AN ABSTRACT OF THE THESIS OF

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In addition to its longstanding recognition as an influential evolutionary process, interspecific hybridization is increasingly regarded as a potential threat to the genetic integrity and survival of rare plant species, manifested through gamete wasting, increased pest and disease pressures, outbreeding depression, competitive exclusion, and genetic assimilation. Alternatively, hybridization has also been interpreted as a theoretically beneficial process for rare species suffering from low adaptive genetic diversity and accumulated genetic load. As such, interspecific hybridization, and the underlying pre- and post-mating reproductive barriers that influence its progression, should be considered fundamental components of conservation planning for many rare species, particularly those predisposed to hybridization by various ecological, genetic, and anthropogenic risk factors. In this study I evaluate the nature and efficacy of pre- and post-mating hybridization barriers in the threatened species, *Sidalcea nelsoniana*, which is sympatric (or nearly so) with three other congeners in the scarce native grasslands of the Willamette Valley in western Oregon. These four perennial species share a high risk of hybridization due to their mutual proximity,

common occupation of disturbed habitats, susceptibility to anthropogenic dispersal, predominantly outcrossing mating systems, their capability of long-lived persistence and vegetative expansion, and demonstrated hybridization tendencies among other members of the family and genus. Results show *S. nelsoniana* is reproductively isolated from all three of its congeners by a complex interplay of pre- and post-mating barriers. Although *S. nelsoniana* overlaps with *S. campestris* in the ecological attributes of flowering time, fine-scale geographic distribution, and pollinators, interspecific hybridization is discouraged through the post-mating barrier of sexual incompatibility (expressed primarily as reduced seed set). Hybridization between *S. nelsoniana* and *S. virgata* is limited by the pre-mating barrier of asynchronous flowering (temporal isolation) and the supplemental post-mating barrier of sexual incompatibility, though the completeness of the latter varies in relation to crossing direction and *S. virgata* phenotype. Lastly, although *S. nelsoniana* and *S. cusickii* exhibit full interspecific sexual compatibility and produce fully fertile hybrids, hybridization in this species pair is discouraged by the pre-mating barrier of geographic isolation (the two species are narrowly parapatric).

Additional findings of this study show that 1) pollinators transferred significantly more *S. nelsoniana* pollen to the heterospecific flowers of *S. virgata* and *S. cusickii* than to conspecific flowers (or those of *S. campestris*) in a mixed species array, 2) anthropogenic disturbance is ubiquitous across extant *S. nelsoniana* study populations so cannot be conclusively linked with the breakdown of spatial reproductive barriers, 3) polyploidy is confirmed in the species group and appears to directly influence the observed patterns of interspecific sexual compatibility and the chromosome numbers of hybrids, and 4) F1 hybrids exhibit a mosaic of parental, intermediate, and transgressive phenotypic characters that render hybrid discrimination difficult (especially in light of pronounced parental phenotypic variability).

Ultimately, despite the presence of pre- and post-mating reproductive barriers, hybridization between *Sidalcea nelsoniana* and its local congeners is still possible through human intervention. Preserving the species' current genetic integrity will require prevention of *Sidalcea* dispersal that could lead to the breakdown of spatial, temporal, and sexual barriers between species. Development of useful molecular markers will be needed to help recognize hybrids in the wild, as traditional morphological methods appear inadequate for this endeavor.

Reproductive Isolation and Interspecific Hybridization in the Threatened Species, *Sidalcea nelsoniana*

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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.

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Chapter 1: Introduction

Hybridization: historical, evolutionary, and conservation perspectives

Long overshadowed by the plight of threatened animal species and historically considered "the poor relative of the conservation world" (Ehrenfeld 1995), declining species of plants are now receiving increased recognition as both fundamental and critically imperiled components of global biodiversity (Falk and Hoslinger 1991, Fiedler and Jain 1992, Given 1994). In addition to their economic, pharmaceutical, cultural, intrinsic, and aesthetic values, plants are also acknowledged for their essential role as the primary producers that sustain functioning ecosystems. Indeed, Ellstrand and Elam (1993) conclude that, "because of the key role they play in the earth's ecosystems, plants should have the highest priority in conservation efforts." Towards this end, conservationists around the world are struggling to preserve native plant populations against the rapidly expanding threats of habitat loss, the proliferation of invasive weeds, the impacts of introduced pests and diseases, and innumerable other anthropogenic factors collectively contributing to a global mass extinction event advancing at an estimated 1,000 times the natural background rate (Raven 1999). Identifying and gaining a deeper scientific understanding of the threats facing native plant species are crucial to slowing their progression and developing effective conservation and recovery strategies.

Many of the most pressing threats confronting the world's plant populations are readily identifiable and, at least in principle (though not necessarily in practicality, due to funding and logistic limitations), straightforward to address and resolve. These threats generally fall into four categories collectively termed “the evil quartet:” over-exploitation, habitat destruction, impacts of introduced species, and chains of extinction (Diamond 1989). Whereas this well-known foursome has traditionally commanded most of the conservation spotlight for both animals and plants (Rhymer and Simberloff 1996), research performed over the last two decades has revealed other less familiar and explicit categories of threats likewise meriting serious conservation concern (Falk 1992). Prominent among these "recent arrivals" is interspecific hybridization, or the interbreeding of different species to form hybrid offspring.

Although only now gaining a foothold in mainstream plant conservation activities, interspecific hybridization has been the subject of intensive scientific inquiry and debate for over 300 years, and has long been acknowledged as a profoundly influential force in the processes of speciation. The earliest recorded inquiry into the nature of interbreeding between different plant species appears to date back to 1694, when the German philosopher, Rudolf Jakob Camerarius, first documented the existence of sexual reproduction in plants and questioned "whether a female plant can be fertilized by a male of a different kind...and whether a germ would arise from this crossing and to what extent it would be altered" (from Zirkle 1935). Shortly thereafter, in a letter dated 1716, Massachusetts colonist Cotton Mather made the first recorded observation of spontaneous plant hybridization in his description of hybrids produced in mixed plantings of Indian and yellow corn, as well as gourds and squash (Zirkle 1935).

Broader recognition of hybridization and its possible evolutionary implications was not realized until the 1760 printing of *Disquisitio de Sexu Plantarum* by Linnaeus, whose experiments in crossing recognized species not only led to the production of "mule vegetables," but ultimately inspired the first model of speciation by hybridization. Here, Linnaeus wrote, "For it seems probable that many plants, which now appear different of the same *genus*, may in the beginning have been one plant, having arisen merely from hybrid generation" (from Zirkle 1935). Only six years after Linnaeus' groundbreaking publication, the field of experimental hybridization was further advanced by the work of Joseph Gottlieb Kölreuter, who obtained hybrids between more than fifty species of plants and demonstrated 1) the range of potential fertility expression in hybrids, 2) the possibility of overcoming hybrid sterility through backcrossing, 3) patterns of dominance and recessiveness in inheritance of parental characters in hybrids, and 4) the phenomenon of heterosis in hybrids (Goryunov 1962).

Nearly a century after Linnaeus and Kölreuter, appreciation of hybridization's evolutionary significance was rekindled, this time by the landmark writings of Darwin (1859) and Wallace (1889), which in turn inspired early twentieth century evolutionary literature by influential authors such as Lotsy (1916), Fisher (1930), Dobzhansky (1937), Wright (1940), Mayr (1942), Heiser (1949), Anderson (1949), Stebbins (1950), Clausen (1951), and Grant (1963). In the past few decades, fueled by the development of molecular analytical techniques, there has been yet another resurgence of scientific interest in plant hybridization, especially with regard to its role in phylogenetic relationships and microevolutionary processes (Arnold 1997, Rieseberg and Carney 1998).

This renewed academic attention, however, has been matched by a dawning consciousness of hybridization's significant and potentially harmful implications to the conservation of rare plant species, as reflected by Ellstrand's (1992) admonition that "interspecific gene flow is perhaps the greatest gene flow hazard in plant conservation genetics," and the similarly foreboding conclusion by Wolf et al. (2001) that, based upon simulation models, "...hybridization is perhaps the most rapidly acting genetic threat to endangered species, with extinction often taking place in less than five generations."

How is it that this long-recognized and important evolutionary process poses such a threat to rare plant species? At the most fundamental level, hybridization is believed to promote the decline or eventual extinction of some rare species through reproductive fitness reductions and inhibition of population growth. One of several ways such consequences may come about is through the simple co-occurrence and interbreeding of related species (or parental species and their hybrids), potentially resulting in reproductive interference through pollinator competition, stigmatic clogging by foreign pollen, and overall wasting of gametes on interspecific matings at the expense of "legitimate" conspecific seed production (Waser 1983, Armbruster and Herzig 1984, Armbruster and McGuire 1991, Levin et al. 1996). Such negative reproductive interactions would be especially problematic for rare plant species or populations already constrained by low seed production and depressed recruitment of new individuals.

Reproductive interference may take place even when actual gene transfer and hybrid formation do not occur. However, if interspecific pollen exchange leads to the successful production of hybrids, parental fitness may face further limitations posed by outbreeding depression--the break up of locally coadapted gene complexes caused by the combining of disparate genomes (Dobzhansky 1937, Grant 1963, Riesberg 1991b, Ellstrand 1992, Lynch 1991, Fenster and Dudash 1994, Levin et al. 1996, Siikamäki 1999, Wasser et al. 2000). The adverse impacts of outbreeding depression to rare species can be profound; indeed, Ellstrand and Elam (1993) state, "the dramatic fitness consequences of outbreeding depression may account for the occasional reports of unusually low seed set when an endangered species is sympatric with a common relative." These authors go on to recommend the removal of cross-compatible gene flow sources from rare plant populations under some circumstances, "because of the substantial fitness losses accrued from outbreeding depression."

Whereas outbreeding depression may reduce the fitness of parental species through the production of unfit hybrid progeny, as discussed later, not all interspecific combinations yield such "inferior" products. Production of more vigorous, competitive, and fertile hybrids may not only compound existing fitness-related threats by enhancing the numerical disadvantage of parental species (Levin et al. 1996), but also lead to more severe perils posed by competitive exclusion and genetic assimilation.

Competitive exclusion can become problematic when recombinant hybrid offspring are not only better suited to colonizing novel habitats not already occupied by either parent species, but are also more successful in the habitats of one or both progenitors, thereby causing their suppression and eventual displacement through competition for limiting resources (Harper et al. 1961, Lewontin and Birch 1966, Cruzan and Arnold 1993, Arnold 1997). The competitive pressure exerted on parental species by hybrids is expected to be particularly intense given their inherent genetic relatedness and corresponding ecological similarities. For instance, Darwin (1859) noted:

> "...it is the most closely allied forms—varieties of the same species and species of the same and related genera-which, from having the same structure, constitution and habits, generally come into the severest competition with each other; consequently each new variety or species, during the progress of its formation, will generally press hardest on its nearest kindred, and tend to exterminate them."

Although not explicitly identified in Darwin's passage, interspecific hybrids would presumably rank as high or higher on the "kindred scale" than even varieties of the same species. Accordingly, experimental studies have demonstrated the capacity for parental growth suppression by competitively superior F1 hybrids in variety of plant groups, including *Festuca* (O'Brien et al. 1967), *Hordeum* (Norrington-Davies 1972), and *Anigozanthos* (Hopper 1978).

Even if parental species are not physically displaced by hybrids in co-occupied habitats, numerically superior hybrids can still "swamp" their progenitors with gametes, leading to rampant backcrossing, the formation of hybrid swarms, and the eventual complete genetic assimilation or fusion of one or both parent species into a single polymorphic hybrid taxon (Anderson and Hubricht 1938, Allendorf and Leary 1988, Avise 1994, Levin et al. 1996, Arnold 1997, Ellstrand and Schierenbeck 2000). With regard to this assimilation process, Hopper (1995) concludes, "it would be reasonable to describe one or both of the [parent] species as being 'hybridized out of existence.'"

But can hybrids really be so vigorous and fecund as to bring about these negative consequences and warrant serious conservation concern? Contrary to long-held assumptions that interspecific hybrids are categorically unfit (if not entirely sterile) relative to their parents, research carried out over the past few decades has discredited this generalization, demonstrating that hybrids are frequently as fit, or *more* fit (due to heterosis), than their parents (see review by Arnold and Hodges 1995). Such fitness advantages have even been noted among hybrids that *are* sterile, yet nonetheless capable of outcompeting their fertile parents through vigorous clonal expansion (Harper et al. 1961). Moreover, the degree of hybrid "superiority" can become especially apparent if hybrid fitness is evaluated just a few generations after initial formation, subsequent to the elimination of maladaptive gene combinations through selection (Clausen 1951, Rieseberg and Carney 1998). The lack of categorical weaknesses among hybrids, combined with the inordinate vulnerability of small rare plant populations to directional interspecific gene flow (Ellstrand and Elam 1993), elevates the level of concern about the potential extent and severity of genetic assimilation and other hybridization-related threats to rare native species.

In addition to the manifold threats identified above, an increasing volume of evidence suggests hybridization might also be capable of indirectly impacting rare species through the elevation of pest and disease pressures. Here, hybrids have frequently been shown to exhibit immoderate susceptibility to pests and disease outbreaks, apparently due to the disruption of genetic resistance mechanisms (Eckenwalder 1984, Whitham 1989, Ericson et al. 1993, Fritz et al. 1994, Strauss 1994, Whitham et al. 1994, Floate et al. 1997, Fritz 1999). In turn, these susceptible hybrids can serve as "hybrid bridges" (Floate and Whitham 1993) or "staging areas" (Levin et al. 1996) that encourage pest host shifts to previously unaffected (or less affected) parental species. In this sense, hybrid-facilitated pests could further contribute to the decline of rare species already burdened by other anthropogenic constraints.

Lastly, in the advent of modern agricultural practices, hybridization may also pose a new (and still poorly understood) threat to rare plant species through the possible spread of transgenes from genetically engineered crops, with unknown consequences for weed control, competitive interactions, and other habitat issues facing both rare and more common native plants alike (Ellstrand and Hoffmann 1992, Raybould and Gray 1993, Darmency 1994, Lefol et al. 1995, Snow and Morán-Palma 1997, Ellstrand 2001).

Ultimately, hybridization may play an inconsequential role in the long-term evolutionary trajectories of common native plant species exhibiting broad geographic distributions and ecological amplitudes, large demographic reserves, and high levels of genetic variability. For these species, hybridization may represent little more than what some have likened to mere "evolutionary noise" (Wagner 1970, Rieseberg, Linder and Seiler 1995, Arnold 1997). However, for rare species already suffering a myriad of anthropogenic threats, and susceptible to genetic and reproductive constraints intrinsic to small and fragmented populations, hybridization could tip the scales towards steeper demographic declines or even extinction.

But does hybridization occur with adequate frequency and severity to merit legitimate concern for rare plants? Contrary to the longstanding dogma that interspecific hybridization takes place only rarely in nature, and often only after the direct prompting of human intervention (an idea seeded in the early writings of Kölreuter) (Roberts 1929), hybridization is now understood to represent a common and pervasive phenomenon affecting many rare plant species. For instance, a survey by Ellstrand and Elam (1993) implicates hybridization in the extinction of six different species of plants, finds interspecific mating likely in 19 percent of 743 sensitive plant species in California, and documents hybridization in 10 percent of 93 protected plants in the British Isles. Numerous other researchers have identified interspecific hybridization as a primary threat to a wide variety of rare plant species (i.e., Rogers et al. 1982, Brochmann 1984, Freas and Murphy 1988, McGranahan et al. 1988, Liston et al. 1990, Rieseberg 1991b, Levin et al. 1996, Rhymer and Simberloff 1996, Daehler and Strong 1997, Imper 1997). These examples justify a growing sense of alarm among plant conservationists.

Paradoxically, despite the preceding reproachful portrayal of interspecific hybridization, it has nevertheless been argued that this evolutionary process may not only represent a less pressing threat to rare plants than suggested above, but may even prove *advantageous* to the conservation cause. Proponents of this viewpoint advance hybridization as a natural feature in the evolutionary history of plants, as reflected by Raven's (1976) suggestion that "the formation of hybrids is a consistent feature of the adaptive system of many, if not most, groups of plants," and Knobloch's (1972) assertion that "although mutation has been given the major role in effecting diversity in the natural world..., it is now quite clear to many biologists that the role of hybridization in speciation has been much larger." These characterizations are supported by studies indicating that up to 70 percent of all flowering plant species have hybridization events in their phylogenetic histories (Grant 1981, Stace 1987, Ehrlich and Wilson 1991, Whitham et al. 1991, Soltis and Soltis 1993, Masterson 1994).

Cumulatively, the aforementioned attributes have led some researchers to interpret hybridization as a “creative rather than destructive process” (Arnold 1997), contributing to expansion, rather than reduction, of global biodiversity. Applying these sentiments specifically to rare plants, it has been further proposed that hybridization might play a critical role in the conservation of some threatened and endangered species by infusing their populations with desperately needed genetic variability (Levin and Schaal 1970), which is considered “the raw material for adaptive evolutionary advance" (Lewontin and Birch 1966). Likewise, hybridization has been proposed as a possible mechanism for rare plants to escape the effects of genetic load (Ellstrand and Schierenbeck 2000), which can accumulate through the fixation of deleterious mutations in reproductively isolated populations (Charlesworth et al. 1993, Mills and Smouse 1994, Lande 1995).

Further fueling this positive interpretation of hybridization, hybrids have frequently been shown to exhibit higher levels of quantitative genetic variation (Stebbins 1969, see also review by Rieseberg and Wendel 1993), broader ecological tolerances (Anderson 1949), and greater reproductive fitness (see review by Arnold and Hodges 1995) than their progenitors, to such an extent that hybridization has been advanced as a primary stimulus for the evolution of invasiveness in weedy plants (Ellstrand and Schierenbeck 2000), and a means of injecting "aggressiveness" into rare species (Stebbins 1942). Although the intention of conservationists is certainly not to transform rare native species into invasive weeds, it appears to be the consensus among the aforementioned authors that hybrids could in essence serve as arks, rather than anchors, for sinking rare species, through creation of novel, more adaptive, polymorphic taxa that still contain the integrated genomes of the parental species, but are better suited to survival in modern human-altered ecosystems.

Regardless of the debatable constructive and destructive roles of interspecific hybridization, it is widely believed that a variety of natural crossing barriers generally arise between related species to maintain their reproductive isolation and discourage interspecific hybridization from taking place, or at least from having a significantly deleterious effect. For example, Darwin (1859) and later evolutionists (i.e., Muller 1940) recognized the pervasiveness and importance of reproductive isolation between related species, and suggested that underlying crossing barriers come about as incidental byproducts of divergent evolution in response to competition for limiting resources. Alternatively, Wright (1940) and Stebbins (1950) proposed that interspecific crossing barriers between related species might also arise through random fixation of novel but selectively neutral genes.

In contrast to these “incidental” or "random" explanations of reproductive isolation, Wallace (1889), Fisher (1930), Dobzhansky (1937, 1940) and others have suggested crossing barriers between related species might arise specifically in response to selection against hybridization. Through this process, commonly referred to as “reproductive reinforcement,” selection is expected to favor alleles (and corresponding crossing barrier traits) that confer discrimination in mating between different species, thereby discouraging maladaptive interspecific gene flow and completing the process of speciation. One potential byproduct of reproductive reinforcement is reproductive character displacement, a theory first presented by Brown and Wilson (1956) (though harkening back a century to Darwin's 1859 principle of "character divergence"), and later clarified by Grant (1975), to describe the pattern of greater divergence and effectiveness of crossing barrier traits among related species in areas of sympatry compared to areas where they do not co-occur. Evidence of these selective processes in plants and animals, their evolutionary importance, and the ongoing debate over their function and importance are reviewed by Howard (1993).

In light of the enormous ecological and genetic complexities inherent to plant sexual reproduction, there are numerous potential plant traits on which selection against hybridization can act to develop and maintain crossing barriers between co-occurring species. Levin (1971) and others have divided these manifold crossing barriers into two categories: pre-mating barriers (i.e., ecological factors that restrict interspecific pollen exchange), and post-mating barriers (i.e., multi-stage expressions of sexual incompatibility that restrict hybrid formation after interspecific pollen transfer has occurred). Pre-mating and post-mating reproductive barriers are discussed in greater detail in Chapters 2 and 3, respectively.

If the aforementioned evolutionary processes truly operate to maintain defined species boundaries through reproductive isolation, then why is interspecific hybridization currently taking place and causing such widespread alarm among conservationists? Anthropogenic activities are largely to blame, as humans have effected far-reaching changes in natural plant distribution and mating patterns, leading to the breakdown of reproductive isolating mechanisms and unprecedented opportunities for interspecific gene flow and hybridization. One way this breakdown has been unwittingly achieved is through human-mediated plant dispersal events. Worldwide, the rapid pace of human expansion has been accompanied by corresponding waves of invasions by non-native plants and animals (Harper et al. 1961, Sauer 1988, Wilson 1992, Hodkinson and Thompson 1997, Mooney and Cleland 2001). In turn, these invasions have resulted in the widespread mixing of previously isolated floras lacking common evolutionary histories of interspecific crossing barrier development, facilitating the repeated formation of hybrids (many of them exhibiting more aggressive behavior than their progenitors and capable of becoming problematic invasive weeds) (Heiser 1965, Thompson 1991, Abbot 1992, Arnold 1997, Ellstrand and Schierenbeck 2000). Indeed, as stated by Ellstrand and Schierenbeck (2000), “human-mediated dispersal may magnify the potential for hybridization by increasing the migration distances and the number of independent colonization events several fold as compared with other processes.”

Anthropogenic plant dispersal may occur through the inadvertent transport of “stowaway” propagules (Harper et al. 1961) or via the deliberate pathways of horticultural trade, agricultural practices, and (perhaps ironically) even conservation and restoration activities involving the introduction of native species into new sites—a practice increasingly used to establish and enhance populations of rare native plants (Falk and McMahan 1988, Falk 1992). Human-mediated dispersal is also directly linked to transportation routes and other types of interconnected habitat disturbances (i.e., communication, power, and gas right-of-ways) that are exploited by introduced species as corridors for expansion (Pysek et al. 1995). As a result of these processes, ranges of many native species have become honeycombed with corridors occupied by introduced species, “increasing the surface area of [interspecific] contact and accelerating the ultimate outcome of this contact" (Wolf et al. 2001).

The preceding paragraph hints at the significant role habitat disturbance may play in the frequency and trajectory of hybridization events. The important connection between habitat disturbance and hybridization was made as early as the mid-1700's, when Kölreuter concluded from his own hybridization experiments that hybrid plants are most likely to occur in nature following some type of human intervention or habitat disturbance (Roberts 1929). Nearly two hundred years later, this important association was again recognized by Wiegand (1935), and then by Anderson (1949), who poignantly wrote:

> "When he digs ditches, lumbers woodlands, builds roads, creates pastures, etc., man unconsciously brings about new combinations of light and moisture and soil conditions. At such time he may be said to 'hybridize the habitat,' and it is significant that many of the careful studies of hybridization in the field have been made in such areas."

According to Anderson, the enormous magnitude of human-caused landscape disturbances often result in the homogenization (or "hybridization") of habitats in such a way that species previously separated by differential habitat adaptations are provided new opportunities for migration, geographic intermingling, and subsequent interspecific gene flow. At the same time, Anderson points out that such disturbances also open an array of novel habitat niches better suited to the recruitment and stabilization of recombinant hybrids than to the parental species themselves (see also Anderson and Stebbins 1954).

Since the initial writings of Anderson, the connection between hybridization and habitat disturbance has been corroborated by several influential authors including Kruckeberg (1969), Grant (1981), and Arnold (1997), and has been clearly demonstrated in a wide variety of plant species (Hardin 1975, Harlan 1983, Brochmann 1984, Arnold and Bennett 1993, Levin et al. 1996, Judd et al.

1999, Wendt et al. 2001). For example, Harlan (1983) describes three grass genera, *Botrhriochloa*, *Dichanthium*, and *Capillipedium* that were historically distinct and well-defined...

> "But disturbance of the habitat [deforestation and terracing for farming] and wide transport by human activities have brought *B. bladhii* into contact with members of each of the genera. Hybridization and introgression have followed. The integrity of the genera has broken down. They are in the process of being merged by genetic aggression."

Other examples of disturbance-induced hybridization are provided by Judd et al. (1999), who state that human disturbance in Europe and North America has promoted extensive hybridization in the genera *Amelanchier*, *Crataegus*, *Vaccinium*, and *Rubus*. Moreover, the influence of habitat disturbance is noted in all 28 cases of hybridization reviewed by Ellstrand and Schierenbeck (2000). The association between disturbance and hybridization is particularly well documented in the genus *Helianthus*. Here, Ellstrand and Elam (1993) conclude that many endangered *Helianthus* species face increasing threats posed by hybridization with the weedy annual sunflower, *H. annuus*, which has dramatically expanded its range following human-caused habitat disturbances. Conversely, Heiser (1979) notes a decrease in the frequency of hybridization in several *Helianthus* species as a result of the elimination or reduction of habitat disturbances.

Regrettably, the connection between human activities and hybridization will likely become increasingly apparent in the future, not only due to increased awareness of the problem, but also because "...the key forces conducing to hybridization—anthropogenic species (and subspecies) introductions and habitat modification—are increasing with burgeoning human populations and mobility" (Rhymer and Simberloff 1996).

Given the various adverse (yet also theoretically beneficial) conservation implications of interspecific hybridization, gaining a deeper understanding of the complex ecological and genetic factors that influence its progression is crucial for developing effective conservation and recovery plans for many threatened and endangered species. Such an understanding is especially important for rare species that are intrinsically susceptible or predisposed to hybridization, such as those exhibiting some or all of the following six "high-risk" hybridization attributes:

(1) Belonging to taxonomic groups exhibiting documented examples of interspecific sexual compatibility;

(2) Having multiple sympatric and/or parapatric congeners with which interbreeding might occur;

(3) Exhibiting breeding systems characterized by facultative or obligate outcrossing (as opposed to predominantly self-fertilizing species that would be less prone to interspecific gene exchange) (Levin 1971, Ellstrand and Hoffman 1992);

(4) Possessing modes for asexual reproduction that might promote stabilization and proliferation of hybrids once they are formed, even those that are highly sterile or infrequently produced (Harper et al.

1961, Stebbins 1971, Grant 1981, Grootjans et al. 1987, Emms and Arnold 1997);

(5) Occurring in habitats or landscapes subject to intensive anthropogenic disturbance, which might result in "hybridization of the habitat" (Anderson 1949); and,

(6) Being subject to human-mediated dispersal, such as those involved in commercial trade or targeted for restoration activities.

The Study system

Individually, these six "high-risk" traits are doubtlessly common to many plant species, including those demonstrating no evidence of hybridization at all. Collectively, however, they may constitute a virtual blueprint for interspecific hybridization, and when recognized in rare plant species, should at the very least alert conservationists to the potential for hybridization-related concerns and/or opportunities. An example of one such "blueprint" scenario can be found in the rapidly shrinking native grassland habitats of northwestern Oregon, involving the state- and federally-listed threatened species, Nelson's checkermallow (*Sidalcea nelsoniana*) (Figure 1.1). This rare, herbaceous, perennial species in the mallow family (Malvaceae) exhibits all six of the aforementioned high-risk attributes:

(1) Interspecific sexual compatibility and the ability to form fertile hybrids is known to be widespread in the Malvaceae, a fact that has been lucratively exploited by breeders for the production of novel cotton varieties (Vysotskii 1962). Narrowing the taxonomic focus to the

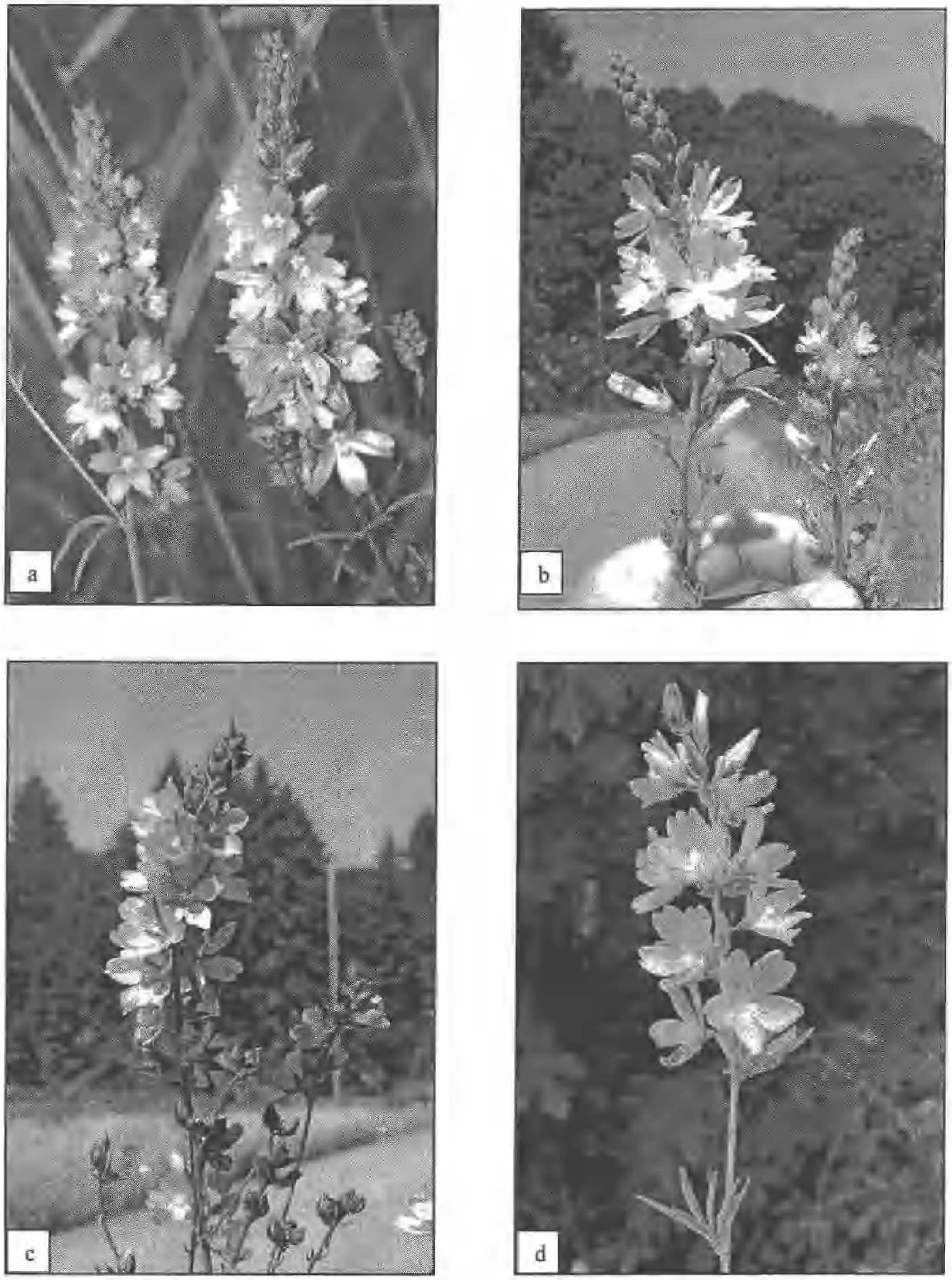


Figure 1.1 Four *Sidalcea* species inhabiting the native grasslands of western Oregon's Willamette Valley: (a) *S. nelsoniana* (female on left, hermaphrodite on right), (b) *S. campestris* (hermaphrodite on left, female on right), (c) *S. cusickii* (female), and (d) *S. virgata* (hermaphrodite).

genus *Sidalcea*, Hitchcock and Kruckeberg (1957) carried out a large-scale *Sidalcea* hybridization program and concluded:

> "On the basis of pollen fertility...and meiotic behavior of the hybrids studied to date, it appears possible that there may be no significant sterility barriers either between species or among the various infraspecific taxa in the larger species complexes."

Furthermore, in contrast to earlier assertions by Roush (1931) that hybridization does not play a significant role in polymorphism within species of *Sidalcea*, these authors go on to advance hybridization as a possible explanation for weak morphological differentiation within many members of the genus. Additional evidence of hybridization in *Sidalcea* is provided by Whittall et al. (2000), who detected molecular (ITS) evidence of allopolyploidy in the genome of tetraploid *S. virgata,* suggesting the species originated through an historic diploid x hexaploid hybridization event.

Narrowing the taxonomic focus yet further, although there is currently no direct evidence of hybridization in *Sidalcea nelsoniana*, Rhymer and Simberloff (1996) state that interspecific hybridization is "suspected of occurring" between *S. nelsoniana* and the sympatric *S. virgata* in western Oregon (the scientific basis of this suspicion is not reported). Ultimately, although this collective information fails to conclusively demonstrate that *S. nelsoniana* is capable of or currently undergoing hybridization, it does at least suggest the strong possibility that it and its relatives might be genetically pre-disposed to such events.

(2) The native grasslands of western Oregon's Willamette Valley are inhabited by three other native *Sidalcea* species with which *S. nelsoniana* could potentially interbreed: the meadow checkermallow (*S. campestris*), Cusick's checkermallow (*S. cusickii*), and the rose checkermallow (*S. virgata*) (Figure 1.1). As shown in Figure 1.1, all four species produce showy floral displays composed of numerous flowers borne on dense columnar racemes. Flowers are typically pink, with the exception of white flowers produced by *S. campestris*. Confident discrimination of these species is notoriously difficult due to indistinct vegetative characters, pronounced within-species variability in pubescence and corolla traits (the latter frequently exacerbated by sexual floral dimorphism), ambiguous habitat affinities, and the absence of other well-defined diagnostic morphological features. As such, if interspecific hybridization were to occur within this group of species in nature it might easily be overlooked, as hybrids would likely blend into the substantial background variation exhibited among phenotypes of these congeners.

(3) *Sidalcea nelsoniana* and its local congeners possess a gynodioecious breeding system, with populations of each species typically containing a mixture of individuals bearing either exclusively female (male-

sterile) or hermaphroditic (perfect) flowers. Because they lack functional anthers, female *Sidalcea* individuals rely on outcrossing for sexual reproduction, whereas hermaphroditic plants are self-compatible and capable of self-pollination (Gisler, unpublished). However, autogamy (within-flower fertilization) is prevented in hermaphroditic individuals by protandry, whereby anthesis typically occurs 1-2 days prior to full emergence and receptivity of the stigmas (Gisler, unpublished). As such, self-pollination in hermaphrodites is limited to geitonogamy (pollen exchange between separate flowers within the same individual). Therefore, female and hermaphroditic individuals alike require insect visitation for seed production, in turn opening the door to interspecific gene flow by potentially non-discriminating pollinators.

(4) Sexual reproduction in all four *Sidalcea* species (and in *S. nelsoniana* in particular) is limited by pre-dispersal seed predation by weevils (Gisler and Meinke 1997), and seedling establishment in most populations appears constrained by invasive grasses and other competitive weeds (Gisler, unpublished). Established *Sidalcea* individuals, however, are evidently long-lived and capable of varying degrees of clonal expansion via rhizomes (especially in *S. virgata*), and/or the lateral expansion of new root crowns (Gisler unpublished). This propensity for clonal expansion and long-term persistence could promote the establishment and spread of hybrids, if or when they are

formed, including those failing to reproduce sexually due to sterility or lack of mating partners (such as might occur during the colonization of new sites).

(5) The native grassland habitats occupied by *Sidalcea nelsoniana* and its Willamette Valley congeners are rapidly vanishing and undergoing severe modification due to intensive agricultural and urban encroachment. It is estimated that only one-tenth of one percent of intact native grassland remains in the Willamette Valley, leading to the area's designation as one of the most endangered ecoregions in North America (Noss and Peters 1995).

These grassland habitats of the four Willamette Valley *Sidalcea* species appear to differ subtly, primarily in terms of soil moisture regimes. Recognizing and describing these subtle habitat differences is confounded, however, by ubiquitous introduced weeds with wide ecological tolerances that displace native wet prairie and upland prairie indicator species. Nevertheless, casual observation suggests *S. nelsoniana* typically occupies the wettest sites (i.e., wetland/wet prairie/open riparian habitats), *S. campestris* and *S. cusickii* usually occupy slightly drier sites (though still seasonally saturated and harboring wetland associates), and *S. virgata* tends to require yet drier, upland prairie habitats.

Due to alteration of hydrologic regimes through the excavation of drainage ditches, underground tile-draining of agricultural fields, artificial land contouring, channelizing of rivers and streams, and construction of various water-impounding structures (i.e., roads, dikes, and railroad grades), habitats for Willamette Valley *Sidalcea* species may indeed have become "hybridized," potentially resulting in historically unprecedented opportunities for interbreeding and the opening of novel niches for hybrid recruitment and establishment (if hybrids are in fact formed).

(6) All four *Sidalcea* species, including the threatened *S. nelsoniana*, are attractive wildflowers suitable for use in landscaping and native plant gardening, rendering them subject to dispersal through horticultural trade and unauthorized collecting. Despite its listing by the State of Oregon as a threatened species, which imposes various commercial trade restrictions (see Oregon Administrative Rules 603-73-100), *S. nelsoniana* is still available for sale at several western Oregon nurseries, presumably because the nursery stock was acquired prior to the species' listing (which would technically be legal, with some restrictions, under state law). The remaining three *Sidalcea* species are not legally protected or subject to administrative trade restrictions, and are likewise available through local nurseries. Given their horticultural value and commercial availability, opportunities now exist for moving these species beyond their natural geographic and ecological ranges, increasing the likelihood for their mutual contact.

An example of this type of contact could, until recently, be observed in the display garden outside of Cordley Hall at Oregon State University in Corvallis, Oregon, where three of the four Willamette Valley *Sidalcea* species were planted together in the same flowerbed (*S. virgata* has since been inexplicably removed from the bed, leaving behind *S. nelsoniana* and *S. campestris*). The author has also observed mixed plantings of native *Sidalcea* species in several other private garden settings in and around the city of Corvallis, an area harboring the largest concentration of extant *S. nelsoniana* populations.

Sidalcea nelsoniana and its local congeners are also subject to dispersal through conservation and habitat restoration activities. These activities range from "unofficial" introductions by ostensibly well-meaning individuals and conservation organizations on private lands, to state- and federally-sanctioned introductions of new populations on public lands (Gisler, unpublished). The latter are proposed in the federal recovery plan for *S. nelsoniana* (U.S. Fish and Wildlife Service 1998), and have already been carried out at several locations in western Oregon. In no case, to the author's knowledge, has any effort been made to identify occurrences of other *Sidalcea* species in restoration areas prior to project implementation, and in several instances *S. nelsoniana* introduction sites have indeed been occupied or closely neighbored by at least one other *Sidalcea* species (Gisler, unpublished). As such, these conservation efforts could unwittingly lead to opportunities for interspecific pollen flow and its unpredictable outcomes.

Study objectives

The objective of the current study is to investigate the nature and efficacy of reproductive isolation in *Sidalcea nelsoniana,* in part to shed light on the ecological and genetic mechanisms underlying sympatric speciation in the Willamette Valley *Sidalcea*s, but more importantly as a means of evaluating the likelihood for interspecific hybridization in *S. nelsoniana* and predicting the conditions that might promote its occurrence. This information will then be used to develop hybridization-related conservation and recovery recommendations for this threatened species.

It should be noted that this study is not aimed at passing judgment on whether hybridization is good or bad for *Sidalcea nelsoniana* (though the contrasting consequences of hybridization will be discussed), or whether hybridization has historically occurred in this species group. Addressing the latter question (i.e., with DNA or isozyme techniques) would not necessarily increase our understanding of the current and future *potential* for hybridization in *S. nelsoniana*, nor assist in predicting the various conditions that might encourage hybridization, regardless of whether it has historically taken place or not. Likewise, such an approach would provide little information about the underlying ecological and genetic mechanisms that might be at work to *prevent* opportunities for interspecific gene flow and hybridization in the species group. Nevertheless, the extent and patterns of past hybridization in *Sidalcea* are certainly of phylogenetic and conservation interest, and it is hoped the current study will stimulate future research into this issue.

As briefly discussed earlier in this chapter, there are numerous ecological and genetic hurdles that must be overcome in order for hybridization to successfully occur between *Sidalcea nelsoniana* and its congeners. First, pollen from one species must be delivered to the stigmas of another. This delivery can only take place if the different species exhibit temporal synchrony in flowering, if they grow in sufficient spatial proximity to permit pollinator-mediated gene flow, if they share the same insect visitors, and if these shared insects actually transfer pollen between species. These ecological, pre-mating factors are investigated and discussed in Chapter 2. Subsequently, even if interspecific pollen exchange successfully takes place, interbreeding species must be sexually compatible to achieve pollen germination and pollen tube growth, fertilization, and formation of viable zygotes, embryos, germinable seeds, and fertile hybrid progeny. Sexual incompatibility (post-mating) barriers to hybridization between *S. nelsoniana* and its congeners are addressed in Chapter 3. This chapter also investigates *Sidalcea* cytogenetics, or more specifically, the nature and role of polyploidy as it relates to interspecific sexual incompatibility and hybridization in this species group. Chapter 3 concludes by addressing the expression of parental phenotypic traits in artificially produced F1 interspecific hybrids, and its implications for our ability to detect the occurrence of *Sidalcea* hybridization in nature (if it exists). Lastly, in Chapter 4 this information on pre- and post-mating reproductive isolation is brought to a common conclusion and used as a foundation for the development of hybridization-related conservation recommendations for *S. nelsoniana*.

Chapter 2: Pre-mating Reproductive Isolation and Interspecific Hybridization in the Threatened Species, *Sidalcea nelsoniana*

Abstract

Understanding the nature and efficacy of pre- and post-mating reproductive isolating mechanisms in rare plant species is important for evaluating their vulnerability to the manifold threats (and receptivity to the theoretical benefits) posed by interspecific hybridization. In this chapter I investigate ecological factors influencing pre-mating reproductive isolation in the threatened species, *Sidalcea nelsoniana*, which inhabits shrinking native grassland habitats with three potential heterospecific mating partners in the Willamette Valley of western Oregon. Inventories of anthropogenic habitat disturbances were also performed at extant *S. nelsoniana* populations to evaluate their potential association with the presence and efficacy of ecological interspecific crossing barriers. Results indicate that although *S. nelsoniana* exhibits geographical and ethological (pollinator) overlap with its congener, *S. virgata*, interspecific hybridization in this species pair is discouraged by narrow temporal isolation arising through asynchronous flowering. Neither temporal nor ethological isolation separate *S. nelsoniana* from *S. cusickii*, though hybridization in this species pair is discouraged by narrow, yet apparently complete, geographical isolation—their respective ranges are parapatric, currently separated by less than 3 km. No pre-mating crossing barriers are detected between *S. nelsoniana* and *S. campestris*, which overlap in flowering times, frequently co-occur in mixed

populations, and share pollinators. Pollinator overlap among the *Sidalcea* species is inferred both through analysis of each species' visiting insect assemblages and documentation of interspecific pollen exchange in an experimental mixed-species array. The results of this study raise the questions of (addressed in Chapter 3) whether post-mating crossing barriers might still intervene in hybrid formation between ecologically overlapping *S. nelsoniana* and *S. campestris*, and whether such genetic barriers might also serve as supplemental obstacles to hybridization between *S. nelsoniana*, *S. virgata*, and *S. cusickii*, should existing pre-mating reproductive isolating mechanisms become broken down by random or anthropogenic events. Lastly, inventories show varied yet ubiquitous anthropogenic habitat disturbances among virtually all extant Willamette Valley *S. nelsoniana* populations (including both those with and without co-occurring congeners), so no conclusions can be confidently drawn regarding the association between disturbance regimes and the lack or breakdown of pre-mating crossing barriers.

Introduction

As discussed in Chapter 1, despite interspecific hybridization's longstanding recognition as a fundamentally important force in the process of speciation (an idea dating back to Linnaeus), only the last two decades have brought about realization of its additional significance as a formidable threat to the genetic integrity and survival of rare plant species throughout the world—a threat with varied and complex manifestations including reproductive interference and gamete wasting, outbreeding depression, elevated pest and disease pressures, competitive exclusion, and genetic assimilation. Alternatively, Chapter 1 also addresses the contrasting interpretation of hybridization as a theoretically beneficial process for rare species suffering the adverse consequences of depressed adaptive genetic variability and accumulated genetic load.

Regardless of its debatable harmful and beneficial conservation implications, hybridization is ultimately thought to be discouraged by a variety of selective and/or random processes (see Chapter 1) that lead to the origin and maintenance of reproductive barriers between interfertile species. However, as indicated in Chapter 1, the natural ecological conditions in which these processes operate to sustain distinct species boundaries have, in many ecosystems, been dramatically, if unwittingly, altered by human modifications to native habitats and natural plant dispersal patterns. The ensuing breakdown of crossing barriers and promotion of hybridization caused by such anthropogenic ecological meddling has been well documented (see Chapter 1); what remains unclear is whether rare species, already limited by a myriad of other human-caused threats, possess the demographic reserves and overall resiliency to withstand such hybridization events. As discussed in Chapter 1, an increasing body of evidence indicates many rare species do not.

Given the importance of interspecific hybridization to the conservation of rare plant species, and the expanding anthropogenic impacts promoting its occurrence, achieving a thorough understanding of reproductive isolation and hybridization potential should be considered a fundamental component of conservation and recovery planning for many threatened and endangered species, particularly those exhibiting various "risk factors" predisposing them to hybridization (see Chapter 1). Such an understanding must begin with, or at least encompass, an analysis of the crossing barriers underlying and directly influencing interspecific hybridization. This task, however, is complicated by the diversity and complexity of plant reproductive characters on which selection (or other random mechanisms discussed in Chapter 1) can act to bring such multi-faceted barriers about. To help clarify the identity and respective roles of reproductive isolating mechanisms, Levin (1971) and others have grouped them into two functional categories: pre-mating (ecological) crossing barriers that restrict opportunities for interspecific pollen exchange, and post-mating (genetic) crossing barriers that limit hybrid production through sexual incompatibility following successful interspecific mating.

In this chapter I investigate the nature and efficacy of pre-mating crossing barriers to hybridization in the threatened species, *Sidalcea nelsoniana* (Malvaceae), which inhabits rapidly shrinking native grassland habitats with three other native congeners (*S. campestris*, *S. cusickii*, and *S. virgata*) in the Willamette Valley of western Oregon (Figure 1.1 in Chapter 1). This information is then used to assess the current level of ecological reproductive isolation in *S. nelsoniana*, predict what conditions might encourage interspecific hybridization now and in the future, and evaluate whether any observed gaps or breakdowns in pre-mating crossing barriers might be attributable to anthropogenic plant dispersal events or habitat modifications, the likes of which have rendered native

Willamette Valley grasslands among the most endangered ecoregions in North America (Noss and Peters 1995).

Three primary categories of pre-mating crossing barriers are addressed in this study: reproductive isolation through asynchronous flowering (temporal isolation), spatial separation of populations due to distributional/habitat differences (geographical isolation), and reproductive isolation arising through pollinator specificity (ethological isolation). Mechanical isolation related to differential floral morphology has also been treated as an additional pre-mating crossing barrier in some hybridization studies, though this factor is not included here because all four *Sidalcea* species share the same general open flower structure (Figure 1.1).

The presence of any one of the aforementioned reproductive barriers would be sufficient to interrupt the pathway for interspecific pollen exchange and thus discourage subsequent hybridization between *Sidalcea nelsoniana* and its congeners. Conversely, *all three* barriers must be successfully overcome for interspecific pollen exchange to take place. An understanding of these pre-mating factors is important, because even if *S. nelsoniana* and its congeners prove to be sexually compatible and capable of forming hybrid offspring under artificial conditions (see Chapter 3), this information would be of little practical conservation or evolutionary consequence if there is no ecological potential for interspecific mating.

The first pre-mating interspecific crossing barrier addressed in this study is temporal isolation, sometimes referred to as phenological or seasonal isolation.

This barrier is significant because even if related species overlap in fine-scale geographic distribution and pollinators (discussed below), interspecific mating cannot take place unless there is also a window of temporal overlap opened by synchronous flowering. Separation of flowering times has been identified as a common evolutionary stimulus of sympatric speciation (Richards 1997) and an important reproductive isolating mechanism in a wide variety of co-occurring species, including *Phlox* and *Taraxacum* (Levin 1971), *Lactuca* (Whitaker 1944), *Agrostis* (McNeilly and Antonovics 1968), *Erigeron* and *Aster* (Armbruster and McGuire 1991), *Iris* (Young 1996), *Spiranthes* (Sun 1996), and numerous short-blooming tropical taxa (Grant 1963). Moreover, asynchronous flowering has been shown to be a direct result of selection against hybridization in maize (Paterniani 1969), and Stace (1975) lists numerous interfertile species pairs in the British flora that are separated by flowering time. Here, Stace also notes that hybridization is most common in areas where overlap of flowering time is greatest. Given the clear importance of temporal isolation in regulating interspecific pollen flow, one objective of this study is to document the seasonal progression of flowering in *Sidalcea nelsoniana* and its three potential heterospecific mating partners, and evaluate the extent to which their respective phenologies may favor or discourage reproductive isolation.

Provided *Sidalcea nelsoniana* and its relatives exhibit flowering synchrony, interspecific pollen exchange still cannot take place between congeners that do not also spatially co-occur. Although it is generally acknowledged that the four *Sidalcea* species in this study exhibit more or less overlapping geographic ranges (and largely overlapping grassland habitat affinities, see Chapter 1) in the Willamette Valley of western Oregon, more precise information is lacking on the degree of local proximity between extant *S. nelsoniana* and its congeners. An understanding of fine-scale distributions is necessary to evaluate the potential influence of human activities on spatial relationships between species and for assessing the overall likelihood of insect-mediated interspecific pollen exchange, in light of the predominantly short flight distances and leptokurtic foraging patterns of most pollinating insects. For instance, Hopper (1995) states, "A growing literature [i.e., Hadley and Levin 1967; Levin and Kerster 1974] is suggesting that most plant gene flow in nature occurs over distances measured in metres rather than kilometres." This view is echoed by Richards (1997), who concludes that, among most bee-pollinated plants, it is usual to find at least 80 percent of between-visit bee transitions extending less than 1m distance, and 99 percent of flights less than 5 meters. Given the predominance of such highly localized foraging distances, another objective of this study is to quantify the proximity between *S. nelsoniana* and its congeners at a scale of "meters rather than kilometers."

Even if *Sidalcea nelsoniana* and its congeners overlap in flowering times and pollinator foraging areas, simple foraging will not result in successful interspecific pollen exchange unless *S. nelsoniana* and its congeners share the same pollinators. As discussed in Chapter 1, Willamette Valley *Sidalcea*s are gynodioecious, producing mixed populations of hermaphroditic and female (male-sterile) individuals. Lack of functional anthers in females and protandry in hermaphrodites (Gisler, unpublished) render individuals reliant upon insect pollination for seed production, and therefore potentially susceptible to carryover of heterospecific pollen grains by non-discriminating insect visitors.

Lack of pollinator sharing, or ethological isolation, has been shown to inhibit interspecific gene flow in a variety of closely co-occurring species (Dobzhansky 1937, Grant 1949, 1981, Stebbins 1950, McNaughton and Harper 1960, Sprague 1962, Waser 1983, Armbruster and Herzig 1984). Moreover, natural selection specifically for ethological isolation has been demonstrated in *Phlox* (Levin and Kerster 1967), and selection against maladaptive interspecific gene flow is proposed as an explanation for the evolution of self-pollination in *Arenaria* (Fishman and Wyatt 1999).

Assortative mating between sympatric plant species may result from differences in assemblages of pollinator taxa attracted to each floral reward source, or they may arise through foraging specificity expressed by individual pollinators within shared pollinator taxa (Levin and Kerster 1973, Levin and Watkins 1984, Waser 1986, Leebens-Mack and Milligan 1998). The tendency for floral constancy to a certain flower type or species during foraging is common among bees, and to a lesser extent lepidopterans (Grant 1963), though interspecific gene flow may still occur depending on pollen longevity and rates of pollen carryover between foraging episodes (Stucky 1985, Thompson and Thompson 1989, Wolfe and Barrett 1989). Given the importance of pollinators as potential vectors for interspecific pollen transfer in *S. nelsoniana*, two additional objectives of this study are to evaluate whether the four *Sidalcea* species share the same assemblages of insect visitors, and whether these visitors discourage hybridization through host-specific foraging behavior.

Combined with data on the nature and efficacy of post-mating barriers discussed in Chapter 3, this information on pre-mating reproductive isolation will supply an ecological context for evaluating the overall potential for interspecific hybridization in *Sidalcea nelsoniana*. Cumulatively, these data will also help lay the foundation for developing hybridization-related conservation and recovery recommendations for this threatened species.

Methods

Temporal isolation

To determine the extent of flowering asynchrony between *Sidalcea nelsoniana* and its three local congeners, and evaluate its potential role as a pre-mating reproductive isolating mechanism, I documented floral development in populations of the four species at approximately two-week intervals throughout the flowering season, beginning in late March and extending through mid-August, 2000. This phenology data was collected at the following number of populations for each species: *S. nelsoniana* (n=24), *S. campestris* (n=28), *S. cusickii* (n=18) and *S. virgata* (n=26). Emphasis in study population selection was given to those located in the mid-Willamette Valley (i.e., northern Lane, Benton, western Linn, and southern Polk and Marion counties), where the species exhibit the greatest overlap and highest likelihood for interspecific contact. Within this geographical area, study populations were selected based upon their accessibility on public lands (primarily roadsides and wildlife refuges). Map locations of all populations used for the temporal isolation study are provided in Appendix 2.1.

Floral development within study populations was determined by recording the percent of inflorescences exhibiting any open flowers at each two-week measurement interval. Although varying considerably by species, age, and sex, *Sidalcea* individuals can produce up to a hundred or more inflorescences, each typically producing 1-12 branched racemes bearing 30-130 flowers. Racemes of all species are indeterminate, with the lowermost flowers opening first and the uppermost flowers opening last. As an unfortunate result of severe population fragmentation and habitat loss, most study populations were small enough to allow complete censusing of flowering inflorescences during each visit. For larger populations (i.e., those containing more than 1000 inflorescences), however, floral development was visually estimated. Random subsampling of inflorescences was not used to estimate flowering in these larger populations because this technique could have overlooked statistically infrequent flowering inflorescences very early and late in the season. Such inflorescences, although rare, are nonetheless significant insofar that they define the absolute start- and end-points of phenological expression in each population and still offer opportunities (albeit probably few) for pollen exchange early and late in the flowering season—the times that, for some species pairs, may represent the greatest or only temporal window of flowering overlap.

Geographical isolation

To determine if geographical isolation constitutes a pre-mating crossing barrier between *Sidalcea nelsoniana* and its local congeners, inventories for heterospecific *Sidalcea*s were performed at 34 extant *S. nelsoniana* populations. These inventories entailed walking outward from *S. nelsoniana* populations in progressively distant radiating circles (or, more accurately, in various radiating shapes, as dictated by land ownership/accessibility patterns and the distribution of public roads). As the previous parenthetical statement suggests, inventories were complicated by the fact that the vast majority of land in the Willamette Valley is privately owned with restricted public access. As such, by necessity, searches were typically limited to areas along, or visible from, public roads. However, due to decades of intensive agricultural and urban land development in the Willamette Valley, there are very few remaining intact native prairie remnants within private landholdings (or anywhere else for that matter), with most known extant *S. nelsoniana* populations persisting along the undeveloped margins of state highways and county roads. As such, with the exception of a few populations located in wildlife refuges, parks, and other areas with relatively contiguous and accessible habitats, roadside inventories typically encompassed the majority of available habitat.

Inventories for heterospecific *Sidalcea*s were performed twice during 2000, once in late April to facilitate detection of *S. virgata*, and once again in mid-June during peak flowering of the remaining species (see Table 2.1 in the results section of this chapter for flowering times). At each *S. nelsoniana* study population data were recorded on the presence or absence of heterospecific *Sidalcea* individuals within 500 m of the population perimeter. This sampling distance was selected as a cutoff for proximity measurements because it represents what may be considered a realistic limit for entomophilous pollen flow in an outcrossing species (Ellstrand and Elam 1993, and other bee foraging references, above). From a practical standpoint, 500 m also represents the approximate maximum distance at which flowering *Sidalcea* species can be recognized inside private lands from adjacent roadsides or property boundaries using binoculars. When *Sidalcea* species were observed inside private lands and there was uncertainty about their identity, landowners were contacted to provide access and species determinations.

When heterospecific *Sidalcea* individuals were discovered within 500 m of *S. nelsoniana*, the distance of their separation was measured using 100 m tapes, or on restricted private lands, through ocular estimation. In addition, notes were made of any observable habitat disturbances at each site, to assess whether human activities might have contributed to "hybridization of the habitat" (see Chapter 1) and any accompanying breakdown of spatial barriers between *Sidalcea* species. Map locations of all *S. nelsoniana* populations used for geographical isolation studies are provided in Appendix 2.2.

Ethological isolation

Two methods were employed to investigate potential pollinator-related interspecific crossing barriers between *Sidalcea nelsoniana* and its local congeners. First, to identify the level of potential host specificity among pollinators of the four *Sidalcea* species, floral visitors were collected from each *Sidalcea* species throughout the flowering season in 2000 at all populations used for temporal isolation studies. Collections were only made of visitors observed directly contacting floral reproductive structures and did not include other insects associated with foliage and/or those using flowers exclusively for perching or shelter. Upon collection, insects were incapacitated in glass jars containing ammonium carbonate and then pinned and stored in sealed boxes for subsequent identification.

Secondly, even if *Sidalcea nelsoniana* and its Willamette Valley congeners exhibit overlap of visiting insect taxa, it does not necessarily follow that individual pollinators (within common taxa) perform indiscriminate *Sidalcea* foraging that leads to interspecific pollen exchange. To determine if non-constant foraging and consequent pollen exchange occurs in nature, an artificial array of mixed-species cut flowering racemes was constructed to document and quantify interspecific pollen transfer. This array also allowed investigation of potential host species-based visitation preferences exhibited by pollinators.

The mixed-species array consisted of 40 *Sidalcea* racemes (10 replicates for each of the four different species) introduced into a natural *S. nelsoniana* population ("Decker Road"-- population #2 in Appendix 2.2) on June 19, 2001. All introduced racemes were single-stemmed, and were comprised exclusively of female flowers that had developed within breathable polyethylene mesh pollinator exclusion bags to prevent stigma contamination by previous pollen deposition. All bagged racemes originated from natural populations located within 20 km of the array. However, because local *S. virgata* populations had already completed flowering by the time the array was constructed (see discussion of flowering times in Results, below), a high elevation (approximately 500 m) population was used to supply bagged female *S. virgata* racemes for the array. As the *S. nelsoniana* population into which racemes were introduced harbors no nearby (within 1 km) heterospecific *Sidalcea* individuals, all *Sidalcea* pollen grains observed on stigmas of array flowers were assumed to have originated from hermaphroditic *S. nelsoniana* individuals within the study site. Exclusive use of female racemes in the experiment not only facilitated the documentation of interspecific pollen receipt (because they produced no pollen themselves), but also prevented inadvertent export of heterospecific pollen into the natural population.

Because cut flowering stems of all four *Sidalcea* species remain fresh and continue to flower when placed in water, and sometimes even form roots and become capable of independent growth if maintained properly (Gisler, unpublished), they served as a more convenient experimental unit than entire transplanted *Sidalcea* individuals. Racemes for the mixed-species array were supplied by randomly assigning cut flowering stems (extracted from pollinator exclusion bags) to 473 ml (16 fl. oz.) clear glass bottles filled with water. Prior to raceme assignments, these bottles were attached to the tops of bamboo garden stakes with transparent packaging tape, and stakes were then inserted into the soil such that all racemes were elevated to a height similar to that of *S. nelsoniana* inflorescences in the surrounding natural population (approximately 90 cm). The purpose of elevating flowers in this manner was to avoid possible discrimination and neglect of experimental racemes by bees, which are known to exhibit vertical constancy when foraging within a patch (Levin and Kerster 1973, Levin and Watkins 1984, Peakall and Handel 1993, Leebens-Mack and Milligan 1998). Grass foliage was taped on to the outside of the glass bottles to camouflage them and minimize their potential visual impacts on pollinator foraging behavior.

All 40 glass bottles were evenly inserted in a circle (approximately 2.5 meters in diameter) surrounding a single large hermaphroditic individual approximately 1.5 meters in diameter and containing 352 flowering racemes (producing approximately 3000 open flowers during the two-day study period). This hermaphroditic individual was naturally isolated from other hermaphrodites in the population by approximately 15 meters, so presumably served as the primary pollen source for the array. The array was constructed around this single hermaphrodite, rather than spaced throughout the spatially and sexually heterogeneous population, to provide more or less consistent pollen access and environmental conditions among all introduced racemes. Racemes were randomly assigned to glass bottles in the early morning (0700), well before peak insect activity, and pollen flow data were recorded after 34 hours.

For each raceme in the array I tallied (*in situ*) the total number of *Sidalcea* pollen grains on stigmas of mature flowers to obtain per flower means for each raceme. Although immature (but still open) flowers may contribute to overall floral displays of racemes, they cannot receive pollen grains because their multiple (7-9) styles are united in a column with their stigmatic surfaces oriented inward, whereas mature flowers possess styles that are exerted radially, exposing their receptive stigmas. *Sidalcea* pollen grains are spherical, large (60-70 microns, Halse et al. 1989), and have a distinctive, uniformly spiny surface that rendered them easily recognizable on stigmas. Pollen grain tallies were performed on the following cumulative number of mature flowers among the 10 racemes of each species: *S. campestris*=80, *S. cusickii*=150, *S. nelsoniana*=148, and *S. virgata*=52 (such differences in floral output are typical of the species).

Presence of *Sidalcea* pollen on flowers in the array was considered positive evidence of interspecific pollen flow (or evidence of *intraspecific* pollen flow, in the case of introduced *S. nelsoniana* array racemes serving as experimental controls). Univariate analysis of variance with post hoc Tukey means comparisons (SPSS 10.0) was used to test differences in mean pollen deposition between the four *Sidalcea* species. The interaction factor of species x floral display size (total open flowers per stem) was included in the full model analysis to test the significance of its effect on differences in mean pollen deposition between species. Pollen deposition data were log-transformed prior to analysis to improve distribution normality.

Results

Temporal isolation

Data on *Sidalcea* flowering times recorded in 2000 reveal almost perfect flowering synchrony in three of the four species: *S. nelsoniana*, *S. campestris*, and *S. cusickii*. Excluding a geographically isolated group of five *S. campestris* populations (discussed below), mean onset of flowering in these species co-occurred within a 10 day period in late May/early June, and mean peak flowering co-occurred within a 13 day period in late June/early July (Table 2.1 and Appendix 2.3). In addition to temporal synchrony, mean *levels* of flowering (percent total inflorescences with open flowers) over time were also nearly identical among this species trio (Figure 2.1). As stated above, the exception to this phenological consistency was a group of *S. campestris* outlier populations, all located east of the Willamette River in western Linn County, which commenced flowering 30-45 days earlier than conspecific populations elsewhere in the species' range (see Appendix 2.3). Given their anomalous phenology and restriction to a small area (where they do not overlap with *Sidalcea nelsoniana*) these populations are acknowledged here but are not included in flowering calculations.

In contrast to the three co-flowering *Sidalcea* species identified above, flowering is *asynchronous* in *S. virgata*, with mean initiation and subsequent peak of flowering 51 and 44 days earlier than that of *S. nelsoniana*, respectively (Table 2.1 and Appendix 2.3). Although these timing differences are pronounced, there is a *minor* degree of overlap (13.5 days) between the mean end of flowering in *S. virgata* study populations and mean start of flowering in *S. nelsoniana* study populations. However, this calculation encompasses all study populations of both species, including those that are widely separated and span various latitudes and elevations. If only closely neighboring *S. virgata* and *S. nelsoniana* populations are considered (i.e., those with more realistic geographical opportunities for pollen exchange), such as the five neighboring heterospecific population pairs

Table 2.1 Mean timing (calendar dates) of start, peak, and end of flowering in *Sidalcea nelsoniana* and its three Willamette Valley congeners in 2000.

Species	**Number study populations**	**Mean start date**	**Mean peak date**	**Mean end date**
S. campestris	22	May 29 (SD 10.3 days)	June 28 (SD 10.7 days)	August 2 (SD 10.0)
S. cusickii	18	June 9 (SD 6.31 days)	July 11 (SD 6.8 days)	August 12 (SD 5.8 days)
S. nelsoniana	24	June 5 (SD 11.2 days)	June 30 (SD 11.2 days)	July 31 (8.3 days)
S. virgata	26	March 16 (SD 12.7 days)	May 17 (SD 12.5 days)	June 18 (SD 14.6 days)

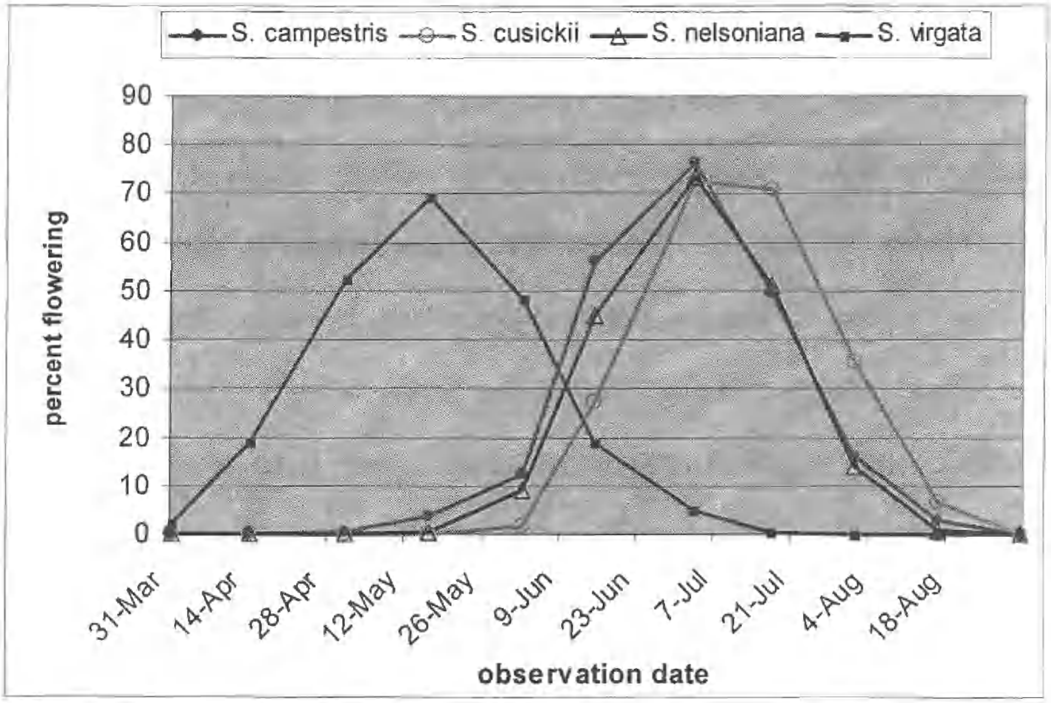


Figure 2.1 Mean percent inflorescences with open flowers over time (measured at approximate two-week intervals) in study populations of *Sidalcea nelsoniana* (n=24 populations), *S. campestris* (n=22 populations), *S. cusickii* (n=18 populations), and *S. virgata* (n=26 populations). Complete flowering data for all populations are provided in Appendix 2.3.

located in and around Finley National Wildlife Refuge in southern Benton County, then asynchrony is even more pronounced (mean overlap of 0 days, range -13 to 13 days) (Table 2.2).

When all *Sidalcea nelsoniana* and *S. virgata* populations are once again considered together, mean levels of flowering (percent inflorescences exhibiting any open flowers) during the brief period of flowering overlap are low, peaking at 30 percent (Figure 2.1). As later addressed in the discussion section of this chapter, the degree of reproductive isolation afforded by flowering asynchrony between *S. nelsoniana* and *S. virgata* may be further elevated by female-biased sex ratios and patterns of sex expression during the period of greatest flowering overlap.

Geographical isolation

Inventories conducted at 34 extant *Sidalcea nelsoniana* populations indicate this species frequently co-occurs with *S. campestris* and *S. virgata* at a fine spatial scale (i.e., less than 500 m), but does not co-occur with *S. cusickii*. As shown in Table 2.3, *S. virgata* is found in proximity to *S. nelsoniana* at eight of the 34 study populations, with the nearest occurrence observed within 65 meters. Geographic overlap is more pronounced in *S. campestris*, which is present at 24 of the 34 *S. nelsoniana* populations, and in 15 of these cases the two species occur in essentially mixed populations (i.e., less than 20 m separation between nearest heterospecific individuals).

Table 2.2 Progression of flowering in closely neighboring populations of *Sidalcea nelsoniana* and *S. virgata* in and around William Finley National Wildlife Refuge in southern Benton County, Oregon. Values represent percent of total inflorescences in each population exhibiting open flowers. Observations were made at approximately two-week intervals. Flowering data for all study populations are provided in Appendix 2.3.

Species	**Site**	**3/31**	**4/14**	**5/1**	**5/16**	**6/2**	**6/15**	**7/3**	**7/17**	**8/1**	**8/16**	**8/31**
S. nelsoniana	Fern Rd	0	0	0	0	4.8	35.7	95.2	52.4	9.5	0	0
S. virgata	Fern Rd.	10.7	60.0	84.0	20.0	12.0	0	0	0	0	0	0
S. nelsoniana	Decker Rd	0	0	0	0	5	25	90	65	10	0	0
S. virgata	Decker Rd	6.0	47.8	91.0	67.2	11.9	0	0	0	0	0	0
S. nelsoniana	Finley Kiosk	0	0	0	0	4.5	22.3	84.3	64.7	11.6	0	0
S. virgata	Finley Kiosk	0	10.5	52.6	90.8	11.8	3.9	0	0	0	0	0
S. nelsoniana	Finley Wdpkr	0	0	0	0	0	16.4	74.5	81.8	10.9	0	0
S. virgata	Finley Wdpkr	0	20.8	70.8	91.7	12.5	0	0	0	0	0	0
S. nelsoniana	Finley Bruce	0	0	0	0	16.7	58.1	87.1	77.4	16.1	0	0
S. virgata	Finley Bruce	12.2	63.4	89.0	61.0	8.5	0	0	0	0	0	0

In contrast, *S. cusickii* is not found in proximity to any extant *S. nelsoniana* populations. Extensive inventories performed during 2000 and 2001 indicate the northernmost limit of *S. cusickii*'s range currently extends to the southern border of William Finley National Wildlife Refuge in southern Benton County, Oregon—the same location that marks the southernmost distribution of *S. nelsoniana*. As such, *S. nelsoniana* and *S. cusickii* are parapatric, separated by less than 3 km, with populations of each species distributed approximately 200 km to the north and south, respectively.

All 34 *Sidalcea nelsoniana* study populations were surveyed for obvious signs of human-caused or other habitat disturbances that might reflect the occurrence of "hybridization of the habitat" (see Chapter 1). As shown in Table 2.3, all but one population exhibited clear evidence of intensive habitat disturbances, routinely involving excavation of roadside drainage ditches, construction of elevated roads and railways, and agricultural practices (i.e., cultivation, ditch and dike construction, ground contouring, and possible underground tiling for improved drainage). As these disturbances are ubiquitous across *S. nelsoniana* study populations, regardless of the presence or absence of congeners, no correlations can be drawn between habitat disturbance and the lack of interspecific geographical reproductive barriers.

Table 2.3 Summary of nearest congener distances (≤ 500 m) and disturbance regimes at 34 *Sidalcea nelsoniana* populations. Population map numbers correspond with those in Appendix 2.2.

Site (map #)	**Co-occurring congeners**	**Distance (m)**	**Disturbance regime**
Fern Rd (1)	*S. virgata*	500	Roadside drainage ditch, adjacent to elevated cut bank and cultivated fields
Decker Rd (2)	None	N/A	Roadside drainage ditch, adjacent to cultivated fields and agricultural ditch
Finley Kiosk (3)	*S. campestris*	6	Water-impounding gravel road and adjacent to cultivated fields
	S. virgata	440	
Finley Wdpkr (4)	*S. virgata*	65	None apparent, although bordered on two sides by cultivated fields
Finley Bruce (5)	*S. campestris*	3	Roadside and agricultural drainage ditches, surrounded by cultivated fields
	S. virgata	215	
Finley McFlnd (6)	none	N/A	Roadside drainage ditch, adjacent to water-impounding dikes
Ridge Dr (7)	*S. campestris*	45	Roadside and agricultural drainage ditches, adjacent to water-impounding railroad grade and cultivated fields
	S. virgata	190	
Miller Cmtry (8)	*S. campestris*	7	Roadside drainage ditch, adjacent to artificially channelized stream and cultivated fields
	S. virgata	250	
Aumsville pit (9)	*S. campestris*	12	Roadside drainage ditch and excavated man-made ponds
Burklnd lumbr(10)	*S. campestris*	70	Roadside drainage ditch, adjacent to channelized stream and cultivated fields
Salem Airport(11)	*S. campestris*	260	Drainage ditch, adjacent to road and airport runway construction, culverts, and buried sewerline

Table 2.3 (Continued)

Site (map #)	**Co-occurring congeners**	**Distance (m)**	**Disturbance regime**
Cordon Rd (12)	none	N/A	Roadside drainage ditch, adjacent to cultivated fields and residential development
SR 22 (13)	*S. campestris*	5	Highway shoulder, adjacent to roadside ditch, drainage culvert, and cultivated fields
SR 99W (14)	*S. campestris*	4	Highway shoulder, adjacent to roadside ditch and cultivated fields
Dyck Rd (15)	*S. campestris*	35	Roadside drainage ditch and artificially channelized stream, adjacent to cultivated fields
VanWell Rd (16)	*S. campestris*	105	Artificially channelized stream and roadside drainage ditch, adjacent to cultivated fields
Guthrie Rd (17)	*S. campestris*	4	Roadside drainage ditch and adjacent to cultivated fields
E.E. Wilson 1 (18)	*S. campestris*	240	Road construction, man-made ponds and drainage patterns, disturbance associated with historic military base operations
Tampico Rd (19)	*S. campestris*	13	Roadside drainage ditch and adjacent to cultivated fields
Lewisburg (20)	*S. campestris*	7	Highway shoulder, roadside drainage ditch, and elevated railroad grade
Walnut Park (21)	*S. campestris*	12	Park maintenance activities, trail construction, adjacent to artificially channelized stream
OSU Horse (22)	*S. campestris*	19	Roadside drainage ditch, paved bike path, adjacent to drainage culvert and horse pasture
OSU Turkey (23)	*S. campestris*	15	Roadside drainage ditch, adjacent to drainage culvert and livestock pasture
Bald Hill (24)	none	N/A	Water-impounding bike path, adjacent to drainage culvert and livestock pasture

Table 2.3 (Continued)

Site (map #)	Co-occurring congeners	Distance (m)	Disturbance regime
Grand Ronde: Brown (25)	*S. campestris*	130	Roadside drainage ditch, otherwise apparently undisturbed
Grand Ronde: Bode (26)	*S. campestris*	6	Located within historically cultivated field, disturbed by excavation/fill removal
Starr Crk (27)	none	N/A	Adjacent to roadside and agricultural drainage ditches, surrounded by cultivated fields
Bellfountain (28)	none	N/A	Roadside drainage ditch and adjacent to cultivated fields
Cabel Marsh (29)	none	N/A	Water-impounding gravel road and man-made pond
Timberhill (30)	*S. campestris*	85	Located in area of historic fill removal and deposition (slated for residential development)
	S. virgata	95	
Adair Rifle (31)	*S. campestris*	10	Water-impounding gravel road and adjacent to drainage culvert
E.E. Wilson 2 (32)	*S. campestris*	55	Road construction, man-made ponds and drainage patterns, disturbance associated with historic military base operations
E.E. Wilson 2 (32)	*S. campestris*	55	Road construction, man-made ponds and drainage patterns, disturbance associated with historic military base operations

Ethological isolation

Pollinator inventories reveal that Willamette Valley *Sidalcea* species attract an impressive abundance and diversity of floral visitors, including at least 17 species of bees, three species of wasps, nine species of flies, six species of beetles, and five species of lepidopterans (Table 2.4). Although numerous pollinator taxa prove common to all four *Sidalcea* species, Table 2.4 also suggests some apparent host- (or possibly habitat-) specificity within insect assemblages. Among the floral visitors documented in this study, *S. nelsoniana* shares 15 taxa with *S. campestris* (with 13 taxa unique to one species or the other), shares 17 taxa with *S. cusickii* (13 taxa unique to one species or the other), and shares 17 taxa with *S. virgata* (18 taxa unique to one species or the other). Photographs of six bee species common to all four *Sidalcea*s, and ranking among the most frequent floral visitors, are provided in Figure 2.2. One of the pictured bees, *Diadasia nigrafrons*, belongs to a genus known to specialize on *Sidalcea* and other members of the Malvaceae (Moldenke, Oregon State University Entomology Department, personal communication).

Data from the experimental mixed-species array demonstrate insect-mediated within-species pollen flow between naturally occurring *Sidalcea nelsoniana* and introduced conspecific control racemes, as well as interspecific pollen transfer to all three heterospecific *Sidalcea* racemes in the array. However, whereas positive evidence of pollen transfer was consistent among all four species, there were markedly variable *levels* of pollen deposition among the array

Table 2.4 Insect taxa observed visiting flowers of *Sidalcea nelsoniana* and its three local congeners (positive visitation indicated by "x" in each corresponding *Sidalcea* species column).

	S. nelsoniana	***S. campestris***	***S. cusickii***	***S. virgata***
Bees				
Family: Anthophoridae				
Ceratina acantha	x	x	x	x
Ceratina micheneri	x	x	x	x
Diadasia nigrafrons	x	x	x	x
Melissodes sp.	x			
Nomada sp. 1				x
Nomada sp. 2	x			x
Synhalonia sp.	x	x	x	x
Family: Apidae				
Apis mellifera	x	x	x	x
Bombus californicus	x	x	x	x
Bombus sitkensis	x	x	x	x
Bombus vosnesenskii	x		x	x
Family: Halictidae				
Agapostemon sp.	x	x	x	x
Dialictus sp.			x	x
Halictus sp.	x		x	x
Lasioglossum sp.	x	x	x	x
Family: Megachilidae				
Megachile sp.		x		
Osmia sp.	x	x	x	x
Wasps				
Family: Ichneumonidae	x			
Family: Polistidae			x	
Family: Tenthredinidae				x
Flies				
Family: Bombylidae				
Bombylius major				x
Bombylius sp.				x
Family: Callaphoridae				
Pollenia sp.		x	x	x
Family: Conopidae				
Zodion sp.				x
Family: Cyrtidae				
Eulonchus tristis	x			
Family: Empididae				
Microphorus sp.				x
Family: Syrphidae				
sp. 1	x		x	x
sp. 2			x	
sp. 3	x	x		

Table 2.4 (Continued)

	S. nelsoniana	***S. campestris***	***S. cusickii***	***S. virgata***
Lepidopterans				
Family: Hesperiidae				
Hesperia juba	x		x	x
Family: Lycaenidae				
Strymon melinus	x	x	x	
Family: Noctuidae				
Acontia flavipennis				x
Family: Nymphalidae				
Vanessa annabella	x	x	x	x
Family: Pieridae				
Pieris rapae		x	x	
Beetles				
Family: Cerambycidae				x
Family: Chrysomelidae				
Diabrotica undecimpunctata	x	x	x	x
Family: Cleridae		x	x	x
Family: Curculionidae				
Macrorhoptus niger		x	x	x
Macrorhoptus sidalceae	x			
Family: Meloidae	x	x		

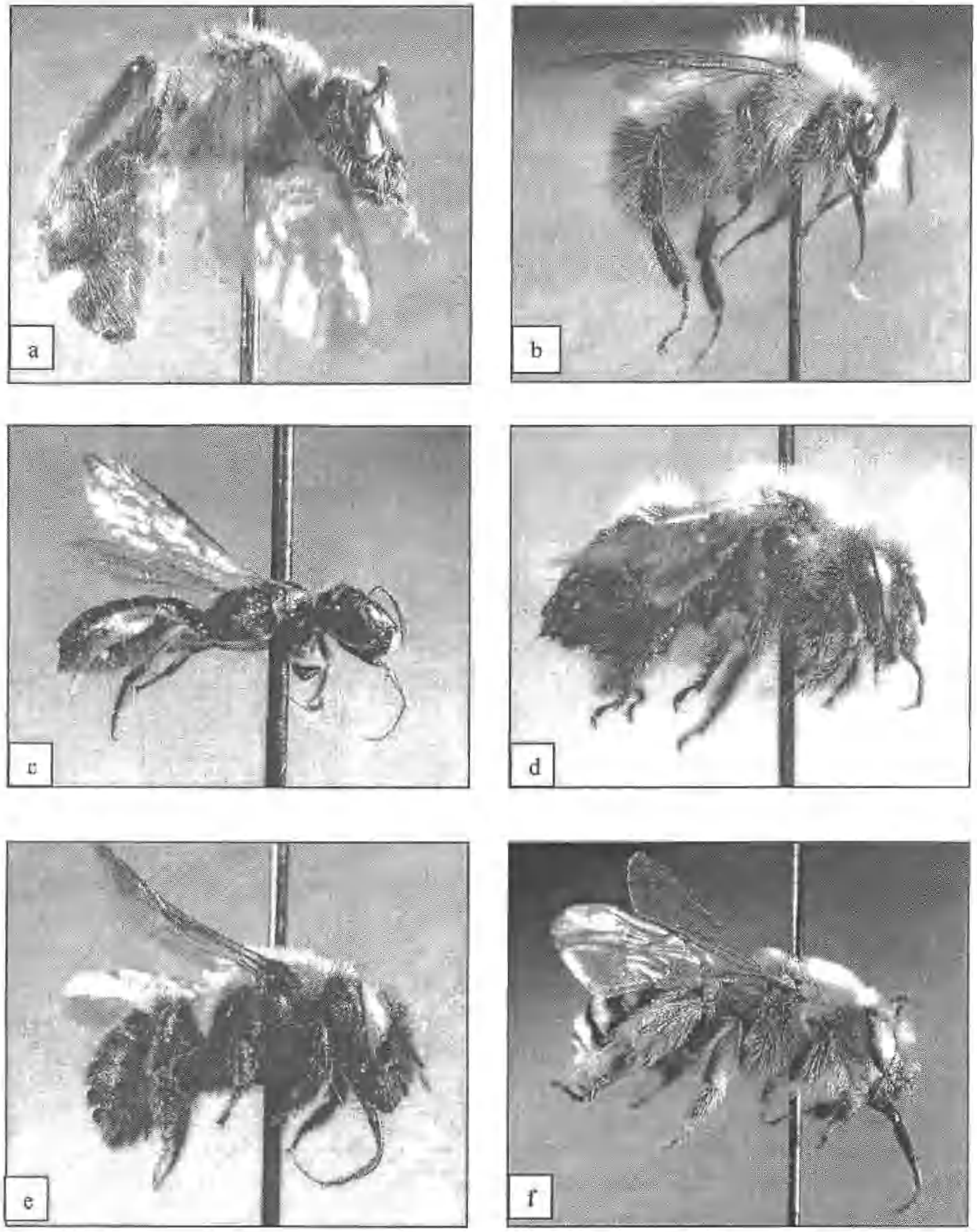


Figure 2.2 Photographs of selected native bee species shared by all four *Sidalcea* study species: (a) *Agapostomon* sp., (b) *Bombus sitchensis*, (c) *Ceratina acantha*, (d) *Diadasia nigrafrons*, (e) *Osmia* sp., and (f) *Synhalonia* sp.

species (Figure 2.3). Here, *S. campestris* racemes consistently received the lowest number of pollen grains in the array, exhibiting only about half the mean per flower number of grains as received by *S. nelsoniana* control racemes. In contrast, *S. cusickii* and *S. virgata* received about two- and four-times the mean per flower number of pollen grains as *S. nelsoniana*, respectively. Analysis supports the significance (p=0.000) of the species effect on mean per flower pollen deposition, and indicates means for all four species are significantly different from each other at the alpha= 0.05 confidence level. Analysis provides no evidence that the observed variability in means between *Sidalcea* species is significantly effected by species-based differences in display size (number of total open flowers per raceme) (p=0.42). Complete data from the experimental array are provided in Appendix 2.4.

Discussion

The results of this study indicate the presence of several intact pre-mating crossing barriers that promote reproductive isolation and discourage interspecific hybridization in the threatened species, *Sidalcea nelsoniana*. With regard to temporal reproductive isolation, *S. nelsoniana* exhibits almost perfect flowering synchrony with both *S. campestris* and *S. cusickii*, so this factor does not serve as a reliable crossing barrier in this species trio. However, *S. nelsoniana* exhibits very little flowering synchrony with *S. virgata*, which completes its flowering season just as the first flowers open in the remaining study species. This lack of

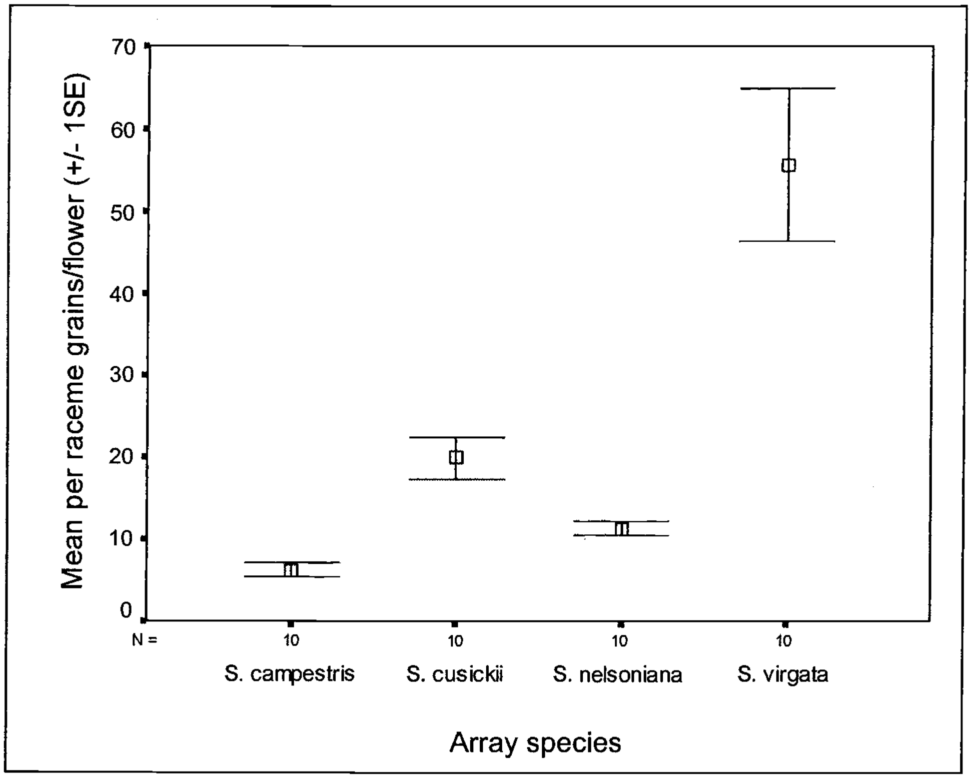


Figure 2.3 Mean per flower number of *Sidalcea nelsoniana* pollen grains transferred by insects to racemes (n=10 racemes per species) in the mixed-species array. Error bars represent +/- 1 SE. Differences between all four means are significant ($p<0.05$).

overlap is most conspicuous among closely neighboring heterospecific populations, whereas populations from different geographical regions sometimes overlap to a slight degree. While it is possible this flowering pattern may reflect the evolution of reproductive character displacement in flowering times, whereby the two species exhibit greater phenological divergence in areas of local sympatry than where they do not co-occur, this pattern might also be attributable simply to inherent environmental differences between populations from different regions and elevations.

Whatever the mechanism(s) behind flowering asynchrony in *Sidalcea nelsoniana* and *S. virgata*, temporal isolation appears to represent an important obstacle to hybridization between these species, especially among neighboring heterospecific populations with the greatest geographical likelihood of pollen exchange. However, as discussed later in Chapter 4, if the observed variability in flowering times within each species is genetically controlled, rather than strictly environmentally determined, temporal isolation could conceivably be narrowed or broken down through anthropogenic mixing of heterospecific populations from different regions.

Beyond asynchronous flowering, two additional phenological factors reinforcing temporal reproductive isolation between *Sidalcea nelsoniana* and *S. virgata* are the complimentary patterns of female sex expression exhibited by the two species during the period of their closest temporal proximity. Here, due to gynodioecious population structures and protandrous floral development in hermaphrodites, the majority of open flowers at the end of *S. virgata*'s flowering season tend to be functionally female. Likewise, the earliest flowers to open in most *S. nelsoniana* populations tend to be those of female individuals; female *S. nelsoniana* appear to exhibit an intrinsic predisposition to earlier flowering than hermaphrodites (typically by 5-15 days), both under wild and common greenhouse conditions (Gisler, unpublished). Moreover, due to female-biased population structures, female individuals are generally far more frequent than hermaphrodites in Willamette Valley *S. nelsoniana* populations; among 29 populations surveyed in 1995, female individuals outnumbered hermaphrodites on average 2.6:1 (range 0.4:1 - 21.5:1), with two populations containing exclusively female individuals (Gisler and Meinke 1996). In light of these combined factors, opportunities for interspecific pollen flow between *S. virgata* and *S. nelsoniana* are even fewer than accorded strictly by their predominantly asynchronous flowering.

Despite the evidence of temporal isolation between *Sidalcea nelsoniana* and *S. virgata*, it is acknowledged that the conclusions drawn here are based on a single year of data collection, and that longer-term observations might reveal some degree of annual variability in flowering times that could (at least occasionally) narrow the seasonal gap between these species. For instance, it is conceivable that increased late-spring precipitation could prolong flowering in *S. virgata* beyond the initiation of flowering in *S. nelsoniana*, though it is equally likely such climatic events might simultaneously delay flowering onset in *S. nelsoniana*, thereby maintaining (though shifting) temporal separation between the two species. Nevertheless, the conclusions drawn here should be considered preliminary pending additional years of data collection.

Geographical reproductive isolation does not appear to represent a reliable barrier to hybridization between *Sidalcea nelsoniana* and its two sympatric congeners, *S. virgata* and *S. campestris*. Inventories show these species commonly occur within 500 m of each other, and *S. campestris* often forms intimately mixed populations with *S. nelsoniana*. In contrast, *S. nelsoniana* and *S. cusickii* are currently separated by approximately 3 km, a distance that divides their entire geographic ranges. Thus, geographical isolation appears to serve as a formidable pre-mating crossing barrier in this species pair.

The narrow, but apparently complete, geographical separation between *Sidalcea nelsoniana* and *S. cusickii* is puzzling, however, because these two species share the most similar wet prairie habitat affinities of the four congeners in this study, and their respective geographic ranges abut in a seemingly homogenous, expansive, topographically and hydrologically uniform floodplain (i.e., the south-central Willamette Valley). Furthermore, the boundary between their parapatric ranges is successfully bridged by numerous other native Willamette Valley taxa, including the other two *Sidalcea* species in this study. Thus, there are no obvious biogeographic explanations why these two species should exhibit non-overlapping distributions.

One possible reason for the observed parapatry in *Sidalcea nelsoniana* and *S. cusickii* is that the two species might have diverged in isolation (i.e., they underwent allopatric speciation) and are coincidentally only now meeting along their expanding fronts. However, the condition of parapatry has long been recognized as a common and widespread phenomenon (Bull 1991); for instance, Charles Darwin (1859) noted that the location of a species border is often determined by the border of a related species. This suggests there may be more going on in the parapatric distributions of *S. nelsoniana* and *S. cusickii* than random divergence and migration events. Rather, it is possible that the maintenance of parapatry in this species pair may result, at least in part, by selection against reproductive interference and/or maladaptive hybridization (see Chapter 1 for a brief discussion of these factors).

Areas co-occupied by multiple *Sidalcea* species in this study were overwhelmingly shown to exhibit obvious signs of human disturbance that may have promoted the mixing (or "hybridization," see Chapter 1) of subtly different microsites, resulting in the weakening of spatial reproductive barriers. For instance, steep-walled roadside ditches and/or abruptly elevated gravel roadbeds and railway grades can provide a mixture of wetland and upland microsites within much more localized areas than would be expected in undisturbed prairie habitats with broader, more gradual hydrologic gradients and natural valley bottom floodplain topographies. Likewise, agricultural ditching and installation of underground drainage tiles could result in complex patterns of wet and dry soils within confined areas. As the four *Sidalcea* species in this study demonstrate subtly different habitat affinities in relation to wetland and upland soil moisture conditions, such disturbances could provide unprecedented opportunities for migration and interspecific proximity. However, drawing conclusions about current species' distributions in relation to historic disturbance events is undoubtedly speculative, and difficult to support with comparative examples of overlap in non-disturbed areas because ecologically pristine habitats were virtually never observed during this study, even within wildlife refuges and parks. As such, the association between observed *Sidalcea* geographical patterns and human disturbance activities remains difficult to confidently assess.

Ethological isolation, the last pre-mating crossing barrier addressed in this study, does not appear to inhibit interspecific pollen exchange between *Sidalcea nelsoniana* and any of its local congeners. Although certain insect taxa appeared specific to each of the different *Sidalcea* species in this study, many other pollinators were common to all, including several large bees (i.e., bumble bees, honey bees, and *Diadasia*). These large bees were the most frequent visitors, were observed carrying the largest pollen loads, and by all accounts appeared to serve as the primary pollinators of the Willamette Valley *Sidalcea*s. In particular, the native solitary bee, *Diadasia nigrafrons*, may be predisposed to indiscriminate *Sidalcea* visitation due to its specialization on this genus (Moldenke, personal communication), which may necessitate broader flight distances and non-constant foraging behavior in order to locate adequate *Sidalcea* pollen and nectar resources for nest provisioning (especially in light of the fragmentation and small size of most extant *Sidalcea* populations). Such indiscriminate foraging behavior was directly observed at two locations (William Finley National Wildlife Refuge and E.E. Wilson Wildlife Area), where the two species co-occur in mixed populations. Ultimately, the observed lack of overlap of occasional small bees, flies, and wasps is probably incidental in terms of its overall impact on reproductive isolation between *S. nelsoniana* and its congeners.

Lack of pollinator isolation is also supported by results of the mixed-species array experiment, which demonstrated insect-mediated pollen flow between *in situ Sidalcea nelsoniana* and introduced racemes of all three congeners. Data from the array indicate these species not only share the same types of pollinators (as demonstrated by the aforementioned pollinator inventories), but may also share the same *individual* pollinators. Although this array experiment does not necessarily reflect natural conditions, insofar that neither *S. cusickii* nor *S. virgata* occur intimately mixed with *S. nelsoniana* in nature, it nonetheless demonstrates that pollinators do not exhibit exclusive floral constancy while foraging under these artificial conditions. Indeed, results of this experiment suggest pollinators might be prone to heterospecific visitation when given the opportunity, given their apparent preference for *S. virgata* and *S. cusickii* over conspecific racemes in the mixed-species array.

The bases for these pollinator preferences for *Sidalcea cusickii* and *S. virgata* (and discrimination against *S. campestris*) relative to *S. nelsoniana* are unknown. The four species tend to vary in the number of flowers per raceme, but analysis provides no evidence that this factor influenced visitation rates. Instead, differential visitation may be related to the size of petals; female flowers of *S. virgata* and *S. cusickii* are both larger than those of *S. nelsoniana*, which exhibits pronounced sexual dimorphism in flower size. Indeed, typical flowers of female *S. virgata* and *S. cusickii* are approximately the same size, or slightly larger, than *hermaphroditic* flowers of *S. nelsoniana* (which in turn exhibit 40-45 percent larger petals than female *S. nelsoniana* flowers) (Gisler, unpublished). Similarly, female flowers of *S. virgata* and *S. cusickii* typically produce larger vestigial anther sacs than *S. nelsoniana* (Gisler, unpublished), and therefore might prove more adept at deceitfully attracting female bees foraging for pollen. Numerous researchers have reported pollinator preferences for larger petaled flowers over shorter petaled flowers within species (Bell 1985, Eckhart 1991, Delph and Lively 1992, Eckhart 1992, Ashman 2000), and petal size is often correlated with nectar and pollen production and may thus serve as a reliable cue to floral rewards (Zimmerman 1988, Young and Stanton 1990, Campbell et al. 1991, Eckhart 1992, Vaughton and Ramsey 1998). Flowers of *S. campestris* received the least pollen of the four species in the array. This might be explained by their white coloration, which may be less attractive to pollinators than the dark pink flowers of the other congeners. Despite their coloration, however, heterospecific pollen transfer still occurred in *S. campestris*, reinforcing the contention (i.e., Richards 1997) that, although color-based discrimination is common among insects, it is rarely adequate to constitute a complete interspecific crossing barrier.

Rare species such as those in this study may be particularly susceptible to pollinator behaviors that lead to interspecific gene flow. Research has shown that floral constancy among pollinators is most pronounced in areas of high nectar source availability, and decreases as resource availability declines, to the point that pollinators are apt to feed upon any suitable plant in a local area when resources are scarce (Levin 1971), including cross-fertile relatives (Ellstrand and

Elam 1993). Due to widespread habitat fragmentation, most extant *Sidalcea* populations in the Willamette Valley contain very few individuals, so the tendency for pollinators to visit other suitable plants, including sympatric congeners, may be inordinately high.

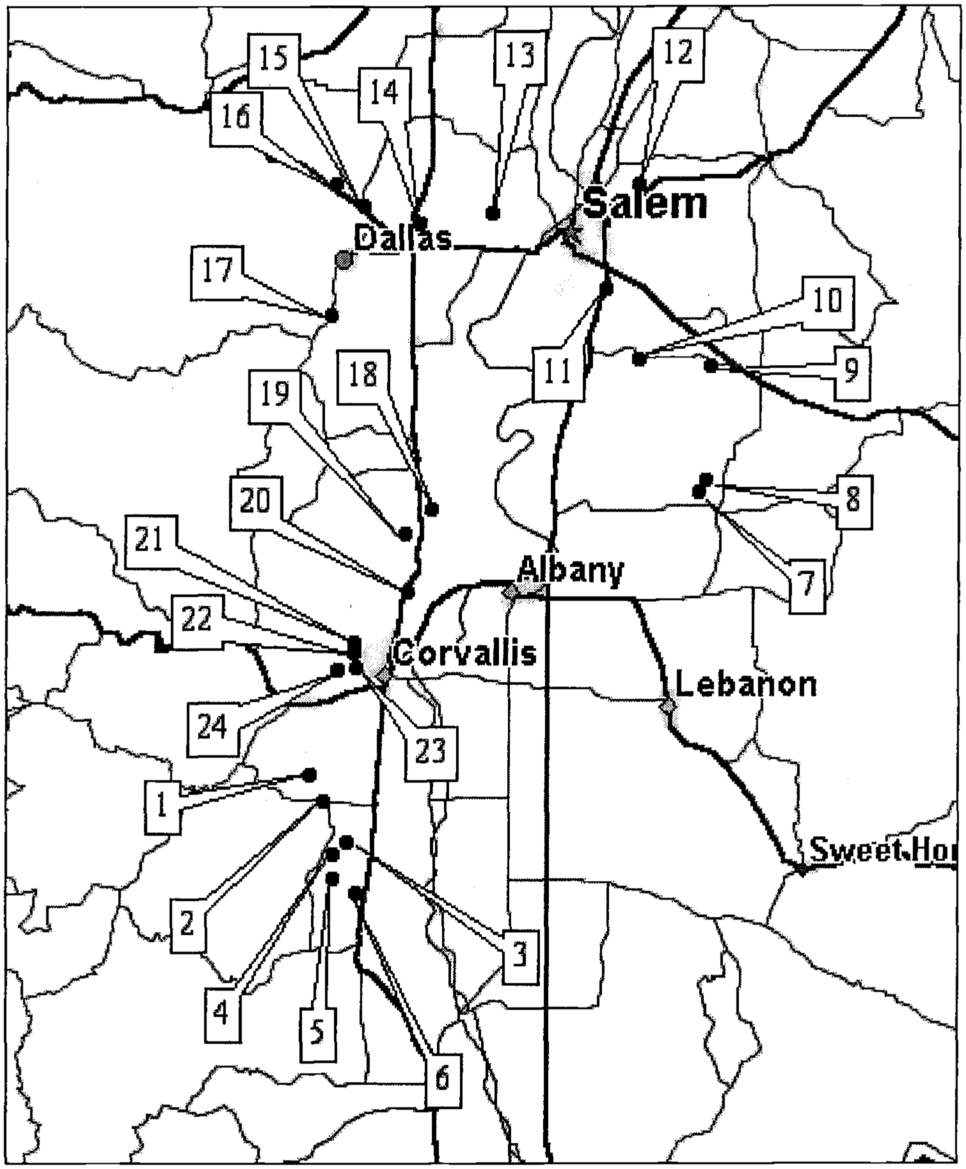
In conclusion, it appears that pre-mating crossing barriers are functioning to promote reproductive isolation in *Sidalcea nelsoniana* by discouraging interspecific pollen exchange with *S. cusickii* (due to geographical isolation) and *S. virgata* (due to temporal isolation). However, reliable pre-mating isolating mechanisms are absent between *S. nelsoniana* and its white-flowered relative, *S. campestris*. Given the lack of pollinator isolation between all four taxa, two important questions remain unanswered: 1) does hybridization take place between *S. nelsoniana* and *S. campestris*, or do post-mating crossing barriers intervene to maintain their mutual reproductive isolation, and 2) might *S. nelsoniana* still hybridize with *S. virgata* and *S. cusickii* if human-caused dispersal or habitat disturbance events result in the breakdown of seemingly intact phenological and spatial crossing barriers? These questions are addressed Chapter 3.

APPENDICES

Appendix 2.1 Map locations of *Sidalcea* study populations used to document flowering times in 2000. For *S. nelsoniana*, *S. campestris*, and *S. virgata*, study populations occupy Benton, Linn, Marion, and Polk Counties, Oregon. For *S. cusickii*, study populations occur in Benton, Douglas, and Lane Counties, Oregon. Precise population locality information is available upon request. Map numbers correspond with population identification numbers in Tables 2.1-2.3 and Appendix 2.3.

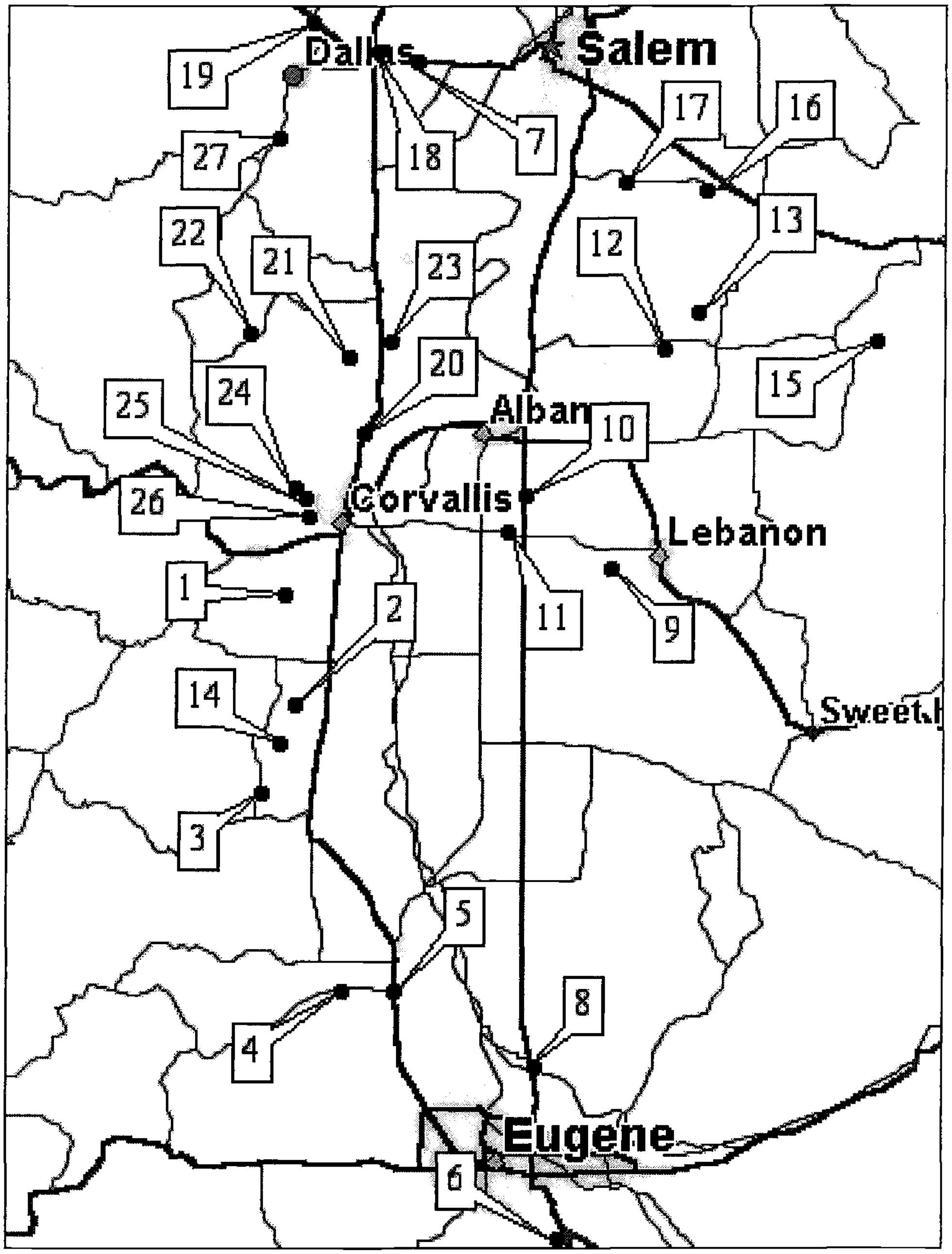
Appendix 2.1 (Continued)

Sidalcea nelsoniana study populations



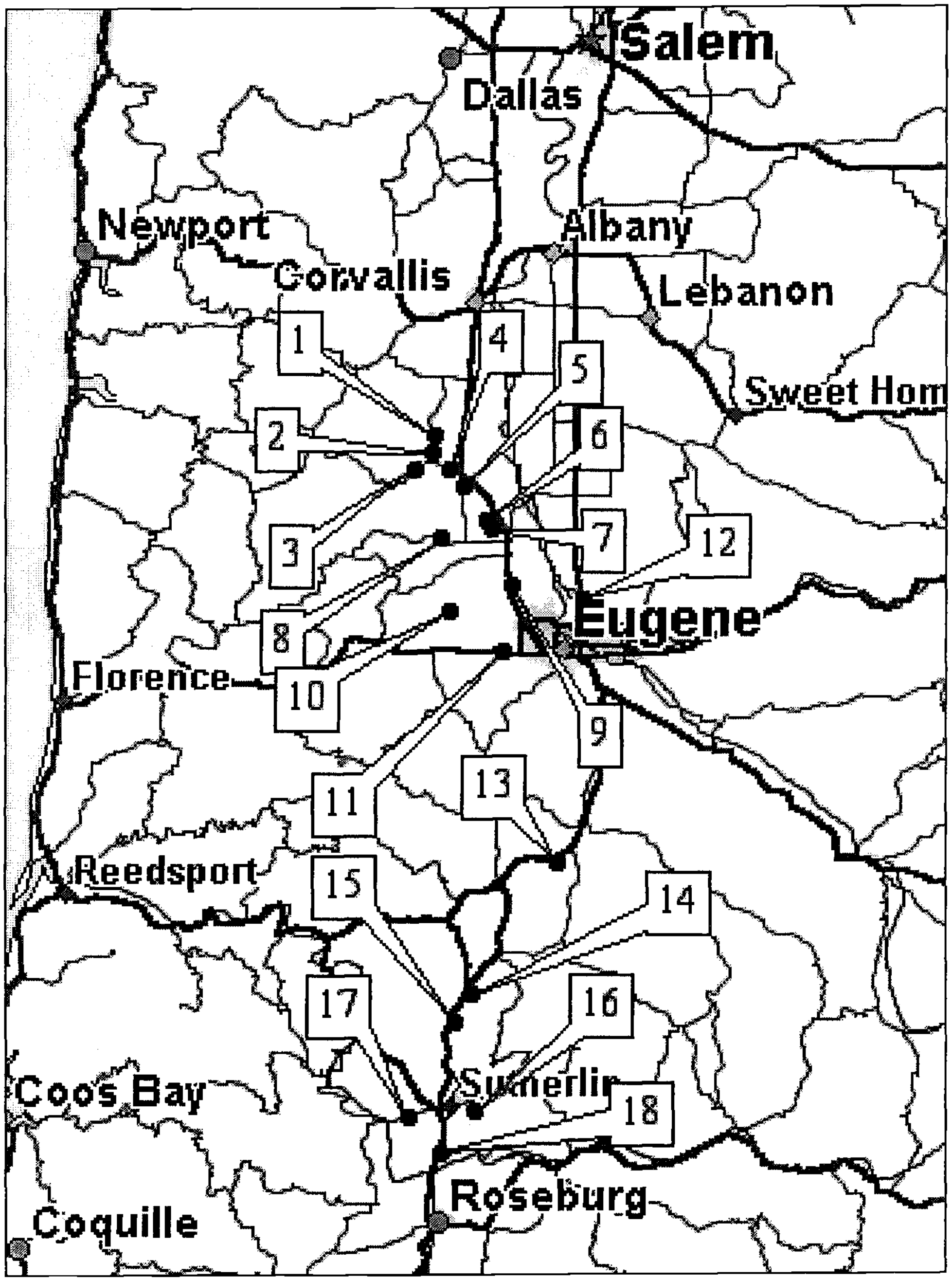
Appendix 2.1 (Continued)

Sidalcea campestris study populations



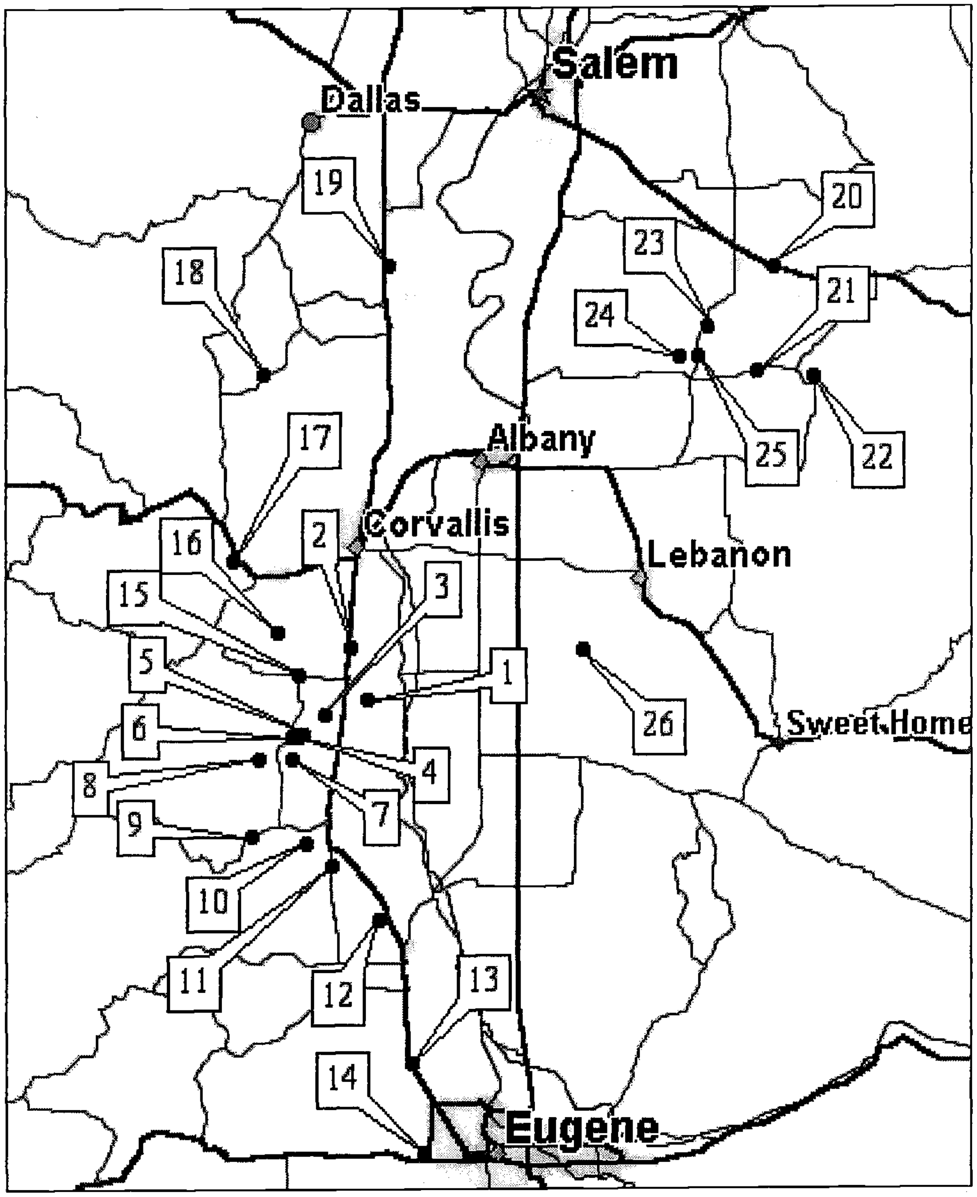
Appendix 2.1 (Continued)

Sidalcea cusickii study populations

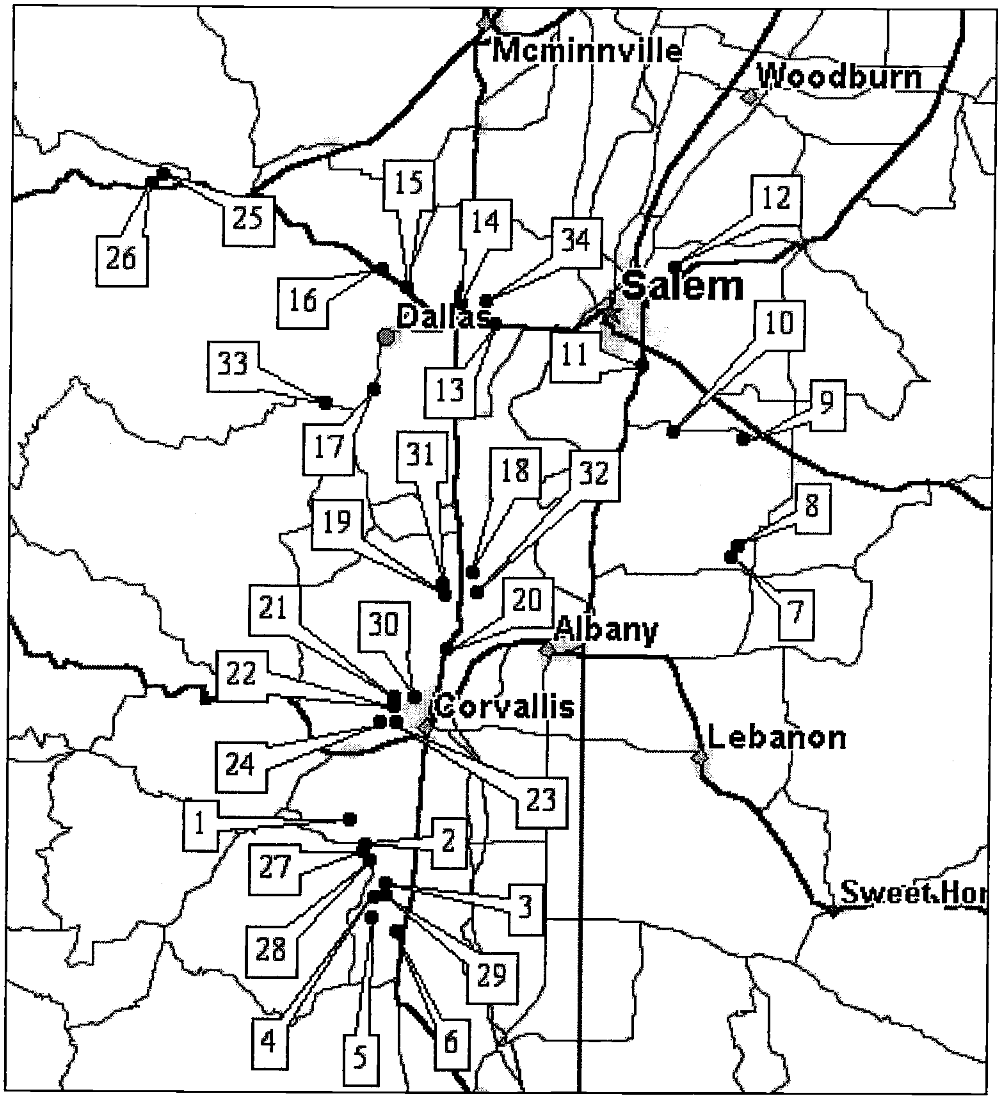


Appendix 2.1 (Continued)

Sidalcea virgata study populations



Appendix 2.2 Map locations of *Sidalcea nelsoniana* study populations used to document spatial isolation from congeners and assess habitat disturbance regimes in 2000. Study populations include those used for flowering time estimates (see Appendix 2.1), as well as 10 additional sites (numbers 25-34). Precise population locality information is available upon request. Map numbers correspond to population identification numbers in Table 2.3.



Appendix 2.3 Data on floral development (percent total inflorescences exhibiting open flowers) at *Sidalcea* study populations in 2000. All values represent complete censuses of inflorescences, except in populations containing >1000 inflorescences, whereupon the percent of flowering inflorescences visually estimated (these populations are labeled "est" in parentheses following corresponding site names). Data were recorded at approximate two-week intervals. Numbers in the "map#" column correspond with site locations shown in Appendix 2.1.

Appendix 2.3 (Continued)

Sidalcea nelsoniana

Site (# racemes)	**map#**	**3/12**	**3/31**	**4/14**	**5/1**	**5/16**	**6/2**	**6/15**	**7/3**	**7/17**	**8/1**	**8/16**	**8/31**
Fern Rd (42)	1	0	0	0	0	0	4.8	35.7	95.2	52.4	9.5	0	0
Decker Rd (est)	2	0	0	0	0	0	5	25	90	65	10	0	0
Finley Kiosk (224)	3	0	0	0	0	0	4.5	22.3	84.3	64.7	11.6	0	0
Finley Wdpkr (55)	4	0	0	0	0	0	0	16.4	74.5	81.8	10.9	0	0
Finley Bruce (31)	5	0	0	0	0	0	16.7	58.1	87.1	77.4	16.1	0	0
Finley McFlnd (est)	6	0	0	0	0	5	25	80	70	15	0	0	0
Ridge Drive (460)	7	0	0	0	0	0	3.2	46.1	80.4	67.0	9.8	0	0
Miller Cmtry (27)	8	0	0	0	0	0	0	48.1	92.6	44.4	3.7	0	0
Aumsville pit (119)	9	0	0	0	0	0	6.7	63.0	79.0	58.8	14.3	0	0
Burkland lmbr (480)	10	0	0	0	0	0	0	5.0	49.0	81.0	12.7	0.8	0
Salem Airport (est)	11	0	0	0	0	0	5	45	80	50	5	0	0
Cordon Rd (17)	12	0	0	0	0	0	35.3	76.5	70.5	29.4	11.8	0	0
SR 22 (268)	13	0	0	0	0	3.4	48.5	90.3	78.7	30.2	1.9	0	0
SR 99W (109)	14	0	0	0	0	0	14.7	47.7	86.2	43.1	11.9	0	0
Dyck Rd (267)	15	0	0	0	0	0	0	16.9	67.4	75.3	4.5	0	0
VanWell Rd (260)	16	0	0	0	0	0	5.0	64.7	83.8	15.8	3.8	0	0
Guthrie Rd (est)	18	0	0	0	0	0	0	0	25	75	65	10	0
Tampico Rd (305)	19	0	0	0	0	0	0	0	28.9	90.2	78.8	7.9	0
Lewisburg (est)	20	0	0	0	0	0	0	15	75	80	30	5	0
Walnut Park (385)	21	0	0	0	0	0	5.5	33.5	84.7	43.4	9.9	0	0
OSU Horse (615)	22	0	0	0	0	0	11.4	82.6	73.2	6.7	0	0	0
OSU Turkey (est)	23	0	0	0	0	0	10	80	60	10	0	0	0
Bald Hill (236)	24	0	0	0	0	0	5.9	79.9	62.7	14.4	1.3	0	0

Appendix 2.3 (Continued)

Sidalcea campestris

Site (# racemes)	**map#**	**3/12**	**3/31**	**4/14**	**5/1**	**5/16**	**6/2**	**6/15**	**7/3**	**7/17**	**8/1**	**8/16**	**8/31**
Bellfountain (106)	1	0	0	0	0	0	6.6	34.9	84.0	66.0	4.7	0	0
Finley Kiosk (35)	2	0	0	0	0	0	8.6	57.1	94.3	48.6	11.4	0	0
Dykstra (51)	3	0	0	0	0	0	3.9	78.4	96.1	64.7	7.8	0	0
Cheshire (220)	4	0	0	0	0	0	8.2	55.9	80.9	47.7	5.0	0	0
Junction City (47)	5	0	0	0	0	0	2.1	63.8	93.6	31.9	10.6	0	0
Camas Swale (est)	6	0	0	0	0	0	5	50	80	70	35	5	0
SR22 (64)	7	0	0	0	0	3.1	23.4	90.6	54.7	12.5	0	0	0
Coburg (70)	8	0	0	0	0	0	2.9	85.7	78.6	22.9	4.3	0	0
Lebanon (389)	9	0	4.6	63.2	82.3	77.4	25.4	12.9	0	0	0	0	0
Freeway Lakes (115)	10	0	17.4	77.4	83.5	20.0	5.2	0.9	0	0	0	0	0
Columbus Rd. (198)	11	0	0	16.2	45.5	75.8	68.2	7.6	0	0	0	0	0
Robinson Dr. (63)	12	0	0	7.9	63.5	90.5	65.1	17.5	1.6	0	0	0	0
Miller Cmtry (40)	13	0	0	5.0	22.5	90.0	80.0	22.5	2.5	0	0	0	0
Bruce Rd. (336)	14	0	0	0	0	0	0	20.5	59.5	44.6	29.5	0	0
Camp Morrison (est)	15	0	0	0	10	65	75	70	15	5	0	0	0
Aumsville (95)	16	0	0	0	0	0	10.5	70.5	84.2	53.7	3.1	0	0
Burkland (48)	17	0	0	0	0	0	6.3	43.8	87.5	62.5	22.9	2.1	0
SR99W (212)	18	0	0	0	0	1.9	48.1	79.7	73.6	13.7	0	0	0
Dyck Rd. (72)	19	0	0	0	0	0	0	11.1	75.0	90.3	30.6	6.9	0
Lewisburg (133)	20	0	0	0	0	0	15.0	55.7	81.2	26.3	3.8	0	0
Tampico (est)	21	0	0	0	0	0	0	15	55	80	75	30	0
Maxfield Crk.(27)	22	0	0	0	0	9.1	22.2	77.8	74.1	42.9	3.7	0	0
E.E. Wilson (est)	23	0	0	0	0	0	5	35	70	65	15	5	0
Walnut Park (35)	24	0	0	0	0	0	11.4	71.4	74.3	54.3	11.4	2.9	0
OSU Horse (270)	25	0	0	0	0	0	5.9	57.8	81.1	77.8	24.1	5.2	0
OSU Turkey (41)	26	0	0	0	0	0	7.3	61.0	95.1	19.5	4.9	0	0
Guthrie Park (116)	27	0	0	0	0	0	7.8	51.7	87.9	77.6	44.8	6.9	0

Appendix 2.3 (Continued)

Sidalcea cusickii

Site (# racemes)	**map#**	**3/12**	**3/31**	**4/14**	**5/1**	**5/16**	**6/2**	**6/15**	**7/3**	**7/17**	**8/1**	**8/16**	**8/31**
Bellfountain (108)	1	0	0	0	0	0	11.1	74.1	90.1	19.4	1.9	0	0
Dykstra (est)	2	0	0	0	0	0	0	5	60	80	50	10	0
Alpine (39)	3	0	0	0	0	0	0	15.4	71.8	76.9	20.5	2.6	0
MacFarland (126)	4	0	0	0	0	0	0	4.0	67.5	84.9	62.7	3.2	0
Monroe (40)	5	0	0	0	0	0	2.1	63.8	93.6	31.9	10.6	0	0
Washburn (est)	6	0	0	0	0	0	0	15	70	80	50	5	0
Cox Butte (240)	7	0	0	0	0	0	0	20.8	75.0	87.5	16.3	4.6	0
Turnbow Ln. (82)	8	0	0	0	0	0	0	19.5	91.5	74.4	60.9	6.1	0
Eugene Air (est)	9	0	0	0	0	0	0	10	60	80	70	15	0
Fern Ridge (118)	10	0	0	0	0	0	0	5.9	66.1	84.7	47.5	11.0	0
Oxbow (est)	11	0	0	0	0	0	0	10	65	80	70	20	0
Coburg (55)	12	0	0	0	0	0	1.8	69.1	72.7	72.7	7.3	3.6	0
Martin Crk (425)	13	0	0	0	0	0	2.4	46.8	88.9	68.2	9.4	0.5	0
Exit 150 (est)	14	0	0	0	0	0	0	10	60	70	40	10	0
Cabin Crk (580)	15	0	0	0	0	0	1.0	15.3	54.3	74.7	21.3	5.3	0
Cooper Crk (461)	16	0	0	0	0	0	10.5	70.5	84.2	53.7	3.1	0	0
Cole Rd (399)	17	0	0	0	0	0	0	20.0	59.0	77.4	55.6	11.3	0
Wilbur (212)	18	0	0	0	0	0	0	11.8	77.8	80.2	42.0	14.2	0

Appendix 2.3 (Continued)

Sidalcea virgata

Site (# racemes)	map#	3/12	3/31	4/14	5/1	5/16	6/2	6/15	7/3	7/17	8/1	8/16	8/31
Smith Loop (45)	1	0	13.3	62.2	86.7	55.5	8.9	0	0	0	0	0	0
Hwy99 (37)	2	0	10.8	51.4	91.9	43.2	13.5	0	0	0	0	0	0
Finley Kiosk (76)	3	0	0	10.5	52.6	90.8	11.8	3.9	0	0	0	0	0
Finley Pond (est)	4	0	0	5	50	80	30	5	0	0	0	0	0
Finley Wdpkr (24)	5	0	0	20.8	70.8	91.7	12.5	0	0	0	0	0	0
Bellfountain (est)	6	0	0	5	45	75	60	15	0	0	0	0	0
Bruce Rd. (82)	7	0	8.5	63.4	89.0	61.0	8.5	0	0	0	0	0	0
Reese Crk (144)	8	0	0	0	0	13.9	59.0	83.3	61.8	4.9	0	0	0
Alsea Falls (21)	9	0	0	0	28.6	81.0	95.2	23.8	9.5	0	0	0	0
Kyle Rd. (478)	10	0	0	6.7	52.3	94.1	66.9	12.6	0	0	0	0	0
Monroe (65)	11	0	0	21.5	72.3	84.6	33.8	9.2	0	0	0	0	0
Ferguson Rd. (49)	12	0	0	12.2	61.2	81.6	51.0	6.1	0	0	0	0	0
Awbry Rd. (58)	13	0	0	17.2	51.7	89.7	44.8	10.3	0	0	0	0	0
Oxbow East (est)	14	0	0	10	65	75	65	5	0	0	0	0	0
Decker (67)	15	0	6.0	47.8	91.0	67.2	11.9	0	0	0	0	0	0
Fern Rd. (75)	16	0	10.7	60.0	84.0	20.0	12.0	0	0	0	0	0	0
Woods Crk. (52)	17	0	0	0	15.4	76.9	78.8	50.0	17.3	3.8	0	0	0
Maxfield Crk (115)	18	0	0	1.7	56.5	90.4	69.6	38.3	6.1	0	0	0	0
Parker Rd. (30)	19	0	3.3	63.3	90.0	43.3	16.6	0	0	0	0	0	0
Old Mehema (est)	20	0	0	0	20	70	80	35	5	0	0	0	0
Shimaneck (214)	21	0	0	0	16.4	44.9	84.1	67.8	7.5	0	0	0	0
Bilyeu Crk. (43)	22	0	0	0	11.6	58.1	93.0	69.8	14.0	0	0	0	0
Scio (123)	23	0	0	0	7.3	69.1	80.5	26.0	6.5	0	0	0	0
Miller Cmtry (91)	24	0	0	6.6	71.4	75.8	44.0	7.8	2.1	0	0	0	0
Ridge Dr. (18)	25	0	0	22.2	67.7	83.3	50.0	11.1	0	0	0	0	0
Brownsville (36)	26	0	0	0	19.4	86.1	80.6	16.7	5.6	0	0	0	0

Appendix 2.4 Floral and pollen flow data for racemes (10 replicates for each of the four species) in the experimental mixed-species array.

Array species	# open flowers	# receptive flowers	Total # pollen grains	Mean grains per receptive flower
S. campestris	13	7	29	4.14
S. campestris	26	18	64	3.56
S. campestris	13	7	38	5.43
S. campestris	18	12	83	6.92
S. campestris	15	7	37	5.29
S. campestris	7	5	61	12.2
S. campestris	12	6	56	9.33
S. campestris	13	8	31	3.88
S. campestris	10	6	26	4.33
S. campestris	7	4	25	6.25
S. cusickii	17	12	193	16.08
S. cusickii	32	20	469	23.45
S. cusickii	13	9	201	22.33
S. cusickii	7	5	177	35.4
S. cusickii	26	18	279	15.5
S. cusickii	35	25	640	25.6
S. cusickii	39	25	580	23.2
S. cusickii	27	17	132	7.76
S. cusickii	18	12	113	9.42
S. cusickii	9	7	142	20.29
S. nelsoniana	21	16	156	9.75
S. nelsoniana	32	21	255	12.14
S. nelsoniana	22	17	200	11.76
S. nelsoniana	34	26	278	10.69
S. nelsoniana	10	5	68	13.6
S. nelsoniana	12	7	79	11.29
S. nelsoniana	43	27	415	15.37
S. nelsoniana	16	11	61	5.55
S. nelsoniana	8	5	57	11.4
S. nelsoniana	20	13	153	11.77
S. virgata	5	3	153	51
S. virgata	10	7	227	32.43
S. virgata	4	3	239	79.67
S. virgata	15	9	344	38.22
S. virgata	7	4	415	103.75
S. virgata	11	7	271	38.71
S. virgata	6	3	229	76.33
S. virgata	9	7	109	15.57
S. virgata	8	5	167	33.4
S. virgata	7	4	348	87

Chapter 3: Post-mating Reproductive Isolation and Interspecific Hybridization in the Threatened Species, *Sidalcea nelsoniana*

Abstract

Understanding the nature and efficacy of pre- and post-mating reproductive isolating mechanisms in rare plant species is important for evaluating their vulnerability to the manifold threats (and receptivity to the theoretical benefits) posed by interspecific hybridization. In Chapter 2, I reported an investigation of ecological factors influencing pre-mating reproductive isolation in the threatened species, *Sidalcea nelsoniana*. In this chapter I assess the influence of *post-mating* crossing barriers on the feasibility of hybrid formation between this species and its three local congeners in the Willamette Valley of western Oregon. Results from reciprocal interspecific crosses reveal almost complete sexual incompatibility between *S. nelsoniana* and *S. campestris*, strong (but incomplete) post-mating crossing barriers between *S. nelsoniana* and *S. virgata* (depending on crossing direction and *S. virgata* phenotype), and no significant post-mating obstacles to hybridization between *S. nelsoniana* and *S. cusickii*. Species pairs exhibiting reduced sexual compatibility produced very few seeds, but when seeds were produced they were consistently viable among all F1 hybrid and conspecific lines. Evidence of hybrid sterility was only observed in one of three lines tested, though fertility of the remaining five hybrid lines could not be conclusively evaluated due to exclusive production of female progeny. Chromosome counts shed light on several important hybridization issues in the

species group, including 1) confirmation of earlier reports of polyploidy (including diploids, tetraploids, and hexaploids) among parental *Sidalcea* species, 2) indication of a positive relationship between sexual incompatibility and the magnitude of ploidy differences between heterospecific mating partners, and 3) suggestion of several ploidy-related mechanisms underlying observed and predicted hybrid fertilities. Lastly, morphological data indicate that F1 *Sidalcea* hybrids exhibit a varied mosaic of phenotypic character states encompassing those that are similar to one or both parents, some that are intermediate between parents, and others that are extreme or "transgressive" to parents. These results, combined with pronounced morphological variability exhibited *within* each of the study species, suggest that detection of *Sidalcea* hybrids in nature (if present) will likely be more complicated than simply searching for obvious patterns of phenotypic intermediacy within or around extant populations.

Introduction

In chapter 2, pre-mating crossing barriers were investigated in the threatened species, *Sidalcea nelsoniana*, an herbaceous perennial sharing rapidly shrinking and increasingly degraded grassland habitats in western Oregon with three other native congeners, *S. campestris*, *S. cusickii*, and *S. virgata* (Figure 1.1 in Chapter 1). This work revealed the absence of pre-mating barriers between *S. nelsoniana* and *S. campestris*, with the two species sharing flowering times, fine-scale geographic distributions, pollinators, and pollen transfer. This finding raises the question whether post-mating reproductive barriers may still intervene to prevent hybrid formation following successful interspecific pollen exchange in this species pair. Similarly, despite apparently intact pre-mating crossing barriers observed between *S. nelsoniana* and *S. cusickii* (geographical isolation) and *S. virgata* (temporal isolation), it remains uncertain to what extent post-mating crossing barriers might still serve as supplemental obstacles to hybrid formation if these ecological barriers become circumvented through chance events or anthropogenic activities.

One objective of this chapter is to assess the extent of post-mating reproductive isolation between *Sidalcea nelsoniana* and its congeners by investigating interspecific sexual incompatibility and hybrid formation through a series of artificial reciprocal cross-pollinations. Together with the ecological data reported in chapter 2, this information will help complete an evaluation of reproductive isolation and hybridization potential for the threatened *S. nelsoniana*.

Post-mating interspecific crossing barriers encompass multi-faceted expressions of sexual incompatibility that restrict the production and survival of hybrid progeny following successful pollen exchange between different species. These post-mating barriers can take effect prior to actual fertilization, as when heterospecific pollen grains fail to germinate and/or penetrate the stigmatic surface due to interspecific recognition and incompatibility responses (Grant 1963; Heslop-Harrison 1982; De Nettancourt 1984, Harder et al. 1993), and when complications affect pollen tube growth and ovule penetration (Thompson 1930, Buchholz et al. 1935, Levin 1970, Williams et al. 1986, Williams and Rouse 1988, Arnold 1997). If heterospecific pollen tubes successfully enter ovules and fertilization (or in the case of plants, double fertilization) takes place, hybrid lethality may yet result from a variety of factors including chromosomal imbalances in the endosperm, genic incompatibilities, and negative epistatic interactions arising from the combination of disparate genomes (Thompson 1930, Dobzhansky 1937, 1951; Brock 1955, Greenshields 1954, Stebbins 1958, Grant 1963, Johnston et al. 1980, Abbo and Ladizinsky 1994, Rieseberg 1997, Judd et al. 1999, Fishman and Willis 2001). Some authors also include hybrid sterility (attributable to reduced pairing of homologous chromosomes and/or unbalanced gametes) and later generation hybrid breakdown as late-stage post-mating crossing barriers (Dobzhansky 1937, Grant 1963, Stebbins 1971).

One critical factor underlying the expression of post-mating crossing barriers is polyploidy—the occurrence of different multiples of base chromosome sets among related taxa. Sexual incompatibility between species with different chromosome numbers has long been recognized as an important barrier to hybridization between different species, typically arising through the mechanisms of ploidy-related pollen tube failure and incomplete endosperm formation (Thompson 1930, Johnston et al. 1980), as well as disrupted or unbalanced pairing of chromosomes at meiosis leading to hybrid sterility (Jackson, 1976). When hybrids are formed between heteroploid parents, their chromosome numbers frequently differ from either progenitor, sometimes resulting in their instantaneous reproductive isolation and the subsequent onset of sympatric speciation (Winge 1917, Dobzhansky 1937, Clausen et al. 1945, Stebbins 1950, Lewis 1967, Judd et al. 1999, McArthur and Sanderson 1999). These attributes, along with the ubiquitous occurrence of polyploidy across vascular plant families (Averett 1980, Goldblatt 1980) no doubt fueled Stebbins' (1971) assessment of polyploidy as "The most wide-spread and distinctive cytogenetic process which has affected the evolution of higher plants." Given the influential role of polyploidy in regulating the occurrence and trajectory of hybridization events, additional objectives of this study are to identify the ploidy levels of parental *Sidalcea* species and their F1 interspecific hybrids, and explore the relationship between post-mating crossing barrier expression and polyploidy in this group of species.

If both pre- and post-mating crossing barriers are successfully overcome in rare plant species, whether due to human or natural processes, detection of resulting hybrids in the wild may be critically important for identifying populations in need of mitigation or containment. Conversely, hybrids may need to be identified to preserve and monitor the possible "constructive" consequences of hybridization (see Chapter 1). In many cases, however, it can be difficult to immediately recognize hybrids in the wild, especially among groups of parental species exhibiting high morphological variability (Judd et al. 1999), and after several generations of backcrossing have taken place (Rhymer and Simberloff 1996).

Detection of hybrids in nature can be further complicated by the fact that F1 and later generation hybrids are not always intermediate between their parents (as is often presumed), but instead frequently exhibit an unpredictable mosaic of phenotyes comprised of parental, intermediate, and/or transgressive (extreme to either parent) characters (Rieseberg and Ellstrand 1993, Judd et al. 1999). To help predict the feasibility of recognizing *Sidalcea nelsoniana* hybrids, and assess their patterns in parental phenotypic expression, this chapter will compare diagnostic traits of parental *Sidalcea* individuals with those of their F1 hybrid progeny.

Methods

Interspecific sexual incompatibility

Reciprocal cross-pollinations were performed between *Sidalcea nelsoniana* and its three congeners to assess the nature and efficacy of post-mating crossing barriers in the species group. All crosses involving *S. virgata* were performed using two different forms--or phenotypes--of the species that were suspected of corresponding with two different cytotypes. This suspicion was based upon personal observation of voucher specimens from earlier chromosome counts reported by Hitchcock and Kruckeberg (1957) that suggested diploid and tetraploid *S. virgata* cytotypes might correspond with small and large phenotypes, respectively. Given the potential influence of polyploidy on the expression of post-mating reproductive isolation, both large and small *S. virgata* phenotypes were included in an effort to incorporate both cytotypes into the reciprocal crossing study.

Sidalcea individuals used for reciprocal crosses were cultivated from seed in the greenhouse at Oregon State University. Here, seed coats were scarified with sandpaper to promote imbibition and break dormancy. Following germination on moist filter paper, 12 seedlings of each species were transplanted into four-inch plastic pots filled with a peat-based potting mix. At the same time these seedlings were planted, a single mature *Sidalcea nelsoniana* hermaphrodite, previously cultivated from seed, was separated into 3 divisions to provide genetically identical sires for interspecific crosses. Seeds of each species originated from the following source populations: *Sidalcea campestris* (E.E. Wilson--site # 23 in Appendix 2.1), *S. cusickii* (Dykstra Rd.--site # 2 in Appendix 2.1), *S. nelsoniana* (E.E. Wilson--site # 18 in Appendix 2.1), *S. virgata* (small phenotype) (Parker Rd., site # 19 in Appendix 2.1), and *S. virgata* (big phenotype) (Maxfield Creek Rd., site # 18 in Appendix 2.1). Throughout cultivation, plants were kept in water-filled trays, fertilized every two weeks with Peter's 20-20-20 liquid fertilizer, and provided with 14-hour photoperiods using high pressure sodium lamps.

Reciprocal crosses were facilitated by the simultaneous flowering of all species approximately 14 weeks after planting, with the exception of the two *S. virgata* phenotypes, which exhibited healthy and vigorous growth but never produced flowering stems (a behavior consistent with past, and equally futile, efforts by the author to produce flowering *S. virgata* plants in the greenhouse). As such, reciprocal crosses involving the putative diploid and tetraploid *S. virgata* cytotypes necessitated the use of flowering individuals in wild populations.

Because members of the genus *Sidalcea* are gynodioecious, hermaphroditic individuals were necessarily employed as the sires for reciprocal crosses, and female (male-sterile) individuals as the pollen recipients. Lacking functional anthers, females lend themselves as pollen recipients in crossing experiments because, unlike hermaphrodites, they need not be emasculated to prevent self-fertilization through autogamy or geitonogamy. The author has never observed apomictic seed production among pollen-excluded *Sidalcea* females, so any resulting seed production in this study was attributable to the crossing treatments. As an added precaution to prevent incidental pollination in the greenhouse by uninvited insects, all female racemes were covered with breathable polyethylene mesh pollinator exclusion bags for the duration of the study. The same mesh bags were also used to exclude pollinators from *S. virgata* females in wild populations.

Reciprocal crosses were performed by removing entire fused stamen columns (typical of the Malvaceae family) of hermaphroditic flowers with forceps and then brushing the dehiscent anthers across the outspread stigmatic surfaces of receptive female flowers. A single hermaphroditic flower produced adequate pollen to saturate the stigmas of four to five female flowers. Three *Sidalcea nelsoniana* hermaphrodites (all genetically identical clones obtained through divisions) were propagated to supply pollen for crosses involving *S. nelsoniana* sires, whereas, due to the incomplete factorial design of the crossing experiment, one hermaphroditic individual of each congener provided sufficient pollen for reciprocal crosses with *S. nelsoniana* dams. Each reciprocal cross was replicated 30 times on each species (10 times each on 3 maternal individuals), including both *S. virgata* phenotypes, for a total of 240 interspecific crosses. Conspecific crosses were also performed as experimental controls on the same maternal individuals, and these were likewise replicated 30 times, for a total of 150 control crosses. All crossing treatments were randomly assigned to flowers of each female and were labeled by tying color-coded threads around their pedicels.

Seed set, calculated as the proportion of filled seeds to total available ovules, was recorded for fruits (multiple-seeded schizocarps) formed by all conspecific and reciprocal interspecific crosses. As the response variable (seed set) encompassed limited value possibilities by virtue of its calculation as a proportion, logistic regression was used to test for a significant species crossing combination effect, and also test for potential data overdispersion and maternal effects. To determine the extent and pattern of interspecific incompatibility expressed as reduced seed set, two-sample proportions parameters tests (a Chi-square procedure) were performed to determine equality of seed set means between individual interspecific species crossing combinations and their corresponding conspecific parental "control" crosses. Analyses were performed using SPlus 6.1 for Windows.

Next, seeds produced through interspecific and conspecific crosses were subjected to germination trials to determine if post-mating crossing barriers might be expressed through reduced seed viability. Seeds were prepared in the same manner described above for the cultivation of parental species. When available, up to 10 seeds were randomly selected from each of the three maternal lines for each interspecific and conspecific crossing combination, and were then assigned to independent germination trays. For several interspecific crossing combinations, fewer than 10 seeds were successfully produced by each maternal line (see Table 3.2 in the results section, below), so in these instances all available seeds (aside from a few stored in reserve) were used for the germination trials.

Seed germination, the proportion of seeds exhibiting emerged radicles and cotyledons in each germination tray, was recorded two weeks after sowing. As with seed set data, logistic regression was used to test for a significant species crossing combination effect on germination, and to test for potential data overdispersion and maternal effects. Next, two-sample proportions parameters tests were performed to determine equality of germination proportions between each interspecific crossing combination and its corresponding conspecific parental "control" crosses. Analyses were performed using SPlus 6.1 for Windows.

Following seed germination, up to 12 (when available) hybrid seedlings from each crossing combination were randomly selected from the three maternal lines and transferred from germination trays to pots and cultivated in the greenhouse to supply individuals for fertility estimates and morphological measurements (described below). Male and female fertilities were simultaneously assessed for each F1 hybrid line by crossing one randomly selected hermaphrodite (when available) with one randomly selected female of the same hybrid line. Each cross was replicated 10 times using the same crossing procedures described above for reciprocal interspecific crosses. Positive seed set was considered indicative of both male and female fertility. In cases of negative seed production, pollen grains of hermaphrodites were collected and stained with analine blue lactophenol (following Kearns and Inouye 1993) to gain additional information about which sex exhibited hybrid sterility.

Polyploidy

To investigate the relationship between post-mating crossing barriers and polyploidy in *Sidalcea nelsoniana*, and gain a better understanding of the cytology of the species group and their hybrids, I performed counts of chromosomes in the somatic cells of their root tips. Here, fine white root tips were collected from parental individuals and F1 hybrids in the greenhouse. For both *S. virgata* morphotypes that failed to flower in the greenhouse, rhizome fragments were collected from individuals used for interspecific crosses in wild populations and were then rooted in water in the greenhouse to gain fresh root tips. Chromosome counts were performed on root samples taken from two individuals of each parental species and F1 hybrid line.

To obtain chromosome counts, root tips were rinsed in distilled water and placed for two hours in a solution of colchicine-OH-Quinoline-DMSO for arresting metaphases of root tip cells. Root tips were then removed from the colchicine solution and stained and fixed for 48 hours in a 2% solution of Aceto-Orcein. Following staining and fixing, root tips were boiled in 45% acetic acid for approximately five seconds and then placed under a dissecting microscope for extraction of meristematic cells and slide preparation. After squashing cells, slides were rapidly passed over an alcohol flame and then examined under 100 x magnification for performing chromosome counts. Voucher photographs of chromosomes for each parental species and interspecific hybrid line were made using a digital camera.

Hybrid morphology

To help assess the general feasibility of recognizing *Sidalcea* hybrids in the wild and determine the extent to which parental traits are expressed in hybrid phenotypes, I recorded 10 morphological characters from the parental individuals used in interspecific crosses (n=1 hermaphrodite and 3 females per parent species) and compared them with the same traits measured on all (up to 12) cultivated F1 progeny from each hybrid line. Although parental individuals were necessarily cultivated prior to their offspring, both generations were grown using the same procedures and table space in the greenhouse, thereby offering more or less common garden conditions for purposes of morphological trait comparisons (with the exception of *S. virgata* parents, which had to be measured in wild populations because they did not flower in the greenhouse).

The morphological traits used in this study include two quantitative traits (petal length and number flowers per raceme), and eight categorical qualitative traits (Table 3.1), and encompass those previously recognized as diagnostic in floras and monographs (i.e., Hitchcock and Kruckeberg 1957, Peck 1961, Hitchcock and Cronquist 1973), as well as those considered important for the Willamette Valley species group based upon personal observations by the author. Certain vegetative traits, such as plant height and number and shape of leaves, frequently vary between *Sidalcea* species in the wild, but were not included in this study due to their seemingly atypical plasticity in the greenhouse (perhaps brought about by the artificial lighting and cultivation regime they experience) (Gisler unpublished).

Due to the limited number of parental and F1 hybrid individuals available for morphological measurements, combined with the predominance of qualitative diagnostic characters, exclusive production of female individuals in some hybrid lines, and the need to separate data by plant sex (due to sexual dimorphism), morphological data provide insufficient sample sizes for meaningful statistical analyses. Nevertheless, for comparative purposes, these data are presented in the form of descriptive tables and bivariate plots.

Table 3.1 Explanation of qualitative morphological traits measured on parental *Sidalcea* species and their interspecific hybrids (see Table 3.4 and Appendix 3.3 for resulting data).

Morphological trait	Explanation of categorical levels codes
Flower color	1=white 2=very light pink 3=light pink 4=pink 5=dark pink
Habit	1=stems fully erect 2=stems slightly decumbent at base (10°-45°) 3=stems strongly decumbent at base (>45°)
Sepal shape	1=long and marginally rolled 2=short and evenly tapered 3=short and widened above the base before tapering
Sepal color	1=no anthocyanic pigmentation 2=slight pigmentation, primarily along midvein and margins 3=strong pigmentation throughout
Stem hairs	1=glabrous 2=short-simple 3=long-simple 4=mixed simple + forked 5=forked 6=stellate
Upper leaf surface	1=glabrous 2=sparsely hairy 3=densely hairy
Glaucus stems	1=flowering stems not glaucus 2=flowering stems glaucus
Raceme taper (in bud stage)	1=tapered to a point 2=blunt to slightly widened at top

Results

Interspecific sexual incompatibility

Production of seeds, the first manifestation of sexual incompatibility addressed in this study, was significantly affected by species crossing combination ($p=0.02$) after accounting for potential nested maternal line effects (which were not significant, $p>0.25$). Seed set for conspecific "control" crosses was consistent among all parental species, with no evidence of significant differences between *Sidalcea nelsoniana* and its congeners at the alpha= 0.05 confidence level (Table 3.2). In contrast, seed set among interspecific crossing combinations was highly variable, exhibiting both significant and non-significant differences in pairwise comparisons with conspecific seed set in *S. nelsoniana* (Table 3.2). Despite this variability, at least *some* seeds were produced by all species crossing combinations. Seed set data for all crosses are provided in Appendix 3.1.

Although seed set proved to be extremely low for reciprocal crosses between *Sidalcea nelsoniana* and *S. campestris* (Table 3.2), sexual compatibility in this species pair initially seemed much higher than reflected by the data because fruits and seeds appeared to develop normally. For example, ovaries became fully swollen, fruits remained firmly attached to stems (normally *Sidalcea* fruits with unfertilized ovules do not swell and promptly disarticulate from stems after flowering [Gisler, unpublished]), and seeds had all outward appearances of viability. However, later it was discovered during scarification that most of the

Table 3.2 Seed set and seed germination (measured as proportions) for parental conspecific crosses (in bold type) and reciprocal interspecific crosses with *Sidalcea nelsoniana*. Values followed by different letters are significantly different from *S. nelsoniana* conspecific crosses (alpha=0.05). As discussed in the text, significance determinations for some germination comparisons (namely, those involving crosses yielding low seed sample sizes) should be interpreted with caution as they may not be appropriate for statistical approximations. For *S. virgata*, "(small)" and "(big)" refer to small and large phenotypes, respectively.

Sire species	**Dam species**	**Proportion seed set (out of *n* total ovules)**	**Proportion germination (out of *n* total seeds)**
S. nelsoniana	***S. nelsoniana***	0.70 (217) a	0.90 (30) a
S. campestris	***S. campestris***	0.63 (247) a	0.73 (30) a
S. nelsoniana	*S. campestris*	0.03 (256) b	0.63 (08) a
S. campestris	*S. nelsoniana*	0.04 (227) b	0.75 (08) a
S. nelsoniana	***S. nelsoniana***	0.70 (217) a	0.90 (30) a
S. cusickii	***S. cusickii***	0.67 (260) a	0.97 (30) a
S. nelsoniana	*S. cusickii*	0.67 (251) a	0.93 (30) a
S. cusickii	*S. nelsoniana*	0.68 (211) a	0.93 (30) a
S. nelsoniana	***S. nelsoniana***	0.70 (217) a	0.90 (30) a
***S. virgata* (small)**	***S. virgata* (small)**	0.62 (218) a	0.77 (30) a
S. nelsoniana	*S. virgata* (small)	0.04 (212) b	0.25 (08) b
S. virgata (small)	*S. nelsoniana*	0.49 (219) b	0.87 (30) a
S. nelsoniana	***S. nelsoniana***	0.70 (217) a	0.90 (30) a
***S. virgata* (big)**	***S. virgata* (big)**	0.64 (214) a	0.80 (30) a
S. nelsoniana	*S. virgata* (big)	0.12 (216) b	0.39 (18) b
S. virgata (big)	*S. nelsoniana*	0.03 (214) b	0.57 (07) a

seeds were solely comprised of empty seed coats lacking developed embryo and endosperm tissue. As such, seed set data were revised following a complete inventory of all seeds for developed embryos, ultimately demonstrating very low (but not entirely absent) production of filled seeds from bi-directional crosses between *S. nelsoniana* and *S. campestris*. This "deceptive" phenomenon of empty seed coat formation was also observed among fruits formed by *S. nelsoniana*♂ x *S. virgata*♀ (large morphotype) crosses.

Following seed production, seed viability (germinability) was investigated as the next level of post-mating crossing barrier expression between *Sidalcea nelsoniana* and its local congeners. Unlike seed production, which varied significantly depending upon species crossing combination, there was no evidence of a significant species combination effect on seed germination (p=0.14), after accounting for insignificant (p=0.13) nested maternal effects. In other words, germination rates for seeds from interspecific crosses neither varied significantly among different heterospecific pairings, nor between heterospecific and conspecific pairings (Table 3.2 and Appendix 3.2). However, analysis of germination data was complicated by very low seed counts yielded by some interspecific crossing combinations, limiting seed availability for germination trials. Although Chi-square tests using logistic regression residuals showed no evidence of binomial data overdispersion, and elimination of hybrid lines with low seed counts from the germination data set did not significantly affect the outcome of analyses, caution is warranted when interpreting germination results with differential (and low) seed counts.

Efforts to evaluate post-mating crossing barriers expressed as reduced hybrid fertility were unexpectedly complicated by exclusive production of female hybrid progeny in five of the eight bi-directional interspecific crossing combinations. As such, hermaphrodite x female crosses could not be performed as planned to simultaneously estimate male and female fertilities for all hybrid lines. Because females produce no pollen, pollen staining techniques and/or self-pollination could not be used as an alternate method of fertility estimation for these hybrid lines. Likewise, backcrossing F1 hybrid females with their hermaphroditic progenitors was rejected as a fertility estimation method because any resulting lack of seed production might have been attributable to interspecific crossing barriers rather than female sterility *per se*. As such, hybrid fertility could only be conclusively assessed in the three hybrid lines that yielded both female and hermaphroditic individuals (*S. nelsoniana*♂ x *S. cusickii*♀, *S. cusickii*♂x *S. nelsoniana*♀, and *S. virgata* (small phenotype)♂ x *S. nelsoniana*♀).

Crosses performed within these three hybrid lines yielded the following mean seed set rates (calculated as proportions, n=10 crosses each): *S. nelsoniana*♂ x *S. cusickii*♀ = 0.67 (SD 0.12), *S. cusickii*♂ x *S. nelsoniana*♀ = 0.67 (SD 0.07), and *S. virgata* (small phenotype)♂ x *S. nelsoniana*♀ = 0 (SD 0). For bi-directional *S. nelsoniana* x *S. cusickii* hybrid lines, seed set levels were virtually identical to those of conspecific crosses reported above in Table 3.2, and therefore serve as positive indication of unreduced male and female hybrid fertility. For the *S. virgata* x *S. nelsoniana* hybrid line exhibiting negative seed set, pollen staining performed on the hermaphroditic hybrid sire proved inconclusive because 100 percent of grains stained positive for viability, including those from *very* old (i.e., several weeks) withered flowers that were almost certainly dead. As such, in this hybrid line it remains uncertain if negative seed set resulted from male and/or female sterility.

Polyploidy

Somatic chromosome numbers of root tip cells from parental *Sidalcea* species are consistent with counts previously reported by Hitchcock and Kruckeberg (1957), indicating a basal diploid chromosome number of $2n=20$ and the occurrence of diploid, tetraploid, and hexaploid species in the species group (Table 3.3 and Figure 3.1). The single deviation from previous counts was exhibited by *S. virgata*, which proved to be tetraploid for both the large *and* small phenotypes used in the current study.

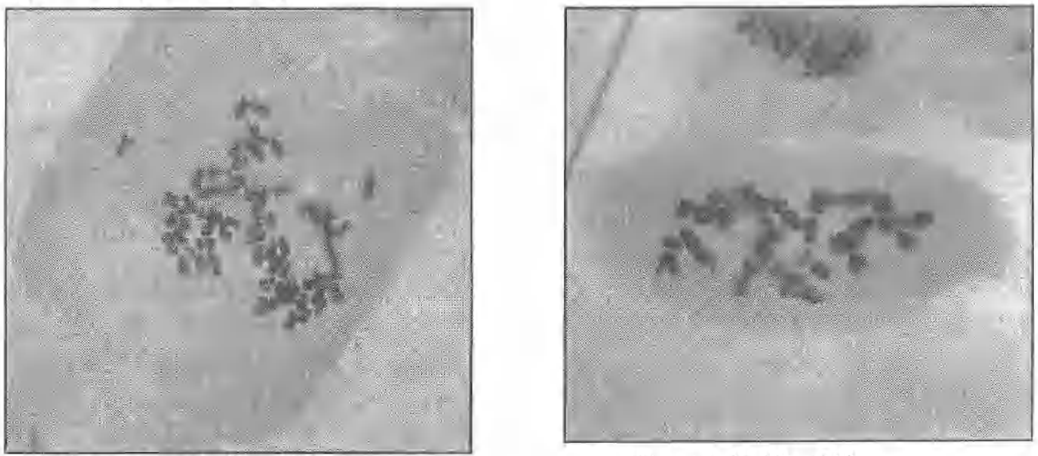
Despite the unexpected homoploidy of *Sidalcea virgata* individuals used in this study, crosses between *S. nelsoniana* and the two tetraploid *S. virgata* phenotypes yielded hybrids with different chromosome numbers. F1 hybrids produced between diploid *S. nelsoniana* and the large *S. virgata* phenotype were triploid in both crossing directions, whereas hybrids produced between *S. nelsoniana* and the small *S. virgata* phenotype were triploid when *S. virgata*

Table 3.3 Somatic chromosome numbers of parental *Sidalcea* species (in bold type) and their interspecific hybrids. For *S. virgata*, "(small)" and "(big)" refer to small and large morphotypes, respectively.

Taxon	Somatic chromosome number
S. nelsoniana	2n=20
S. campestris	2n=60
S. nelsoniana♂x *S. campestris*♀	2n=40
S. campestris♂ x *S. nelsoniana*♀	2n=40
S. nelsoniana	2n=20
S. cusickii	2n=20
S. nelsoniana♂ x *S. cusickii*♀	2n=20
S. cusickii♂ x *S. nelsoniana*♀	2n=20
S. nelsoniana	2n=20
***S. virgata* (big)**	2n=40
S. nelsoniana♂ x *S. virgata* (big)♀	2n=30
S. virgata (big)♂ x *S. nelsoniana*♀	2n=30
S. nelsoniana	2n=20
***S. virgata* (small)**	2n=40
S. nelsoniana♂ x *S. virgata* (small)♀	2n=40
S. virgata (small)♂ x *S. nelsoniana*♀	2n=30

Figure 3.1 Voucher photographs of somatic chromosomes of parental *Sidalcea* species and their interspecific hybrids. Chromosome counts were performed under a microscope (100x), allowing more accurate counts than available from the following voucher photographs that do not effectively capture the slight three-dimensional orientation and layering of chromosomes.

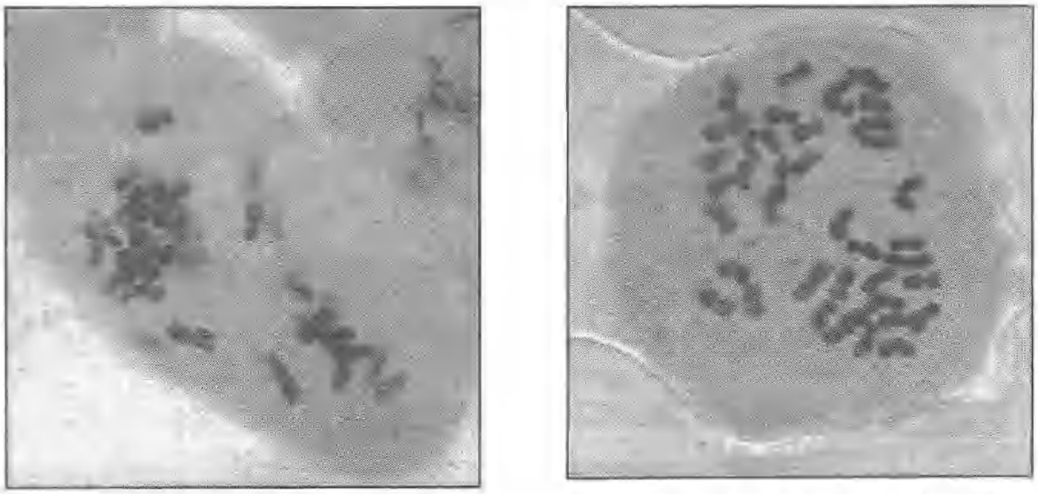
Figure 3.1 (Continued)



S. campestris (2n=60)

S. cusickii (2n=20)

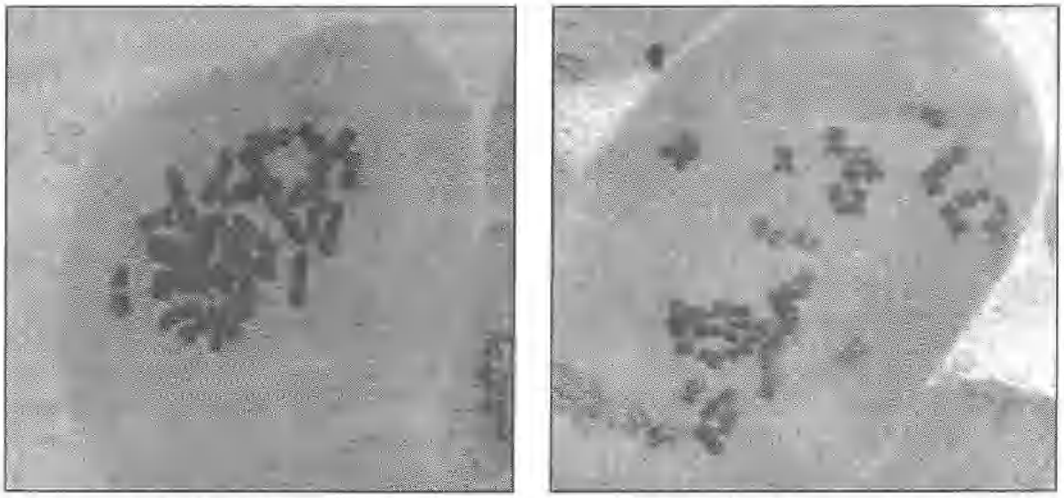
S. nelsoniana (2n=20)



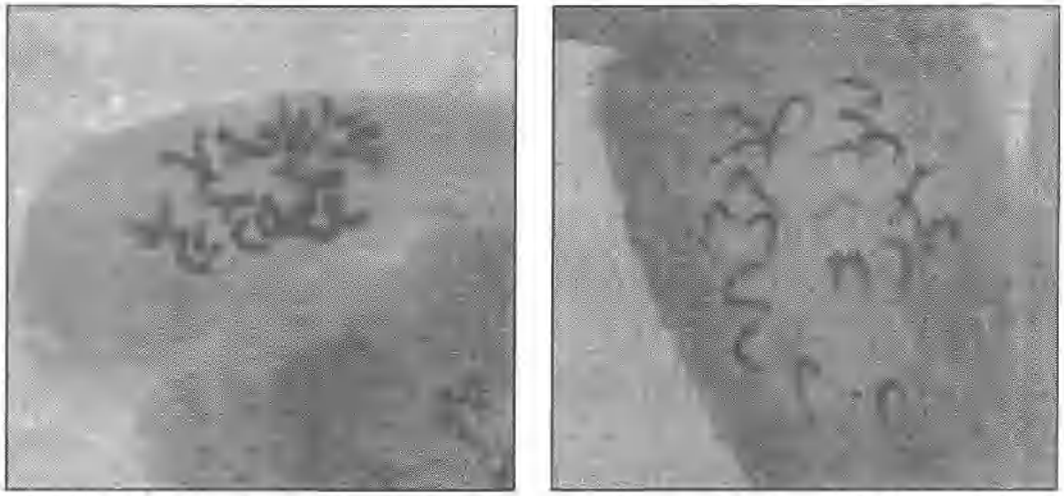
S. virgata(large phenotype) (2n=40)

S. virgata(small phenotype) (2n=40)

Figure 3.1 (Continued)

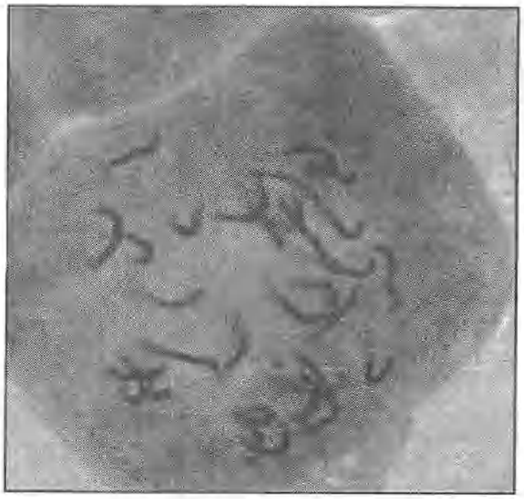


S. campestris♂ x *S. nelsoniana*♂(2n=40) *S. nelsoniana*♂ x *S. campestris*♀ (2n=40)

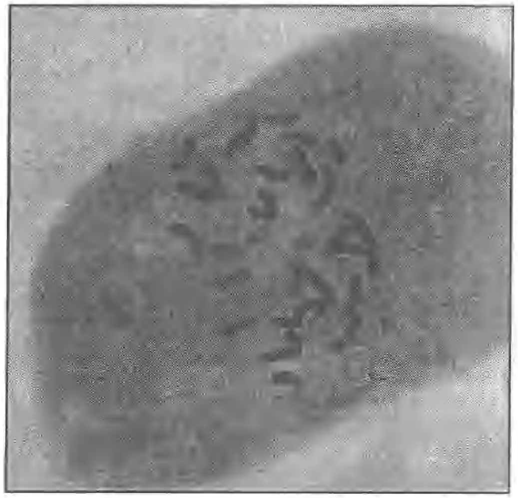


S. cusickii♂ x *S. nelsoniana*♀ (2n=20) *S. nelsoniana*♂ x *S. cusickii*♀ (2n=20)

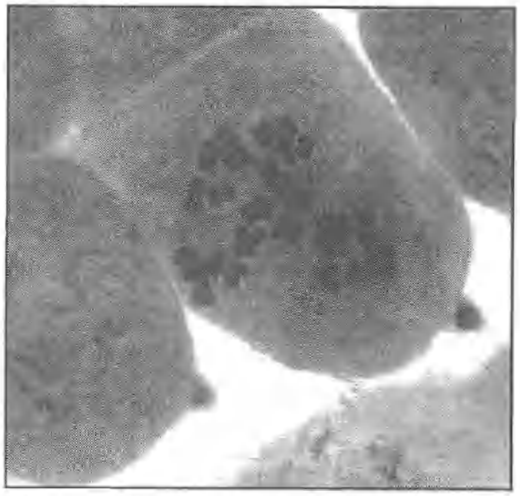
Figure 3.1 (Continued)



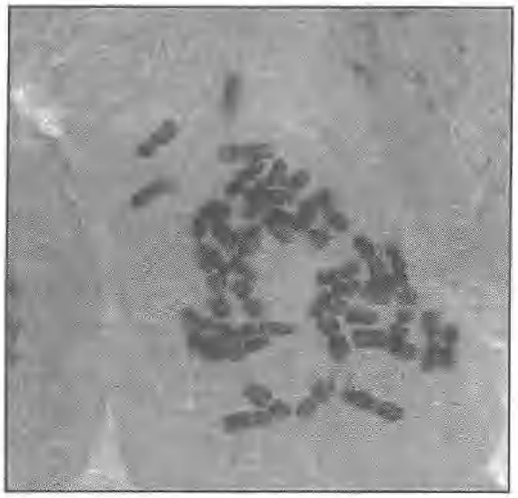
S. virgata(large phenotype)♂ x
S. nelsoniana♀ (2n=30)



S. nelsoniana♂ x *S. virgata*(large
phenotype)♀ (2n=30)



S. virgata(small phenotype)♂ x
S. nelsoniana♀ (2n=30)



S. nelsoniana♂ x *S. virgata*(small
phenotype)♀ (2n=40)

served as the sire and tetraploid when *S. nelsoniana* served as the sire. Directional differences were not observed among the remaining interspecific crosses, which exhibited "predicted" ploidy levels that were intermediate to those of their progenitors (Table 3.3 and Figure 3.1).

Hybrid morphology

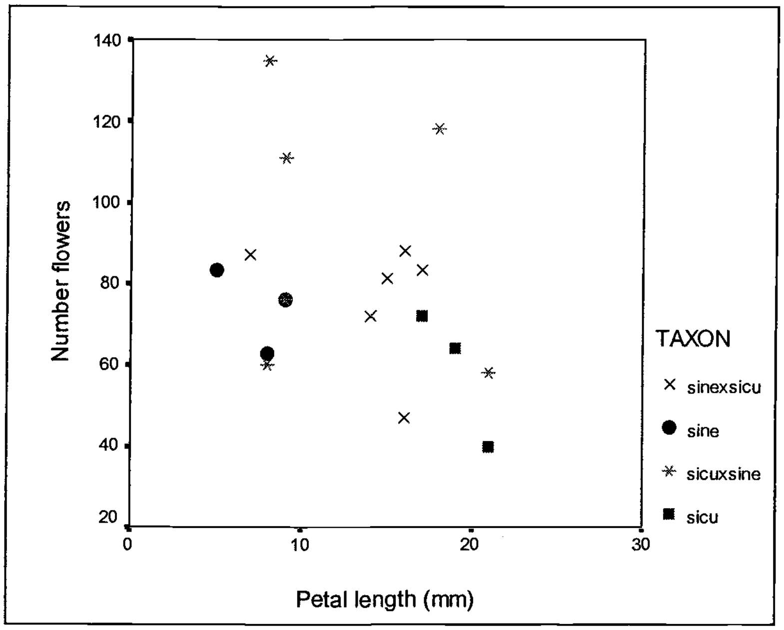
Results of morphological measurements performed on parental *Sidalcea* species and their F1 interspecific hybrids demonstrate that hybrids exhibit a wide range of phenotypic character states, including some that are similar to one or both parents, some that are intermediate between both parents, and others that are more extreme than either parent (i.e., transgressive traits). For instance, in reciprocal crosses between *S. nelsoniana* and *S. campestris*, hybrid flower color matched the white-flowered *S. campestris* in eight cases, the pink-flowered *S. nelsoniana* in two cases, and was intermediate in only a single case. However, stem pubescence in these hybrids matched *S. campestris* in zero cases, *S. nelsoniana* in 10 cases, and in one case was extreme to both parents (i.e., forked hairs instead of exclusively simple hairs).

Hybrid stem pubescence traits were especially variable in reciprocal crosses between *Sidalcea nelsoniana* (bearing short simple hairs) and *S. virgata* (small morphotype) (producing stellate hairs). Here, among the 14 F1 hybrids, four resembled *S. virgata*, three resembled *S. nelsoniana*, and seven differed from either parent by producing forked (and/or simple + forked) stem pubescence. This forked pubescence is classified here as an extreme trait, though it might similarly be interpreted as an intermediate character state between simple and stellate pubescence. Perhaps the most obvious, or at least visually striking, examples of extreme character states were three white-flowered hybrids produced between the two pink-flowered species, *S. nelsoniana* and *S. virgata* (small phenotype) (pictured in Appendix 3.4).

Morphological data for the two quantitative traits measured in this study are shown as bivariate plots for parent species and their F1 hybrids (separated by sex due to sexual dimorphism) (Figure 3.2), and data on the expression of categorical traits in hybrids, relative to their parents, are summarized in Table 3.4. Complete qualitative morphological data sets are provided in Appendix 3.3, and voucher photographs of each hybrid line are provided in Appendix 3.4.

Although hybrid phenotypes were comprised of an unpredictable and variable array of parental traits, for the most part hybrids appeared "normal," that is without any obvious evidence of developmental instability or other phenotypic irregularities. The only exceptions to this generalization were hybrids arising from *S. virgata* (small phenotype)♂ x *S. nelsoniana*♀ crosses. Here, among the 12 resulting F1 hybrids, four exhibited floral irregularities in the form of extra, missing, and/or fused petals (Figure 3.3).

Figure 3.2 Bivariate plots of quantitative traits measured on parental *Sidalcea* species and their F1 interspecific hybrids, separated by sex.



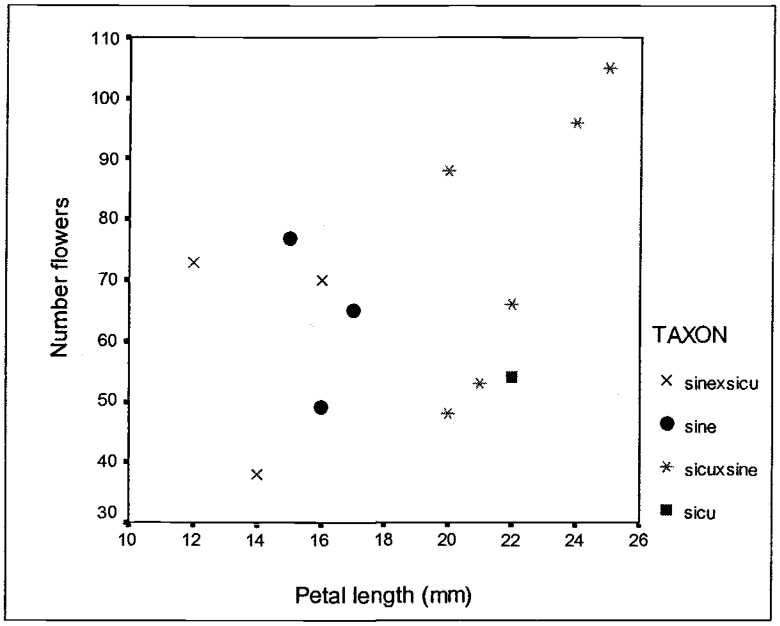
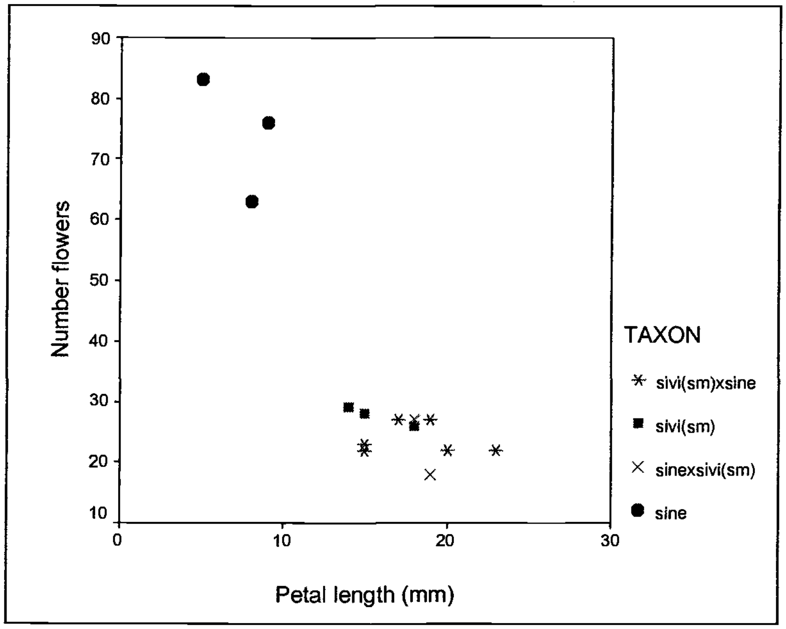


Figure 3.2 (Continued) Petal length versus number of flowers for female (**top**) and hermaphroditic (**bottom**) parental *Sidalcea nelsoniana* (sine), *S. cusickii* (sicu), and their F1 hybrids. Sexes are separated due to their dimorphism in the measured traits.



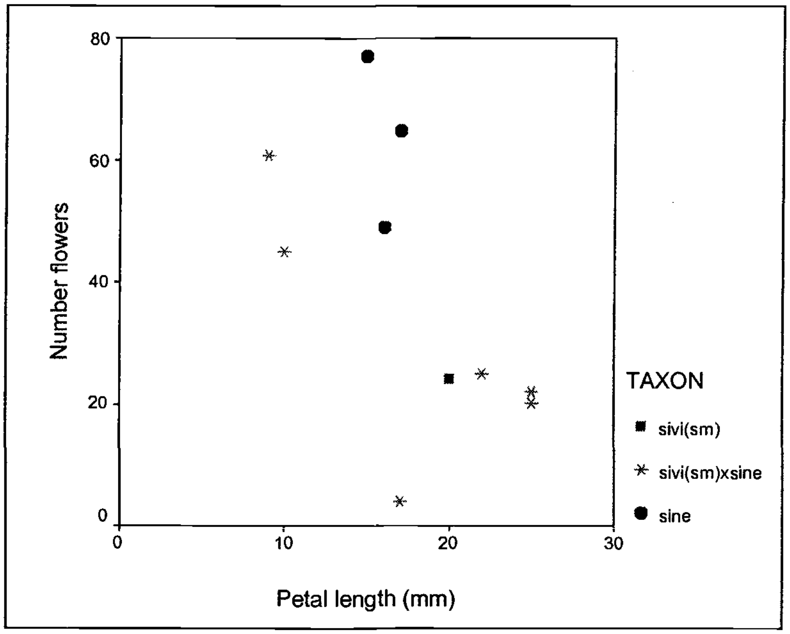
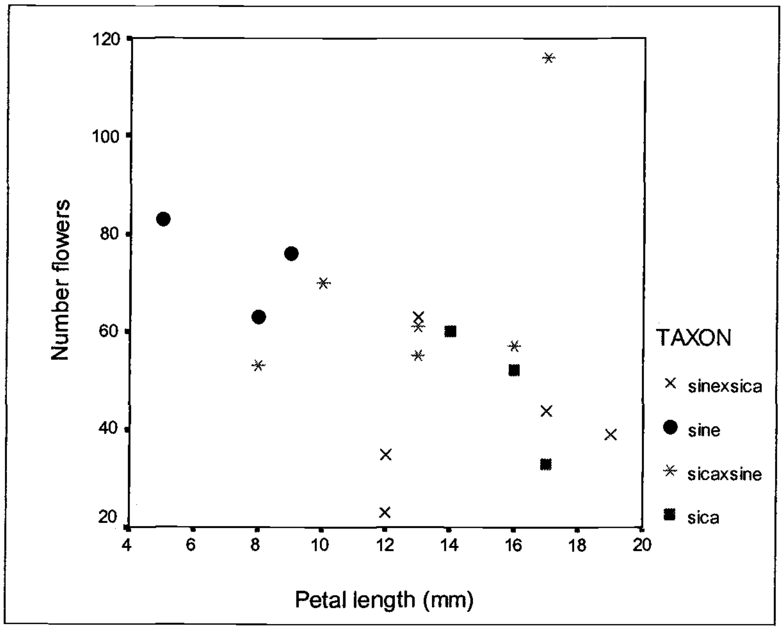


Figure 3.2 (Continued) Petal length versus number of flowers for female (**top**) and hermaphroditic (**bottom**) parental *Sidalcea nelsoniana* (sine), small phenotype *S. virgata* (sivi(sm)), and their F1 hybrids. Sexes are separated due to their dimorphism in the measured traits. No hermaphroditic hybrids were produced when *S. nelsoniana* was the sire in crosses with *S. virgata*.



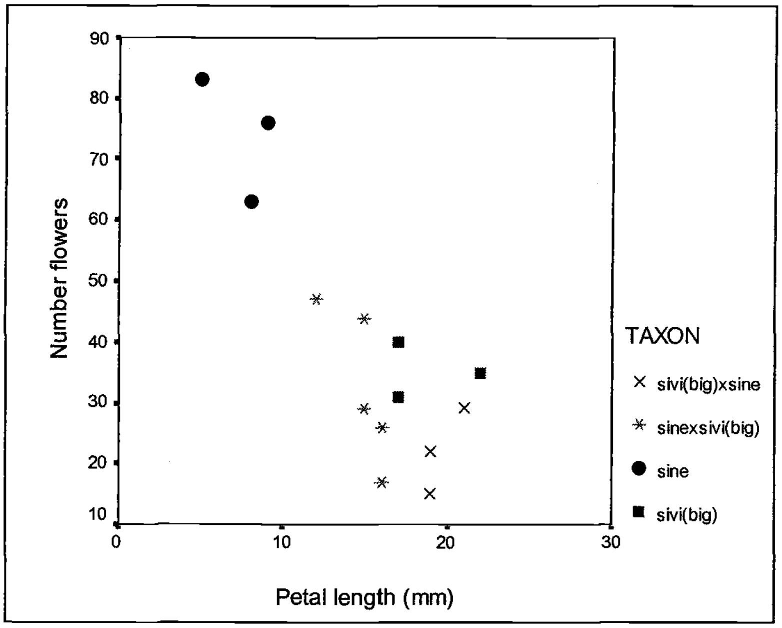


Figure 3.2 (Continued) Petal length versus number of flowers for two species pairs producing exclusively female hybrid progeny. **Top**: female parental *Sidalcea nelsoniana* (sine), *S. campestris* (sica) and their F1 hybrids. **Bottom**: female parental *S. nelsoniana* (sine), large phenotype *S. virgata* (sivi(big)), and their F1 hybrids.

Table 3.4 Summary of F1 *Sidalcea* hybrid phenotypes for eight qualitative morphological traits, categorized in relation to parental trait expression. Categories are: **PS**=resembling parental sire ("*" indicates lack of applicability due to trait homomorphy between parents), **PD**=resembling parental dam ("*" indicates lack of applicability due to trait homomorphy between parents), **PB**=resembling both parents when parents are homomorphic ("*" indicates lack of applicable homomorphic parental character state), **I**=intermediate between parents ("*" indicates lack of applicable intermediate character state), and **E**=extreme to both parents). The number of hybrids individuals in each category are provided in corresponding parentheses. For hybrids involving *S. virgata*, "(big)" and "(sm)" refer to large and small parental phenotypes, respectively. Complete morphological datasets are provided in Appendix 3.3.

Hybrid taxon	**Flower color**	**Stem habit**	**Sepal shape**	**Sepal color**	**Stem hairs**	**Leaf hairs**	**Glaucus stem**	**Raceme taper**
S. campestris♂ x *S. nelsoniana*♀	(4) PS (2) PD (*) PB (0) I (0) E	(*) PS (*) PD (6) PB (*) I (0) E	(*) PS (*) PD (6) PB (*) I (0) E	(*) PS (*) PD (6) PB (*) I (0) E	(0) PS (6) PD (*) PB (*) I (0) E	(2) PS (4) PD (*) PB (*) I (0) E	(*) PS (*) PD (6) PB (*) I (0) E	(*) PS (*) PD (6) PB (*) I (0) E
S. nelsoniana♂ x *S. campestris*♀	(0) PS (4) PD (*) PB (1) I (0) E	(*) PS (*) PD (5) PB (*) I (0) E	(*) PS (*) PD (5) PB (*) I (0) E	(*) PS (*) PD (5) PB (*) I (0) E	(4) PS (0) PD (*) PB (*) I (1) E	(0) PS (5) PD (*) PB (*) I (0) E	(*) PS (*) PD (5) PB (*) I (0) E	(*) PS (*) PD (5) PB (*) I (0) E
S. cusickii♂ x *S. nelsoniana*♀	(*) PS (*) PD (12) PB (*) I (0) E	(*) PS (*) PD (12) PB (*) I (0) E	(5) PS (7) PD (*) PB (*) I (0) E	(6) PS (6) PD (*) PB (0) I (0) E	(5) PS (7) PD (*) PB (*) I (0) E	(10) PS (2) PD (*) PB (*) I (0) E	(4) PS (8) PD (*) PB (*) I (0) E	(11) PS (1) PD (*) PB (*) I (0) E
S. nelsoniana♂ x S. *cusickii*♀	(*) PS (*) PD (9) PB (*) I (0) E	(*) PS (*) PD (9) PB (*) I (0) E	(1) PS (8) PD (*) PB (*) I (0) E	(6) PS (2) PD (*) PB (1) I (0) E	(7) PS (2) PD (*) PB (*) I (0) E	(6) PS (3) PD (*) PB (*) I (0) E	(9) PS (0) PD (*) PB (*) I (0) E	(6) PS (3) PD (*) PB (*) I (0) E

Table 3.4 (Continued)

Hybrid taxon	Flower color	Stem habit	Sepal shape	Sepal color	Stem hairs	Leaf hairs	Glaucus stem	Raceme taper
S. nelsoniana♂ x S. *cusickii*♀	(*) PS (*) PD (9) PB (*) I (0) E	(*) PS (*) PD (9) PB (*) I (0) E	(1) PS (8) PD (*) PB (*) I (0) E	(6) PS (2) PD (*) PB (1) I (0) E	(7) PS (2) PD (*) PB (*) I (0) E	(6) PS (3) PD (*) PB (*) I (0) E	(9) PS (0) PD (*) PB (*) I (0) E	(6) PS (3) PD (*) PB (*) I (0) E
S. virgata(big) ♂ x *S. nelsoniana*♀	(*) PS (*) PD (3) PB (*) I (0) E	(3) PS (0) PD (*) PB (*) I 0) E	(3) PS (0) PD (*) PB (*) I (0) E	(0) PS (3) PD (*) PB (0) I (0) E	(3) PS (0) PD (*) PB (0) I (0) E	(0) PS (3) PD (*) PB (*) I (0) E	(0) PS (3) PD (*) PB (*) I (0) E	(*) PS (*) PD (3) PB (*) I (0) E
S. nelsoniana♂ x *S. virgata*(big) ♀	(*) PS (*) PD (4) PB (*) I (1) E	(2) PS (3) PD (*) PB (*) I (0) E	(0) PS (5) PD (*) PB (*) I (0) E	(5) PS (0) PD (*) PB (0) I (0) E	(0) PS (5) PD (*) PB (0) I (0) E	(5) PS (0) PD (*) PB (*) I (0) E	(5) PS (0) PD (*) PB (*) I (0) E	(*) PS (*) PD (5) PB (*) I (0) E
S. virgata(sm) ♂ x *S. nelsoniana*♀	(*) PS (*) PD (9) PB (*) I (3) E	(9) PS (3) PD (*) PB (*) I (0) E	(12) PS (0) PD (*) PB (*) I (0) E	(*) PS (*) PD (12) PB (*) I (0) E	(4) PS (3) PD (*) PB (0) I (5) E	(7) PS (5) PD (*) PB (*) I (0) E	(4) PS (8) PD (*) PB (*) I (0) E	(*) PS (*) PD (12) PB (*) I (0) E
S. nelsoniana♂ x *S. virgata*(sm) ♀	(*) PS (*) PD (2) PB (*) I (0) E	(0) PS (2) PD (*) PB (*) I (0) E	(0) PS (2) PD (*) PB (*) I (0) E	(*) PS (*) PD (2) PB (*) I (0) E	(0) PS (2) PD (*) PB (0) I (0) E	(0) PS (2) PD (*) PB (*) I (0) E	(1) PS (1) PD (*) PB (*) I (0) E	(*) PS (*) PD (2) PB (*) I (0) E

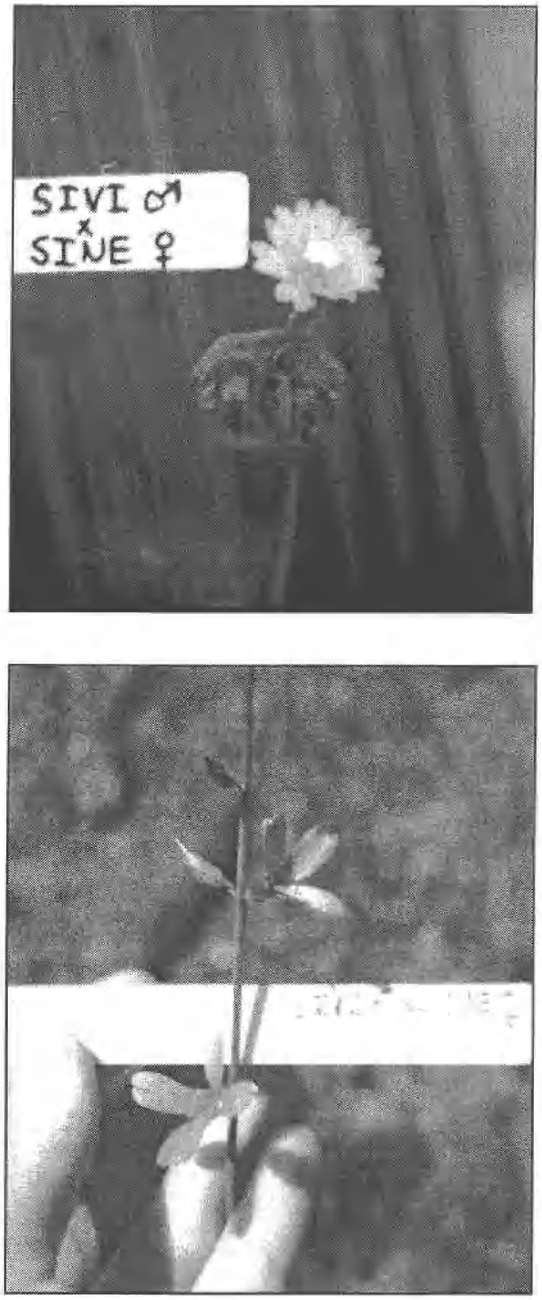


Figure 3.3 Examples of floral irregularities observed in *Sidalcea virgata* (small phenotype)♂ x *S. nelsoniana*♀ F1 hybrids. **Top**: hermaphroditic flower with three extra petals. **Bottom**: female flowers lacking their fifth petals.

Discussion

The extent to which post-mating crossing barriers inhibit hybrid formation between *Sidalcea nelsoniana* and its local congeners varies among the different interspecific crossing combinations. Sexual incompatibility appears almost complete between *S. nelsoniana* and its white-flowered relative, *S. campestris,* as very few seeds are ever formed when these two species are reciprocally crossed. Likewise, seeds are rarely produced through crosses between *S. nelsoniana* and *S. virgata,* with the exception of directional crosses involving *S. virgata* (small phenotype) sires; within these crosses, interspecific seed set is nearly as high as (though still significantly lower than) "legitimate" conspecific seed production levels. Such directional differences in crossing success within a species pair is common in many groups of plants, especially when species with different chromosome numbers are crossed—a phenomenon often attributable to differential pollen grain germination, pollen tube growth success, and endosperm formation (Thompson 1930). In contrast to the aforementioned examples, post-mating crossing barriers appear non-existent between *S. nelsoniana* and *S. cusickii,* both of which produce ample viable seeds and fertile hybrid progeny through reciprocal crosses.

Germinability of seeds proved fairly constant across all conspecific and interspecific crossing combinations, suggesting that seed production, not seed viability, is the most important post-mating limiting step to hybrid formation in this species group. As hybrid fertility could not be tested in most of the hybrid lines due to exclusive production of female progeny, the frequency of hybrid sterility and its potential role in limiting hybrid fitness remains unknown. Exclusive yields of female hybrids in some crosses probably resulted from cytoplasmically controlled male sterility inheritance, whereby female individuals (in this study serving as pollen recipients in all reciprocal crosses) always give rise to female progeny. This pattern was observed among interspecific crosses involving *Sidalcea campestris* and *S. virgata* dams. In contrast, a mixture of female and hermaphroditic progeny were produced by interspecific crosses involving *S. nelsoniana* and *S. cusickii* dams, suggesting male sterility inheritance in these species is probably controlled by cytoplasmic *and* nuclear genes. However, the same *S. nelsoniana* dams produced exclusively female progeny when crossed with *S. virgata* (large phenotype) and *S. campestris* sires. The observed variability could be due to chance sex ratios, small sample sizes, nuclear-cytoplasmic interactions, and/or other genic interactions between the different parent species.

Results of chromosome counts suggest that polyploidy may play an important role in post-mating reproductive isolation in *Sidalcea nelsoniana*, insofar that interspecific sexual incompatibility is most pronounced between the most dissimilar cytotypes (diploid x hexaploid), somewhat lower between closer cytotypes (diploid x tetraploid), and virtually nonexistent between the two diploid species. This positive relationship between sexual incompatibility and the magnitude of mating pair heteroploidy is commonly observed in plants, and may frequently arise through chromosomal imbalances in F1 endosperms (Riera-Lizarazu, Oregon State University Department of Crop and Soil Science, personal communication). These findings support the widely held belief that polyploidy commonly serves as an agent of reproductive isolation and subsequent sympatric speciation among related species. However, in the case of *S. nelsoniana* and *S. virgata*, such post-mating reproductive isolation is incomplete.

Incomplete reproductive isolation between species with different chromosome numbers is thought to often result from the production of unreduced gametes, a routine occurrence among flowering plants that can lead to successful interbreeding between different cytotypes and the origin of polyploid species (Avers 1957, Lewis 1967, De Wet 1980, Bretagnolle and Thompson 1995). In this study, unreduced gametes (i.e., pollen) produced by diploid hermaphroditic *Sidalcea nelsoniana* might have accounted for the tetraploid hybrid progeny yielded through crosses with female tetraploid *S. virgata* (small phenotype). Here, if unreduced *S. nelsoniana* pollen was involved in crosses, two sets of chromosomes would have been ostensibly contributed by each heterospecific parent, yielding balanced chromosome numbers in their F1 hybrids. Examples of tetraploid offspring arising through diploid x tetraploid interspecific crosses are not uncommon, and are, again, typically attributed to unreduced gametes in the diploid parents (Zohary and Nur 1958, Marks 1966, Lewis 1967, Dunford 1970). If unreduced *S. nelsoniana* pollen indeed contributed to the tetraploid hybrids produced with *S. virgata*, it is unknown why such pollen did not also affect interspecific crosses with large phenotype *S. virgata* dams, or any of the other interspecific crosses, given their common paternity. Perhaps in these crosses, unreduced pollen grains were simply outperformed by normal haploid pollen grains (if present) in the maternal styles.

As hinted in the preceding sentence, unreduced gametes were apparently *not* involved in the reciprocal crosses between *Sidalcea nelsoniana* dams and *S. virgata* (small phenotype) sires, as resulting hybrids in this directional cross were triploid—the "predicted" outcome of diploid x tetraploid crosses. Here, two sets of chromosomes were ostensibly contributed by the haploid gametes of hermaphroditic tetraploid *S. virgata* and one set from female diploid *S. nelsoniana*. Likewise, normally reduced haploid gametes were apparently involved in bi-directional crosses between *S. nelsoniana* and the large phenotype tetraploid *S. virgata*, as their resulting hybrids were also triploid.

Sterility observed in triploid hybrids from *S. virgata*♂ x *S. nelsoniana*♀ crosses may have been attributable to unbalanced chromosome pairing at meiosis caused by its triploid condition (Lewis 1967, Jackson 1976). Although fertility tests could not be conducted on triploid hybrids resulting from reciprocal crosses with the large phenotype *S. virgata* (due to exclusive production of females), these hybrids may also have proved sterile via similar meiotic complications.

As in the example above, normally reduced haploid gametes were probably also involved in the formation of tetraploid hybrids through crosses between diploid *S. nelsoniana* and hexaploid *S. campestris* (one set of chromosomes contributed by *S. nelsoniana* and three sets from *S. campestris*). Reduced haploid gametes may have contributed to the formation of diploid hybrids produced through bi-directional crosses between homoploid *S. nelsoniana* and *S. cusickii* as well. Hybrids formed by the latter proved fertile, as balanced numbers of chromosomes were ostensibly available for pairing during meiosis. Likewise, balanced chromosomes may have been present in the tetraploid hybrids produced by *S. nelsoniana* and *S. campestris*, though once again fertility could not be confirmed among the exclusively female progeny.

Despite the extreme infrequency of hybrids successfully formed through crosses between *Sidalcea nelsoniana* and its congeners, *S. campestris* and *S. virgata* (large phenotype), such rare events could still be of evolutionary and conservation significance. Indeed, although the formation of F1s often represents a formidable barrier to hybridization, hybrid production (even on rare occasions) is commonly followed by rapid and extensive introgression (Arnold 1997, Rieseberg and Carney 1998). In addition, infrequent F1 hybrids, even those that are highly sterile, can serve as "bridges" for the production of advanced hybrid generations that possess relatively fit genotypes (Rieseberg and Wendel 1993, Arnold and Hodges 1995).

This scenario is exemplified by the sunflower species, *Helianthus annuus* and *H. petiolaris*, which produce highly sterile early generation hybrids when artificially crossed but have nonetheless given rise to at least three hybrid species in the wild, apparently due to the recovery of fertility through backcrossing with parent species (Rieseberg and Carney 1998). The breakdown of chromosomal sterility barriers has likewise been suggested in studies by Stebbins and Daly (1961), Hauber and Bloom (1983), Meyn and Emboden (1987), and Ungerer et al. (1998), all similarly demonstrating dramatically increased fertility of advanced generation hybrids compared to the largely sterile F1s that spawned them.

As such, the importance of triploid hybrids produced by *Sidalcea nelsoniana* and the large phenotype *S. virgata* should likewise not be discounted simply on grounds of their demonstrated sterility. Generally, although triploids often exhibit reduced fertility, they are rarely, if ever, *completely* sterile (Avers 1957, Lewis 1967), and as stated by Rieseberg, Linder and Seiler (1995), "...most chromosomal sterility barriers (even those involving several chromosomes) should not be impermeable to gene flow." Moreover, triploid individuals have frequently been shown capable of serving as important intermediates that eventually yield fertile tetraploid or diploid offspring when backcrossed with their parents (Lewis 1967). And as discussed in the preceding paragraph, fertility of largely sterile hybrids can also be regained over successive generations, even without chromosomal additions attributable to backcrossing.

In *Sidalcea*, the potential significance of rare and/or sterile hybrids is further amplified by the fact that these species are long-lived perennials, and in some cases are prone to vigorous asexual expansion. As such, F1 hybrids (even highly sterile ones) may become stabilized through clonal growth and experience many years of repeated opportunities to mate and form advanced generations of hybrids (Harper et al. 1961, Stebbins 1971, Grant 1981, Grootjans et al. 1987, Emms and Arnold 1997). Such conferred advantages to hybrid formation would presumably be even greater among the more commonly produced and fully fertile hybrids formed between *S. nelsoniana* and *S. cusickii*, if such are ever formed outside the artificial setting of this study.

Despite evidence of at least some degree of sexual compatibility and hybrid formation between *Sidalcea nelsoniana* and all three of its congeners, there are several reasons why these data, taken alone, may overestimate the practical likelihood of hybridization in the wild. First, as discussed in Chapter 2, there are several important pre-mating crossing barriers in place (namely temporal and geographical isolation) that discourage interspecific gene flow between *S. nelsoniana* and two of its three Willamette Valley congeners. Moreover, there remains the fact that this study employed artificial hand-pollinations with the specific goal of creating hybrids...pollinations that saturated stigmas with pure loads of heterospecific pollen. Although such methods are useful for the objectives of producing hybrids and investigating levels of interspecific sexual compatibility, they do not reflect natural or ecologically realistic constraints intrinsic to insect-mediated pollinations in the wild. Here, even if pollinators move between heterospecific individuals within mixed, or between neighboring, populations, production of hybrids would likely be limited by 1) previous deposition of conspecific pollen, 2) dilution of heterospecific pollen by mixed loads of conspecific pollen, and 3) outperformance of heterospecific pollen tubes by those from conspecific grains.

The role of pollen competition as a possible barrier to hybridization has been recognized for a long time. For example, Charles Darwin (1859) believed pollen competition to be widespread, writing:

> "It is well known that if pollen of a distinct species be placed on the stigmas of a flower, and its own pollen be afterward, even after a considerable interval of time, placed on the same stigma, its action is so strongly prepotent that it generally annihilates the effect of the foreign pollen."

This observation has since been shown to hold true in a wide variety of species pairs that exhibit greater germination and growth of conspecific pollen relative to that of congeners in mixed pollen loads (Buchholz et al. 1935, Heiser et al. 1969, Smith 1970, Kiang and Hamrick 1978, Carney et al. 1994, Rieseberg, Desrochers and Youn 1995, Klips 1999). Therefore, although it is useful to consider the hybridization potential for species under artificial conditions, it should be noted that the current data do not reflect the likely "filtering" effect of mixed heterospecific and conspecific pollen loads.

Looking at the expression of parental *Sidalcea* morphological traits in hybrids, F1 hybrids mostly exhibit a mixture of characters similar to one or both parents, though in some cases certain traits are intermediate or transgressive to those of their parents. This mirrors Rieseberg's (1995) statement that, contrary to the popular belief that hybrids are always intermediate between their parents, "Hybrids are actually a mosaic of parental, intermediate, and extreme characters."

The high proportion of parental characters expressed by the *Sidalcea* hybrids may be due to the fact that many morphological differences between closely related species are generally under simple genetic control (Rieseberg and Ellstrand 1993, Rieseberg 1995), combined with the phenomenon of segregation distortion, whereby hybrid progeny receive more alleles from one parent than would be predicted under Mendelian rules of inheritance (Rieseberg and Carney 1998).

Ultimately, although it is interesting to see what *Sidalcea* hybrids look like relative to their parents, and contemplate the possible genetic mechanisms influencing hybrid phenotypes, it is highly probable that, in nature, hybrid traits would be obscured by the pronounced morphological variability exhibited within each of the study species. This variability (see Appendix 3.5 for photographic examples) often complicates the basic task of discerning parental *Sidalcea*s, so would likewise be expected to render hybrid identification problematic, especially since *Sidalcea* hybrids are not consistently characterized by predictable morphological intermediacy. Such complications would probably become even more pronounced after several generations of hybrid-parent backcrossing, which would ostensibly lead to even more extensive blurring of already ambiguous diagnostic traits.

Exclusive reliance on morphological characters for hybrid identification has been criticized because of the propensity for character plasticity in many plant groups (Paige and Chapman 1993) and because of the unpredictable nature of parental character expression in hybrids (Rieseberg and Carney 1998). Future molecular work is clearly needed to help resolve the taxonomy of Willamette Valley *Sidalcea*s and clarify the extent of past and current hybridization in nature, considering 1) the potential for hybridization in *Sidalcea* documented in this study, 2) published suspicions about the importance of hybridization in speciation within the genus (Hitchcock and Cronquist 1957), 3) the taxonomic confusion arising from intraspecific morphological variability in Willamette Valley *Sidalcea*s, 4) the ambiguity of interspecific sexual compatibility between tetraploid *S. virgata* phenotypes, and 5) the potential conservation implications (good and bad) of hybridization. However, this work should be undertaken with the caveat that the expression of molecular markers often differs minimally between parental and hybrid genotypic lines (Rieseberg and Carney 1998), and marker proportions in hybrids can be strongly biased by selection, potentially leading to faulty genealogical assignments (Rieseberg and Linder 1999).

APPENDICES

Appendix 3.1 Seed set data for conspecific parental *Sidalcea* crosses and reciprocal interspecific crosses with *S. nelsoniana*. Seed set is calculated as the proportion of total seeds to total available ovules among 10 replicated crosses for each maternal line (*n*= 3 maternal lines per species crossing combination). For *S. virgata*, "(sm)" and "(big)" refer to small and large phenotypes, respectively.

Species crossing combination	**Maternal line #**	***n* total ovules**	***n* total seeds**	**Seed set**
S. nelsoniana ♂ x *S. nelsoniana* ♀	1	74	63	0.85
S. nelsoniana ♂ x *S. nelsoniana* ♀	2	71	45	0.63
S. nelsoniana ♂ x *S. nelsoniana* ♀	3	72	44	0.61
S. campestris ♂ x *S. campestris* ♀	4	84	57	0.68
S. campestris ♂ x *S. campestris* ♀	5	79	50	0.63
S. campestris ♂ x *S. campestris* ♀	6	84	49	0.58
S. cusickii ♂ x *S. cusickii* ♀	7	88	56	0.64
S. cusickii ♂ x *S. cusickii* ♀	8	84	52	0.62
S. cusickii ♂ x *S. cusickii* ♀	9	88	66	0.75
S. virgata (sm) ♂ x *S. virgata* (sm) ♀	10	76	49	0.64
S. virgata (sm) ♂ x *S. virgata* (sm) ♀	11	70	42	0.6
S. virgata (sm) ♂ x *S. virgata* (sm) ♀	12	72	45	0.63
S. virgata (big) ♂ x *S. virgata* (big) ♀	13	70	42	0.6
S. virgata (big) ♂ x *S. virgata* (big) ♀	14	73	50	0.68
S. virgata (big) ♂ x *S. virgata* (big) ♀	15	71	46	0.65
S. nelsoniana ♂ x *S. campestris* ♀	4	85	1	0.01
S. nelsoniana ♂ x *S. campestris* ♀	5	85	4	0.05
S. nelsoniana ♂ x *S. campestris* ♀	6	86	3	0.03
S. campestris ♂ x *S. nelsoniana* ♀	1	77	0	0
S. campestris ♂ x *S. nelsoniana* ♀	2	78	4	0.05
S. campestris ♂ x *S. nelsoniana* ♀	3	72	6	0.08
S. nelsoniana ♂ x *S. cusickii* ♀	7	83	60	0.72
S. nelsoniana ♂ x *S. cusickii* ♀	8	82	55	0.67
S. nelsoniana ♂ x *S. cusickii* ♀	9	86	53	0.62
S. cusickii ♂ x *S. nelsoniana* ♀	1	73	59	0.81
S. cusickii ♂ x *S. nelsoniana* ♀	2	69	46	0.67
S. cusickii ♂ x *S. nelsoniana* ♀	3	69	38	0.55
S. nelsoniana ♂ x *S. virgata* (sm) ♀	10	75	7	0.09
S. nelsoniana ♂ x *S. virgata* (sm) ♀	11	68	1	0.01
S. nelsoniana ♂ x *S. virgata* (sm) ♀	12	69	0	0
S. virgata (sm) ♂ x *S. nelsoniana* ♀	1	77	25	0.32
S. virgata (sm) ♂ x *S. nelsoniana* ♀	2	74	40	0.54
S. virgata (sm) ♂ x *S. nelsoniana* ♀	3	68	43	0.63
S. nelsoniana ♂ x *S. virgata* (big) ♀	13	72	13	0.18
S. nelsoniana ♂ x *S. virgata* (big) ♀	14	72	4	0.06
S. nelsoniana ♂ x *S. virgata* (big) ♀	15	72	9	0.13
S. virgata (big) ♂ x *S. nelsoniana* ♀	1	72	0	0
S. virgata (big) ♂ x *S. nelsoniana* ♀	2	72	2	0.03
S. virgata (big) ♂ x *S. nelsoniana* ♀	3	70	5	0.07

Appendix 3.2 Seed germination data for conspecific parental *Sidalcea* crosses and reciprocal interspecific crosses with *S. nelsoniana*. Seed germination is calculated as the proportion of total germinated seeds to total seeds tested for each maternal line. Variability in sample sizes was due to low seed set in some maternal lines (see Appendix 3.1). For *S. virgata*, "(sm)" and '(big)" refer to small and large phenotypes, respectively.

Species crossing combination	**Maternal line #**	***n* seeds tested**	***n* seeds germinated**	**Seed germination**
S. nelsoniana ♂ x *S. nelsoniana* ♀	1	10	9	0.90
S. nelsoniana ♂ x *S. nelsoniana* ♀	2	10	8	0.80
S. nelsoniana ♂ x *S. nelsoniana* ♀	3	10	10	1.00
S. campestris ♂ x *S. campestris* ♀	4	10	8	0.80
S. campestris ♂ x *S. campestris* ♀	5	10	7	0.70
S. campestris ♂ x *S. campestris* ♀	6	10	7	0.70
S. cusickii ♂ x *S. cusickii* ♀	7	10	10	1.00
S. cusickii ♂ x *S. cusickii* ♀	8	10	9	0.90
S. cusickii ♂ x *S. cusickii* ♀	9	10	10	1.00
S. virgata (sm) ♂ x *S. virgata* (sm) ♀	10	10	7	0.70
S. virgata (sm) ♂ x *S. virgata* (sm) ♀	11	10	8	0.80
S. virgata (sm) ♂ x *S. virgata* (sm) ♀	12	10	8	0.80
S. virgata (big) ♂ x *S. virgata* (big) ♀	13	10	8	0.80
S. virgata (big) ♂ x *S. virgata* (big) ♀	14	10	9	0.90
S. virgata (big) ♂ x *S. virgata* (big) ♀	15	10	7	0.70
S. nelsoniana ♂ x *S. campestris* ♀	4	1	0	0
S. nelsoniana ♂ x *S. campestris* ♀	5	4	3	0.75
S. nelsoniana ♂ x *S. campestris* ♀	6	3	2	0.67
S. campestris ♂ x *S. nelsoniana* ♀	1	0	n/a	n/a
S. campestris ♂ x *S. nelsoniana* ♀	2	3	2	0.67
S. campestris ♂ x *S. nelsoniana* ♀	3	5	4	0.80
S. nelsoniana ♂ x *S. cusickii* ♀	7	10	9	0.90
S. nelsoniana ♂ x *S. cusickii* ♀	8	10	10	1.00
S. nelsoniana ♂ x *S. cusickii* ♀	9	10	9	0.90
S. cusickii ♂ x *S. nelsoniana* ♀	1	10	8	0.80
S. cusickii ♂ x *S. nelsoniana* ♀	2	10	10	1.00
S. cusickii ♂ x *S. nelsoniana* ♀	3	10	10	1.00
S. nelsoniana ♂ x *S. virgata* (sm) ♀	10	7	2	0.29
S. nelsoniana ♂ x *S. virgata* (sm) ♀	11	1	0	0
S. nelsoniana ♂ x *S. virgata* (sm) ♀	12	0	n/a	n/a
S. virgata (sm) ♂ x *S. nelsoniana* ♀	1	10	7	0.70
S. virgata (sm) ♂ x *S. nelsoniana* ♀	2	10	9	0.90
S. virgata (sm) ♂ x *S. nelsoniana* ♀	3	10	9	0.90
S. nelsoniana ♂ x *S. virgata* (big) ♀	13	10	5	0.50
S. nelsoniana ♂ x *S. virgata* (big) ♀	14	3	0	0
S. nelsoniana ♂ x *S. virgata* (big) ♀	15	5	2	0.40
S. virgata (big) ♂ x *S. nelsoniana* ♀	1	0	n/a	n/a
S. virgata (big) ♂ x *S. nelsoniana* ♀	2	2	2	1.00
S. virgata (big) ♂ x *S. nelsoniana* ♀	3	5	2	0.40

Appendix 3.3 Qualitative morphological traits measured among parental *Sidalcea* species and their interspecific hybrids. Data are grouped by each reciprocal species crossing combination, and separated by sex (F=female and Hm=hermaphrodite) to account for sexual dimorphism in some traits. For *S. nelsoniana*, data are only provided for one of the three hermaphroditic sires used for crosses, as all three were monomorphic for qualitative traits due to their common origin via division of a single individual.

Appendix 3.3 (Continued)

Explanation of categorical codes for qualitative morphological traits measured on parental *Sidalcea* species and their interspecific hybrids.

Morphological trait	Explanation of categorical level codes
Flower color	1=white 2=very light pink 3=light pink 4=pink 5=dark pink
Habit	1=stems fully erect 2=stems slightly decumbent at base (10°-45°) 3=stems strongly decumbent at base (>45°)
Sepal shape	1=long and marginally rolled 2=short and evenly tapered 3=short and widened above the base before tapering
Sepal color	1=no anthocyanic pigmentation 2=slight pigmentation, primarily along midvein and margins 3=strong pigmentation throughout
Stem hairs	1=glabrous 2=short-simple 3=long-simple 4=mixed simple + forked 5=forked 6=stellate
Upper leaf surface	1=glabrous 2=sparsely hairy 3=densely hairy
Glaucus stems	1=flowering stems not glaucus 2=flowering stems glaucus
Raceme taper (in bud stage)	1=tapered to a point 2=blunt to slightly widened at top

Appendix 3.3 (Continued)

Qualitative morphological traits for **female** parental *Sidalcea nelsoniana* (sine), *S. campestris* (sica), and F1 hybrids (hybrids were exclusively female).

Taxon	**Sex**	**Flower color**	**Habit**	**Sepal shape**	**Sepal color**	**Stem hairs**	**Leaf hairs**	**Glaucus**	**Raceme taper**
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sica	F	1	1	2	1	3	3	2	1
sica	F	2	1	2	1	3	3	2	1
sica	F	2	1	2	1	3	3	2	1
sine♂ x sica♀	F	1	1	2	1	2	3	2	1
sine♂ x sica♀	F	3	1	2	1	4	3	2	1
sine♂ x sica♀	F	1	1	2	1	2	3	2	1
sine♂ x sica♀	F	2	1	2	1	2	3	2	1
sine♂ x sica♀	F	2	1	2	1	2	3	2	1
sica♂ x sine♀	F	1	1	2	1	2	3	2	1
sica♂ x sine♀	F	2	1	2	1	2	3	2	1
sica♂ x sine♀	F	2	1	2	1	2	2	2	1
sica♂ x sine♀	F	1	1	2	1	2	2	2	1
sica♂ x sine♀	F	4	1	2	1	2	2	2	1
sica♂ x sine♀	F	4	1	2	1	2	2	2	1

Appendix 3.3 (Continued)

Qualitative morphological traits for **female** parental *Sidalcea nelsoniana* (sine) *S. cusickii* (sicu), and F1 hybrids.

Taxon	**Sex**	**Flower color**	**Habit**	**Sepal shape**	**Sepal color**	**Stem hairs**	**Leaf hairs**	**Glaucus**	**Raceme taper**
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sicu	F	4	1	3	3	1	1	1	2
sicu	F	4	1	3	3	1	1	1	2
sicu	F	5	1	3	2	1	1	1	2
sine♂ x sicu♀	F	4	1	3	1	2	2	2	2
sine♂ x sicu♀	F	4	1	3	3	2	2	2	2
sine♂ x sicu♀	F	4	1	3	1	1	1	2	2
sine♂ x sicu♀	F	4	1	3	1	2	2	2	1
sine♂ x sicu♀	F	4	1	2	1	1	1	2	1
sine♂ x sicu♀	F	4	1	3	1	2	2	2	1
sicu♂ x sine♀	F	4	1	2	1	1	1	1	2
sicu♂ x sine♀	F	4	1	2	1	1	1	2	2
sicu♂ x sine♀	F	4	1	2	3	2	2	2	2
sicu♂ x sine♀	F	4	1	3	3	1	1	2	1
sicu♂ x sine♀	F	4	1	3	1	1	1	2	2
sicu♂ x sine♀	F	4	1	3	3	1	1	1	2

Appendix 3.3 (Continued)

Qualitative morphological traits for **hermaphroditic** parental *Sidalcea nelsoniana* (sine), *S. cusickii* (sicu), and F1 hybrids.

Taxon	**Sex**	**Flower color**	**Habit**	**Sepal shape**	**Sepal color**	**Stem hairs**	**Leaf hairs**	**Glaucus**	**Raceme taper**
sine	Hm	4	1	2	1	2	2	2	1
sicu	Hm	4	1	3	3	1	1	1	2
sine♂ x sicu♀	Hm	5	1	3	3	2	1	2	1
sine♂ x sicu♀	Hm	4	1	3	1	2	2	2	1
sine♂ x sicu♀	Hm	5	1	3	2	2	2	2	1
sicu♂ x sine♀	Hm	4	1	2	3	1	1	2	2
sicu♂ x sine♀	Hm	4	1	2	1	1	1	2	2
sicu♂ x sine♀	Hm	4	1	2	3	1	1	2	2
sicu♂ x sine♀	Hm	4	1	3	1	1	1	2	2
sicu♂ x sine♀	Hm	4	1	3	3	1	2	2	2
sicu♂ x sine♀	Hm	4	1	2	1	1	1	2	2

Appendix 3.3 (Continued)

Qualitative morphological traits for **female** parental *Sidalcea nelsoniana* (sine), large phenotype *S. virgata* (sivi(big)), and F1 hybrids (hybrids were exclusively female).

Taxon	**Sex**	**Flower color**	**Habit**	**Sepal shape**	**Sepal color**	**Stem hairs**	**Leaf hairs**	**Glaucus**	**Raceme taper**
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sivi(big)	F	4	2	1	2	6	3	1	1
sivi(big)	F	4	2	1	2	6	3	1	1
sivi(big)	F	4	3	1	2	6	3	1	1
sine♂ x sivi(big)♀	F	4	1	1	1	6	2	2	1
sine♂ x sivi(big)♀	F	4	1	1	1	6	2	2	1
sine♂ x sivi(big)♀	F	3	3	1	1	6	2	2	1
sine♂ x sivi(big)♀	F	4	3	1	1	6	2	2	1
sine♂ x sivi(big)♀	F	4	3	1	1	6	2	2	1
sivi(big) ♂ x sine♀	F	4	3	1	1	6	3	2	1
sivi(big) ♂ x sine♀	F	4	2	1	1	6	3	2	1
sivi(big) ♂ x sine♀	F	4	2	1	1	6	3	2	1

Appendix 3.3 (Continued)

Qualitative morphological traits for **female** parental *Sidalcea nelsoniana* (sine), small phenotype *S. virgata* (sivi(sm)), and F1 hybrids.

Taxon	**Sex**	**Flower color**	**Habit**	**Sepal shape**	**Sepal color**	**Stem hairs**	**Leaf hairs**	**Glaucus**	**Raceme taper**
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sine	F	4	1	2	1	2	2	2	1
sivi(sm)	F	5	2	1	1	6	3	1	1
sivi(sm)	F	4	3	1	2	6	3	1	1
sivi(sm)	F	5	3	1	1	6	3	1	1
sine♂ x sivi(sm)♀	F	4	3	1	1	6	3	2	1
sine♂ x sivi(sm)♀	F	4	2	1	1	6	3	1	1
sivi(sm) ♂ x sine♀	F	4	1	1	1	5	3	2	1
sivi(sm) ♂ x sine♀	F	3	3	1	1	5	3	2	1
sivi(sm) ♂ x sine♀	F	4	1	1	1	2	3	2	1
sivi(sm) ♂ x sine♀	F	4	3	1	1	5	3	2	1
sivi(sm) ♂ x sine♀	F	4	3	1	1	6	3	1	1
sivi(sm) ♂ x sine♀	F	5	3	1	1	6	3	2	1

Appendix 3.3 (Continued)

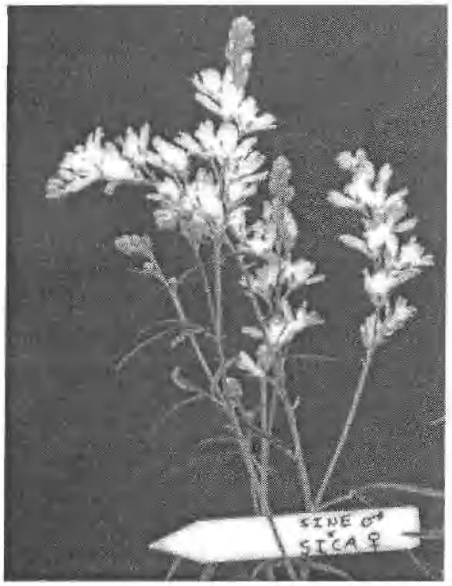
Qualitative morphological traits for **hermaphroditic** parental *Sidalcea nelsoniana* (sine), small phenotype *S. virgata* (sivi(sm)), and F1 hybrids.

Taxon	**Sex**	**Flower color**	**Habit**	**Sepal shape**	**Sepal color**	**Stem hairs**	**Leaf hairs**	**Glaucus**	**Raceme taper**
sine	Hm	4	1	2	1	2	2	2	1
sivi(sm)	Hm	4	3	1	2	6	3	1	1
sivi(sm) ♂ x sine♀	Hm	4	3	1	2	4	2	2	1
sivi(sm) ♂ x sine♀	Hm	4	2	1	1	2	2	2	1
sivi(sm) ♂ x sine♀	Hm	4	3	1	1	2	2	1	1
sivi(sm) ♂ x sine♀	Hm	2	1	1	1	5	3	1	1
sivi(sm) ♂ x sine♀	Hm	5	3	1	1	5	2	2	1
sivi(sm) ♂ x sine♀	Hm	2	3	1	1	5	2	1	1

Appendix 3.4 Voucher photographs of F1 interspecific *Sidalcea* hybrids produced through reciprocal crosses. Photographs of parental *Sidalcea* species used in crosses are provided in Figure 1.1.

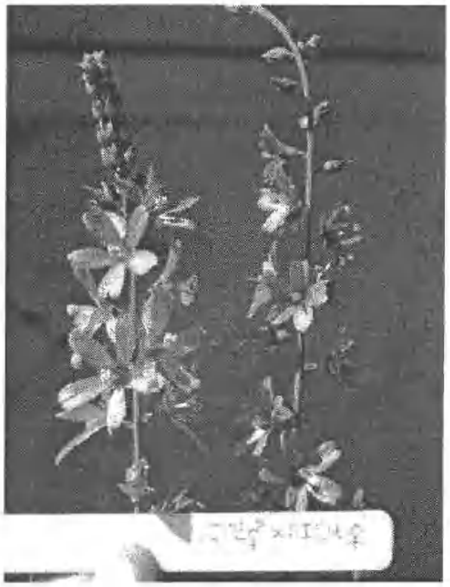
Appendix 3.4 (Continued)

S. campestris♂ x *S. nelsoniana*♀



S. nelsoniana♂ x *S. campestris*♀

S. cusickii♂ x *S. nelsoniana*♀



S. nelsoniana♂ x *S. cusickii*♀

Appendix 3.4 (Continued)

S. virgata(large phenotype)♂ x
S. nelsoniana♀

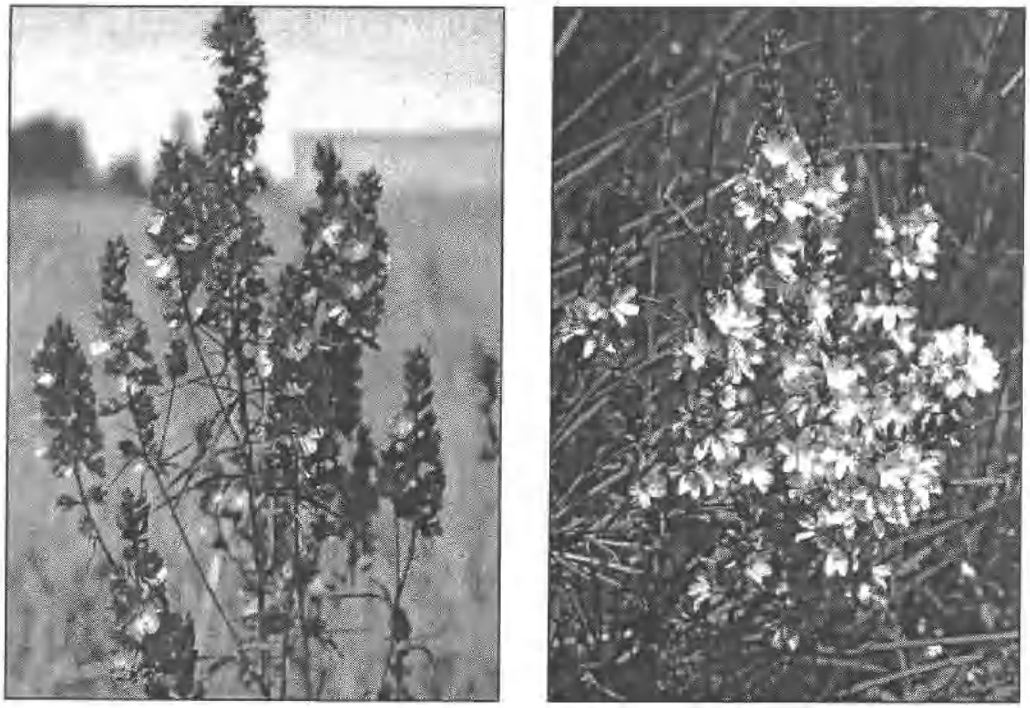
S. nelsoniana♂ x *S. virgata*(large
phenotype)♀

S. virgata(small phenotype)♂ x
S. nelsoniana♀

S. nelsoniana♂ x *S. virgata*(small
phenotype)♀

Appendix 3.5 Photographic examples of intraspecific morphologic variability exhibited by parental *Sidalcea* species. This variability, combined with sexual dimorphism is several traits, often complicates confident species identification and would likely prevent (or at least confound) the immediate recognition of interspecific hybrids, if such are produced.

Appendix 3.5 (Continued) Intraspecific (and in this case within-population) morphologic variability in *Sidalcea campestris*. **Left**: two individuals exhibiting variable flower color ranging from white (typical) to light pink (resembling darker flowered congeners). **Right**: two individuals exhibiting pronounced differences in the size of female flowers--large female flowers (approximately 3 cm in diameter) are typical, whereas smaller flowers resemble those more commonly observed in *S. nelsoniana*.



Appendix 3.5 (Continued) Intraspecific morphologic variability in *Sidalcea cusickii.* **Left**: individual exhibiting typical pink-dark pink flower color. **Right**: individual exhibiting very light pink flowers, more typical of those observed in *S. campestris*.

Appendix 3.5 (Continued) Intraspecific morphologic variability in *Sidalcea nelsoniana.* **Left**: two individuals (within the same population) exhibiting differences in flower color, ranging from pink (typical) to pale pink/white (the latter exhibiting coloration more typical of *S. campestris*). **Right**: two female-flowered individuals exhibiting differences in raceme architecture, the more compact raceme being more typical of those usually observed in *S. cusickii*.

Appendix 3.5 (Continued) Intraspecific (and in this case within-population) morphologic variability in *Sidalcea virgata*. **Left**: two individuals exhibiting pronounced differences in flower color, ranging from pink (typical) to pale pink/white (the latter resembling coloration more typical of *S. campestris*). **Right**: two female-flowered individuals exhibiting pronounced differences in flower size, the larger being typical of the species and the smaller being of a size more commonly observed in *S. nelsoniana*.

Chapter 4: Summary, Conclusions, and Conservation Recommendations

The native grasslands of western Oregon's Willamette Valley are a rapidly shrinking ecosystem harboring, among numerous rare endemic species, the threatened *Sidalcea nelsoniana* and three other native *Sidalcea* congeners. Given their shared occupation of these unique habitats, the question arises whether (and by what underlying ecological and genetic mechanisms) *S. nelsoniana* and its congeners are capable of maintaining mutual reproductive isolation and stable species boundaries? This question is particularly poignant in light of several biogeographic attributes expected to encourage interspecific pollen exchange and hybridization in this species group (see Chapter 1):

1) Congeners exhibiting closely neighboring and/or overlapping geographic distributions;
2) Occupation of habitats subjected to a variety of intensive anthropogenic disturbances;
3) Susceptibility of *Sidalceas* to human dispersal through conservation/restoration activities and horticultural trade;
4) Exhibiting predominantly outcrossing mating systems;
5) Capability of long-lived persistence and asexual expansion;
6) Previously documented evidence of interspecific sexual compatibility and hybridization among other members of the family and genus; and,
7) Published "suspicions" of hybridization in *S. nelsoniana*.

Moreover, given increasing concerns about the multi-faceted adverse (yet theoretical beneficial) conservation implications of interspecific hybridization discussed in Chapter 1, the additional question arises whether this process really merits serious consideration in the development of conservation strategies and recovery plans for *S. nelsoniana* and its increasingly threatened relatives.

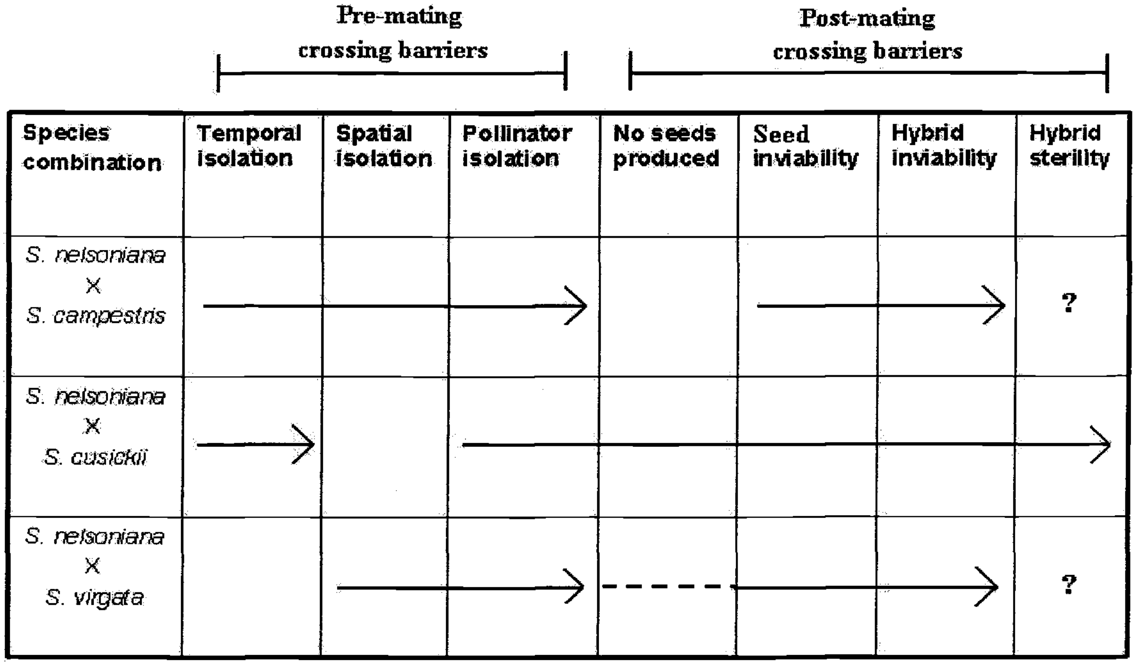
This study confronts these important conservation issues by evaluating the nature and efficacies of pre- and post-mating reproductive isolating mechanisms in *Sidalcea nelsoniana*, identifying the conditions most likely to encourage and/or discourage the occurrence of interspecific hybridization, and providing a scientific foundation for the development of hybridization-related conservation strategies for *S. nelsoniana* and its congeners.

The long history of scientific inquiry into interspecific hybridization and its more recently realized implications for rare plant conservation are discussed in Chapter 1. In Chapter 2, I explore pre-mating barriers to hybridization in *S. nelsoniana* (i.e., temporal, geographic, and ethological reproductive isolation) and their potential disruption by contemporary habitat disturbances. In Chapter 3, I evaluate post-mating hybridization barriers (i.e., multi-stage expressions of interspecific sexual incompatibility), investigate the nature and role of polyploidy in *Sidalcea* hybridization, and lastly, assess the expression of parental morphological characteristics in F1 hybrid phenotypes and its implications for detecting hybrids using traditional morphometric techniques. The purpose of this final chapter is to bring the results of these separate investigations to a common conclusion, and consider their collective implications for the conservation of *S. nelsoniana* and its Willamette Valley congeners.

The findings of this study reveal a complex interplay of pre- and post-mating barriers that appear to promote reproductive isolation, and discourage hybridization, between *Sidalcea nelsoniana* and all three of its local relatives (*S. campestris*, *S. cusickii*, and *S. virgata*). It is noteworthy, however, that the nature and function of these reproductive barriers differ between each respective species pair (Figure 4.1), such that they only function in concert to keep *S. nelsoniana* simultaneously isolated from the entire species group.

For example, reproductive isolation between *Sidalcea nelsoniana* and *S. cusickii* appears to hinge solely on a single pre-mating crossing barrier. Reciprocal crossing studies in Chapter 3 reveal these species are fully sexually compatible (likely promoted by their homodiploidy), and ecological studies in Chapter 2 demonstrate their overlap in pollinators, pollen transfer, and timing of flowering. However, *S. cusickii* is the only species in this study that fails to co-occur with *S. nelsoniana* at a fine geographic scale; inventories reveal their ranges are narrowly parapatric, currently separated by less than 3 km. Therefore, despite the absence of other pre- and post-mating crossing barriers, geographic separation functions alone as a significant obstacle to hybridization between *S. nelsoniana* and *S. cusickii.*

In contrast, reproductive isolation between *Sidalcea nelsoniana* and *S. campestris* relies exclusively on the existence of *post-mating* crossing barriers. Ecological studies in Chapter 2 show these two species exhibit synchronous



	Pre-mating crossing barriers			Post-mating crossing barriers			
Species combination	**Temporal isolation**	**Spatial isolation**	**Pollinator isolation**	**No seeds produced**	**Seed inviability**	**Hybrid inviability**	**Hybrid sterility**
S. nelsoniana X *S. campestris*	→	→	→		→	→	?
S. nelsoniana X *S. cusickii*	→		→	→	→	→	→
S. nelsoniana X *S. virgata*		→	→	- - - - -	→	→	?

Figure 4.1 Diagram summarizing pre- and post-mating barriers to hybrid formation (barriers indicated by blank cells) identified between *Sidalcea nelsoniana* and its three local congeners. Dashed line reflects incomplete interspecific sexual compatibility within *S. virgata*, and cells with question marks reflect uncertainty due to lack of fertility estimates (see text).

flowering, pollinator overlap, interspecific pollen transfer, and fine-scale geographic co-occurrence (indeed, they frequently form mixed populations), yet reciprocal crosses between these species yielded *extremely* low levels of seed production. Sexual incompatibility between *S. nelsoniana* and *S. campestris* may be attributable to chromosomal incompatibilities related to the pronounced heteroploidy inherent in this diploid x hexaploid species crossing combination (see Chapter 3). Given the manifold constraints posed by conspecific pollen competition (see references in Chapter 3), hybrid seed production between these species in nature would be expected to be even lower than the meager levels yielded through the artificial crosses in this study.

Lastly, reproductive isolation between *Sidalcea nelsoniana* and *S. virgata* appears to be maintained by a combination of both pre- *and* post-mating reproductive barriers. Ecological studies in Chapter 2 demonstrate that, despite overlap in pollinators and fine scale geographic distributions, these two species exhibit little or no overlap in flowering times. Flowering asynchrony is especially pronounced among neighboring heterospecific populations (those with the greatest geographical likelihood for gene exchange), and is further reinforced by patterns of female-biased sex expression in both species during the period of their closest temporal proximity (see Chapter 2).

In addition to the pre-mating barrier of temporal isolation, crossing studies in Chapter 3 indicate that post-mating barriers provide a supplemental "back-up" mechanism to discourage hybridization between *Sidalcea nelsoniana* and *S. virgata*, should interspecific pollen exchange occur though the breakdown of temporal isolation via random environmental or anthropogenic processes. The apparent exception to this species boundary safeguard is the directional crossing combination of *S. nelsoniana* dams paired with *S. virgata* (small phenotype) sires, which yields only slightly reduced seed production compared to "legitimate" conspecific matings. As in crosses between *S. nelsoniana* and *S. campestris*, chromosomal incompatibilities related to polyploidy may be responsible for the lack of seed production in remaining diploid *S. nelsoniana* x tetraploid *S. virgata* crossing combinations.

In light of these findings, should the conclusion be drawn that interspecific hybridization poses no threat to *Sidalcea nelsoniana*? After all, this study provides compelling evidence of significant pre- and post-mating reproductive barriers between *S. nelsoniana* and all of its local congeners, a pattern possibly reflecting Dobzhansky's (1937) process of reproductive reinforcement, or the result of other selective and/or random processes described in Chapter 1. Which of these processes, or combination thereof, truly accounts for this seemingly "tidy" system of reproductive isolation remains unknown. Regardless of the evolutionary stimulus, however, it is by no means certain if such a system can be expected to persist in a landscape subject to routine anthropogenic habitat disturbance and plant dispersal events. All four *Sidalcea* congeners in this study are available for sale through native plant vendors and utilized in local conservation and habitat restoration activities, so are therefore subject to human dispersal beyond their current ranges. Likewise, inventories in Chapter 2 demonstrate the ubiquitous occurrence and severity of anthropogenic habitat disturbances among extant *S. nelsoniana* populations. As discussed in Chapter 1, these kinds of dispersal and disturbance regimes can have profound effects on the frequency and trajectory of interspecific hybridization episodes.

Ultimately, such disturbance and dispersal issues may be of little evolutionary or conservation significance with regard to hybridization between *Sidalcea nelsoniana* and *S. campestris* because this species pair already lacks pre-mating crossing barriers and exhibits almost complete sexual incompatibility. However, the representatives of these two species used for crossing experiments in this study were sampled from only two closely neighboring Benton County populations (one population for each species). It is possible (though perhaps unlikely) that levels of interspecific sexual compatibility and/or levels of polyploidy might vary across populations, thereby increasing chances for successful hybridization if genotypes from regionally disparate populations are ever combined through human activities. And even if mixing of *S. nelsoniana* and *S. campestris* doesn't lead to the formation of interspecific hybrids *per se*, it could nevertheless result in negative reproductive interactions between species (i.e., reduced conspecific seed set through pollinator competition, gamete wasting, and stigmatic interference) (see Chapter 1). Currently, however, it is unknown if such negative interactions really take place in populations where these species co-occur, nor is it understood to what degree negative interactions might even be offset by increased pollinator attraction to their combined nectar and pollen resources (particularly in small, fragmented populations). The nature and balance of such reproductive interactions between *S. nelsoniana* and *S. campestris* represents an interesting area of future research with potentially important conservation implications.

Anthropogenic dispersal and disturbance events could have more significant implications for the hybridization potential between *Sidalcea nelsoniana* and *S. virgata*. Although asynchronous flowering limits opportunities for interspecific pollen exchange in this species pair, this asynchrony is most conspicuous between neighboring populations from similar elevations, whereas more widely separated populations exhibit minor amounts of flowering overlap. As such, if flowering times are genetically controlled, then anthropogenic mixing of genotypes from disparate regions or elevations could result in the formation of brief temporal windows for pollen exchange between the species. In contrast, if flowering times are influenced by environmental factors, such temporal windows might also open in response to habitat disturbance events that alter hydrologic regimes in ways that extend flowering time in *S. virgata* and/or advance flowering in *S. nelsoniana*. Until recently, an example of this scenario could be readily observed in the display garden outside Cordley Hall at Oregon State University, where *S. nelsoniana* and *S. virgata* were planted together in the same bed. Here, perhaps due to dispersal from different geographic source populations, or more likely in response to artificial irrigation, both species flowered simultaneously over several consecutive years. This particular phenomenon can no longer be observed, however, as *Sidalcea virgata* has inexplicably been removed from the garden.

Even if human activities lead to the eventual breakdown in pre-mating temporal barriers between *Sidalcea nelsoniana* and *S. virgata*, however, interspecific sexual incompatibility is still expected to serve as a supplemental barrier to the formation of hybrids in this species pair (see Chapter 3). But the degree to which this supplemental effect takes place remains somewhat uncertain, primarily due to the variability in interspecific sexual compatibility observed between small and large *S. virgata* phenotypes, and between crossing directions within the small phenotype *S. virgata* (see Chapter 3). This variability confounds the development of hybridization predictions for this species pair, and illustrates the need for future research to clarify the cytology and taxonomy of *S. virgata* in the Willamette Valley. In the meantime, the results of this study demonstrate at least some level of sexual compatibility between these two species, suggesting that the breakdown of pre-mating temporal barriers could lead to the onset of interspecific hybridization under some circumstances.

Sidalcea nelsoniana and *S. cusickii* represent the two species most susceptible to human-induced hybridization. Given the lack of any significant post-mating barriers preventing the formation of fully fertile F1 hybrids, and the lack of pre-mating temporal and ethological barriers, reproductive isolation between these species rests solely on their extremely narrow geographic separation. Any latitudinal migration or anthropogenic dispersal of these species beyond their respective parapatric ranges (currently abutting at the southern border of the William Finley National Wildlife Refuge in southern Benton County), could promote the onset of interspecific pollen exchange and ensuing hybridization. As such, to maintain the current genetic integrity of both species, special care should be taken when planning and implementing *Sidalcea* introduction and restoration activities in the southern Benton County area.

Until this point, this chapter has objectively focused on the nature of reproductive isolation in *Sidalcea nelsoniana* and the various ecological and anthropogenic circumstances that might lead to its breakdown. Although *S. nelsoniana* and its local congeners offer an interesting and model system in which to conduct such investigations, this system represents more than a biological laboratory for academic inquiry. Rather, because this unique species assemblage has been driven by habitat loss and other anthropogenic factors towards the brink of extinction, this research is ultimately aimed at providing meaningful and practical conservation guidelines that will promote the long-term survival of these *Sidalcea*s within their heavily impacted native grassland ecosystem.

However, whereas the objective "academic inquiry" components of this study are fairly straightforward and contribute added perspective to the fields of reproductive ecology and sympatric speciation, the broader interpretation of study results for development of conservation guidelines for *Sidalcea nelsoniana* is complicated by starkly contrasting ideas about how interspecific hybridization impacts conservation. As discussed in Chapter 1, interspecific hybridization is considered by some to represent more of a constructive and creative process than a destructive one. For instance, Rhymer and Simberloff (1996) state, "...sometimes particularly vigorous hybrids thrive in habitats inimical to both parental taxa, in ways that would normally be construed as benefits to conservation." Following this line of reasoning, hybridization could be seen as a tool for introducing adaptive genetic variability into *S. nelsoniana* populations which may be suffering the adverse genetic consequences of small sizes and fragmentation. Resulting hybrids might exhibit increased reproductive fitness and thrive in a wider range of human-altered niches than their declining progenitors, thereby increasing the chances of preserving *S. nelsoniana*'s genome (in one form or another) in new, more adaptive polymorphic hybrid lines.

If conservation goals are weighted towards this "hybridization is beneficial" viewpoint, then hybridization between *Sidalcea nelsoniana* and its congeners (if it occurs) should at the very least be allowed to take place unhindered, with little need to track and monitor its progression. A more proactive approach would be to actively encourage *Sidalcea* hybridization through prescribed mixing of parental species and development of breeding programs to overcome interspecific post-mating barriers.

However, as discussed in Chapter 1, hybridization can also be interpreted as having a much "darker side," posing serious and irreversible threats to rare species in ways *not* normally construed as benefits to conservation. These threats can become manifest in numerous ways (recall gamete wasting and reproductive interference, increased pest and disease pressures, competitive exclusion, and genetic assimilation). Certainly such adverse consequences are most pronounced among rare species that are subject to hybridization across most, or the entirety, of their known distributions, but they are also significant for species susceptible to isolated hybridization events. As pointed out by Burgman et al. (1993), extinction probabilities of separate populations are independent, and the chance of species extinction is an inverse function of the number of populations. Therefore, in the case of *Sidalcea nelsoniana*, which suffers from low extant population numbers and threats posed by a myriad of anthropogenic pressures, even isolated loss of populations through hybridization events could increase the overall likelihood of its extinction.

If the consequences of interspecific hybridization in *Sidalcea nelsoniana* are interpreted in this negative light, then active human intervention (in the form of removing hybrids and/or transplanting congeners to separate locations) may be warranted to reduce or eliminate the risk of interspecific hybridization. For instance, Levin et al. (1996) recommend that, for some rare plant species "... contact with a cross-compatible congener may constitute an environmental perturbation whose consequences are soon irreversible. Therefore, isolation from cross-compatible congeners should be a key goal in rare plant conservation programs." Likewise, Ellstrand and Elam (1993) make the following recommendation:

> "If evidence suggests a high risk of interspecific gene flow, then management steps must be swift and sure because of the speed at which genetic assimilation can occur and because of the substantial fitness losses accrued from outbreeding depression. Eradication of the gene flow source and/or transplantation are the only solutions for the problem."

This approach of intervention has already been implemented in an effort to preserve the gene pools of native cutthroat trout (Allendorf and Leary 1988), and similar measures are recommended by Rieseberg et al. (1989) for the world's only population of the tree, *Cercocarpus traskiae.* Here, to preserve the genetic integrity of remaining *C. traskiae* individuals against continued hybridization, the authors recommend the elimination of sympatric *C. betuloides* and establishment of cuttings representing the five 'pure' *C. traskiae* trees in other areas where the risk of hybridization is minimal. If such intervention is ever considered necessary for *Sidalcea nelsoniana*, significant advances will first need to be made in our ability to reliably identify hybrids and discern them from their variable progenitors, lest non-hybrid individuals be sentenced to an unjust end. Along these lines, Whitham et al. (1991) contend that genetic data is often lacking to discriminate between hybrids and parental species, and quote Nabhan et al. (1989) in recommending that "a plant should be considered 'innocent' of being a hybrid until proven 'guilty,' particularly if it is already listed." For *S. nelsoniana*, such proof will likely lie in the development of molecular markers, given the complications in identifying *Sidalcea* hybrids using traditional morphological methods (see Chapter 3).

Ultimately, the debate over interspecific hybridization's place in rare plant conservation will probably continue to evolve as new information is gained through additional research and management experience. Indeed, as stated by Arnold (1997), "...natural hybridization will likely continue to play an increasingly important role in the endangered ecosystems of our biosphere. Whether this latter role is one that contributes to the extinction or preservation of species remains to be seen." Eventually, once we have "seen" these roles, our final conclusion may simply mirror Rieseberg's (1991b) assessment that

"...hybridization can have both beneficial and harmful consequences for the conservation of biological diversity." It will therefore be the challenge of future researchers of *Sidalcea nelsoniana* and other rare species to tease apart these beneficial and harmful consequences so that we might effectively address their respective exploitation and amelioration for the common goals of conservation. Until such progress comes about, however, it may be wise (at least for *S. nelsoniana*) to conservatively side with the prevention of hybridization and avoidance of its potentially adverse consequences, an approach consistent with Rieseberg's (1991b) opinion: "...I can think of no justification for exchanging several distinct rare plant species, each with its own unique growth form and habitat requirements, for a single widespread compilospecies."

Towards this end, the current study has identified 1) the nature and efficacy of pre- and post-mating barriers to hybridization in *Sidalcea nelsoniana*, 2) the species combinations and ecological conditions most likely to promote *Sidalcea* hybridization, 3) the phenotypic confusion hindering hybrid detection in the wild using conventional morphometric techniques, and 4) steps needed to preserve the current genetic integrity of *S. nelsoniana* against human-induced hybridization events.

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