.80 mm, $p < 0.0001$). Based on the distribution and the comparison of means, length of apical appendage does not provide evidence that clearly indicated whether these plants were self-pollinated or backcrosses.

The two putative YX backcrosses had mean apical appendage lengths of 3.67 mm and 3.89 mm (μ = 3.78 mm, σ = 0.16), which was much closer to the hybrid groups. The putative hybrids from the field had a mean length of 2.78 mm (σ = 0.69 mm) and hybrids produced through controlled crossing had a mean length of 2.30 mm (σ = 0.53 mm).

Figure 3.5. Length of apical bract appendage for the 10 groups analyzed.



MX progeny (μ = 0.67 mm, σ = 0.48 mm) were not significantly different from the meadow knapweed parent population (μ = 1.30 mm, σ = 0.35 mm, p = 0.3024) or the self-pollinated meadow knapweed (μ = 0.41 mm, σ = 0.53 mm, p = 0.8552). The MX progeny group was not different (p = 0.0713) from the hybrids collected in the field (μ = 2.78 mm, σ = 0.69 mm). The MX progeny does show a significant difference when compared to the hybrids generated through controlled crossing (μ = 2.30 mm, σ = 0.53 mm, p = 0.0253). Based on this character, it appears more likely that these MX progeny are the result of self-pollination, rather pollination by hybrids.

The plants which germinated from seeds from hybrid flowers after being pollinated by meadow knapweed (HX_m) (μ = 1.14, σ = 0.40) were compared to the hybrid and meadow knapweed groups. When compared to the hybrids collected in the field (μ = 2.78 mm, σ = 0.69 mm, p = 0.1688) and the hybrids generated through controlled crossing (μ = 2.30 mm, σ = 0.53 mm, p = 0.1359), there was a difference in apical appendage length. There was no difference with the meadow knapweed parent population (μ = 1.30 mm, σ = 0.35 mm, p = 0.8138) and the meadow knapweed progeny from hybridization attempts (μ = 0.41 mm, σ = 0.53 mm, p = 0.6153). These plants have

mean apical bract appendage lengths closer to meadow knapweed than hybrids, which may mean meadow knapweed was the pollen parent. The HX_m progeny showed no difference with either hybrids or meadow knapweed for this character. This character provided suggestive, though inconclusive, support to the hypothesis that these plants were backcrosses with meadow knapweed as the pollen parent.

Table 3.10. Mean apical appendage length for the parent species, hybrids, self-pollinated progeny, and backcross progeny.

Group	n	Mean Length (mm)
Putative hybrids	5	2.78 (σ = 0.69)
F_1 hybrids	23	2.30 (σ = 0.53)
Yellow starthistle parent	30	17.17 (σ = 3.34)
Yellow starthistle self-pollinated (Ch 2)	34	12.21 (σ = 3.80)
Putative YX self-pollinated	30	10.69 (σ = 3.44)
Putative YX backcrosses	2	7.93 (σ = 7.19)
Meadow knapweed parent	45	1.30 (σ = 0.35)
Meadow knapweed self-pollinated (Ch 2)	3	0.41 (σ = 0.53)
Meadow knapweed self-pollinated (MX)	23	0.67 (σ = 0.48)
HX_m (Hybrid seed pollinated by meadow knapweed)	19	1.14 (σ = 0.40)
HX_y (Hybrid seed pollinated by yellow starthistle)	1	2.33

The plant germinated from a hybrid seed after being pollinated by yellow starthistle (HX_y) had mean length of apical appendages of 2.33, which was within the range of the hybrids (putative μ = 2.78 mm and F_1 μ = 2.30 mm) and was not close to yellow starthistle (parent population μ = 17.17 mm and self-pollinated μ = 12.21 mm), indicating this plant was likely self-pollinated.

Number of Lobes per Basal Leaf: The mean number of lobes per basal leaf of the putative self-pollinated YX progeny (μ = 11.97, σ = 2.42) was compared to the yellow starthistle groups and the hybrid groups. This group was different from the yellow starthistle parent population (μ = 9.65, σ = 2.67, $p < 0.0001$), but was not different from the self-pollinated yellow starthistle progeny (Chapter 2) (μ = 11.00, σ = 2.74, $p = 0.1163$).

The mean number of lobes per basal leaf of the putative self-pollinated YX progeny was different from the mean number of lobes per basal leaf of the hybrids collected in the field (μ = 4.10, σ = 1.01, $p < 0.0001$). The mean number of lobes per basal leaf of the putative self-pollinated YX progeny was also different from the hybrids

generated through controlled crossing ($\mu = 5.04$, $\sigma = 2.56$, $p < 0.0001$). These comparisons provide evidence that these plants were self-pollinated. Both the self-pollinated yellow starthistle from the hybridization attempts ($\mu = 11.00$) and the putative self-pollinated YX progeny ($\mu = 11.97$) had a slightly greater mean number of lobes per leaf than the yellow starthistle parent population ($\mu = 9.65$). This character does not provide as strong evidence that these plants were self-pollinated as did the other characters, but included with the other characters, it appeared that these YX progeny were self-pollinated.

Figure 3.6. Rosette leaves of yellow starthistle parent, yellow starthistle self-pollinated progeny, putative backcross germinated from yellow starthistle seed (YX_bc2), and *C.* × *kleinii* hybrid.

When the mean number of lobes per basal leaf of the YX plants which appeared to be backcrosses between yellow starthistle and hybrids ($\mu = 4.93$, $\sigma = 1.13$) was compared to yellow starthistle parent population, the self-pollinated yellow starthistle

from hybridization crosses, and the self-pollinated YX progeny, there were differences from all of these groups ($p < 0.0001$ for each comparison).

When the mean number of lobes per basal leaf of the putative YX backcrosses was compared to the mean number of bracts per head of the hybrids collected in the field ($\mu = 4.10$, $\sigma = 1.01$, $p = 0.8569$) and the hybrids generated through controlled crossing ($\mu = 5.04$, $\sigma = 2.56$, $p = 0.6022$), there was also no difference. The difference with yellow starthistle and the lack of difference with hybrid provide evidence that these plants were backcrosses.

The mean number of lobes per basal leaf for the MX progeny ($\mu = 3.77$, $\sigma = 1.98$) was not different from the meadow knapweed parent population ($\mu = 3.53$, $\sigma = 1.27$, $p = 0.6704$) or the self-pollinated meadow knapweed progeny ($\mu = 2.53$, $\sigma = 0.61$, $p = 0.3446$). The MX group showed no difference from the meadow knapweed groups or the hybrids collected in the field ($\mu = 4.10$, $\sigma = 1.01$, $p = 0.7636$). There was a difference when compared to the hybrids generated through controlled crossing ($\mu = 5.04$, $\sigma = 2.56$, $p = 0.0213$). The hybrids from the field have a sample size of $n = 5$, so the lack of difference may be influenced by the small sample size. Because the MX progeny were not different from meadow knapweed or hybrids, this character provides evidence either that they were self-pollinated meadow knapweed or backcrosses.

The mean number of lobes per basal leaf of plants which germinated from hybrid seed after being pollinated by meadow knapweed (HX_m) ($\mu = 1.94$, $\sigma = 1.21$) was compared to the hybrid and meadow knapweed groups. When compared to the hybrids collected in the field ($\mu = 4.10$, $\sigma = 1.01$, $p = 0.0455$) and the hybrids generated through controlled crossing ($\mu = 5.04$, $\sigma = 2.56$, $p < 0.0001$), there was a difference. The p-value of 0.0455, while below 0.05, is in a region which is suggestive of a difference, but not conclusive.

The HX_m progeny were also compared to the meadow knapweed groups. The HX_m progeny were different from meadow knapweed parent population ($\mu = 3.53$, $\sigma = 1.27$, $p = 0.0064$), but were not different from the self-pollinated meadow knapweed progeny ($\mu = 2.53$, $\sigma = 0.61$, $p = 0.6509$). The HX_m had a lower mean number of lobes

than the other groups. While the mean was closer to the meadow knapweed means for lobes, two-way comparisons of this character did not provide clear evidence for these plants being either self-pollinated or backcrosses. The plant germinated from a seed from a hybrid flower after being pollinated by yellow starthistle (HX_y) had mean number of lobes of 5.00. This was within the range of the hybrids, indicating that this plant was self-pollinated.

The mean number of lobes per basal leaf was included in the analysis to investigate whether land managers could use the number of lobes to identify hybrids or backcrosses in the field prior to the plants producing flower heads. There was a large amount of within-group variability for number of lobes per basal leaf. The number of lobes could be used to differentiate hybrids and backcrosses from yellow starthistle, but could not be used to differentiate backcrosses from hybrids or meadow knapweed.

3.8 Discussion

For the characters tested, small sample sizes limited the power of the statistical tests. The small sample size led to the statistical tests having low power, so it was not clear whether the plants which grew from hybrid seeds were backcrosses or were self-pollinated hybrids. Three of the four morphological characters depend on the plant having capitula. The descriptive morphological criteria used by Roché and Susanna's (2010) to characterize the *C.* × *kleinii* hybrid also depend on the plants having developed capitula.

The most significant result of the backcrossing experiment was that hybrids can produce viable seed. If these were backcrosses, this experiment showed that under controlled greenhouse conditions, hybrids can be pollinated at a low level by meadow knapweed (0.66%) and an even lower level by yellow starthistle (0.06%). The plants from the hybrid seed pollinated by meadow knapweed had a genome size range of 3.34 – 4.22 (μ = 3.96 pg, σ = 0.35 pg). The plant from the hybrid seed pollinated by yellow starthistle pollen had a genome size of 2.63 pg. This difference in genome size makes it likely that at least one or the other of these groups was backcrossed. Even if one or the

other of these groups was self-pollinated hybrids, the difference in genome size indicated they were not both self-pollinated hybrids.

Figure 3.7. Flowers of plants (HX_m) germinated from seeds from *C.* × *kleinii* hybrids pollinated by meadow knapweed (*C.* × *moncktonii*).

Figure 3.8. YX_bc1, germinated from a seed from a yellow starthistle flower pollinated by *C.* × *kleinii* hybrid.

Figure 3.9. YX_bc2, germinated from a seed from a yellow starthistle flower pollinated by *C.* × *kleinii* hybrid.

Figure 3.10. YX_bc3, germinated from a seed from a yellow starthistle flower pollinated by *C.* × *kleinii* hybrid.

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4. DISCUSSION

4.1 Hybridization and Backcrossing

Yellow starthistle and meadow knapweed produced hybrids through controlled crossing (Chapter 2). When compared with putative hybrids collected in the field, these hybrids were not different for any of characters tested. Based on this analysis, it appears the putative hybrids are hybrids between yellow starthistle and meadow knapweed.

Based on the morphological similarities between the putative hybrids and meadow knapweed, Roché and Susanna (2010) hypothesized that meadow knapweed was more likely to be the maternal parent of the hybrids. In the crossing experiments, yellow starthistle was the maternal parent of all the hybrids produced (n = 30). However, the lack of hybrids produced from meadow knapweed maternal parents does not prove that meadow knapweed cannot serve as the maternal parent under field conditions. The two crossing methods used in these studies under greenhouse conditions present a different set of circumstances than those found in the field. These experiments do show that yellow starthistle can serve as the maternal parent.

Two management concerns raised by the presence of the putative hybrids in the area are both dependent on the hybrids being fertile. The first concern is that *C.* × *kleinii* hybrids will evolve into a new species. The second is that gene flow could occur between the parent species through backcrossing. For either speciation or backcrossing to occur, *C.* × *kleinii* would need to produce viable pollen, viable seeds, or both. The backcrossing experiments showed that hybrids can produce viable offspring when pollinated with either meadow knapweed or yellow starthistle pollen (Chapter 3). Hybrid pollen can pollinate yellow starthistle to produce viable backcrossed individuals. *Centaurea* × *kleinii* does produce viable pollen and seed at very low rates.

Three factors limit the likelihood of *C.* × *kleinii* developing into a species. The first is the distance between hybrids located in the field. Yellow starthistle, meadow knapweed, and *C.* × *kleinii* depend on pollination for reproduction. They do not spread via rhizomes or stolons. The hybrids that have been located in the field thus far occur too far for a pollinator to carry pollen from one hybrid to another. If two hybrids were to

germinate and flower in a population of meadow knapweed and yellow starthistle plants, much more pollen from the parent species would be delivered to the hybrid flowers by pollinating insects than pollen from the other hybrid. *C.* × *kleinii* hybrids would need to occur at a much greater frequency than has been observed to date to become a species.

The second factor limiting the likelihood that hybrids will develop into a species is that the hybrids identified in the field thus far have appeared within or adjacent to meadow knapweed populations, with individual yellow starthistle plants at the same site. As long as hybrids occur singly and surrounded by members of the parent species, it is unlikely that the *C.* × *kleinii* hybrids will develop into a species.

Hypothetically, a situation could exist or develop where hybrids germinated in a niche where they can survive, but the parent species are less successful. Due to increased genetic variation, decreased genetic load, or transgressive traits, hybrids could colonize new habitats where the parent species are outcompeted. Genetic load is the difference between the optimal genotype and the average genotype found in a population. Transgressive segregation can lead to some traits which in the hybrid are more extreme than in either of the parent species. Linder and Reisenberg (2004) call speciation based on hybrids successfully competing in a new habitat, while the parent species do not, diploid hybrid speciation. The hybrid would then become isolated enough from parent species pollen, receive sufficient amounts of *C.* × *kleinii* pollen, and speciate. However, all the known examples of *C.* × *kleinii* occur with both meadow knapweed and yellow starthistle. It is unknown what type of habitat would be required to facilitate *C.* × *kleinii* speciation.

A third factor, ploidy level, makes it unlikely *C.* × *kleinii* would develop into a viable species. Yellow starthistle is diploid ($2n = 2x = 16$) and meadow knapweed is tetraploid ($2n = 4x = 44$), so most of the hybrids are likely triploid. Triploid hybrids are likely to be sterile or have very low levels of fertility.

A total of 1886 seeds were produced when 42 hybrid-hybrid crosses were attempted, though most of these seeds were not fully developed. None of the seeds from

these crosses germinated (Table 3.1). In the backcrossing experiment (Chapter 3), 20 of the 8688 hybrid seeds pollinated with either yellow starthistle pollen or meadow knapweed pollen germinated and seedlings established (0.32%). Despite this low level of fertility, the three factors (distance, frequency, and ploidy level) make the possibility of speciation unlikely for the level at which hybrids are currently occurring. With careful management and monitoring of known hybrid populations, it should be possible to prevent speciation.

A very low level of hybridization may mean a greater likelihood of gene flow between parent species rather than speciation. At each of the locations where hybrids have been located in the field, the hybrids will receive pollen load primarily from meadow knapweed. In each location, yellow starthistle is also located within a few meters of the hybrid, so some yellow starthistle pollen is likely to reach *C.* × *kleinii* hybrids through insect pollinators. Based on the pollen load on the hybrids in the field and the lack of seed germination in the hybrid-hybrid cross attempts, backcrossing is a greater management concern than speciation.

Backcrossing could lead to gene flow of meadow knapweed alleles into the yellow starthistle gene pool, or vice versa. Expression of many of these alleles would have no effect, a neutral effect, or a deleterious effect on individuals. Some alleles, however, may produce individuals that are more fit for certain environments. This infusion of alleles into the parent species populations could lead to the development of an ecotype which proves to be more competitive and spread into new habitats in Oregon.

4.2 Life cycle

One trait which could be affected by backcrossing is life cycle. Meadow knapweed is perennial and yellow starthistle is an annual. The hybrids appear to be perennial. Three of the putative hybrids were collected from 2004-2007 and have survived under greenhouse conditions for more than six years. The growing conditions in a greenhouse are different from growing conditions in the field, so the life cycle of the

plants in the greenhouse may not align with what occurs in the field, but these plants appear to be perennial in habit and form.

Among the yellow starthistle parent generation used in this crossing experiment (n = 268), none of the plants produced a second rosette, stem, or flowers after the plant went through its life cycle. All died within fourteen months of germination, as did the self-pollinated yellow starthistle plants.

Among the meadow knapweed parent generation used in this crossing experiment (n = 322), eight died after becoming established. The rest (n = 316) maintained a live rosette, and most periodically produced flowers. In the field, meadow knapweed produces flowers on a yearly basis. Twelve meadow knapweed plants collected during the period 2004-2007 with the putative hybrids survived over six years under greenhouse conditions.

Though the hybrids produced through controlled crossing germinated from yellow starthistle seeds, the hybrids exhibited physiology which suggests they are perennial. After they produced flowers, the hybrids have either maintained a live rosette or produced new rosette leaves. Eight hybrids produced new stems after a period of months. This pattern is similar to meadow knapweed and the putative hybrids. None of the hybrids which produced flowers died after flowering. The hybrids produced through controlled crossing appear to be perennial under greenhouse conditions. As with the putative hybrids, it is not certain that results quantified under greenhouse conditions would translate to field conditions.

One putative hybrid was located in 2012 at the same location (± 6 m) (42°29’59”N 124°15’33”W, in Curry County, OR) where a putative hybrid was identified in 2006. This may be the same plant, but the presence at the same location is not sufficient evidence to show that the two reports are of the same plant.

4.3 *Centaurea* Phylogeny

The hybridization between yellow starthistle and meadow knapweed occurs despite the difference in base number of chromosomes and ploidy levels. The genus *Centaurea* has complicated, unresolved phylogenetic relationships. It has been hypothesized that reticulation played a role in the evolution of *Centaurea* species (Suárez-Santiago et al. 2007). The hybridization found between meadow knapweed and yellow starthistle indicates that hybridization can occur between member of this genus with different base numbers of chromosomes and different ploidy levels.

Garcia-Jacas et al. (2006) found that hybridization, even among distantly-related species within different sections within *Centaurea*, contributed to the difficulty in elucidating the phylogenetic relationships within the genus. Within the Jacea group, black knapweed, brown knapweed, and, by extension, meadow knapweed, are placed within a different clade than yellow starthistle. Meadow knapweed has 37 sister species more closely related to it than yellow starthistle. Yellow starthistle is placed in a clade with 58 other species, all more closely related to it than any species outside the clade, such as meadow knapweed.

The finding that meadow knapweed and yellow starthistle can hybridize indicates that this phylogenetic organization is incorrect or that hybridization can occur across wide ranges of phylogenetic distance.

4.4 Identification of *C.* × *kleinii* in the Field

Because both meadow knapweed and yellow starthistle are invasive species in North America, hybridization between these two species has implications for management. The development of hybrids into a separate species appears to be a remote possibility, but backcrossing and gene flow are possible if hybrids are allowed to produce seed or pollen. Both possibilities could have negative consequences and should be prevented. There are locations in southwestern Oregon where the species occur in close proximity, but where hybrids have not yet been identified. Some of these areas were surveyed in 2012, but only one hybrid plant was found in a location where hybrids had

not previously been found (Figure 1.1). The area where two species co-occur should be monitored for hybrids and any hybrids located should be killed.

Differentiating *C.* × *kleinii* from meadow knapweed depends on the presence of bracts or flowers. The number of lobes per rosette leaf was tested to determine if this character could be used to identify hybrids while they are still in the rosette stage, prior to the development of capitula. This character would be ideal for monitoring, because identification could be done throughout the spring and early summer, prior to flowering. However, there was no clear distinction between meadow knapweed and *C.* × *kleinii* for number of lobes per rosette leaf. There is also no clear distinction for the shape of the leaves, coloration, size, or hairs. Both have rosette leaves which are flat and entire to lobed. Both meadow knapweed and hybrids have shallow lobes which are not deeply cleft. Hybrid rosettes have characteristics which distinguish them from yellow starthistle. Yellow starthistle leaves are crisped, or wavy. The lobes are deeply cleft. Yellow starthistle leaves also have more lobes per leaf (typically >8) than hybrids.

Identification of hybrids can be done once bracts have developed on the capitula, typically in July or early August in southwest OR. Hybrids can be clearly differentiated from meadow knapweed or yellow starthistle based on the shape of the bract, the number of bract appendages, and flower color. The period between bract development and emergence of the inflorescence is the best time to identify hybrids, in order to prevent pollination.

4.5 Recommendations

There are currently 14 species in the genus *Centaurea* listed as Noxious Weeds within at least one state. As hybridization is hypothesized to have played a role in the evolution of the genus, it is important to limit the hybridization among those species which have already become invasive in North America.

The *C.* × *kleinii* hybrids are currently distributed very infrequently within meadow knapweed and yellow starthistle populations. It is very important to continue to monitor and control all hybrids that appear in the field before they produce seed or

pollen. There is often a lag period from the time a species is first introduced to the time when population explodes and the species become invasive. The evolution of increased competitive ability (EICA) hypothesis suggests that a lag period may allow natural selection to work on a species or ecotype for a number of generations (Blossey and Nötzold 1995).

Yellow starthistle is widespread across parts of the western U.S. Meadow knapweed is also widespread, but within a more limited range. Both species began a rapid expansion of range decades after first being introduced. These species may continue to expand and come to co-occur in areas other than southwest OR. Locations where the two species grow in close proximity should be carefully monitored for the presence of hybrids. Should the hybrid be allowed to undergo pollination and seed production in the field, backcrossing or speciation could occur. It is much easier and more cost-effective to prevent backcrossing or speciation than to wait until the hybrid is widespread and then try to manage it.

Based on this evidence, it is recommended that a weed control method be used which kills the entire plant. If the plant is hand-pulled, it is important to pull the entire plant. Cutting or mowing the aboveground growth may prevent pollen and seed production, but will not kill the plant. If chemical control is used, it is recommended to use herbicides which will kill the entire plant.

At one location (42°29’59”N 124°15’33”W, in Curry County, OR), a putative hybrid was identified. This location was scouted because a hybrid had been identified at this location in 2006. The GPS unit (Garmin eTrex Legend H) reported a tolerance of ± 6 m. It is not clear if this was the same plant that was identified in 2006, or if it was a different hybrid at the same site. If this is the same plant, it was not controlled when it was initially identified.

The results of these experiments showed hybridization and backcrossing occur at low levels. A logical extension of this research would be to investigate the ecology and management of *C.* × *kleinii*. Further research could investigate the hybrid’s ecological

tolerance to competition, soil moisture, or other factors compared to the parent species. Further research should investigate whether hybrids transgress parent species for traits which allow them to be competitive in habitats where the parent species are not competitive. An additional avenue of research should investigate whether biological control agents which attack one or the other of the parent species attack the hybrids or backcrossed plants. Pollinator preference could be compared among the parent species and hybrids. In the crossing experiments presented here, only one backcrossed plant flowered in time to include it in crossing experiments. Introgression could be investigated further than the progeny of backcrossed plants.

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APPENDICES

Appendix A

Mean Genome Size Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	122.3777600	20.3962933	1681.35	<.0001
Error	143	1.7347173	0.0121309		
Corrected Total	149	124.1124773			

R-Square	Coeff Var	Root MSE	genomesize Mean
0.986023	4.498951	0.110140	2.448133

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: genomesize

i/j	MK_parent	MK_f_1	YS_parent	YS_f_1	F_1_hybrid	Put_hybrid	5x put_hyb
MK_parent		2.605422	77.25549	84.43267	40.63796	20.72436	-7.27932
		0.0101	<.0001	<.0001	<.0001	<.0001	<.0001
MK_f_1	-2.60542		41.99805	42.56015	20.85691	15.28465	-7.92949
	0.0101		<.0001	<.0001	<.0001	<.0001	<.0001
YS_parent	-77.2555	-41.9981		-1.61296	-36.6175	-16.7501	-26.9022
	<.0001	<.0001		0.1090	<.0001	<.0001	<.0001
YS_f_1	-84.4327	-42.5602	1.612961		-39.1709	-16.4266	-26.7019
	<.0001	<.0001	0.1090		<.0001	<.0001	<.0001
F_1_hybrid	-40.638	-20.8569	36.61753	39.17092		1.012054	-17.6014
	<.0001	<.0001	<.0001	<.0001		0.3132	<.0001
Put_hybrid	-20.7244	-15.2847	16.75006	16.42663	-1.01205		-16.4852
	<.0001	<.0001	<.0001	<.0001	0.3132		<.0001
5x put_hyb	7.279324	7.929493	26.90224	26.70192	17.60138	16.48522	
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	

Appendix B

Mean Number of Bracts per Head Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	8901.30449	1780.26090	50.30	<.0001
Error	134	4742.47508	35.39161		
Corrected Total	139	13643.77957			

R-Square	Coeff Var	Root MSE	bracts Mean
0.652408	19.60889	5.949084	30.33871

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: bracts

i/j	MK_parent	MK_f_1	YS_parent	YS_f_1	F_1_hybrid	Put_hybrid
MK_parent		0.825343	12.25642	13.92188	5.541119	3.843132
		0.4106	<.0001	<.0001	<.0001	0.0002
MK_f_1	-0.82534		3.958065	4.435364	1.512041	1.806842
	0.4106		0.0001	<.0001	0.1329	0.0730
YS_parent	-12.2564	-3.95806		1.096324	-5.29881	-2.23001
	<.0001	0.0001		0.2749	<.0001	0.0274
YS_f_1	-13.9219	-4.43536	-1.09632		-6.45666	-2.82234
	<.0001	<.0001	0.2749		<.0001	0.0055
F_1_hybrid	-5.54112	-1.51204	5.298814	6.456661		0.793143
	<.0001	0.1329	<.0001	<.0001		0.4291
Put_hybrid	-3.84313	-1.80684	2.230009	2.82234	-0.79314	
	0.0002	0.0730	0.0274	0.0055	0.4291	

Appendix C

Mean Number of Appendages per Bract Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6897.082982	1379.416596	202.02	<.0001
Error	134	914.945500	6.827951		
Corrected Total	139	7812.028482			

R-Square	Coeff Var	Root MSE	appendages Mean
0.882880	21.63172	2.613035	12.07964

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: appendages

i/j	MK_parent	MK_f_1	YS_parent	YS_f_1	F_1_hybrid	Put_hybrid
MK_parent		0.836625	25.32597	26.51125	9.139015	3.901799
		0.4043	<.0001	<.0001	<.0001	0.0002
MK_f_1	-0.83662		9.034271	9.173949	3.003431	1.835499
	0.4043		<.0001	<.0001	0.0032	0.0686
YS_parent	-25.326	-9.03427		0.218744	-13.0863	-8.55004
	<.0001	<.0001		0.8272	<.0001	<.0001
YS_f_1	-26.5113	-9.17395	-0.21874		-13.6367	-8.73721
	<.0001	<.0001	0.8272		<.0001	<.0001
F_1_hybrid	-9.13902	-3.00343	13.08634	13.63671		-1.01978
	<.0001	0.0032	<.0001	<.0001		0.3097
Put_hybrid	-3.9018	-1.8355	8.550044	8.737209	1.019782	
	0.0002	0.0686	<.0001	<.0001	0.3097	

Appendix D

Mean Length of Bract Appendages Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	6158.422503	1231.684501	211.43	<.0001
Error	134	780.623611	5.825549		
Corrected Total	139	6939.046114			

R-Square	Coeff Var	Root MSE	length Mean
0.887503	32.13260	2.413617	7.511429

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: length

i/j	MK_parent	MK_f_1	YS_parent	YS_f_1	F_1_hybrid	Put_hybrid
MK_parent		0.617317	-27.8995	-19.7201	-1.52169	-1.30331
		0.5381	<.0001	<.0001	0.1304	0.1947
MK_f_1	-0.61732		-11.4678	-8.05123	-1.23505	-1.34532
	0.5381		<.0001	<.0001	0.2190	0.1808
YS_parent	27.8995	11.46776		8.363424	22.31988	12.34167
	<.0001	<.0001		<.0001	<.0001	<.0001
YS_f_1	19.72011	8.051229	-8.36342		15.15282	8.07283
	<.0001	<.0001	<.0001		<.0001	<.0001
F_1_hybrid	1.521691	1.235055	-22.3199	-15.1528		-0.45466
	0.1304	0.2190	<.0001	<.0001		0.6501
Put_hybrid	1.303306	1.345318	-12.3417	-8.07283	0.454656	
	0.1947	0.1808	<.0001	<.0001	0.6501	

Appendix E

Mean Number of Lobes Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1485.787739	297.157548	62.83	<.0001
Error	133	629.018989	4.729466		
Corrected Total	138	2114.806728			

R-Square	Coeff Var	Root MSE	lobes Mean
0.702564	31.37818	2.174734	6.930719

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: lobes

i/j	MK_parent	MK_f_1	YS_parent	YS_f_1	F_1_hybrid	Put_hybrid
MK_parent		0.771495	-11.9385	-15.211	-3.01641	-0.53281
		0.4418	<.0001	<.0001	0.0031	0.5951
MK_f_1	-0.7715		-5.40677	-6.54394	-2.00896	-0.97385
	0.4418		<.0001	<.0001	0.0466	0.3319
YS_parent	11.93851	5.406774		-2.66462	7.363414	5.30543
	<.0001	<.0001		0.0087	<.0001	<.0001
YS_f_1	15.211	6.543944	2.664616		9.987757	6.740895
	<.0001	<.0001	0.0087		<.0001	<.0001
F_1_hybrid	3.016414	2.008962	-7.36341	-9.98776		1.057896
	0.0031	0.0466	<.0001	<.0001		0.2920
Put_hybrid	0.532807	0.973848	-5.30543	-6.7409	-1.0579	
	0.5951	0.3319	<.0001	<.0001	0.2920	

Appendix F

Mean Genome Size Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	269.7491647	29.9721294	1888.01	<.0001
Error	269	4.2703772	0.0158750		
Corrected Total	278	274.0195419			

R-Square	Coeff Var	Root MSE	lobes Mean
0.984416	4.942481	0.125996	2.549247

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: genomesize

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_self	YS_F_1	YX_backcross	F_1_hybrid	Put_hyb
HX		4.395254	6.796578	4.926893	63.86724	71.80161	68.07873	18.15993	35.67873	19.87175
		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
MK_par	-4.39525		2.562557	2.277548	67.53341	79.08732	73.8074	16.05852	35.52395	18.11634
	<.0001		0.0109	0.0235	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
MX	-6.79658	-2.56256		0.883815	68.4111	81.74975	75.66986	14.82639	34.77103	17.12522
	<.0001	0.0109		0.3776	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
MK_F_1	-4.92689	-2.27755	-0.88381		36.71289	38.38033	37.20425	10.28496	18.23221	13.36119

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: genomesize

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_self	YS_F_1	YX_backcross	F_1_hybrid	Put_hyb
	<.0001	0.0235	0.3776		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
YS_par	-63.8672	-67.5334	-68.4111	-36.7129		-0.28795	-1.40998	-25.4829	-32.0095	-14.6422
	<.0001	<.0001	<.0001	<.0001		0.7736	0.1597	<.0001	<.0001	<.0001
YX_self	-71.8016	-79.0873	-81.7498	-38.3803	0.287953		-1.40238	-26.7692	-37.3343	-15.0194
	<.0001	<.0001	<.0001	<.0001	0.7736		0.1620	<.0001	<.0001	<.0001
YS_F_1	-68.0787	-73.8074	-75.6699	-37.2043	1.409981	1.402376		-25.6635	-34.2415	-14.3595
	<.0001	<.0001	<.0001	<.0001	0.1597	0.1620		<.0001	<.0001	<.0001
YX_bc	-18.1599	-16.0585	-14.8264	-10.285	25.48295	26.7692	25.66349		5.793134	4.630924
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001
F_1_hybrid	-35.6787	-35.5239	-34.771	-18.2322	32.00947	37.33431	34.24153	-5.79313		0.884694
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.3771
Put_hyb	-19.8717	-18.1163	-17.1252	-13.3612	14.64218	15.01943	14.35946	-4.63092	-0.88469	
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.3771	

Appendix G

Mean Number of Bracts per Head Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	21302.97908	2366.99768	67.96	<.0001
Error	204	7105.33221	34.83006		
Corrected Total	213	28408.31128			

R-Square	Coeff Var	Root MSE	bract Mean
0.749885	18.09360	5.901700	32.61762

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: bract

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
HX		2.204204	-3.0033	1.769222	11.9889	2.760675	12.02897	13.23854	6.563427	4.833176
		0.0286	0.0030	0.0784	<.0001	0.0063	<.0001	<.0001	<.0001	<.0001
MK_par	-2.2042		-5.98518	0.831969	12.35483	2.005406	12.40467	14.03366	5.585608	3.873989
	0.0286		<.0001	0.4064	<.0001	0.0462	<.0001	<.0001	<.0001	0.0001
MX	3.003299	5.98518		3.307354	16.04259	4.046791	16.08498	17.49383	10.05764	6.810102
	0.0030	<.0001		0.0011	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
MK_F_1	-1.76922	-0.83197	-3.30735		3.989844	1.044085	4.009245	4.470975	1.524181	1.821349

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: bract

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
	0.0784	0.4064	0.0011		<.0001	0.2977	<.0001	<.0001	0.1290	0.0700
YS_par	-11.9889	-12.3548	-16.0426	-3.98984		-2.0031	0.0455	1.105127	-5.34136	-2.24791
	<.0001	<.0001	<.0001	<.0001		0.0465	0.9638	0.2704	<.0001	0.0257
YX_bc	-2.76067	-2.00541	-4.04679	-1.04409	2.003097		2.019184	2.390959	-0.02373	0.450613
	0.0063	0.0462	<.0001	0.2977	0.0465		0.0448	0.0177	0.9811	0.6527
YX_self	-12.029	-12.4047	-16.085	-4.00925	-0.0455	-2.01918		1.058226	-5.38375	-2.27223
	<.0001	<.0001	<.0001	<.0001	0.9638	0.0448		0.2912	<.0001	0.0241
YS_F_1	-13.2385	-14.0337	-17.4938	-4.47098	-1.10513	-2.39096	-1.05823		-6.5085	-2.845
	<.0001	<.0001	<.0001	<.0001	0.2704	0.0177	0.2912		<.0001	0.0049
F_1_hybrid	-6.56343	-5.58561	-10.0576	-1.52418	5.341358	0.023734	5.383747	6.508501		0.799511
	<.0001	<.0001	<.0001	0.1290	<.0001	0.9811	<.0001	<.0001		0.4249
Put_hyb	-4.83318	-3.87399	-6.8101	-1.82135	2.247913	-0.45061	2.272234	2.845	-0.79951	
	<.0001	0.0001	<.0001	0.0700	0.0257	0.6527	0.0241	0.0049	0.4249	

Appendix H

Mean Number of Appendages per Bract Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	11443.90306	1271.54478	187.58	<.0001
Error	204	1382.82358	6.77855		
Corrected Total	213	12826.72664			

R-Square	Coeff Var	Root MSE	appendage Mean
0.892192	20.55830	2.603564	12.66430

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: appendages

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
HX		1.199622	2.155686	1.334207	21.55307	3.745727	21.96529	22.25416	8.642308	4.325744
		0.2317	0.0323	0.1836	<.0001	0.0002	<.0001	<.0001	<.0001	<.0001
MK_par	-1.19962		1.326803	0.839668	25.4181	3.399066	25.93086	26.60769	9.172259	3.915992
	0.2317		0.1861	0.4021	<.0001	0.0008	<.0001	<.0001	<.0001	0.0001
MX	-2.15569	-1.3268		0.261617	20.38981	2.870616	20.82589	21.13481	6.819482	3.051929
	0.0323	0.1861		0.7939	<.0001	0.0045	<.0001	<.0001	<.0001	0.0026
MK_F_1	-1.33421	-0.83967	-0.26162		9.067134	2.14231	9.266727	9.20732	3.014356	1.842176

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: appendages

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
	0.1836	0.4021	0.7939		<.0001	0.0334	<.0001	<.0001	0.0029	0.0669
YS_par	-21.5531	-25.4181	-20.3898	-9.06713		-4.84018	0.468089	0.21954	-13.1339	-8.58114
	<.0001	<.0001	<.0001	<.0001		<.0001	0.6402	0.8264	<.0001	<.0001
YX_bc	-3.74573	-3.39907	-2.87062	-2.14231	4.840183		5.005677	4.933656	-0.14282	-0.72947
	0.0002	0.0008	0.0045	0.0334	<.0001		<.0001	<.0001	0.8866	0.4666
YX_self	-21.9653	-25.9309	-20.8259	-9.26673	-0.46809	-5.00568		-0.26295	-13.57	-8.83135
	<.0001	<.0001	<.0001	<.0001	0.6402	<.0001		0.7929	<.0001	<.0001
YS_F_1	-22.2542	-26.6077	-21.1348	-9.20732	-0.21954	-4.93366	0.262955		-13.6863	-8.76899
	<.0001	<.0001	<.0001	<.0001	0.8264	<.0001	0.7929		<.0001	<.0001
F_1_hybrid	-8.64231	-9.17226	-6.81948	-3.01436	13.13394	0.142823	13.57003	13.68632		-1.02349
	<.0001	<.0001	<.0001	0.0029	<.0001	0.8866	<.0001	<.0001		0.3073
Put_hyb	-4.32574	-3.91599	-3.05193	-1.84218	8.581145	0.729469	8.831349	8.768991	1.023492	
	<.0001	0.0001	0.0026	0.0669	<.0001	0.4666	<.0001	<.0001	0.3073	

Appendix I

Mean Length of Apical Appendages Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	8166.446105	907.382901	163.54	<.0001
Error	204	1131.897674	5.548518		
Corrected Total	213	9298.343779			

R-Square	Coeff Var	Root MSE	length Mean
0.878269	35.57448	2.355529	6.621402

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: length

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
HX		-0.23578	0.646748	0.503275	-23.2016	-1.50584	-13.8218	-16.2553	-1.49724	-1.38084
		0.8138	0.5185	0.6153	<.0001	0.1337	<.0001	<.0001	0.1359	0.1688
MK_par	0.235784		1.033903	0.63254	-28.5875	-1.45979	-16.9197	-20.2064	-1.55922	-1.33545
	0.8138		0.3024	0.5277	<.0001	0.1459	<.0001	<.0001	0.1205	0.1832
MX	-0.64675	-1.0339		0.18272	-25.2685	-1.79044	-15.3456	-17.9884	-2.25401	-1.8129
	0.5185	0.3024		0.8552	<.0001	0.0749	<.0001	<.0001	0.0253	0.0713
MK_F_1	-0.50327	-0.63254	-0.18272		-11.7506	-1.56878	-7.20887	-8.24978	-1.26551	-1.37849

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: length

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
	0.6153	0.5277	0.8552		<.0001	0.1182	<.0001	<.0001	0.2071	0.1696
YS_par	23.20158	28.58751	25.26854	11.75056		7.782075	10.65119	8.569669	22.8703	12.64602
	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001
YX_bc	1.505836	1.459791	1.790438	1.568778	-7.78207		-4.01631	-4.86061	0.888835	0.508429
	0.1337	0.1459	0.0749	0.1182	<.0001		<.0001	<.0001	0.3751	0.6117
YX_self	13.82182	16.91972	15.34563	7.208874	-10.6512	4.016311		-2.40933	12.94739	6.952716
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.0169	<.0001	<.0001
YS_F_1	16.25529	20.20642	17.98841	8.249775	-8.56967	4.860606	2.409325		15.5265	8.271909
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0169		<.0001	<.0001
F_1_hybrid	1.497243	1.559216	2.254009	1.265511	-22.8703	-0.88883	-12.9474	-15.5265		-0.46587
	0.1359	0.1205	0.0253	0.2071	<.0001	0.3751	<.0001	<.0001		0.6418
Put_hyb	1.38084	1.335446	1.812896	1.378494	-12.646	-0.50843	-6.95272	-8.27191	0.465868	
	0.1688	0.1832	0.0713	0.1696	<.0001	0.6117	<.0001	<.0001	0.6418	

Appendix J

Mean Number of Lobes per Rosette Leaf Analyzed Using Generalized Linear Models (PROC GLM in SAS 9.3)

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	2934.656571	326.072952	72.67	<.0001
Error	202	906.440891	4.487331		
Corrected Total	211	3841.097462			

R-Square	Coeff Var	Root MSE	lobes Mean
0.764015	31.13100	2.118332	6.804575

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: lobes

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
HX		-2.75541	-2.78412	-0.45325	-12.4241	-1.56416	-16.04	-15.0453	-4.99207	-2.01287
		0.0064	0.0059	0.6509	<.0001	0.1193	<.0001	<.0001	<.0001	0.0455
MK_par	2.755411		-0.42625	0.792036	-12.2564	-0.56586	-16.7152	-15.616	-3.09673	-0.54699
	0.0064		0.6704	0.4293	<.0001	0.5721	<.0001	<.0001	0.0022	0.5850
MX	2.784116	0.426245		0.947357	-10.0293	-0.40648	-13.8643	-12.7737	-2.32126	-0.30115
	0.0059	0.6704		0.3446	<.0001	0.6848	<.0001	<.0001	0.0213	0.7636
MK_F_1	0.453248	-0.79204	-0.94736		-5.55073	-0.9653	-7.3418	-6.71818	-2.06245	-0.99978

Least Squares Means for Effect group
t for H0: LSMean(i)=LSMean(j) / Pr > |t| Dependent Variable: lobes

i/j	HX	MK_par	MX	MK_F_1	YS_par	YX_backcross	YX_self	YS_F_1	F_1_hybrid	Put_hyb
	0.6509	0.4293	0.3446		<.0001	0.3355	<.0001	<.0001	0.0404	0.3186
YS_par	12.42411	12.25638	10.02926	5.550731		3.395796	-4.19143	-2.73556	7.559467	5.446689
	<.0001	<.0001	<.0001	<.0001		0.0008	<.0001	0.0068	<.0001	<.0001
YX_bc	1.564157	0.565858	0.406481	0.965302	-3.3958		-4.88515	-4.35311	-0.52202	0.180554
	0.1193	0.5721	0.6848	0.3355	0.0008		<.0001	<.0001	0.6022	0.8569
YX_self	16.04001	16.71524	13.86427	7.341805	4.191427	4.885148		1.577146	11.41275	7.687421
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		0.1163	<.0001	<.0001
YS_F_1	15.0453	15.616	12.77369	6.718179	2.735562	4.353111	-1.57715		10.25369	6.920374
	<.0001	<.0001	<.0001	<.0001	0.0068	<.0001	0.1163		<.0001	<.0001
F_1_hybrid	4.99207	3.096727	2.321255	2.062452	-7.55947	0.522021	-11.4127	-10.2537		1.086063
	<.0001	0.0022	0.0213	0.0404	<.0001	0.6022	<.0001	<.0001		0.2787
Put_hyb	2.012873	0.546993	0.301153	0.999777	-5.44669	-0.18055	-7.68742	-6.92037	-1.08606	
	0.0455	0.5850	0.7636	0.3186	<.0001	0.8569	<.0001	<.0001	0.2787	