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Ecological and Evolutionary Correlates of Recent Allopolyploidy

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by

Shana R. Welles

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ABSTRACT OF THE DISSERTATION

Evolutionary and Ecological Correlates of Recent Allopolyploidy

by

Shana R. Welles

Doctor of Philosophy, Graduate Program in Plant Biology
University of California, Riverside, August 2015
Dr. Norman C. Ellstrand, Chairperson

Invasive species are a major component to global change. Recent work suggests that evolutionary processes are often involved in the formation of invasive species. Hybridization and whole genome duplication are evolutionary processes that can contribute to the evolution of invasive species; multiple invasive species are the result of hybridization and whole genome duplication in the same lineage (allopolyploid species). Through this work we evaluate the potential invasiveness of a newly formed allopolyploid species, *Salsola ryanii*, which evolved by hybridization between *S. tragus* (an invasive species native to Russia and China) and *S. australis* (an invasive species native to Australia and/or South Africa). We investigate the potential invasiveness of this newly formed through three studies 1) a study of how the range of this newly formed species has shifted in the decade following initial discovery; 2) a common garden comparison of plant size, mass and phenology between *S. ryanii* and its progenitors; 3) a

study of the number of independent evolutionary origins of *S. ryanii* as determined through an analysis of genetic structure.

Through a systematic collection of *Salsola* sp in California we documented a rapid range expansion from three to fifteen populations within the decade following initial discovery. This range expansion included expansion from two floristic provinces to six different floristic provinces. We compare the range expansion in *S. ryanii* to other systems for which similar data is available and demonstrate that *S. ryanii* has experienced the most rapid range expansion of any newly formed allopolyploid invasive for which data is available. A dramatic range expansion provides support for the hypothesis that *S. ryanii* has the potential to become a problematic weedy and invasive species.

In the common garden comparison of *S. ryanii* and its progenitors we document a significant increase in mass and plant size in the newly formed allopolyploid species. Which of the progenitor species had the larger mass and size was dependent on year. The high fitness of *S. ryanii* regardless of year suggests a strong potential for S. ryanii to replace one or both of its progenitors in all or part of their range. *Salsola ryanii*'s large plant size makes it a more problematic weed and the high seed production associated with a large plant size increases the potential for spread.

Number of independent evolutionary origins of potentially invasive newly formed allopolyploid species is linked to evolutionary potential; species resulting from multiple origins which admix with each other have potentially greater genetic diversity and evolutionary potential than species resulting from a single origin. We use an analysis of genetic structure to determine whether *S. ryanii* results from a single or multiple origins. From the analysis of genetic structure it appears that *S. ryanii* is the result of at least three independent origin events followed by wide dispersal and admixture. A species resulting from multiple origins followed by admixture theoretically has a stronger potential to become an invasive species than a species resulting from a single origin.

Together these studies provide strong empirical support for the hypothesis that allopolypolyploidization can lead to the evolution of increased invasiveness; specifically this work provides strong evidence that *S. ryanii* is likely to become more invasive than its invasive progenitors. Documentation of range extension of *S. ryanii* provides land managers with information about new areas where this species is present. Land managers can utilize this information to control the spread of this newly formed invasive species. It additionally provides a needed first common garden comparison between a newly formed allopolyploid species and its progenitors and adds to our understanding of allopolyploid origins.

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CHAPTER 1

General Introduction

OVERVIEW

Invasive species are a significant component of global change (VITOUSEK *et al.* 1996). Invasive species can arise in two ways, through introduction (intentional or accidental) from a distant source or through *in situ* evolution. Evolution of new invasive species *in situ* can occur through hybridization or polyploidy; polyploidy and in some cases hybridization results in immediate reproductive isolate from the progenitor species (GRANT 1981). In addition to reproductively isolating newly formed species from their progenitors, hybridization and polyploidy have both also been proposed to be important in the evolution of invasive species (Ellstrand and Schierenbeck, 2000: Pandit et al 2011). Allopolyploidy (hybridization fixed by whole genome duplication) is also common amongst invasive species (SCHIERENBECK AND ELLSTRAND 2009).

In addition to surveys of invasive species demonstrating that allopolyploidy is common among invasive species, early studies of plant evolution by allopolyploidy hypothesized that increased fitness or ecological success and increased ability to adapt to novel or disturbed habitats, are associated with allopolyploidization (STEBBINS 1947; STEBBINS JR 1950; EHRENDORFER 1980; LEWIS 1980; LEVIN 1983); traits which are also associated with invasiveness (VAN KLEUNEN *et al.* 2010).

Invasiveness of new species which arise via allopolyploid potentially limited due to genetic variability of the species (SOLTIS *et al.* 2004). Genetic variation can contribute to the adaptability of species (FISHER 1958; SAKAI *et al.* 2001) and thereby contribute to their ability to persist and spread within an introduced range (LEE 2002; PARKER *et al.* 2003). In newly formed allopolyploid species, the presence of multiple independent origin events followed by admixture between independent origins can lead to increased invasion success as potentially beneficial, novel gene combinations are generated by genetic heterogeneity (KELLER AND TAYLOR 2010; VERHOEVEN *et al.* 2011)..

For newly formed potentially invasive species, investigating whether the newly formed species has attributes (increased plant size, increased plant mass, increased in range size since initial formation) associated with invasiveness is important to predicting whether newly formed species are likely to be invasive. Additionally, determining the future evolutionary potential of newly formed potentially invasive species is also relevant to predicting future invasiveness. In addition to determining whether species newly formed species are likely to become invasive, it is also important to establish whether specific evolutionary process (in this case allopolyploidy) are broadly associated with evolution of invasiveness.

STUDY SYSTEM

Salsola ryanii (2n=54) is a newly formed weedy allohexaploid derivative of *S. tragus* (2n=36) and *S. australis* (2n=16), which formed *in situ* in California in the last 20-100 years *(*H*RUSA AND* G*ASKIN* *2008)*. Neither of *S. ryanii's* progenitors are native to California. *Salsola tragus*' native range extends from North Africa and Western Russia, through Asia into Northeast Siberia and Northeast China. *Salsola australis* is a weed in California and Arizona and is likely native to Australia or South Africa (BORGER *et al.* 2008).

OBJECTIVES

This body of work explores the evolution and ecology of a newly formed potentially invasive species in California. Three independent objectives contribute to understand the evolutionary ecology of *S. ryanii*.

Objective 1. Determine how the range of S. ryanii has shifted in the decade following the initial discovery of the species.

Objective 2. Assess how the fitness and phenotypes of S. ryanii compared to its progenitors when grown in a common environment.

3) Estimate the number of independent origin events that were involved in the formation of S. ryanii

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Chapter 2

Caught in act: rapid range expansion in the newly formed allopolyploid weed in the genus *Salsola* (Amaranthaceae)

ABSTRACT

Newly formed species (neospecies) can experience a variety of demographic fates, ranging from rapid invasive expansion to rapid extinction. Here we investigate the fate of the neospecies *Salsola ryanii* ten years after its initial discovery in the Central Valley of California, USA. This species is an allopolyploid derived through hybridization between the invasive species, *S. australis* and *S. tragus*. Through systematic collections and molecular analysis, we show that *S. ryanii* has undergone a dramatic geographic and environmental range expansion in a decade. However, it has not yet expanded beyond the ranges of its parents. Nonetheless, it has every indication of being just as invasive as its highly invasive parents. We are not aware of any plant neospecies whose range spontaneously experienced such a dramatic expansion.

INTRODUCTION

The fate of newly formed species (neospecies) after one or several generations can vary dramatically. At one extreme (probably most common), a new species rapidly goes extinct; at the other extreme, the neospecies establishes, rapidly expands its range and becomes a dominant species (Levin, 2000). Determination of how the range of a neospecies changes through time is important to determining its long-term sustainability and even the likelihood of whether the neospecies is likely to become invasive (Nuebert and Parker, 2004; Sakai et al., 2001).

The fate of new species is typically difficult to document. Most new species evolve via incremental changes, creating a continuum between the progenitor species and the neospecies, making it difficult to characterize the early range dynamics. In contrast, new species that arise via quantum speciation involve a genetically discrete speciation event (Scudder, 1974). To illustrate, a new species that evolves via polyploidy can easily be distinguished by an increase of chromosome number relative to its ancestors, which causes the neospecies to be reproductively isolated from its progenitor species. Similarly, species evolved via hybridization often hold the alternate alleles that differentiate the parental taxa (Grant, 1981). Due to the unique characteristics associated with polyploid speciation, these species provide unique opportunities to study early range dynamics as well as to better understand polyploidy and hybridization as evolutionary forces.

Given that polyploidy causes rapid speciation, it is not surprising that the few studies documenting the spread of newly formed species usually involve cases of polyploid speciation. Perhaps the best-known case involves Ownbey's (1950) *Tragopogon*s: two New World allopolyploid species (*T. mirus* and *T. miscellus*) that evolved via hybridization between introduced diploid Old World species (Ownbey, 1950). The first surveys identified 1 population of *T. mirus* and 2 populations of *T. miscellus*. Thirteen years later, follow-up surveys found that both species had experienced modest range expansions (to 4 and 3 populations, respectively) (Brehm and Ownbey, 1965). Later research showed continued range expansion such that by 1990 *T. mirus* was present in 10 populations and *T. miscellus* in 38 (Novak et al., 1991).

In addition to facilitating speciation, hybridization and polyploidy have both also been proposed to be important in the evolution of invasive species (Ellstrand and Schierenbeck, 2000: Pandit et al 2011). In a recent review Schierenbeck and Ellstrand (2009) documented 24 invasive species resulting from hybridization, nine of which result from allopolyploidy. Given the documented importance of allopolyploidy in the evolution of new species and invasive species, an ideal system for investigating the early range dynamics would involve (1) an annual species that reproduces within its first year so that range changes can be measured over a reasonable time, and (2) a species that is extremely new, so that it can be "caught in the act" of early range changes.

The recently evolved *Salsola ryanii* (Amaranthaceae) fits those two qualifications. It is an annual allohexaploid (2n=54) hybrid of *S. tragus* (2n=18) and *S. australis* (2n=18). (Hrusa and Gaskin, 2008; Ayres et al, 2009). *Salsola ryanii* is currently known from three populations in the Central Valley of California, USA (Figure 1), from two geographic regions of the California Floristic Province, as assigned by *The Jepson Manual* (Baldwin et al., 2012). Two of these populations were documented in a 2002 survey and the third was documented in 2008 (Akers et al., 2002; Hrusa and Gaskin, 2008). To our knowledge there is no documentation of *S. ryanii* prior to 2002. It is unknown whether *S. ryanii* has expanded from the three previously known populations or whether it has the potential to become invasive (Ayres et al., 2009; Hrusa and Gaskin, 2008). Hrusa and Gaskin (2008) predict that *S. ryanii*'s ecological intermediacy will prevent it from expanding its range drastically or becoming invasive.

Neither progenitor species of *S. ryanii* is native to California. *Salsola tragus*' native range extends from North Africa and Western Russia, through Asia into Northeast Siberia and Northeast China. The first known introduction of *S. tragus* into North America occurred in South Dakota in the 1870's, likely through contamination of agricultural seed (Young, 1988). The number of introductions of *S. tragus* into the US is unknown but the introduction source was likely Russia (Young, 1988). *S. tragus* has a 2n chromosome number of 36, compared to a base chromosome number for the genus of 2n=18, suggesting that *S. tragus* is of tetraploid origin (Ayres et al., 2009). *Salsola australis* is a weed that is invasive in California and Arizona, and is likely native to

Australia or South Africa (Borger et al., 2008; plants.usda.gov). *Salsola australis* is morphologically very similar to *S. tragus* and was not recognized as a distinct species until recently (Ryan and Ayres, 2000). Given that *S. australis* was an unrecognized cryptic species assigned to *S. tragus* until recently, it is not known exactly when *S. australis* was introduced into North America.

All three *Salsola* species occur on highly disturbed habitats (S. Welles, Personal observation). The well-known *S. tragus* is considered a problematic weed in 48 US states(plants.usda.gov) and has been described as having the most rapid spread of any introduced species (Rilke, 1999). In this study we broadly collected *Salsolsa sp.* within California to determine how the range of *S. ryanii* has shifted since the prior surveys.

MATERIALS AND METHODS

Sample collection: In the summer of 2012, individuals belonging to the genus *Salsola* were systematically collected throughout regions of California where *Salsola* species are present. Four collections were sampled from each of 26 55mi x 55 mi square quadrats unless four populations that were an adequate distance apart could not be located. All collections were a minimum 10 miles apart from each other. Up to 20 individuals were collected per population. Plants of different sizes were intentionally sampled. Locations of all collections were tracked using a Garmin GPSmap 62s unit.

Following collection, up to 0.4 g vegetative tissue from each sample was cut and frozen in preparation for DNA extraction. In these species, fruits are not mature and viable until dried, so green plant tissue and mature fruits could not be collected at the same time. Locations where *S. ryanii* was collected were resampled when possible during fruit maturation season, to help rule out the presence of sterile homoploid hybrid swarms.

Sample identification: DNA was extracted from all samples using a modified CTAB procedure (Ryan and Ayers, 2000). DNA was quantified using a nanodrop-1000. All samples were diluted to 50 ng/µl. Inter simple sequence repeats (ISSR) markers were used to genotype individuals using primers 810, 835, 840 and 890 as described in Ayers et al. (2009). Bands were scored as either present or absent. Individuals with species-specific bands belonging to both of the parental species were determined to be the allopolyploid species (S. *raynii*) as described by Ayers et al. (2009). This method provides a conservative identification tool; because an individual must have all progenitor bands to be classified as hybrid any misidentified individuals are likely to be hybrids misclassified as one of the progenitor species as opposed to one of the progenitors misclassified as hybrid. With this method it is difficult to distinguish homoploid from polyploid hybrids. Given the difference in chromosome numbers between the two progenitor species, homoploid hybrids would face problems in meiosis and therefore be sterile (Grant, 1981). For this reason we resampled from each of the sites where hybrids were identified, we revisited during fruiting to confirm that the hybrids that were identified were actually the allohexaploid species *S. ryanii* and not homoploid hybrids.

One of the challenges with studies like these is the difficulty in ensuring that initial populations are limited to what has been reported in previous studies. This limitation exists here as in other similar studies, but given the thoroughness of previous studies (Akers et al., 2002; Hrusa and Gaskin, 2008) we feel confident in following the previously established method of using previously reported distributions (Novak et al., 1991; Ingram and Noltie, 1995).

RESULTS

Of the 53 collections that were made, 15 contained *S. ryanii,* 21 contained *S. tragus* and 26 contained *S. australis*. Of the 15 collections that contained *S. ryanii*, four contained only individuals identified as *S. ryanii*. Locations of *S. ryanii* populations include areas of California's Central Valley, where original populations were identified (Hrusa and Gaskin, 2008), as well as Southern California and more coastal regions of Northern California (Figure 1, Table 1). We were able to resample 12 of these populations the following year; in the other three sites, it appeared that weed control methods, such as tilling and herbicides, had been used to eliminate all *Salsola*. Seeds from all collected plants germinated, indicating that they are alloploid *S. ryanii*, as homoploid hybrid plants would not produce viable seed.

Populations of *S. ryanii* that had previously been mapped were present in two geographic regions of the California Floristic Province (Baldwin et al., 2012): the Sacramento Valley region and the San Joaquin Valley region. Collections identified as *S. ryanii* in this study were located in six different geographic regions: the two geographic regions where the previous collections were and four geographic regions where *S. ryanii* had not previously been documented (see Table 1).

DISCUSSION

Salsola ryanii has rapidly and dramatically expanded its geographic range in a single decade. To our knowledge this is the fastest documented range expansion of a newly formed allopolyploid species, perhaps the most dramatic known range expansion of any plant neospecies. Earlier work suggested that *S. ryanii*'s morphology, which is intermediate between its progenitors, would prevent it from being as well adapted as either of its progenitor species and greatly limit potential range expansion, preventing *S. ryanii* from becoming a widespread weed (Hrusa and Gaskin, 2008). The dramatic range expansion of *S. ryanii* within the last decade belies that prediction regarding *S. ryanii*, that its morphology would limit its success as a widespread weed, contrasts with its dramatic range expansion within the last decade. . *Salsola ryanii*'s range has not yet expanded beyond the ranges of its progenitors. However, given the broad range of both progenitor species, it is hard to predict the potential relative invasiveness of this neospecies. Expansion beyond the range of *S. tragu*s seems unlikely since *S. tragus* is present in 48 US states (plants.usda.gov). It is too early to determine whether the neospecies will continue to co-exist with or eventually replace its progenitor species.

This range expansion could be occurring via two possible non-mutually exclusive mechanisms; propagule dispersal or multiple evolutionary origins. The three populations that were previously documented were likely each the result of multiple origins (Hrusa and Gaskin, 2008). In other allopolyploid systems multiple independent origins have also been well documented (Soltis and Soltis, 1991, Meimberg et al., 2009). This genus is also documented to have high levels of dispersal (Mitchell and Wilcox, 1988). The parents vary in their dispersal; *S. tragus* exhibits the "tumbling" dispersal that is characteristic of the genus, while *S. australis* is also highly dispersed via wind but does not "tumble" (Mitchell and Wilcox, 1988, Borger et al., 2008). Dispersal mode in *S. ryanii* has not been studied, but the similarities between the progenitors and the neospecies suggest that *s. ryanii* is also highly dispersed. It is likely that the range expansion documented here is the result of a combination of additional independent origins as well as propagule dispersal, however the data presented here are not sufficient to determine the relative importance of these two different mechanisms.

Along with its geographic expansion, *S. ryanii* has enjoyed broad ecological niche expansion. *Salsola ryanii* has maintained its range within the geographic regions of the California floristic province where it was documented in previous surveys, and has expanded its distribution into previously unoccupied geographic regions of the California floristic province. Currently *S. ryanii* has not established in a habitat that is not already occupied by one or both of its parents, however there were a number of sites where only *S. ryanii* was documented, this suggests that *S. ryanii* is possible exploiting different microhabitats within the broader geographic range of its progentiors. *Salsola ryanii's* dramatic sevenfold increase in detected populations and tripling of geographic floristic regions in ten years is in stark contrast with the few other well-studied neoallopolypoid species. The two neopolyploid North American *Tragopogon* species represent the very best studied examples. The more rapidly expanding of the two, *T. mirus*, expanded from one to three locations in the first thirteen years following initial documentation (Brehm and Ownbey, 1965).

We can also compare the range expansion of *S. ryanii* to the well-studied neoallopolyploid, *Senecio cambrensis*, derived from hybridization between *S. squalidus* and *S. vulgaris* (Ashton and Abbot, 1992). *Senecio cambrensis* has evolved twice, independently in both Wales, UK and in Edinburgh, Scotland (Ashton and Abbot, 1992; Harris and Ingram, 1992). The Welsh origin experienced modest range expansion from one to ten populations between initial discovery in 1950 and follow-up surveys in 1995. In contrast, within 10 years after initial discovery the Edinburgh population went extinct (Ingram and Noltie, 1995; Abbott and Forbes 2002).

Allopolyploidy has long been recognized as an important evolutionary mechanism in most major plant groups (Grant, 1971). Recent phylogenomic analyses have revealed that the vast majority of seed plants and angiosperms, if not all, have one or more allopolyploid ancestors (Jiao et al., 2011; Wood et al., 2009). Despite the frequency of polyploidy in plant evolution, current evidence fails to support any special evolutionary advantage to polyploid lineages and suggests that they have a relatively low diversification rate (Mayrose et al., 2011). The rapid spread of *S. ryanii*, both within the floristic providence where it initially evolved and into new floristic provinces, provides evidence that allopolyploidy can produce species with broadened ecological amplitude rapidly after initial species formation.

In comparison with other well-studied cases of range expansion of neospecies, it is clear that *S. ryanii* represents an extreme example. Given this range expansion in just a decade it seems likely that the range of *S. ryanii* will continue to expand and is likely to become an important an invasive species. These results strongly contradict the predictions in early studies of *S. ryanii* that it would not likely become invasive (Hrusa and Gaskin 2008). Given the dramatic range increase that has been documented in California, it is also possible that *S. ryanii* could become an invasive species in other countries through either dispersal of seed of *S. ryanii* from California or through recurrent formation in other locations where progenitor species co-occur.

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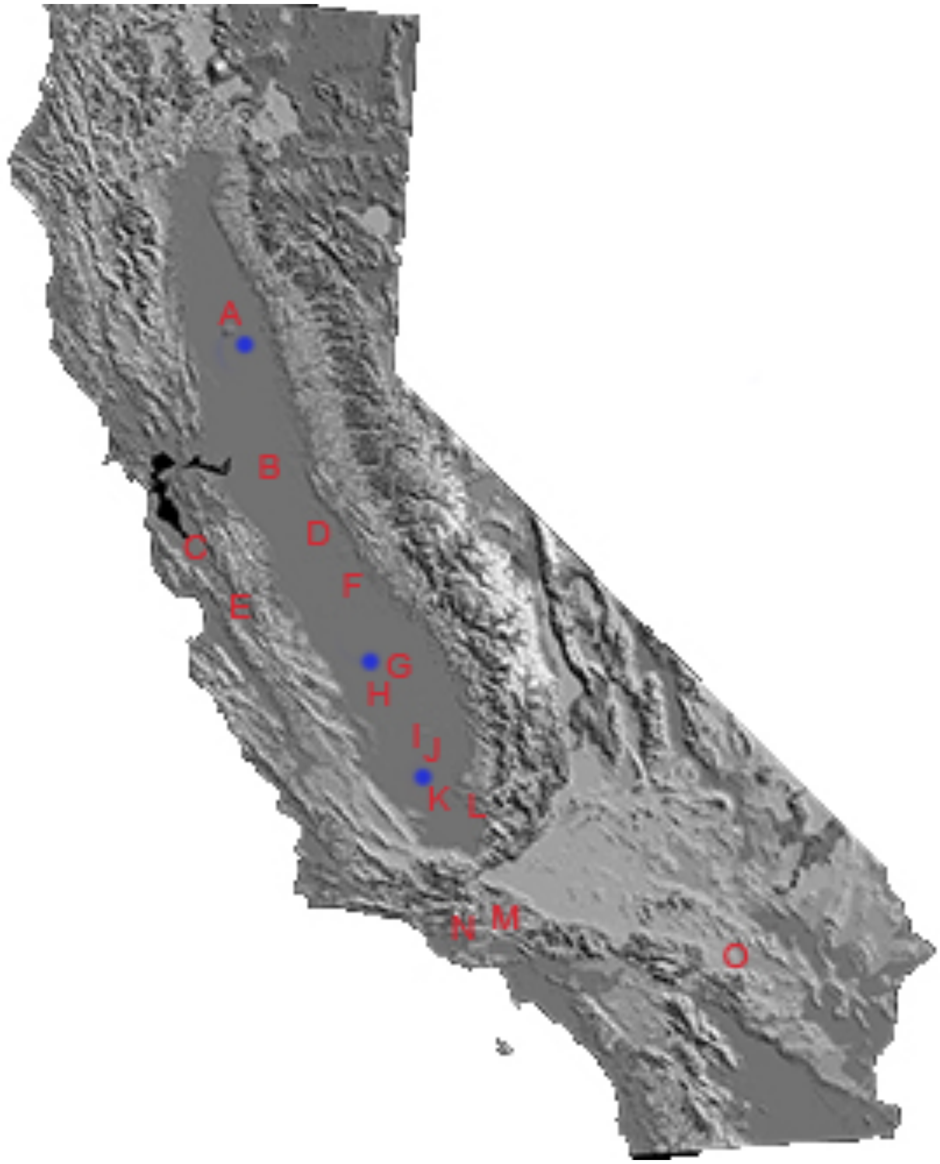
Table 2.1 Location and species make-up of collections that include *S. ryanii*. Map ID letters correspond to letters on Figure 1 representing collection locations. Percentages represent the species identification for all individuals identified from that particular collection. Only collections which contain *S. ryanii* are resented here.

Map ID	% *S. ryanii*	% *S.tragus*	% *S. australis*	Individuals collected	Seeds recollected	California floristic province region[1]
A	100	0	0	14	Yes	Sacramento Valley
B	33	66	0	14	Yes	San Joaquin Valley
C	100	0	0	10	Yes	San Francisco Bay Area
D	93	7	0	15	Yes	San Joaquin Valley
E	8	83	8	11	Yes	San Francisco Bay Area
F	100	0	0	19	No	San Joaquin Valley
G	75	0	25	16	Yes	San Joaquin Valley
H	100	0	0	9	Yes	San Joaquin Valley
I	50	0	50	6	Yes	San Joaquin Valley
J	87	0	13	15	Yes	San Joaquin Valley
K	25	50	25	6	Yes	San Joaquin Valley
L	60	40	0	15	No	Tehachapi Mountain Area
M	30	0	70	10	Yes	Western Traverse Ranges
N	50	0	50	20	Yes	Central Coast
0	89	0	11	9	No	Modoc Plateau

[1] Geographic regions of the California Floristic Province (Jepson 2012)

Figure 2.1

Map of *Salsola ryanii* distribution. Blue dots indication populations that were identified previously. Red letters indicate newly identified populations. Letters correspond to letters in Table 1.



Chapter 3

Evolution of fitness and other traits associated with allopolyploidization in *Salsola ryanii*

ABSTRACT

Allopolyploidy is a prominent feature of plant evolution and has been the focus of considerable research. However, common garden comparisons testing hypotheses about fitness and phenotype shifts associated with allopolyploidy are lacking. We document a significant increase in plant mass and size associated with a recent allopolyploidization event in *Salsola ryanii.* We also review expected phenotypic effects of both hybridization and whole genome duplication, and compare phenotypes (plant size and weight and phenology) observed in this common garden comparison with expectations of hybrid and polyploid lineages.

INTRODUCTION

Allopolyploidy (hybridization fixed by whole genome duplication) is an important component of plant evolution (OTTO AND WHITTON 2000; SOLTIS *et al.* 2009; MADLUNG 2013). All extant angiosperm species have at least one round of polyploidization in their evolutionary past (WENDEL 2000; JIAO *et al.* 2011; MCGRATH AND LYNCH 2012). Although it is often difficult to determine whether polyploidization events in the distant past were the result of allo- or auto-polyploidy, recent evidence suggests that the majority of polyploidization events within angiosperms are allopolyploid (SCARPINO *et al.* 2014). Allopolyploidy appears to be equally important in the evolution of certain other plants, such as ferns (BARRINGTON *et al.* 1989). Given the apparent importance of allopolyploidy in plant evolution, studies of phenotypic shifts associated with allopolyploidization are crucial for understanding the evolutionary significance of this important process (Madlung 2013, Otto and Whitton 2000, Soltis et al. 2009). However, studies of plant trait changes associated with allopolyploidy are largely lacking.

Early studies of plant evolution by allopolyploidy hypothesized that increased fitness or ecological success, as well as increased ability to adapt to novel or disturbed habitats, would be associated with allopolyploidization (STEBBINS 1947; STEBBINS JR 1950; EHRENDORFER 1980; LEWIS 1980; LEVIN 1983). Based on similar arguments, both polyploidy and hybridization have been proposed as a pathways to the evolution of invasiveness (ABBOTT 1992; ELLSTRAND AND SCHIERENBECK 2000; PANDIT *et al.* 2006;

PANDIT *et al.* 2011). A recent review of invasive hybrid species documents numberous invasive species of allopolyploid origin (SCHIERENBECK AND ELLSTRAND 2009).

Correlations have also been established between polyploidy and both weedy and invasive traits. In the California flora, after accounting for life history differences between annual and polyploid species, polyploidy was found to be significantly more common amongst weeds than it is in families overall (HEISER CB AND WHITAKER 1948). There are also numerous cases in which multiple cytotypes exist within the native range of a species, but only the polyploid variants have become invasive (i.e. *Centaurea maculosa, Solidago gigantea*) (TREIER *et al.* 2009; SCHLAEPFER *et al.* 2010). Furthermore, several floristic surveys identify an association between polyploidy and biological invasion (STEBBINS *et al.* 1965; PANDIT *et al.* 2006; PYŠEK *et al.* 2009; PANDIT *et al.* 2011). Although such studies have provided a strong starting point for understanding how allopolyploidy influences evolution of invasiveness, they lack phenotypic comparison data, thereby limiting our understanding of why allopolyploidization causes shifts in invasiveness.

Phenotype Expectations of Allopolyploid Species

Quantitative phenotypic comparisons of allopolyploid species to their progenitors are exceedingly rare, making predictions about how allopolyploidy affects evolutionary success challenging. As an alternative, comparison data exist for homoploid hybrids and their progenitors, as well as for autopolyploids and their progenitors. These data provide a reasonable point of departure for making predictions about how allopolyploidy might influence phenotypic change.

Autopolyploidy is associated with several consistent phenotypic shifts and has been hypothesized to be associated with increased fitness and ecological success (STEBBINS 1947; STEBBINS JR 1950; EHRENDORFER 1980; LEWIS 1980; LEVIN 1983). A number of trait shifts are broadly associated with polyploidization, including shifts in life-history, plant size, phenology and self-incompatibility. Whole-genome duplication is generally associated with a shift from annual to perennial life-history (SANO 1980), as well as the loss of self-incompatibility mechanisms present within the progenitor species (RICHARDS 1997). Common garden comparisons between polyploid species and their diploid progenitors have also established that polyploid derivatives generally have increased plant size (SCHNITZLER AND MULLER 1998; SCHLAEPFER *et al.* 2010), increased flower size (GARBUTT AND BAZZAZ 1983) and increased seed production (BRETAGNOLLE *et al.* 1995) relative to their progenitors. Whole-genome duplication is also directly associated with an increase in DNA content and therefore an increase in cell size; increased cell size is thought to explain the delayed phenology seen in polyploid species (SMITH 1946; GARBUTT AND BAZZAZ 1983).

Whole-genome duplication has the potential to affect the susceptibility of plants to herbivores. A study of diploid and autotetraploid *Heucherea grossulariifolia* demonstrated greater attack by moths (*Greya politella*) on the tetraploid species than on the diploid progenitor (THOMPSON *et al.* 2004). The hexaploid cytotype of *Aster amellus* also suffers greater herbivore damage by the seed predator *Coleophora obscennella* than its diploid progenitor (MÜNZBERGOVÁ 2006). Further work is needed to determine whether these patterns that are well established in autopolyploid species also hold for allopolyploid species.

Studies of hybrid lines compared with their parental taxa can also inform our predictions of allopolyploid traits. Early studies of homoploid hybrid fitness viewed hybrid lineages as sterile dead-ends or consistently phenotypically intermediate between their progenitor species (MAYR 1942; DARWIN 1959). The theory that hybrids will be consistently phenotypically intermediate is based on the idea that morphological trait differences between the progenitor species are under simple additive quantitative genetic control, leading to hybrids that have intermediate phenotypes (ANDERSON 1949). It has since become clear that hybrid fitness can range greatly from sterile or low-fitness lines with limited evolutionary potential to highly successful lineages with increased fitness compared to their progenitors, sometimes evolving into a niche independent from that of their progenitors (ARNOLD 1997; RIESEBERG *et al.* 2003). The phenotypes of hybrid lineages relative to their ancestors also range from intermediate to transgressive or novel phenotypes (ARNOLD 1997; RIESEBERG *et al.* 2003). Although many hybrid lineages have reduced fitness compared to their progenitor species, multiple processes associated with hybridization (novel gene combinations and/ or expression, increased genetic variation, fixed heterosis and dumping of genetic load) are associated with increased fitness and novel or transgressive phenotypes (STEBBINS 1969; GRANT 1981).

A broad literature survey of homoploid hybrids that included first generation (F_1), later generation, and fixed hybrids was conducted by Rieseberg and Ellstrand (2003) to establish the phenotypic correlates of hybridity. They found that hybrids were no more likely to display intermediate traits than progenitor traits. Instead, hybrids showed an "unexpectedly high" proportion of transgressive or novel phenotypes (RIESEBERG AND ELLSTRAND 1993). Within first generation (F1) hybrids in this study, 10% of characters observed were novel or transgressive compared to the progenitors, whereas 64% of F1 hybrids had at least one trait that was novel or transgressive. In later generation hybrids, 89% exhibited one or more novel or transgressive phenotypes (RIESEBERG AND ELLSTRAND 1993).

In hybrid lineages, susceptibility to herbivory is a well-studied phenotype. Multiple phenotypes have been observed—susceptibility to herbivores may be increased, intermediate or equivalent to that of the progenitors (FRITZ 1999). In cases where progenitor levels of susceptibility are observed, the hybrid phenotype is usually more similar to the more susceptible progenitor (FRITZ 1999). From over a century of research on hybrids, it is clear that hybrid fitness and phenotypes are difficult to predict. Success of hybrid lineages is often dependent on availability of suitable habitat and ability to overcome competition with their progenitors (LEVIN 1975; ARNOLD 1997).

Common garden studies involving both hybrid and autopolyploid species provide a starting point for understanding phenotypes of allopolyploid species, for which such data are lacking. Direct comparisons of phenotypes newly formed allopolyploid species with phenotypes of their progenitors are needed.

Study System

Salsola ryanii, a newly formed allopolyploid species, provides an ideal system to study allopolyploid fitness. *Salsola ryanii* is an annual species that has evolved within the last 25-100 years via hybridization between two introduced species, *S. tragus* and *S. australis*, both of which are invasive in California, USA (AYRES *et al.* 2009). *Salsola tragus* is a tetraploid species (2n=36) that is native to Russia and China; it is one of the world's worst weeds and invasive in 48 U. S. states (HOLM 1997; AYRES *et al.* 2009). Native to Australia and South Africa, *S. australis* is a diploid (2n=18) invasive species in California and Arizona (BORGER *et al.* 2008; AYRES *et al.* 2009). *Salsola ryanii* appears to be at least as invasive as its parents; its reported range in California is now several times larger than when it was first identified and named about fifteen years ago (WELLES AND ELLSTRAND Submitted). Although both progenitors also exist in Arizona, *S. ryanii* has not been observed (GASKIN *et al.* 2006).

The goal of this common garden study was to compare the fitness and phenotypes of the newly formed allopolyploid species, *Salsola ryanii*, to those of its progenitors, *S. tragus* and *S. australis*, under controlled environmental conditions.

MATERIALS AND METHODS

Fitness and phenology comparisons between the newly formed allopolyploid species, *S. ryanii* and its progenitors, *S. tragus* and *S. australis*, were completed using two common garden studies, one in 2012-2013 and the other in 2014-2015. Both studies were conducted at the Agricultural Research Station at the University of California, Riverside, in close proximity to naturalized populations of all three species. Plants were grown from seeds collected the previous growing season from the Central Valley of California, where all three species are present. In all cases not more than one individual of each species was used from a single collection location. All seeds were germinated in flats in a temperature controlled greenhouse and transplanted into the field at the seedling stage in the spring, during the time seedlings typically germinate at nearby sites. Seedlings that did not survive transplantation into the field were replaced. Seedlings were watered using drip irrigation for four weeks to ensure successful establishment. The field was divided into three blocks. Equal numbers of each species were planted in each block to reduce potential block effects, creating a completely randomized block design. Within each block, the position of each individual plant was determined using a random number generator.

The 2012-13 experiment included a total of 119 individuals after mortality, 33 individuals of *S. tragus*, 22 individuals of *S. australis* and 64 individuals of *S. ryanii*. In this experiment we measured survivorship, number of days to flowering, number of days to maturity, level of herbivory, final plant width and height and final plant weight. We focused on these traits because preliminary field observations suggested that they may vary among these three *Salsola* species. Once flowers began to form, surveys of first flowering date were conducted weekly. Level of herbivory was assessed on a zero-to-three scale, with zero being no visible herbivory and 3 being extreme herbivory. Herbivory was assessed twice prior to flowering, one and two months following planting. Damage varied from parts of leaves to the majority of the plant. Most of the herbivore damage observed in this study appeared to be caused by insects; however, there also appeared to be damage caused rabbits.

The 2014-2015 experiment included 87 individuals following mortality 27 individuals of *S. australis*, 23 individuals of *S. tragus* and 37 individuals of *S. ryanii.* In this experiment we measured survivorship, days to maturity, final plant width and height, and final plant weight. Final plant width, height and weight were measured once fruit set was complete but prior to plant desiccation. *Salsola* produces very large numbers of tiny fruits, making it necessary to estimate whole-plant fruit production. During this field season, we also selected one branch from each plant to estimate the relationship between plant weight and fruit set. The total number of fruits present on the branch was counted and the branch was weighed. In both field experiments, weight measurements were made using an AWS H-110 digital hanging scale, which has a maximum capacity of 50 kg. Final height and width were measured at the widest and tallest point for each plant, and an estimate of total plant size was determined by multiplying height by width. To combine data from the two independent field experiments, an average weight and size for each of the field experiments was calculated and each individual plant weight and size was divided by the average for all species from that year to generate a relative weight and size measurements for each individual.

Statistical analysis

Analysis of variance (ANOVA) was used to analyze differences between the allopolyploid species and its progenitors for plant weight, size, flowering time and time to harvest. ANOVA assumptions (heterogeneity of variance and normal distribution) were tested prior to analyses. All analyses were done using SAS 9.2.

RESULTS

Across the two common garden experiments, the alloploid *S. ryanii* had significantly greater mass and overall size than both parental taxa (Table 1). *Salsola tragus*, *S. ryanii*'s tetraploid progenitor, had larger size and mass than the diploid progenitor *S. australis* in the 2012-2013 field season, but this pattern was reversed in the 2014-2015 field season (Table 1). Greater mass and size were indicators of greater fecundity, as subsampling branches revealed statistically significant, positive correlation between weight and fruit number (Pearson's r = 0.62; n = 86; P < 0.0001). We do, however, also see a significant effect of species (p=0.0226); *S. tragus* had the highest fecundity followed by *S. ryanii* and *S. australis* (Figure 1).

In 2012-2013, flowering of all of the species overlapped. *S. tragus* had the largest percentage of early flowering individuals and *S. australis* had the largest percentage of late flowering individuals with *S. ryanii* intermediate between the progenitors (Figure 2). In 2012-2013 *S. australis* had the largest percentage of individuals maturing early, while *S. tragus* and *S. ryanii* matured at similar times (Figure 3). In the 2014-2105 field season all the species matured similarly in time, *S. australis* had a slightly larger percentage of individuals maturing early and *S. tragus* and *S. ryanii* individuals matured with similar timing (Figure 4).

One month following planting, *S. tragus* had the largest percentage of individuals experiencing high levels of herbivory; two months following planting *S. australis* had the largest percentage of individuals experiencing high levels of herbivory (Figure 5). Both one and two months post-planting *S. ryanii* had an intermediate percent of individuals experiencing both the highest and lowest levels of herbivory compared to both of its progenitor species (Figure 5).

DISCUSSION

In this common garden comparison between *S. ryanii* and its progenitors, *S. tragus* and *S. australis,* we observed the evolution of increased plant weight and plant size associated with allopolyploidization within an environment in which all three species are established. Although many studies suggest fitness advantages should associated with evolution by allopolyploidy (EHRENDORFER 1980; LEWIS 1980), to our knowledge this is the first study to document increased fitness of a newly formed allopolyploid species within the shared progenitor habitat. This fitness comparison and previous work indicating a rapid range expansion in this species (WELLES AND ELLSTRAND Submitted) provide evidence that *S. ryanii* has the potential to become to be as successful an invasive species as its parents. More broadly, our data provide support for the hypothesis that allopolyploidization has the potential to increase invasiveness. In this case, the invasive phenotype was not generated *de nov*o by the polyploidization event, since the progenitors themselves are invasive species (AYRES *et al.* 2009). However, the greater fitness of *S. ryanii* compared to its progenitors demonstrates that the allopolyploid has the potential to become at least as invasive as the parental species and may well replace them within their naturalized range in California.

Although *S. ryanii* was consistently the largest of the three species included in this study, which of the progenitors was the largest was dependent on the year. The presence of both progenitors within the same location is potentially maintained through differential success of the different species between years. *Salsola ryanii* appears to have higher mean fitness than both progenitors in both years of our study; if this trend is consistent across future years, there is strong potential for *S. ryanii* to replace its progenitor species.

The transgressive phenotype observed for weight and size in this study is consistent with common garden studies demonstrating increased size associated with autopolyploidy (SCHNITZLER AND MULLER 1998; SCHLAEPFER *et al.* 2010) and common garden studies showing that certain hybrid-derived diploid lineages have increased fitness relative to their progenitors (HEGDE *et al.* 2006). Although *S. ryanii* has a transgressive phenotype compared to its progenitor species for plant weight and size, for phenology and herbivory *S. ryanii* is intermediate between its progenitor species or has the phenotype of one of the progenitors. *Salsola ryanii* also has intermediate susceptibility to herbivory, which is inconsistent with previous studies of autopolyploid species documenting increased susceptibility to herbivory in polyploid lineages (THOMPSON *et al.* 2004; MÜNZBERGOVÁ 2006). Previous studies of herbivore susceptibility in hybrid lineages are mixed (BOECKLEN AND SPELLENBERG 1990; FRITZ *et al.* 1994); therefore it is difficult to interpret our results in the context of previous studies of hybrid lineages.

The intermediate flowering time of *S. ryanii* observed in this study was unexpected, based on previous studies of autopolyploid species. Due to the increased genome size in the hexaploid *S. ryanii,* we would have predicted delayed flowering (SMITH 1946; GARBUTT AND BAZZAZ 1983). The overlapping flowering time of all of the species suggests that all species have the opportunity to pollinate each other. This could present a problem for a newly formed species that is in the minority in most locations where it occurs, because it may receive more incompatible pollen than appropriate pollen (ANTONOVICS 1968; BAACK 2005). Further studies of the mating system of *S. ryanii* and its progenitor species are required to determine whether overlapping phenology is problematic for establishment of the hybrid in this system.

This study begins to fill the gap in our understanding of phenotypic evolution during an allopolyploid speciation event by comparing traits of a newly formed allopolyploid and its progenitor species within a common environment. The comparison between *S. ryanii* and its progenitors provides support for the previously unsupported hypothesis that allopolyploidy is associated with fitness increases. Further common garden comparisons between other newly formed allopolyploid species and their progenitors are needed to establish whether the patterns observed in this system represent broad patterns associated with allopolyploid species.

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Table 3.1. Comparisons of relative size and weight between *S. ryanii* and its progenitors in a common garden study. Year one included 119 individuals; 64 of *S. ryanii*, 22 of *S. australis* and 33 of *S. tragus*. Year 2 included 87 individuals; 37 of *S. ryanii*, 27 of *S. australis*, and 23 of *S. tragus*. For all ANOVA analyses DF=2. Size is plant height * plant width. Relative size and mass were generated by dividing each value by the average for the garden it was grown in.

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	ANOVA		Tukey's HSD		
	P-value	R2	*S. australis* vs. *S. Ryanii*	*S. tragus* vs. *S. ryanii*	*S. tragus* vs. *S. australis*
Relative mass	<.0001	0.1	<.0001	0.0391	0.0059
Relative size	<.0001	0.06	<.0001	0.0356	0.2019
Mass year 1	0.0002	0.15	0.0001	0.8224	0.0037
Size year 1	0.005	0.087	0.0033	0.664	0.0612
Mass year 2	0.0065	0.1	0.0165	0.0165	0.9607
Size year 2	0.0082	0.09	0.1432	0.0066	0.521

	Average Relative Mass	Standard Deviation	Year 1 Average Mass (kg)	Year 1 Standard Deviation	Year 2 Average Mass (kg)	Year 2 Standard Deviation
S. ryanii	1.25453	0.95049	1.36817	0.19935	5.82244	0.77638
S. tragus	0.96393	0.844357	1.24793	0.16555	2.77923	0.67509
S. australis	0.52303	0.747071	0.3915	0.11509	3.08935	0.84775
	Average Relative size	Standard Deviation	Year 1 Average Size (m^2)	Year 1 Standard Deviation	Year 2 Average Size (m^2)	Year 2 Standard Deviation
S. ryanii	1.16219	0.540039	0.89428	0.06466	1.76588	0.197
S. tragus	0.92684	0.859094	0.794977	0.09004	0.85388	0.216
S. australis	0.73129	0.565042	0.468941	0.11028	1.19539	0.226

Figure 3.1. Regression analysis of branch mass to fruit number on the branch with species also included in the model. Lines A is the regression for *S. australis*, R is *S. ryanii* and T is *S. tragus*.

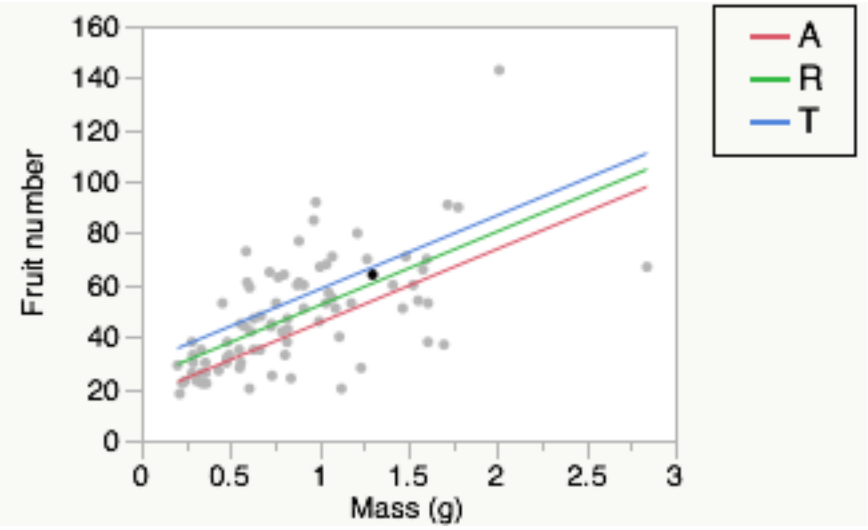


Figure 3.2. Plot of normalized initial flowering during the 2013-2014 field experiment. Percentage of each species that have begun flowering by each number of days post-planting A is *S. australis* (diploid progenitor, n=22), R is *S. ryanii* (allopolyploid, n=64), T is *S. tragus* (tetraploid progenitor, n=33).

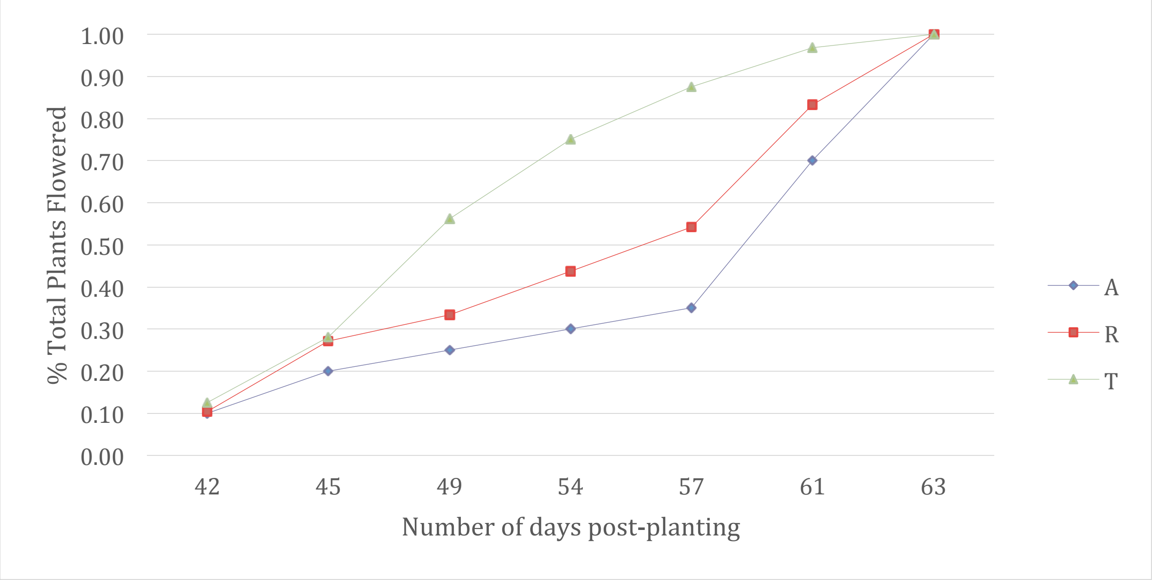


Figure 3.3. Plot of normalized time to maturity for the 2012-2013 field experiment. Percentage of each species that has matured be each number of days post-planting A is *S. australis* (diploid progenitor, n=22), R is *S. ryanii* (allopolyploid, n=64), T is *S. tragus* (tetraploid progenitor, n=33). Plants are considered mature when the plant is dry and seeds become viable.

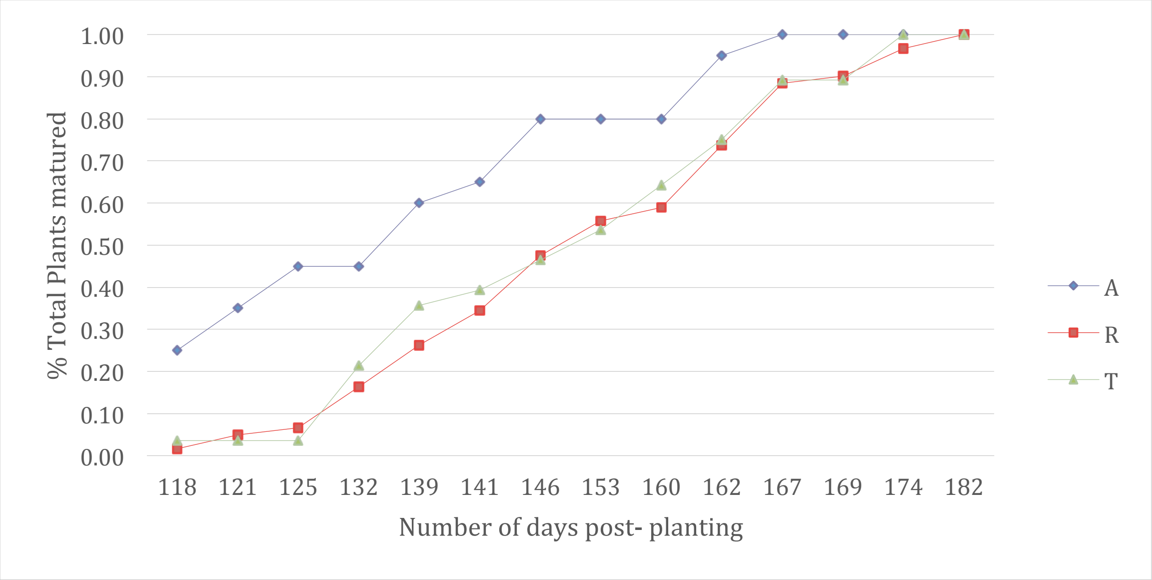


Figure 3.4. Plot of normalized time to maturity for the 2014-2015 field experiment. Percentage of each species that has matured be each number of days post-planting A is *S. australis* (diploid progenitor, n=31), R is *S. ryanii* (allopolyploid, n=41), T is *S. tragus* (tetraploid progenitor, n=26). Plants are considered mature when the plant is dry and seeds become viable

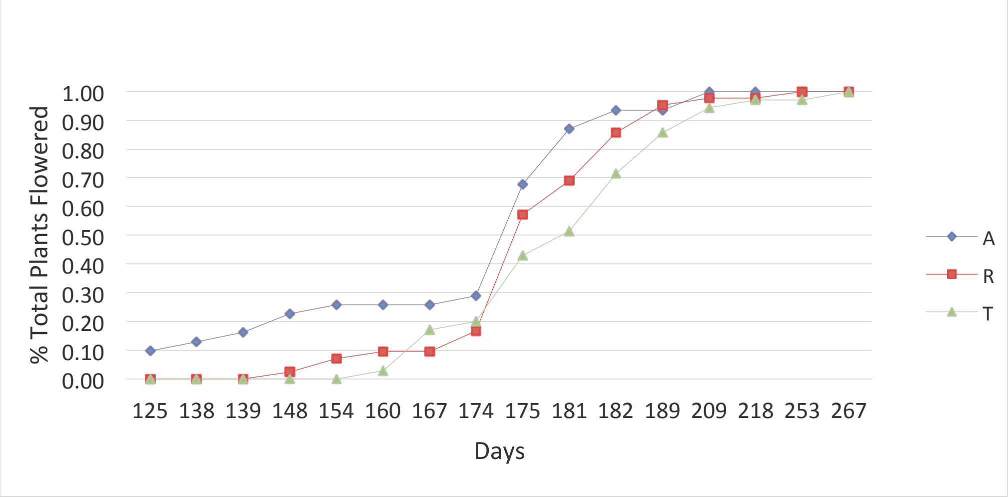
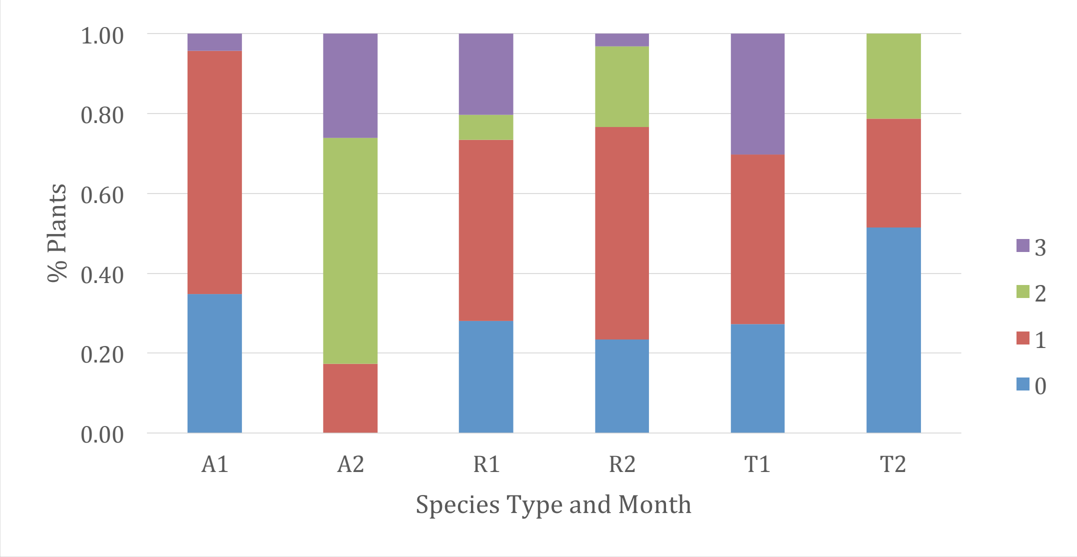


Figure 3.5. Normalized herbivory one and two months post planting. A1 is *S. australis* (diploid progenitor, n=22), one month post-planting, A2 is *S. australis* (diploid progenitor, n=22), two months post-planting, R1 is *S. ryanii* (allopolyploid, n=64) one month post-planting R2 is *S. ryanii* (allopolyploid, n=64) 2 months post-planting, T1 is *S. tragus* (tetraploid progenitor, n=33) one month post-planting and T2 is *S. tragus* (tetraploid progenitor, n=33) two month post-planting. All plants were scores from 0-3, 0 is no evident herbivore damage and 3 is extreme herbivore damage.



Chapter 4

Genetic structure reveals a history of multiple independent origins in the allopolyploid weed *Salsola ryanii*

ABSTRACT

It has recently become clear that many invasive species have evolved *in-situ* via hybridization or polyploidy from progenitors which themselves are introduced species. For species formed by hybridization or polyploidy, the genetic diversity within a newly formed species is influences by the number of evolutionary origins of the species. For recently formed species, an analysis of genetic structure can provide evidence of independent origins. Here we analyze the genetic structure of an allopolyploid species, *Salsola ryanii*, which has formed within the last 20-100 years in California, and utilize these data to determine that this new species is likely the result of multiple allopolyplodization events, leading to increased genetic diversity within the species.

INTRODUCTION

Invasive species play a significant role in global change (VITOUSEK *et al.* 1996). For introduced species to become successful invaders, they need to be able to adapt to changing environmental conditions (SAKAI *et al.* 2001). Genetic variation can contribute to the adaptability of species (FISHER 1958; SAKAI *et al.* 2001) and thereby contribute to their ability to persist and spread within an introduced range (LEE 2002; PARKER *et al.* 2003). For introduced species, the number of founding individuals and the genetic variation present within and between the founding individuals will play a role in the success of the species within the introduced range.

Invasive species can arise in two ways, through introduction (intentional or accidental) from a distant source or through *in situ* evolution. In both cases, the initial number of independent origin events can contribute to the genetic variation available on which selection can act. For invasive species that arise via introduction, the number and origin of introduced propagules will often affect invasion success; multiple introductions of a single species are common, and also generally increase the genetic diversity of the introduced species (DLUGOSCH AND PARKER 2008). In addition to leading to increased genetic diversity, multiple introductions can generate novel gene combinations. In cases where multiple introductions occur from different regions of the native range of a species, admixture between introductions can generate gene combinations that do not exist in the native range (NOVAK 2007; SCHIERENBECK AND ELLSTRAND 2009). It has been hypothesized that admixture between independent introductions leads to increased invasion success as potentially beneficial, novel gene combinations are generated by genetic heterogeneity (KELLER AND TAYLOR 2010; VERHOEVEN *et al.* 2011). In cases where the potential for multiple introductions can be documented, management efforts can be undertaken to reduce the number of multiple introductions or their establishment, thereby reducing the evolutionary potential for a species to become invasive.

Invasive species can arise *in situ* via multiple evolution mechanisms, including hybridization autopolyploidy and allopolyploidy. Both hybridization and polyploidy have been proposed to be associated with the evolution of invasive species (ELLSTRAND AND SCHIERENBECK 2000; PANDIT *et al.* 2011). For invasive species that evolve *in situ*, the number of independent origin events influences the amount of genetic diversity present in the newly formed species and potentially the evolutionary potential of the species. It was originally believed that allopolyploid species (species formed by hybridization fixed by whole genome duplication) are always the result of a single origination event giving rise to a genetically uniform new species (OWNBEY AND MCCOLLUM 1953; SOLTIS *et al.* 1993). The assumption that allopolyploid species arise from a single origin was based on the hypothesis that allopolyploidization events are so infrequent that they would be unlikely to reoccur multiple times within the same lineage (SOLTIS *et al.* 1993). If this were the case, low genetic variation and limited adaptability would be common characteristics of new allopolyploid species.

Number of origins can only be determined when the polyploidization event(s) are recent, because subsequent recombination, diploidization, inter-lineage admixture and mutation all act to erode the molecular footprints generated by the origin event(s). Therefore, for many allopolyploid species it is too late to even crudely estimate whether they have mono- or poly-phyletic origins.

Several recent studies have tested the assumption that allopolyploid species are always the result of a single origin event by molecular genetic analysis of relatively newly formed allopolyploid species, and have reported mixed results. In the well-studied allopolyploid species *Tragopogon mirus* and *T. miscellus* the assumption of a single origin was documented to be incorrect, as both species have polyphyletic origins hundreds of miles apart (SOLTIS AND SOLTIS 1991; SOLTIS *et al.* 2004). Crossing studies of the *Tragopogon* allopolyploids suggest that the lineages from different origins are not compatible with each other, so no admixture occurs between independent origins (SOLTIS *et al.* 2004). Multiple origins have also been demonstrated in a number of other allopolyploid species, including a hybrid of *Aegilops* and *Triticum*, *Senecio cambrensis* and multiple allopolyploid species in the *Glycine tomentella* complex (ASHTON AND ABBOTT 1992; RAUSCHER *et al.* 2004; MEIMBERG *et al.* 2009). In contrast, another well-studied invasive allopolyploid species (*Spartina anglica*) is a clonal species that has been documented to be the result of a single origin event (RAYBOULD *et al.* 1991).

While it is now clear that neither a single origin nor multiple origins can be assumed for allopolyploid species, one could hypothesize that multiple origins would contribute to the invasiveness of a new allopolyploid, just as multiple introductions can contribute to the evolution of invasiveness in introduced species (KELLER AND TAYLOR 2010; VERHOEVEN *et al.* 2011). This prediction has not been fully explored, as among the well-studied allopolyploid invasive species, *T.mirus, T. miscellus*, *S. cambrensis* are all the result of multiple independent origins, whereas *S. anglica* is the result of a single origin. Nonetheless, the number of origins of newly formed allopolyploid species is likely of interest to land managers, other practitioners, and policy-makers who are concerned about the evolution of new invasive species.

In recently formed allopolyploid species there has been very limited time for the development of population structure beyond the structure generated by origin events. Thus, analysis of genetic structure can answer questions about species origins and dispersal or admixture events that occurred after speciation. If the newly formed species is the result of a single origin, we would expect to observe genetic similarity amongst all individuals and little genetic structure observed. Any genetic structure that does exist would have evolved following the recent origin and should be significantly less than what is present in the progenitor species, presuming that they are relatively ancient (>>100 generations) and have similar dispersal patterns. If a newly formed species is the result of multiple origins, the individuals resulting from independent origins will likely be highly genetically distinct from each other (given typical levels of molecular genetic polymorphism). Therefore, molecular genetic analysis should be able to discern the genetic structure generated by the independent origin events.

Assuming that independent origin events occur in discrete locations, some spatial genetic structure should be initially present and erode as different lineages spread via progagule, seed, and pollen dispersal. Using a genetic structure model that allows for admixture can give us insight into (a) potential number of independent origins and (b) potential early interlineage admixture. This approach to identifying independent origin events is likely conservative in terms of the number detected, since independent origins from parental types with similar genetic compositions are less likely to be detected, compared to different origins with parent pairs that are highly differentiation from each other. However, this method is extremely unlikely to assign multiple origins when a single origin is the true history.

Study System

Salsola ryanii (2n=54) is a newly formed weedy allohexaploid derivative of *S. tragus* (2n=36) and *S. australis* (2n=16), which formed *in situ* in California in the last 20-100 years *(HRUSA AND* GASKIN *2008)*. Both of *S. ryanii's* progenitor species are problematic weedy/ invasive species; for this reason, studies into the potential invasiveness of the derivative species are crucial. The range of *S. ryanii* extends through multiple floristic provinces of California including the Sacramento and San Joaquin Valleys, San Francisco Bay area, Central Coast, Western Transverse Range, and Modoc Plateau (Welles and

Ellstrand, Submitted). Hrusa and Gaskin (2008) hypothesize that this newly formed species is the result of multiple independent origin events within the Central Valley of California based on the disjunct geographic distribution that was observed, but this hypothesis has not been confirmed with genetic data.

Neither of *S. ryanii's* progenitors are native to California. *Salsola tragus*' native range extends from North Africa and Western Russia, through Asia into Northeast Siberia and Northeast China. The first known introduction of *Salsola tragus* into North America occurred in South Dakota in the 1870's, likely through contamination of agricultural seed (YOUNG 1988). The number of introductions of *S. tragus* into the US is unknown, but the introduction source was likely Russia (YOUNG 1988). *Salsola australis* is a weed in California and Arizona and is likely native to Australia or South Africa (BORGER *et al.* 2008). *Salsola australis* is morphologically very similar to *S. tragus* and was not recognized as a distinct species until recently (RYAN AND AYRES 2000). Given that *S. australis* was an unrecognized cryptic species assigned to *S. tragus* until recently, little is known of the introduction of *S. australis* into North America.

In this study we attempted to 1) determine whether *S. ryanii* is the result of single or multiple origin events utilizing an analysis of its genetic structure, and 2) investigate dispersal and admixture that have occurred following the origin(s).

MATERIALS AND METHODS

Sampling

Plant samples for the micorosatellite (SSR) analysis were collected throughout the range of *S. ryanii* in California as described in Welles and Ellstrand (Submitted). A total of 135 *Salsola ryanii* individuals were collected from 25 locations, 165 individuals of *S. tragus* were sampled from 20 locations, and 226 individuals of *S. australis* were sampled from 25 locations. All collection locations were at least 16 km apart from each other, but multiple species were often sampled from the same location. The location of each collection was noted using a Garmin GPS map 62s unit.

DNA Extraction and Microsatellite Analysis

DNA was extracted from each sample using a modified CTAB extraction method (DOYLE 1991). The species of each sample was identified using inter simple sequence repeat (ISSR) markers, as described in Welles and Ellstrand (Submitted). DNA was extracted from each sample using a CTAB extraction, with the incubation step following grinding with liquid nitrogen omitted (DOYLE 1991), and amplified using five microsatellite primers develop for *Salsola* species by (MCGRAY *et al.* 2008). Polymerase chain reaction (PCR) was carried out according to protocols established by McGray et al (2008). Following amplification samples were analyzed using the ABI 3130XL Genetic Analyzer. Alleles were binned using GeneScan. For the two polyploid species, when fewer than the number potential number of unique alleles were detected (6 for *S. ryanii* and 4 for *S. tragus*) all the remaining alleles were coded as missing data. For the diploid species (*S. australis*), when only one allele was detected it was coded as homozygous locus.

Genetic Structure Analysis

Population structure of all three species (*S. ryanii, S. tragus* and *S. australis*) was estimated using STRUCTURE (PRITCHARD *et al.* 2000). The population structure of each species was analyzed independently using a 5000 burn-in period, 50000 MCMC replicates following the burn-in period and a model including admixture. The appropriate number of genetic clusters was determined using a nonparametric Wilcoxon test (ROSENBERG *et al.* 2001) and confirmed using second-order rate of change of likelihood (EVANNO *et al.* 2005).

RESULTS

Optimal number of genetic clusters (K) was 3 for *S. ryanii*, 2 for *S. australis* and 1 for S. tragus. In this case the allopolyploid species, *S. ryanii,* has the largest number of genetic clusters of the three species. Figure 1 and Table 1 show three genetic clusters of *S. ryanii* that are geographically dispersed throughout the range of *S. ryanii*, with each of the genetic clusters occurring in multiple floristic provinces. A total of 36% of the 135 total *S. ryanii* individuals appear to be admixed. We considered an individual admixed if it was less than 90% assigned to a single genetic cluster (Table 1), the other individuals

(64%) are dispersed through the three genetic clusters. The green cluster (A) of Figure 1 is represented by the most individuals (34%), and the other two clusters have roughly the same number, 16% and 13% (Table 1). The Sacramento and San Joaquin Valleys have the largest percentage of individuals that appear to be admixed however; all of the floristic provinces with the exception of the South Coast Range and the Peninsular range have some admixed individuals (Table 1).

With the number of loci used in this study, no population structure was detected for the tetraploid parent *S. tragus*. A STRUCTURE plot is not presented for *S. tragus* because a STRUCTURE plot with K=1 is uninformative. For *S. australis* the optimal number of genetic clusters was 2, with each cluster occurring in each of the floristic provinces (Figure 2). In the Sacramento Valley, San Joaquin Valley and South Coast Range (Central California Provinces) there appears to be limited admixture, with most individuals being assigned to a single genetic cluster (Figure 2). The South Coast, Peninsular Range and Western Transverse Range (Southern California Provinces) largely contain individuals that appear to be admixed.

DISCUSSION

In this study, the patterns that we observe for the newly formed allopolyploid species *S. ryanii* are consistent with expectations of a species formed through multiple independent origins. Based on our data, it appears that there are at least three origins of *S. ryanii* that have subsequently dispersed and recombined to form the observed genetic structure. We believe that this is a conservative estimate; and so further work should reexamine the question with a larger number of more polymorphic markers to determine if there are additional distinct origins that are cryptic in this analysis. The finding of multiple origins of *S. ryanii* is consistent with predictions made by Hrusa and Gaskin (2008) and with results of some other invasive allopolyploid species (SOLTIS AND SOLTIS 1991; ASHTON AND ABBOTT 1992; SOLTIS *et al.* 2004)

In addition to observing multiple genetic clusters, we detected other patterns that reinforce the hypothesis of multiple origins; an increased number of genetic clusters compared with the progenitor species, and dispersal of the multiple genetic clusters throughout the species' range. We observe a larger number of genetic clusters in *S. ryanii* than in either of the progenitor species. If the genetic clusters were generated following a single origin we would expect to observe a number of genetic clusters equal or less than what is observed in the progenitor species, which are likely not affected by origin events and only affected by mutation and gene flow. In *S. ryanii* we observe the multiple genetic clusters spread out over the geographic range of the new species. The geographic intermixing of genetic clusters of *S. ryanii* suggests multiple independent origin events followed by dispersal as opposed to a single origin event followed by dispersal and subsequent generation of population structure.

The probability of a high level of dispersal in *S. ryanii* based on the genetic cluster analysis is consistent with the known dispersal abilities of the progenitor species and with previous work demonstrating that the range of S. ryanii has expanded rapidly following formation (WELLES AND ELLSTRAND Submitted-a). *Salsola tragus* is a highly dispersed species, which spreads by its well-known "tumbling" strategy. The observed lack of geographic differentiation of *S. tragus* found in this study is also consistent with expectations for a species with very high dispersal ability (BOHONAK 1999). *Salsola australis* also has appears to have a some level of wind dispersal but lacks both the round plant shape and the formation of an abscission layer at the base of the plant following fruit maturity that make *S. tragus* a highly dispersed species (S. Welles, Personal observation). The morphological differences are consistent with the differences in population structure observed: *S. australis* has very limited population structure but more structure than is observed in the closely related highly dispersing species *S. tragus*.

Given the substantial fraction of "admixed" *S. ryanii* individuals detected, it appears that descendants of the multiple independent origins have begun to hybridize. We also observe that multiple S. ryanii lineages are often currently located within the same or nearby collection sites, leading to the potential for additional admixture in the future. The admixture is potentially continuing in *S. ryanii* has already created populations with increased genetic diversity and increased evolutionary potential. The mating system of *Salsola* has not been well documented, but from this work it appears as though there is admixture via outcrossing of a rate higher than that of highly selfing invasives

(SCHEMSKE AND LANDE 1985). Our result contrasts with what has been found for the multiple lineages of *Tragopon* sp. which are the result of multiple origins but do not admix with each other (SOLTIS *et al.* 2004).

Our finding of multiple origins followed by dispersal and admixture together, with previous studies demonstrating increased fitness and a rapid range expansion, suggest a strong potential for *S. ryanii* to become a problematic weed species (WELLES AND ELLSTRAND Submitted-c; WELLES AND ELLSTRAND Submitted-b). *Salsola ryanii* appears to have increased evolutionary potential compared to its progenitors. It has the advantages associated with allopolyploidy (fixed heterozygosity and potential for neo- or sub-functionalization of duplicate genes) (COMAI 2005) in addition to multiple independent origins with subsequent admixture. Due to these advantages, it is possible that *S. ryanii* could replace one or both of its progenitors in all or part of their range, as has occurred in the hybrid invasive California Wild radish (HEGDE *et al.* 2006). Future work should address the biology of this newly formed species to determine how problematic a weed this species will be as its range continues to expand and what the best management practices are for this species.

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Figure 4.1. STRUCTURE plot for *S. ryanii*. Black lines within the plot separate collections. The bars on the bottom represent different floristic regions. Within floristic provinces collections are organized north to south.

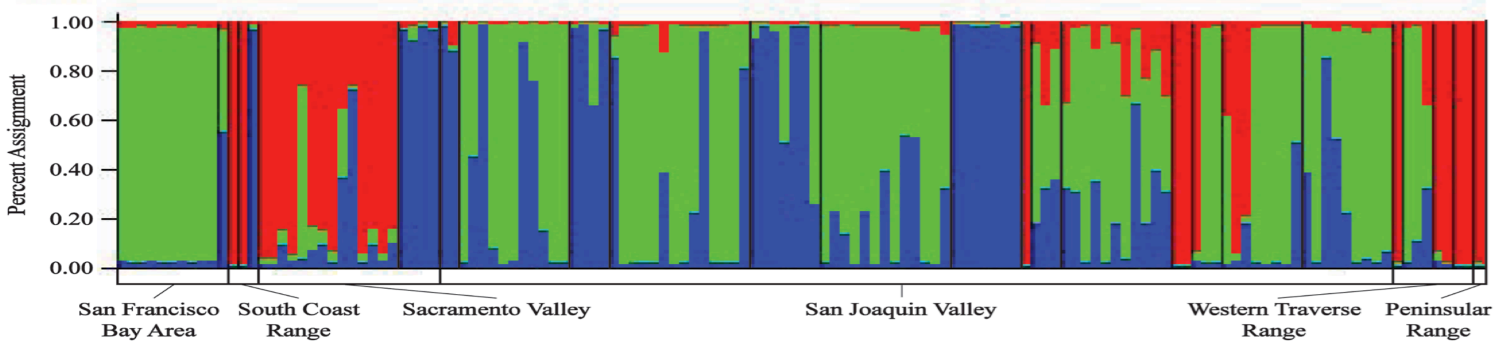
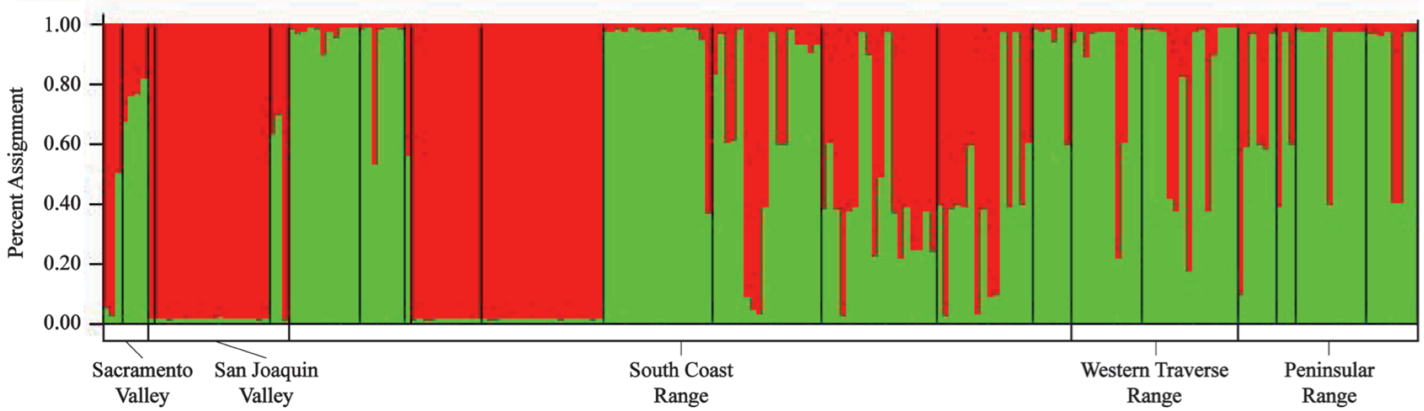


Table 4.1. Summary of STRUCTURE analysis (Figure 1) for *S. ryanii*. Cluster A is the green cluster, B is the blue cluster and C is the red cluster in Figure 1. Collections presented in the same order as in Figure 1, divided by floristic province and presented North to South within each province. SFBA= San Francisco Bay Area, SCR= South Coast Range, SCV= Sacramento Valley, SJV= San Joaquin Valley, WTR= Western Traverse Range and PR= Peninsular Range.

Collection	# of individuals	% Cluster A (#)	% Cluster B (#)	% Cluster C (#)	% admixed (#)
SFBA 1	10	100% (10)	0	0	0
SFBA 2	1	0	0	0	100% (1)
SFBA Total	11	91% (10)	0	0	9% (1)
SCR 1	1	0	0	100% (1)	0
SCR 2	2	0	0	100% (1)	0
SCR Total	2	0	0	100% (2)	0
SCV 1	1	0	100% (1)	0	0
SCV 2	14	0	0	43% (6)	57% (8)
SCV 3	4	0	100% (4)	0	0
SCV Total	19	0	26% (5)	32% (6)	42% (8)
SJV 1	2	0	100% (2)	0	0
SJV 2	11	55% (6)	9% (1)	0	36% (4)
SJV 3	4	75% (3)	0	0	21% (1)
SJV 4	14	64% (9)	7% (1)	0	29% (4)
SJV 5	7	0	71% (5)	0	29% (2)
SJV 6	13	46% (6)	0	0	54% (7)
SJV 7	7	0	100% (7)	0	0
SJV 8	1	0	0	100% (1)	0
SJV 9	3	0	0	0	100% (3)
SJV 10	11	18% (2)	0	0	82% (9)
SJV 11	2	0	0	100% (2)	0
SJV 12	3	66% (2)	0	33% (1)	0
SJV 13	8	50% (4)	0	0	50% (4)
SJV 14	9	56% (5)	0	0	46% (4)
SJV Total	95	37% (35)	17% (16)	3% (3)	40% (38)
WTR 1	1	0	0	100% (1)	0
WTR 2	3	33% (1)	0	0	66% (2)
WTR 3	2	0	0	100% (2)	0
WTR 4	2	0	0	100% (2)	0
WTR Total	8	13% (1)	0	63% (5)	25% (2)
PR 1	2	0	0	100% (2)	0
S. ryanii Total	135	34% (46)	16% (21)	13% (18)	36% (49)

Figure 4.2. STRUCTURE plot for *S. australis*. Black lines within the plot separate collections. The bars on the bottom represent different floristic regions. Within floristic province collections are organized north to south.



Chapter 5

Overall Conclusions

Allopolyploidy has long been recognized as an important evolutionary mechanism in most major plant groups (GRANT 1981). Recent phylogenomic analyses have revealed that the vast majority of seed plants and angiosperms, if not all, have one or more allopolyploid ancestors (WOOD *et al.* 2009; JIAO *et al.* 2011). Despite the frequency of polyploidy in plant evolution, available evidence has failed to support any special evolutionary advantage to polyploid lineages and suggests that they have a relatively low diversification rate (MAYROSE *et al.* 2011).

The rapid spread of *S. ryanii* in the decade following initial discovery provides evidence that allopolyploidy can produce species with that broadened ecological amplitude rapidly after initial species formation. In comparison with other well-studied cases of range expansion of neospecies, it is clear that *S. ryanii* represents an extreme example of range expansion in a newly formed allopolyploid species.

In addition to a rapid range expansion, we have also observed the evolution of increased plant weight and plant size associated with allopolyploidization. Although many studies suggest fitness advantages should associated with evolution by allopolyploidy (EHRENDORFER 1980; LEWIS 1980), to our knowledge this is the first study to document increased fitness of a newly formed allopolyploid species within the shared progenitor habitat. The greater fitness of *S. ryanii* compared to its progenitors demonstrates that this allopolyploid species has the potential to become at least as invasive as the parental species and may well replace them within their naturalized range in California. This study begins to fill the gap in our understanding of phenotypic evolution during an allopolyploid speciation event by comparing traits of a newly formed allopolyploid and its progenitor species within a common environment. The comparison between *S. ryanii* and its progenitors provides support for the previously unsupported hypothesis that allopolyploidy is associated with fitness increases. Further common garden comparisons between other newly formed allopolyploid species and their progenitors are needed to establish whether the patterns observed in this system represent broad patterns associated with allopolyploid species.

Although the fitness of this newly formed allopolyploid species has increased fitness compared to its progenitors within the range of both of the progenitors, at this point it has not expanded outside the range of its progenitors. Expansion beyond the range of the progenitors is more likely within a species that has genetic diversity for evolution to act on. In allopolyploid an species, multiple origins are likely to generate a species with relatively high genetic diversity (SOLTIS *et al.* 2004). The patterns that we observe for the newly formed allopolyploid species *S. ryanii* are consistent with expectations of a species formed through multiple independent origins followed by dispersal and admixture. The finding of multiple origins of *S. ryanii* is consistent with predictions made by Hrusa and Gaskin (2008) and with results of some other invasive allopolyploid species (SOLTIS AND SOLTIS 1991; ASHTON AND ABBOTT 1992; SOLTIS *et al.* 2004)

Together our findings of a rapid range expansion of the *S. ryanii,* evolution increased plant size and mass compared to its progenitors and multiple independent evolutionary origins of *S. ryanii* followed by dispersal and admixture suggest strong potential for *S. ryanii* to become a problematic weed species. *Salsola ryanii* has the advantages associated with allopolyploidy (fixed heterozygosity and potential for neo- or sub-functionalization of duplicate genes) (COMAI 2005) in addition to multiple independent origins with subsequent admixture. Due to these advantages and evidence demonstrating many traits associated with invasiveness, it is possible that *S. ryanii* could replace one or both of its progenitors in all or part of their range, and become a problematic weedy or invasive species. Future work should address the biology of this newly formed species to determine how problematic a weed this species will be as its range continues to expand and what the best management practices are for this species. Based on the results of this work, it seems likely that the range of *S. ryanii* will continue to expand and is likely to become an important an invasive species. More broadly, our data provide support for the hypothesis that allopolyploidization has the potential to lead to the evolution of new species with increased invasiveness compared to its progenitors.

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