

THE MAINTENANCE OF GYNODIOECY

IN *SIDALCEA HENDERSONII*

by

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## Abstract

I investigated the genetic and ecological factors contributing to the maintenance of females in populations of the gynodioecious plant, *Sidalcea hendersonii*. Crossing experiments indicated that male sterility is controlled by a dominant nuclear allele. High frequencies of female plants in the majority of populations surveyed, in combination with nuclear determination of sex, elevates the theoretical requirements for female fitness in this species. Females did have higher fitness, producing more surviving offspring than hermaphrodite plants in an experimental population, and outcrossed hermaphrodite plants out-performed self-pollinated hermaphrodites. These results suggest that female advantage is the product of both maternal effect and obligate outcrossing. However, no inherent fitness advantages were evident in natural populations where females and hermaphrodites did not differ in viable seed production. Ecological factors may play an important role in the maintenance of gynodioecy. Though flower size differences between the sexes (thought to be related to pollinator visitation) did not affect seed production, seed predation did. In populations where females were abundant, weevil larvae destroyed significantly more seeds from hermaphrodite plants and substantially reduced hermaphrodite seed production overall. This study provides the first evidence that sex-related predation may be responsible for high female frequencies in natural populations of a gynodioecious species.

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## Chapter 1

### Introduction

#### Gynodioecy

Mating patterns affect genetic architecture and variability and thus influence the evolutionary potential of plant populations. Mating systems themselves are under genetic control and can respond to selection in an infinite variety of subtle and interrelated ways (Richards, 1986).

Most angiosperms are hermaphroditic, bearing both male and female reproductive structures on an individual plant. In a small percentage of plant species, however, the sexes are separated. Plant populations which consist of female and perfect flowered hermaphroditic individuals are termed gynodioecious, while the coexistence of separate male and female individuals characterizes dioecious populations. Gynodioecy is considered to be derived from a hermaphroditic condition often as an intermediate state in the evolution of dioecy (Lloyd, 1974a; Charlesworth and Charlesworth, 1978; Delannay *et al.*, 1981; Shultz, 1994). There are, however, a number of families and genera in which gynodioecy is common and dioecy is rare or nonexistent.

The existence of male sterile individuals in gynodioecious species has puzzled many evolutionary biologists including Darwin (1877). Female plants have a

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reproductive disadvantage compared to hermaphrodites since they have only one avenue through which to contribute genes to the next generation, their ovules, while hermaphrodites can contribute genes through both pollen and ovules (Lewis, 1941). Females may be able to compensate for the loss of male function either by producing more seeds or higher quality seeds than hermaphrodites (Ashman, 1992a). In most cases, female plants are, in fact, better mothers than hermaphrodites (Richards, 1986).

The level of compensation required for females depends on the genetic control of male sterility. Theoretical models predict that when the inheritance of male sterility is nuclear, females must produce at least twice as much seed as hermaphrodites for male sterility genes to persist, but if maternally-inherited cytoplasmic factors are involved, females require smaller advantages in seed production (Lewis, 1941; Lloyd, 1974b; Charlesworth, 1981; Delannay *et al.*, 1981; Schultz, 1994).

Two principal hypotheses have been put forth to explain the maintenance of females in gynodioecious populations. The outcrossing hypothesis suggests that the progeny of females have higher fitness because female flowers are obligately outcrossed, while the seeds of hermaphrodite flowers may result from self-fertilization. Consequently, the progeny of self-compatible hermaphrodites may be less variable than female progeny and could exhibit inbreeding depression resulting from an accumulation of homozygous deleterious alleles (Lewis, 1941; Ganders, 1978; Charlesworth and Charlesworth, 1978). According to outcrossing models and empirical data (Schultz and Ganders, 1996), when selfing and inbreeding depression

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are high, a male sterility gene can invade hermaphrodite populations even when females have the same seed production as hermaphrodites (Charlesworth and Charlesworth, 1978).

The resource allocation hypothesis states that because female plants do not expend resources on anther and pollen production, they may allocate more resources to seed production than hermaphrodites. Outcrossing advantages and maternal-sex effects can operate simultaneously or at different life cycle stages (*e.g.*, Shykoff, 1988; Ashman, 1992a).

Ecological and genetic forces together determine fitness. Although many genetic models for the maintenance of male sterility invoke ecological parameters (Ross in Kesseli and Jain, 1984), ecological effects are rarely specifically identified or examined in isolation, perhaps because they are difficult to quantify or separate from genetic factors. Ecological factors can play a significant role in mating system evolution, and, in some cases, may even overshadow an initial genetic disposition. For example, in species that depend on pollinators for reproduction, enhanced allocation of resources to seeds and improved vigor in progeny from outcrossing is of no advantage to female plants if their flowers are never pollinated. Also, in natural environments the seeds can encounter a number of obstacles to survival, such as climatic stress, disease, and seed predation. The quantity of seeds produced by the sexes is irrelevant if the seeds are never able to germinate.

**Study plant: *Sidalcea hendersonii* Wats. (Malvaceae)**

TAXONOMY

The genus *Sidalcea* A. Gray, comprises a group of annual and perennial herbs inhabiting western North America from the Rocky Mountains west to the Pacific coast, and from British Columbia south into Mexico. Roush (1931) suggested that *Sidalcea* arose at the end of the Pleistocene and migrated north from Mexico through the Rocky Mountains and along the Sierra Nevada Mountains. Two generic names in the Malvaceae, *Sida* (Gr. water-lily) and *Alcea* (mallow) were combined to form the name "*Sidalcea*," which is differentiated from these and other genera of the Malvaceae by two or more series of stamens emerging from the top of the androecial column (Roush, 1931; Hitchcock and Kruckeberg, 1957).

At least 21 species of *Sidalcea* and 25 subspecies have been described, and the genus is still considered to be highly variable and difficult to resolve (Hitchcock and Kruckeberg, 1957; Hickman, 1993; Dorr, 1993; Dimling, 1991; Wikeem and Newman, 1985). Differentiating species is difficult because taxonomic interpretations rely on vegetative characters and variation in floral structure is minimal (Hitchcock and Kruckeberg, 1957). Intersectional interfertility with fully fertile F1 hybrids further complicates species delimitation (Hitchcock and Kruckeberg, 1957).

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*Sidalcea hendersonii*, the most northerly species, has the characteristic shallow-lobed basal leaves contrasted with deeply dissected cauline leaves, showy purple-pink flowers on terminal racemes, and rhizomes of perennial members of its genus (Figure 1.1).

*S. hendersonii* is conspicuously tall, reaching 15 decimeters in height and is distinguished from other members of the genus by glabrous foliage, smooth carpels, and often a purplish hue to the stems and tips of calyx lobes. The phylogenetic affinity of this species is unclear; while Roush (1931) considered *S. hendersonii* to most closely resemble *S. candida* and *S. oregana*, Hitchcock and Kruckeberg (1957) placed *S. hendersonii* with *S. cusickii* on the basis of pubescence, flower size, and carpel characters.

Several species of *Sidalcea* are gynodioecious including *S. hendersonii*, *S. nelsoniana*, *S. cusickii*, *S. virgata*, *S. campestris*, *S. oregana*, *S. malvaeflora*, *S. multifida*, *S. hartwegii*, *S. hickmanii*, and *S. malachroides* (see Hitchcock and Kruckeberg, 1957 for authorities). The documentation of gynodioecy in *Sidalcea* is limited and often anecdotal, so the number of gynodioecious species may be greater than this list. In *Sidalcea hendersonii*, hermaphroditic flowers contain both functional anthers and ovaries, are self-compatible but protandrous. Female flowers have functional ovaries, and their styles are usually more exerted than in hermaphrodite flowers. Female flowers of *Sidalcea hendersonii* possess vestigial stamens which lack anthers; male sterility is complete.



**Figure 1.1** *Sidalcea hendersonii*.

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### GENERAL ECOLOGY

*Sidalcea hendersonii* inhabits low elevation wet meadows and tidal marshes from southwestern British Columbia to Oregon. The long-term existence of *S. hendersonii* in the lower Fraser Valley was inferred from pollen dating to approximately 3700 years (Mathewes and Clague, 1994). This species typically grows in association with marsh species such as *Potentilla anserina* subsp. *pacifica*, *Juncus* spp., *Carex* spp., *Sium suave*, *Oenanthe sarmentosa*, *Lathyrus palustris*, and *Alisma plantago-aquatica*.

In British Columbia *Sidalcea hendersonii* is listed as rare, with "R2" status which indicates a species with few to several populations, each consisting of a relatively large number of individuals (Straley *et. al.*, 1985). Several factors contribute to the plant's rarity including human encroachment into wetland habitats, displacement by aggressive invasives such as *Lythrum salicaria*, and insect seed predation.

The flowering season extends from the beginning of June through September. Flowers are pollinated by several different insects including *Bombus* (bumblebee), *Apis* (honeybee), and *Vanesa* (butterfly) species.

### FEMALE FREQUENCY IN *SIDALCEA*

The frequency of female plants in gynodioecious *Sidalcea* species seems to be unusually high. In most gynodioecious species, females comprise less than 35% and often less than 10% of the individuals (Richards, 1986), but in *Sidalcea* populations,

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females often reach frequencies of 50%. The average proportion of females in populations of *S. nelsoniana* from the Willamette Valley, Oregon was 60% (Glad *et al.*, 1994), and 50% in a Californian population of *S. oregana* ssp. *spicata* (Ashman, 1992a).

### **Objectives**

The primary objective of this thesis is to determine the relative importance of several factors which could contribute to the maintenance of females in *Sidalcea hendersonii* populations. Genetic control of male sterility, inbreeding depression in hermaphrodite progeny, maternal effect, and ecological factors related to pollinator and seed predator activities are all considered.

These factors are addressed in separate chapters, except for inbreeding depression and maternal effect. Although some of the measurements discussed in different chapters share the same method of determination, the methods are included once in the most appropriate chapter.

Chapter 2 reports the results for genetic control of male sterility. Since the degree of female compensation depends on the mode of inheritance of male sterility, this chapter establishes female fitness requirements.

Chapter 3 describes the sexually dimorphic flowers of *S. hendersonii*. In addition to sex-specific functions, females typically have smaller flowers than hermaphrodites

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in gynodioecious species. These dimorphic features can affect pollinator activity and influence seed-set differences between the sexes. In addition to describing flower size in relation to gynodioecy, sources of flower size variation are also evaluated. While not directly applicable to the primary objective of this thesis, this evaluation is still valuable because *S. hendersonii* was reported to have the unusual combination of three flower forms, suggesting a novel genetic system controlling flower size.

Chapter 4 evaluates fitness differences in the sexes in relation to inbreeding depression and maternal effect. Chapter 5 reveals a striking discovery of sex-related seed predation by weevils. And, Chapter 6 concludes by evaluating overall differences in seed production between the sexes incorporating both abortion rates and the impact of seed predation. The implications of breeding system evolution in relation to these and other fitness measures are also discussed.

An appendix lists brief population descriptions of all of the *S. hendersonii* populations examined in the study in addition to a few other small populations that were not extensively surveyed. This section includes population locations and more detailed ecological information.

## Chapter 2

### Genetic Control of Male Sterility

#### Introduction

The hermaphroditic state is generally regarded as the ancestral condition from which gynodioecy evolves (Darwin, 1877; Charlesworth and Charlesworth, 1978). Females arise in hermaphroditic populations through spontaneous male sterility mutations. In a few species, sexuality has been shown to be influenced by environmental changes, but for the vast majority of gynodioecious species male sterility is genetically determined. Genetic male sterility is widespread among flowering plants having been documented in 617 plant species (Kaul, 1988). Its distribution throughout the plant kingdom suggests that gynodioecy has arisen independently in many lineages. Male sterility is significantly more common than female sterility, possibly due to a greater vulnerability of the male sporophyte and gametophyte to internal and external perturbations (Kaul, 1988).

Male sterility mutations can act before, after, or during microgametogenesis resulting in a diversity of male sterile forms. For example, females may have anthers which fail to dehisce pollen, their pollen may be inviable or underdeveloped, or the anthers themselves may be deformed, or completely aborted (Kaul, 1988). The genetic basis of male sterility has been identified for many gynodioecious species.

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Sex expression may be controlled by nuclear genes, cytoplasmic genes, or by cytoplasmic and nuclear gene interactions.

### NUCLEAR CONTROL

Nuclear male sterility is determined by nuclear genes alone, segregating normally according to Mendelian principles. If a dominant allele controls sterility, females have a heterozygous genotype (Mm) and hermaphrodites have a homozygous recessive genotype (mm). Since pollen donors will always contribute a recessive allele to their progeny, a homozygous dominant genotype (MM) is unattainable without mutation (Ganders, 1978). Half of the progeny of females will be female and half will be hermaphrodite, and all of the progeny of hermaphrodites will be hermaphrodite.

If recessive alleles control sterility, females (mm) will either produce half female and half hermaphrodite progeny or all of their progeny will be hermaphrodite depending on whether the father has a homozygous (MM) or heterozygous (Mm) genotype. Approximately one-quarter of the progeny of hermaphrodites will be female when both hermaphrodite parent's genotypes are heterozygous. Numerous examples of both dominant and recessive expression of male sterility have been documented in higher plants, but recessive gene control is six times more common than dominant control (Kaul, 1988).

Epistatic effects have been documented for *Centranthus ruber*, *Origanum vulgare*, *Phaseolus vulgaris*, and *Cortaderia richardii* in which a dominant allele

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causes male sterility but its expression is regulated by another gene which ultimately controls sex expression (citations in Ganders, 1978; Kaul, 1988).

### CYTOPLASMIC CONTROL

Cytoplasmic genes are maternally transmitted in most plants. The zygote in higher plants typically receives all cytoplasmic contents, including extra-nuclear genes located in cytoplasmic organelles, from its female parent. Cytoplasmic inheritance is non-Mendelian. If the inheritance of male sterility is cytoplasmic, all of the progeny of a male sterile mother will be male sterile.

Kaul (1988) suggested that cytoplasmic male sterility may be a transition state in the evolution of cytoplasmic-nuclear male sterility and therefore should not be considered a distinct type of gynodioecy. In fact, no cases of exclusively cytoplasmic inheritance are currently thought to exist (Ashman, 1992a). Previously reported cases of cytoplasmic control in gynodioecious *Cirsium oleraceum*, *Cirsium acaule*, *Satureja hortensis*, and *Plantago lanceolata* have subsequently been determined to have cytoplasmic-nuclear inheritance (Ross, 1978; Ashman, 1992a).

### CYTOPLASMIC-NUCLEAR CONTROL

Cytoplasmic-nuclear inheritance of male sterility involves an interaction between cytoplasmic genes for male sterility and nuclear fertility-restoring genes. Individuals with a male sterile cytoplasm will be hermaphrodite if they have the appropriate

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nuclear fertility-restoring allele, and female if they do not (Charlesworth and Ganders, 1979). Cytoplasmic-nuclear male sterility has been documented in gynodioecious *Nemophila menziesii* (Ganders, 1978) and *Plantago lanceolata* (Van Damme, 1983) among others.

Cytoplasmic-nuclear inheritance is potentially complex since multiple sterile cytoplasm types and multiple restorer loci can occur with this mode of inheritance. Due to this complexity, few studies have attempted to define the precise mechanism of genetic control. Often when segregation patterns do not conform to Mendelian ratios, a cytoplasmic influence on sex determination is assumed.

The majority of naturally occurring gynodioecious species have cytoplasmic-nuclear sex determination (Ross, 1978; Van Damme, 1983; Nordborg, 1994). Kaul (1988) suggested that the introduction of a nuclear gene into a population would be quicker and easier than replacement of a sterile cytoplasm, which might explain the frequent occurrence of cytoplasmic-nuclear gynodioecy. It has also been argued that populations with purely nuclear control of male sterility are rare because they may evolve towards dioecy (Charlesworth, 1981). This idea will be discussed in more detail in Chapter 6.

### **Objective**

The objective of this study was to attempt to determine the genetic basis of male sterility in *Sidalcea hendersonii*.

## Methods

In September 1994, *Sidalcea hendersonii* seed was collected at random from female (male sterile) and hermaphrodite individuals from Ladner Marsh and Comox populations. After scarification and cold-treatment, seeds were planted in the U.B.C. greenhouse and transferred to insect-free growth chambers just prior to flowering in June 1995.

A variety of crosses were performed between female and hermaphrodite plants and between separate hermaphrodite individuals. Hermaphroditic plants were also self-pollinated. Stamens at anthesis were completely removed with forceps from hermaphrodite flowers and used in hand pollinations. Anthers were brushed across receptive stigmas until each of the style branches was thoroughly coated with pollen. When hermaphrodite flowers were used as seed parents, the emasculation process also helped to prevent contamination from self pollen. Typically the style emerged from the androecium 2-3 days following emasculation, at which point pollen was applied to hermaphrodite flowers selected as seed parents. Between treatments the forceps were cleaned of pollen with a 90% ethanol solution.

Each dam (a female or hermaphrodite seed parent) received pollen from several different sires (hermaphrodite pollen donors), although single flowers received pollen from one donor only. Each sire was crossed to several different dams. The progeny from all crosses performed between any pair of parents is considered a family, while all progeny of any one seed parent constitutes a maternal family.

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The seed produced from these crosses was harvested in the fall of 1995. Thirty families with ample seed stock were selected for genetic interpretation and statistical comparisons.

The seed from the selected families, which totaled 1,103 seeds (660 seeds from female families and 443 seeds from hermaphrodite families), was planted in growth chambers at U.B.C., January 1996. When flowering commenced four months later, the sex of each individual was scored.

Relatively uncomplicated nuclear gene models were considered first (*ie.*, dominant or recessive single locus models). Observed progeny sex ratios were compared to expected Mendelian ratios, and differences were analyzed using a chi-square test.

**Results**

Less than half of the seed from experimental crosses germinated, and only one fourth of those seedlings produced flowers after 13 months. Crosses to female mothers yielded a 1:1 ratio of hermaphrodite to female offspring, while hermaphrodite mothers gave rise to hermaphrodite offspring only (Table 2.1).

Cross	# Female progeny	# Hermaphrodite progeny	$\chi^2$	P
Female × Hermaphrodite	40	41	= 0.0123	> 0.5
Hermaphrodite × Hermaphrodite	0	44	NA	NA

**Table 2.1** Sex of offspring from experimental crosses between female and hermaphroditic plants and between hermaphrodites including self-pollinations (N=125). No significant difference between # female progeny and # hermaphrodite progeny from crosses to female mothers: accept 1:1.

**Discussion**

GENETIC CONTROL OF MALE STERILITY

The inheritance of male sterility appears to conform entirely to Mendelian ratios, suggesting that sterility is controlled by nuclear genes which are not influenced by cytoplasmic factors. Male sterility is most likely controlled by a single dominant allele. The expected phenotypic ratio for the progeny of females would be 1:1 male-steriles to hermaphrodites, exactly as observed for the progeny of female *Sidalcea hendersonii* plants. The sex trait of self-pollinated hermaphrodites bred true, indicating a homozygous genotype for hermaphrodites and lending further support to

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this hypothesis. Although most gynodioecious species display cytoplasmic-nuclear inheritance of sex, nuclear dominant systems have been reported for at least fourteen other gynodioecious species (citations in Kaul, 1988; Ganders, 1978; Kohn, 1989).

This relatively simple result is surprising since a cytoplasmic-nuclear interaction was reported to control male sterility in the closely related species *Sidalcea oregana* ssp. *spicata* (Ashman, 1992a). In Ashman's breeding experiment, females produced strongly female-biased progenies, and hermaphrodites were capable of bearing female offspring. Crosses were performed for two generations, and sample size appeared to be substantial. Such experimental circumstances may have improved the ability to detect cytoplasmic genetic effects. The number of genetic factors which affect sexuality may be underestimated since the finite sample of plants used to gather genetic data may not contain all of the genetic variation for those traits (Frank in Eckhart, 1992a). In the current study, many combinations of crosses between individuals were performed, and the seed used in the breeding experiment was collected from two widely separated populations. These measures might increase the likelihood of encountering such genetic variation for the sex trait if it existed.

Mechanisms of sex determination, however, are not necessarily always the same for related species; in fact, different male sterility genes have been detected within families and even within species. For example, both nuclear and cytoplasmic-nuclear male sterility mutations have been identified in *Plantago lanceolata* (Kaul, 1988).

In addition to *Sidalcea oregana*, several other gynodioecious *Sidalcea* species are known (e.g., *S. nelsoniana*, *S. cusickii*, *S. virgata*, and *S. campestris*), suggesting that gynodioecy is not a recent development in the genus. The genetic control of an apparently well established male sterility system might be expected to be relatively complex. Alternatively, the frequency of gynodioecy could also indicate that *Sidalcea* species are simply more susceptible to male sterility mutations, and the recent appearance of nuclear controlled gynodioecy is certainly possible.

Another explanation for the inconsistency is that the effects of cytoplasmic genes in *S. hendersonii* populations have become masked over time, or that the cytoplasmic genes themselves have been lost. Some models suggest that cytoplasmic-nuclear gynodioecy is a nonequilibrium state inclined to evolve towards nuclear male sterility (Charlesworth and Ganders, 1979; Delannay *et al.*, 1981). If a male sterile cytoplasm becomes fixed in a population, male sterility would appear to be controlled by nuclear genes (Charlesworth and Ganders, 1979).

#### MAINTENANCE OF GYNODIOECY

Many theoretical models have been formulated to investigate the evolution of gynodioecy and predict the conditions under which male sterility mutations are maintained in gynodioecious populations (e.g., Lewis, 1941; Ross and Shaw, 1971; Lloyd, 1974a, Charlesworth and Charlesworth, 1978; Charlesworth and Ganders, 1979; Webb, 1981; Delannay *et al.*, 1981; Schultz, 1994). The selection pressure

## Chapter 2. Male Sterility

required to maintain male sterility differs depending on the location of the sex-determining genes (Lewis, 1941). Cytoplasmic and cytoplasmic-nuclear control of male sterility permit the maintenance of gynodioecy under conditions where nuclear control cannot (Maurice *et al.*, 1994). Most models predict that when the inheritance of male sterility is nuclear, females must produce at least twice as much seed as hermaphrodites for gynodioecy to persist. When cytoplasmic or both cytoplasmic and nuclear genes determine sex, females require smaller advantages in seed production to be maintained (Lewis, 1941; Lloyd, 1974b; Charlesworth and Ganders 1979; Charlesworth, 1981; Delannay *et al.*, 1981). According to these models, female *Sidalcea hendersonii* plants should be twice as fit as hermaphrodites since nuclear controlled male sterility genes are being maintained. The requirements for female maintenance should be the same if a fixed sterile cytoplasm interacts with (nuclear/recessive) fertility restoring alleles.

### **Conclusions**

Male sterility in *Sidalcea hendersonii* appears to be controlled by a single dominant nuclear allele which is apparently not influenced by cytoplasmic factors. The maintenance of this male sterility allele in populations of *S. hendersonii* suggests at least a twofold fertility advantage for females.

## Chapter 3

### Sexually Dimorphic Flowers

#### Introduction

Male-sterility in gynodioecious species is frequently correlated with a reduction in corolla size (Darwin, 1877; Baker, 1948; Lloyd and Webb, 1977; Ashman and Stanton, 1991; Eckhart, 1992a; Delph, 1996). In a recent review of flower size in sexually dimorphic plants, Delph (1996) reported that 98% of the 131 gynodioecious and gynomonoeious (hermaphrodite plants with female as well as hermaphrodite flowers) species considered exhibited larger flowers on hermaphrodite plants than female plants. For the remaining two percent, females and hermaphrodites did not differ in corolla size. The mechanism responsible for the dimorphism is not completely understood, but several hypotheses have been investigated (reviewed below).

#### DEVELOPMENTAL HYPOTHESES

Darwin (1877) doubted that natural selection played a role in the flower size dimorphism commonly observed in gynodioecious species and suggested that this pattern was due to an intimate developmental relationship between stamens and petals.

### Chapter 3. Sexually Dimorphic Flowers

“It seems ... probable that the decreased size of the female corollas ... is due to a tendency to abortion spreading from the stamens to the petals” (Darwin, 1877).

A close developmental association between petals and stamens has indeed been documented (Hardenack *et al.*, 1994; Ainsworth *et al.*, 1995), and an evolutionary relationship between the two structures is a popular theme in angiosperm evolutionary studies.

Experimental evidence suggests that hormones associated with pollen development are at least partially responsible for the relationship between flower size and sex. Plack (1957) found that by emasculating hermaphroditic flowers of *Glechoma hederacea* in the bud, she obtained flowers with corollas reduced to the size of female corollas. Furthermore, she found that the corolla of emasculated flowers could be restored to normal size by administering gibberellic acid, a hormone associated with male sex expression. Studies of “intermediate” plants, those with partially reduced male function, provide additional evidence in support of hormonal effects. A positive correlation was found between petal size and the number of functional anthers in intermediate individuals of gynodioecious *Geranium maculatum* (Agren and Willson, 1991). Hormones related to pollen production, however, may not completely explain sexual dimorphism. In a survey of 436 dioecious and monoecious species, Delph (1996) reported that in 46% of the dimorphic cases, female flowers were larger than male flowers suggesting that strict developmental homology between pollen production and petal size does not exist.

### Chapter 3. Sexually Dimorphic Flowers

#### ADAPTIVE HYPOTHESES

For natural selection to influence a flower size dimorphism, flower size must be genetically determined but not "subject to rigid genetic or developmental constraints" (Stanton and Preston, 1988). Adaptive and developmental arguments are not mutually exclusive since with significant genetic variation for response to or production of hormones, selection may act on the developmental relationship between petal growth and sexual identity (Stanton and Galloway cited in Delph, 1996). Progeny analysis of *Raphanus sativus* (Stanton and Preston, 1988) and gynodioecious *Phacelia linearis* (Eckhart, 1993) revealed highly significant heritability of corolla size and significant genetic variability for flower size among maternal families, suggesting that natural selection could influence flower size variation.

Pollinators may play a critical role in the evolution of sexually dimorphic characters in entomophilous plant populations since pollinators can ultimately affect fitness and gene flow. Pollinator discrimination among floral forms has been shown to affect male reproductive success more profoundly than female reproductive success (Stanton *et al.*, 1986; Horovitz, 1978; Horovitz and Harding, 1972; Campbell *et al.*, 1991) leading evolutionary biologists to hypothesize that allocation to pollinator attractants, such as a showy perianth, may be more important to male function than to female function (Bell, 1985; Charnov and Bull, 1986; Stanton *et al.*, 1986; Delph, 1996). According to the sexual selection theory, female fitness is limited by resource availability, while male fitness is limited by mating opportunities (Bateman cited in

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Eckhart, 1993; Delph, 1996). For instance, increased levels of pollination do not necessarily increase seed-set for plants in resource-limited conditions (*e.g.*, Lee and Bazzaz, 1982). Also, while female fitness is affected by the ability to receive pollen, hermaphrodite fitness is affected by the ability to both donate and receive pollen; thus female fitness may be maximized by fewer pollinator visits than hermaphrodites (Bell, 1985; Stanton *et al.*, 1986; Delph, 1996).

Alternatively, if a size dimorphism in a gynodioecious species is the result of pleiotropy, the observed pattern may have no direct adaptive significance and could even be counter-adaptive (Lloyd and Webb, 1977). For example, a gene responsible for male-sterility may also reduce petal size even when female fitness is compromised. While Eckhart (1992a) considered the possibility of linkage disequilibrium between sex determining genes and those controlling corolla size in *Phacelia linearis*, he concluded that there was a pleiotropic effect on corolla size.

#### OTHER SOURCES OF VARIATION

Maternal and environmental effects are seldom considered sources of variation which could potentially play a role in flower size dimorphism. The progeny of female and hermaphroditic plants may differ in petal size due to maternal effects which result from maternal genotype and/or maternal environment. Maternal environment, the conditions produced by the mother plant while the developing ovules are still

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dependent, varies among individuals, and could affect later developmental processes in the seed progeny through differential resource allocation to ovules and other floral structures. For example, mother plants that allocate more resources to seeds may produce seeds which become larger plants; and larger plants may, in turn, be able to acquire more resources for producing larger flowers. Quantitative genetic analyses are required to detect effects of maternal genotype on petal size, but basic comparisons of flower size differences in the progeny may provide information about cytoplasmic inheritance.

The external environment can also introduce sources of variation in floral size. Differences in soil nutrients, moisture, and sunlight can all affect the growth and development of flower parts. Environmental effect is a legitimate consideration when evaluating gynodioecious species since female and hermaphroditic plants have sometimes been observed growing in different subhabitats within a population (Freeman in Webb, 1981; Kaul, 1988). Also, levels of phenotypic plasticity, morphological variation in response to environmental change, may be different for females and hermaphrodites.

#### MATING SYSTEM AND SEXUALLY DIMORPHIC FLOWERS

Since pollinators can affect fitness and gene flow in plant populations, their activities may also influence mating system dynamics. Studies have shown that larger flowers

### Chapter 3. Sexually Dimorphic Flowers

attract more pollinators, and hermaphroditic flowers in gynodioecious species receive more pollinator visits than females (Uno, 1982; Bell, 1985; Ashman and Stanton, 1991; Eckhart, 1991; Delph and Lively, 1992). While enhanced pollination may not necessarily improve seed-set in resource-limited plants, fertilization does depend on pollination, so insufficient pollination will affect seed-set. If hermaphroditic plants receive more pollinators, they could improve their seed-set relative to females which could in turn influence the maintenance of females in these gynodioecious populations. The relationship between flower size and seed-set will be evaluated in Chapter 4.

#### FLOWER SIZE IN *SIDALCEA HENDERSONII*

Most gynodioecious species have been shown to consist of only large-flowered hermaphroditic plants and small-flowered females, and no other discrete size classes for the sex morphs have been identified. Neither large and small-flowered female nor large and small-flowered hermaphroditic forms have been observed coexisting in a population. Interestingly, three floral forms have been described for *Sidalcea hendersonii*:

*A remarkable feature is the occurrence of 3 kinds of flowers. In addition to the large 1 inch, perfect flowers...occasional flowers in the spike-like racemes are but ½ inch wide (though perfect), and there are also similar small blooms that are imperfect, lacking anthers (Clark, 1973).*

### Chapter 3. Sexually Dimorphic Flowers

While different forms of hermaphrodite flowers on separate plants would be a highly unusual occurrence, Clark's observation that distinct large and small hermaphroditic flowers occur on the same plant is even less probable. The presence of such a unique polymorphism, if it existed, would permit genetic analyses of the relationship between flower size and sex in gynodioecious species that previously were not possible.

However, the nature of the different flower forms in *Sidalcea hendersonii* appears to be ambiguous and requires clarification via flower measurements and statistical comparisons.

#### **Objectives**

The objectives of this study were to:

- 1) document whether *Sidalcea hendersonii* flowers are sexually dimorphic in size, and determine if a third form, hermaphroditic plants with small flowers, exists in these populations;
- 2) locate other sources of petal length variation by examining maternal and environmental effects. For maternal effect, determine if seed-parent sex influences petal size in offspring following experimental breeding. For environmental effect, compare petal size for fertilized experimental plants with unfertilized plants in wild populations; and,
- 3) compile a data base for examining the relationship between flower size and seed production (Chapter 4).

### Methods

In July and August of 1995 and 1996, six British Columbia populations of *Sidalcea hendersonii* were surveyed near Campbell River, Comox, Duncan, Port Alberni, and Sayward on Vancouver Island, and Ladner in the delta of the Fraser River. An experimental population maintained in growth chambers and a population from Kamloops B.C. of *Sidalcea oregana*, a closely related species, were included for comparison. Although a gynodioecious breeding system has been documented for *S. oregana* ssp. *spicata* (Ashman and Stanton, 1991), the Kamloops population included in this study consisted entirely of hermaphroditic individuals and proved particularly interesting for mating system comparisons.

The length of each petal from attachment to apex was measured as an estimate of flower size. Two petals from separate flowers were pseudorandomly selected from at least 30 female and 30 hermaphrodite individuals, as they were encountered in each population. Individuals were sampled on the extreme borders of these populations as well as from central locations. Only open, fresh flowers were measured; this timing typically corresponded with anthesis in hermaphrodite flowers and with stigmatic receptivity in female flowers. Since the position of open flowers on an inflorescence for each plant varied considerably, no attempts were made to sample flowers from a standard position on the stem.

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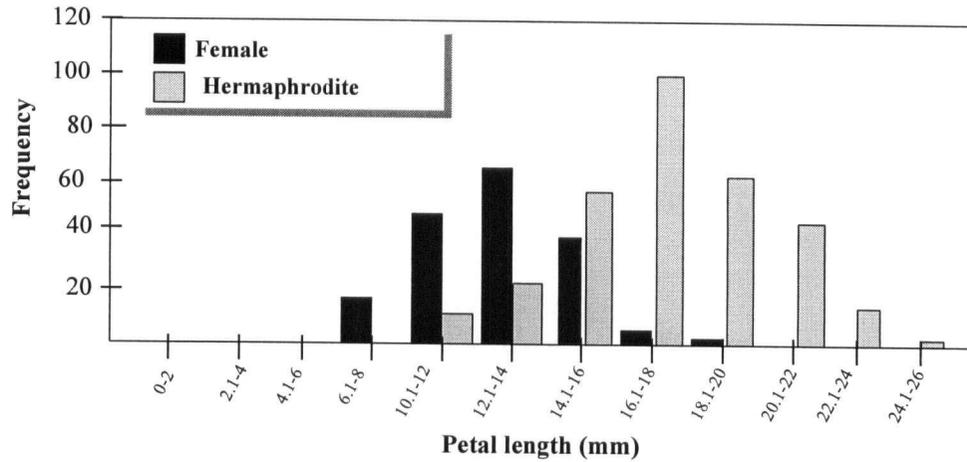
For the experimental population, two petals from the first flower were measured for every individual that flowered. The breeding design is described in Chapter 2 and the experimental conditions are included in Chapter 4.

Individual scores were based on the average of the two petal measurements from each plant. Student's t-test statistics were used to compare the mean petal length of females and hermaphrodites and to compare population means. For all variables, the data were normally distributed, and transformation was not required. In the few cases where variance was unequal, a nonparametric Mann-Whitney Rank Sum Test was calculated. All statistical analyses were performed with SigmaStat® v.2.0 (Jandel Scientific, 1995).

## **Results**

### FLORAL FORMS

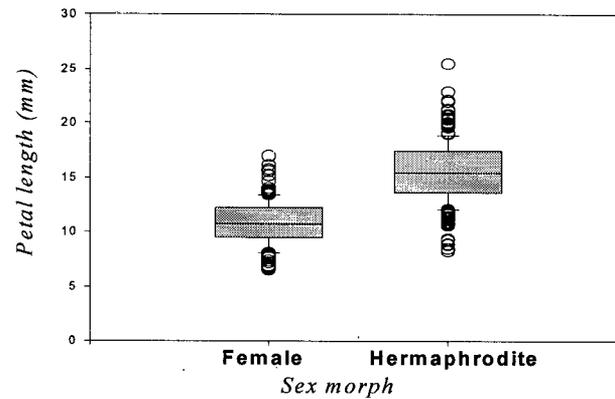
It is clear from the frequency distribution in Figure 3.1 that hermaphroditic individuals express a single, continuous flower-size phenotype. Data for both sexes were normally distributed, and in all populations examined, female flowers had significantly smaller petals than hermaphroditic flowers (Figs. 3.1, 3.2, 3.3; Table 3.1).



**FIGURE 3.1** Frequency distribution of petal-size classes for both sexes of *Sidalcea hendersonii* in all wild populations surveyed in 1995 and 1996.

Population	Mean Petal length Females (mm)	N	Standard Deviation	Mean Petal length Hermaphrodites (mm)	N	Standard Deviation	Significance
Campbell R.	11.28	51	1.59	16.60	49	2.25	$p < 0.001$
Comox	11.90	45	2.25	16.99	45	3.01	$p < 0.001$
Duncan	9.27	30	1.56	12.18	30	1.72	$p < 0.001$
Ladner	11.14	32	1.76	16.90	78	2.16	$p < 0.001$
Port Alberni	8.12	33	1.81	11.19	33	2.59	$p < 0.001$
Sayward	9.52	31	1.31	15.31	35	2.44	$p < 0.001$
Experimental	15.68	40	1.94	17.69	83	3.04	$p < 0.001$

**TABLE 3.1** Average petal length and T-test statistics for *Sidalcea hendersonii* populations.



**Figure 3.2** A comparison of average petal length for female and hermaphrodite plants in wild populations of *S. hendersonii*. Boxes contain the middle 50% of the data. The line dividing a box represents the median value. The T-bar represents the value of the largest observation excluding outliers, and the open circles signify outlying data points (Koopmans, 1987).



**Figure 3.3** Flowers from separate female (right) and hermaphroditic (left) *Sidalcea hendersonii* individuals grown from seed collected at Ladner Marsh, B.C. Photograph courtesy of Helen Kennedy.

### Chapter 3. Sexually Dimorphic Flowers

#### MATERNAL EFFECT

No significant difference ( $p=0.780$ ) in petal length was observed between hermaphrodite offspring from female mothers ( $17.59 \text{ mm} \pm 3.2$ ,  $N=34$ ) and hermaphrodite offspring from hermaphrodite mothers ( $17.78 \text{ mm} \pm 3.0$ ,  $N=44$ ).

#### ENVIRONMENTAL EFFECT

Experimental plants had significantly larger petals than plants from natural populations ( $p<0.001$ ). Petals of females were, on average, 4.88 (mm) longer in the experimental population than wild populations ( $15.68 \text{ mm} \pm 1.94$ ,  $N=40$ , and  $10.80 \text{ mm} \pm 2.01$ ,  $N=537$  respectively). Average petal length of experimental hermaphrodite plants was approximately 2.2 (mm) longer than wild hermaphrodites ( $17.69 \text{ mm} \pm 3.04$ ,  $N=83$  and  $15.54 \text{ mm} \pm 2.7$ ,  $N=330$ ).

#### INTER-SPECIFIC COMPARISON

Since females were not present in the Kamloops population of *Sidalcea oregana*, only hermaphrodite petal measurements were compared. The average petal measurements for hermaphroditic plants in all wild populations of *S. hendersonii* ( $15.54 \text{ mm} \pm 2.7$ ,  $N=330$ ) were significantly larger than those of *S. oregana* ( $14.29 \text{ mm} \pm 1.89$ ,  $N=93$ ;  $p<0.001$ ).

## Discussion

### FLORAL FORMS

Sexually dimorphic flowers are widespread among gynodioecious species, and *Sidalcea hendersonii* flowers are no exception. The flower size results clearly indicate that only two forms of flowers exist in British Columbia populations of *S. hendersonii*: large-petalled hermaphrodite and small-petalled female flowers, exactly as reported for other species of *Sidalcea* (Ashman and Stanton, 1991). Flower size may vary over the flowering season (Eckhart, 1991) and possibly with position on an inflorescence, and since the flowering season for *S. hendersonii* extended from the beginning of June through September in some populations, it is possible that Clark (1973) was comparing early and late flowers of individual plants and their neighbors. Since hermaphroditic flowers obtain a much larger final size, differences in large and small flowers on a hermaphroditic plant may be more pronounced than differences between flowers on a female plant.

### MATERNAL EFFECT

Comparing petal size among same-sex progeny of both females and hermaphrodites allows maternal effect to be examined without interference from sex-specific effects. Since hermaphrodite mothers in this study never gave rise to female progeny (Chapter 2), only the hermaphrodite progeny of the two sexes was compared. Hermaphrodite offspring from female mothers and hermaphrodite offspring from hermaphrodite

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mothers did not differ in petal length, indicating that while the sex of the individual is an important determinant of petal size, an individual's petal size is not influenced by the sex of its seed parent. Granted, since parent sexual identity affects the outcome of sex in the progeny, flower size in sexually dimorphic plants is indirectly affected by parent sex.

Differences in maternal environment between the sex classes were not detected through petal size measurements of the progeny. Had maternal effects been evident, female effects on hermaphrodite flowers would, if anything, reduce the differences between the sexes. These negative results do not rule out the hormonal/developmental control of flower size since hermaphrodite progeny had pollen and large flowers regardless of parentage. Cytoplasmic factors do not appear to influence petal size. Heritability of petal size could not be estimated since parent petal length measurements were not available for this study. Without quantitative genetic analysis, the evolution of flower size in response to selection could not be considered.

#### ENVIRONMENTAL EFFECT

Plants grown in growth chambers had significantly larger petals than plants which were surveyed from wild populations suggesting that the external environment could affect petal size. The flowers of experimentally grown plants may have larger petals due to superior growth conditions, such as regular light, water, and fertilizer, moderate

temperatures, and lack of root competition. Levels of phenotypic plasticity and differences in subhabitats for the sex morphs were not investigated.

Flower size was similar among all natural populations surveyed, except at Port Alberni (where *Sidalcea hendersonii* grew a short distance from a major logging operation) and Duncan. This consistency is surprising since many environmental factors, including climate, soil type, and growing season, differed for each population (Appendix A).

Within the experimental population, petal length for female and hermaphroditic plants was significantly different (Table 3.1), indicating that although petal size is influenced by the environment, environmental effect is not an important factor affecting the flower size dimorphism in *Sidalcea hendersonii*.

#### INTER-SPECIFIC COMPARISON

The hermaphroditic population of *Sidalcea oregana* had significantly smaller petals than hermaphroditic plants in *S. hendersonii* populations. This difference in petal size may simply be due to the fact that two different species of *Sidalcea* are being compared. However, average petal length of *Sidalcea oregana* flowers in California populations was 8.77 (mm) for hermaphrodites and 5.72 (mm) for females (Ashman and Stanton, 1991), while average petal length was 14.38 (mm) for *S. oregana* hermaphrodites at Kamloops. The Kamloops population is considerably north of the California population and east of *S. hendersonii* populations; environmental

differences may be great enough to explain the discrepancy in petal size observed for Kamloops plants. The Kamloops population could also be genetically different from the California populations since the comparison is between the extremes of the geographical range.

### **Conclusions**

Gynodioecious *Sidalcea hendersonii* plants are distinctly sexually dimorphic for flower size, although the cause of this dimorphism is unknown. While several hypotheses exist to explain the different forms of flowers in female and hermaphroditic plants of gynodioecious species, no single mechanism is likely responsible for the complex relationship between sex and flower size. This study examined other potential sources of flower size variation, and showed that while maternal effect on flower size was insignificant, environmental effects clearly do contribute to petal size variation but do not influence the relationship between flower size and sex.

## Chapter 4

### Inbreeding Depression and Maternal Sex Effect

#### Introduction

The maintenance of gynodioecy depends, in theory, on the ability of females to compensate for the loss of male function (Lewis, 1941; Lloyd, 1974c; Charlesworth and Charlesworth, 1978). Females may increase their fitness relative to hermaphrodites by improving seed production and/or seed quality (Ashman, 1992a). Hypotheses which attempt to explain sources female advantage are described below.

#### OUTCROSSING ADVANTAGE

According to the outcrossing hypothesis, females have higher fitness because their flowers are obligately outcrossed, while the progeny of hermaphrodites may arise from self-fertilization which can cause a reduction in fitness through inbreeding depression (Lewis, 1941; Ganders, 1978; Charlesworth and Charlesworth, 1978). Inbreeding depression results from an accumulation of deleterious recessive alleles which can strongly influence reproductive yield and growth (Hartl and Clark, 1989).

The outcrossing hypothesis has been extensively tested with population genetics models (*e.g.*, Lewis, 1941; Ross and Shaw, 1971; Lloyd, 1974c; Charlesworth and

#### Chapter 4. Inbreeding Depression and Maternal Effect

Charlesworth, 1978; Charlesworth and Ganders, 1979; Webb, 1981; Delannay *et al.*, 1981; Schultz, 1993). The frequency of females at equilibrium ( $p$ ) can be predicted by:

$$p = \frac{(1 - 2f + 2fs\delta)}{(2 - 2f + 2fs\delta)} \quad [4-1]$$

where  $s$  is the selfing rate of hermaphrodites,  $\delta$  is the inbreeding depression in the progeny from selfing; and  $f$  is the seed production of hermaphrodites relative to females (modified from Charlesworth and Charlesworth, 1978). From this equation, the frequency of females increases as  $s$  and  $\delta$  increase or  $f$  decreases. Even in the absence of differences in seed production, females may be maintained at equilibrium when selfing and inbreeding are high, *i.e.*,  $s\delta > 0.5$  (Lloyd, 1974c; Ganders, 1978; Charlesworth and Charlesworth, 1978).

Equation 4-1 also illustrates that since the lowest value for  $f$  is zero, female frequencies are confined between zero and 0.5, and that females cannot exceed hermaphrodites in a population at equilibrium (Lewis, 1941; Charlesworth and Charlesworth, 1978). When female frequencies approach 50%, the seed fertility of hermaphrodites is negligible, and the population may be cryptically dioecious (Schultz, 1994).

In several gynodioecious species, the frequency of females can be explained by high inbreeding depression and/or high selfing rate in hermaphrodites (Sun and

#### *Chapter 4. Inbreeding Depression and Maternal Effect*

Ganders, 1986; Kohn, 1988; Sakai *et al.*, 1989; Kalisz, 1989; Molina-Freaner and Jain, 1993; Schultz, 1993; Schultz and Ganders, 1996).

##### MATERNAL SEX EFFECT

Maternal effect which results from maternal genotype and/or maternal environment may be another source of female advantage (Van Damme and Van Delden, 1984; Ashman, 1992a). The resource allocation hypothesis states that female plants may allocate more resources to ovules than hermaphrodites do because females need not expend resources on anther and pollen production. Thus, seeds which develop on a female plant could experience a different maternal environment from seeds which develop on a hermaphrodite plant. Developmental advantages associated with maternal parentage have been reported for *Sidalcea oregana* ssp. *spicata* (Ashman, 1992a), *Cucurbita foetidissima* (Kohn, 1989), and *Phacelia linearis* (Eckhart, 1992b). Ashman (1994) examined resource partitioning between the sexes, monitoring the allocation of biomass, nitrogen, phosphorus, and potassium. Females, it was demonstrated, did indeed allocate larger proportions of their reproductive currency to seeds. Advantages associated with maternal genotype could be the result of pleiotropic effects of the sex determining genes (Lloyd, 1974c; Van Damme *et al.*, 1984; Ganders, 1978) or overdominance (Ross, 1977; Van Damme *et al.*, 1984).

Some studies have reported that inbreeding depression was significant during certain life history stages while maternal effect was more important at other stages

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(*e.g.*, Shykoff, 1988; Kohn, 1988; Ashman, 1992a). Since genetic composition can influence maternal ability, it is difficult to separate outcrossing effect from maternal resource allocation differences (Kesseli and Jain, 1984).

##### FITNESS AND FLOWER SIZE

Larger flowers tend to attract more pollinators, and the relatively large hermaphroditic flowers in several gynodioecious species have been reported to receive more pollinator visits than females (Uno, 1982; Bell, 1985; Ashman and Stanton, 1991; Eckhart, 1991). By attracting more pollinators with their larger flowers, hermaphrodites may enhance their fertilization rates and seed production relative to females. Alternatively, larger flowers could represent a resource drain to ovules, reducing hermaphrodite seed production relative to females. Some investigators hypothesize that hermaphrodites sacrifice seed production to attract pollinators and improve their male reproductive success (Eckhart, 1993).

##### FITNESS AND FLOWERING TIME

Female and hermaphrodite plants may bloom at different times; in fact, earlier flowering of females is common (*e.g.*, Baker, 1948; Uno, 1982; Ashman, 1992b). Female fitness can be affected by differences in flowering time since the quantity of pollen available changes over the flowering season as the number of flowering hermaphrodites changes (Ashman, 1992b).

### Objectives

Studies investigating the maintenance of gynodioecy regularly compare females, self-pollinated hermaphrodites, and outcrossed hermaphrodites for various components of fitness (e.g., Kesseli and Jain, 1984; Kohn, 1988; Shydoff, 1988; Sakai *et al.*, 1989; Kalisz, 1989; Jollis and Chenier, 1989; Agren and Willson, 1991; Eckhart, 1992b; Ashman, 1992a; Shultz, 1993). The objectives of this study are to determine whether female plants produce more viable seeds than hermaphrodite plants in natural populations; evaluate the effect of flower size on seed-set; and experimentally examine the effects of inbreeding and maternal parentage on fitness and phenology.

### Methods

#### FIELD STUDY

The relative frequency of females in a population was estimated by scoring plants randomly as encountered. Fruiting racemes from randomly selected female and hermaphrodite individuals were collected in wild *Sidalcea hendersonii* populations in August of 1995 and 1996. Racemes were also collected from *Sidalcea oregana* plants in Kamloops B.C.

To estimate seed production, the number of fruits on each raceme and the total number of seeds contained in a fruit (from a sample of ten fruits on each plant) were counted. Seeds were classified as viable or aborted; aborted seeds were dry inside,

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distinctly flattened, and smaller than viable seed. Viable seeds of several different female and hermaphrodite individuals were weighed, and the values averaged for each sex.

For each plant, the proportion of viable seed was calculated by dividing the number of viable seeds in a capsule by the total number of seeds in that capsule. Because flower size may influence seed-set, petal length measurements (from Chapter 3) were compared to the proportion of viable seed with the Pearson product-moment correlation coefficient. Separate correlation tests were performed for female and hermaphrodite plants since the comparison of sexually dimorphic flowers could produce spurious results. Student's t-test statistics were used to compare average values of females and hermaphrodites for all seed measures.

#### EXPERIMENTAL STUDY

Seed collected from wild *Sidalcea hendersonii* populations was scarified with sandpaper and then sealed in plastic bags along with moist peat mixed with a fungicide (No Damp® (Oxine Benzoate 2.5%, Plant Products Co. Ltd., Brampton Ont.)) and cold stratified at 9° C for 14 days. Plants were watered as needed, and fertilized biweekly with a standard commercial fertilizer. Pesticides and insecticidal soap were applied periodically to control whiteflies and aphids. In November 1994, germinated seed was planted into 4 inch pots in the U.B.C. Department of Botany greenhouses,

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and was later transferred to growth chambers for experimental pollination. The growth chamber was maintained at 20°C with an 8L:16D hour photoperiod.

Crosses were performed between female and hermaphrodite plants and between separate hermaphrodite individuals to estimate both levels of inbreeding depression and to determine the genetic control of male sterility (Chapter 2). Outcrossed hermaphrodite flowers were emasculated at anthesis to prevent self-pollination, and to standardize treatments, several of the self-pollinated flowers were also emasculated.

To insure an adequate seed crop, as many flowers as possible were pollinated, so the number of flowers receiving pollen on a plant depended on the individual's flower production. Seed was harvested during the fall of 1995, and viable seed (and total seed/fruit) was counted and weighed.

In January 1996, the seed from the thirty selected families was scarified and planted (two per pot) into 1.5 inch pots of moist MetroMix ® soil (W.R. Grace, Ajax, Ont) mixed with a fungicide. The pots were covered with clear plastic and stored at 5° C for three weeks then moved to growth chambers. The seedlings were exposed to the same environmental conditions as the parent plants except that the growth chamber temperature and daylight hours were increased to accelerate germination and flowering (23° C, 12L:12D hour photoperiod). To reduce position effects, individual pots were randomized and rotated every two weeks for four months, at which point the plants were too large and fragile to rotate. When both seeds in a pot germinated, one

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seedling was transplanted to a new pot, and in March, all seedlings were transplanted into larger four-inch pots.

Germination was monitored every 1-2 days for three months, and germinating seeds were marked with wooden picks for later detection of seedling mortality. Survival was reported after 5 months. The proportion of plants which flowered within 13 months, the day of flowering, and the petal length (Chapter 3) were also recorded. Due to space limitations, some of the plants were moved to a separate growth chamber, but once moved, these plants were not used for fitness measures.

Differences in seed production and flowering time among female, outcrossed hermaphrodite, and self-pollinated hermaphrodite parents were evaluated with a one-way analysis of variance ANOVA (Sokal and Rohlf, 1969). Differences between progeny fitness were evaluated with a chi-square test. Multiplicative fitness functions (after Sakai *et al.*, 1989) were estimated for each maternal parent group as the product of seed production and progeny performance at all measured stages (overall fitness = proportion of viable seed produced by parent x proportion of seed that germinated x proportion of seedlings that survived x proportion of progeny that flowered).

## Results

### FIELD STUDY

#### *Female frequency:*

Female frequency in *Sidalcea hendersonii* populations ranged from 14 to 54%, but in the majority of these populations, females approached levels of 50% (Table 4.1).

POPULATION	N	PERCENT FEMALES
Campbell River	100	44
Comox Spit	365	54
Duncan	175	49
Ladner Marsh, Delta	300	21
Port Alberni	66	52
Sayward	337	14

**Table 4.1** Frequency of females in natural populations of *Sidalcea hendersonii* in British Columbia. These populations were located from herbarium specimens at the University of British Columbia (UBC), and the Royal British Columbia Museum (V).

#### *Seed production:*

No consistent pattern of sex-related differences in fecundity was evident in natural populations. The number of fruit on a raceme was not significantly different between female and hermaphrodite plants in any of the populations. Females did produce significantly more seeds per fruit than hermaphrodites for most populations, but frequently many of the seeds were inviable. At Campbell River and Duncan, female fruit contained more viable seeds than hermaphrodite fruit, but hermaphrodite fruit contained more viable seeds at Port Alberni (Table 4.2).

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Population	sex	N	Mean # fruits per raceme	sig.	Mean # seeds per fruit	sig.	Mean # viable seeds/fruit	sig.
Campbell R.	F	40	25.5 (12.3)	NS	7.2 (0.8)	p=0.009	4.0 (1.5)	p=0.04
	H	42	22.4 (10.6)		6.7 (0.9)		3.3 (1.3)	
Comox	F	34	25.7 (09.5)	NS	7.3 (0.5)	p=<0.001	3.2 (0.9)	NS
	H	30	23.9 (10.7)		6.8 (0.4)		3.2 (0.8)	
Duncan	F	30	25.7 (13.5)	NS	7.1 (0.7)	p=<0.001	5.7 (1.1)	p=0.004
	H	30	24.7 (09.9)		6.4 (0.5)		4.9 (0.9)	
Ladner	F	38	30.2 (12.9)	NS	6.4 (0.6)	p=<0.001	2.4 (0.7)	NS
	H	33	25.4 (12.3)		5.7 (0.4)		2.7 (0.8)	
Port Alberni	F	30	15.6 (05.2)	NS	7.6 (0.4)	NS	0.4 (0.5)	p=<0.001
	H	28	16.5 (07.6)		7.5 (0.6)		0.9 (0.6)	
Sayward	F	34	23.4 (09.8)	NS	7.3 (0.5)	p=<0.001	3.1 (1.5)	NS
	H	36	20.0 (09.0)		6.7 (0.6)		2.7 (1.2)	

**Table 4.2** Mean values for fruit and seed production in natural populations of *Sidalcea hendersonii*. Data reported for Port Alberni and Sayward is from 1996 (data not available for 1995), and data reported for the remainder of populations is from 1995 (results were similar in 1996). The standard deviation is listed in parentheses.

*Seed weight:*

Sex-related differences in seed weight were not statistically significant for wild collected seed ( $p=0.567$ ). The average weight for female seed was 4.24 mg ( $N=121$ ) and hermaphrodite seed was 4.21 mg ( $N=75$ ).

*Interspecific comparison:*

Individuals from the hermaphrodite population of *Sidalcea oregana* near Kamloops produced twice as many viable seed compared to both female and hermaphrodite *Sidalcea hendersonii* plants. *S. oregana* plants produced an average of 6.6 viable

#### *Chapter 4. Inbreeding Depression and Maternal Effect*

seeds per fruit ( $N=30$ ) (mean total seeds/fruit=6.8) while female and hermaphrodite *S. hendersonii* plants produced an average of 3.3 ( $N=176$ ) (mean total seeds/fruit=7.2) and 3.0 ( $N=179$ ) (mean total seeds/fruit=6.7) viable seeds per fruit respectively ( $p<0.001$ ).

##### *Relationship between petal length and seed-set:*

In general, no relationship between petal length and the proportion of viable seed was observed. However, at Duncan there was a marginally significant positive relationship between petal length and viable seed production within hermaphrodite plants ( $r=0.384$ ;  $p=0.040$ ), and at Comox there was a marginally significant negative relationship within the females ( $r= -0.358$ ;  $p=0.038$ ).

#### EXPERIMENTAL STUDY

##### *Parent seed production and progeny performance:*

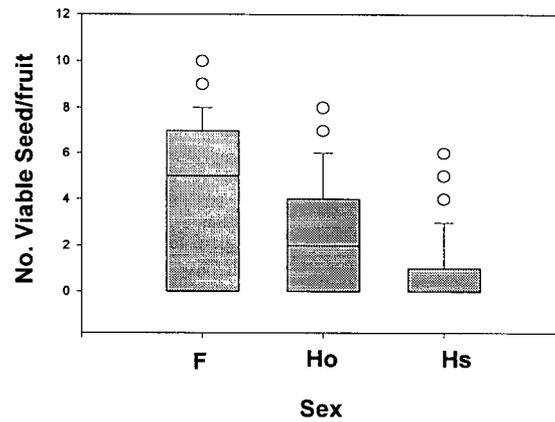
Female plants in the experimental study produced significantly more viable seed than both outcrossed and self-pollinated hermaphrodite plants, and the progeny of female parents had superior germination, seedling survival, and flowering success. On average, the progeny of all three parent groups flowered at essentially the same time. Fitness measures for progeny of outcrossed hermaphrodite plants were higher than for progeny of self-pollinated hermaphrodites for most traits except for the total number

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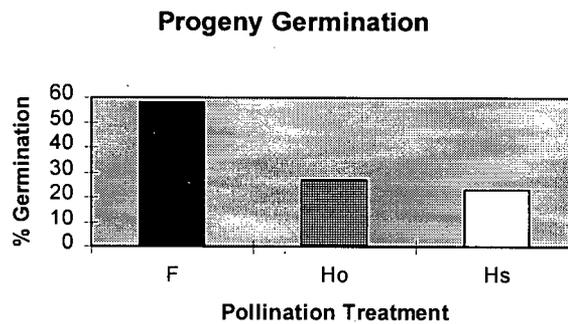
of seeds/fruit, but the number of viable seeds/fruit is a more informative measure of fitness (Figs. 4.1, 4.2, 4.3, and 4.4; Table 4.3).

Pollination Treatment	N	Mean # seeds per fruit	Mean # viable seeds/fruit	Proportion of seeds to germinate	Proportion seedlings to survive	Proportion of progeny to flower	Days to flower
Female outcrossed	334	8.10 (0.85)	4.35 (3.25) prop. viable seeds=0.54	383/660 = .58	376/383 = .98	81/126 = .57	136
Hermaph. outcrossed	141	7.12 (0.83)	2.27 (2.38) prop. viable seeds=0.32	97/361 = .27	88/97 = .91	36/97 = .37	135
Hermaph. selfed	50	6.85 (1.04)	0.87 (1.57) prop. viable seeds=0.13	23/82 = .23	19/23 = .83	8/35 = .35	133
<b>STATISTICS</b>		ANOVA p<0.001	ANOVA p<0.001	X <sup>2</sup> =102.35 p<0.001	X <sup>2</sup> =22.61 p<0.001	X <sup>2</sup> =10.38 p=0.006	ANOVA p=0.880
<b>SUMMARY</b>		(F>Ho=Hs)	(F>Ho>Hs)	(F>Ho>Hs)	(F>Ho>Hs)	(F>Ho>Hs)	(F=Ho=Hs)

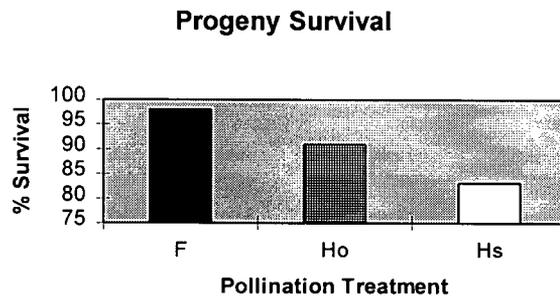
**Table 4.3** Performance comparisons in the experimental population of *Sidalcea hendersonii*. 'Days to flower' is the average number of days for the progeny to flower in the first year. Chi Square tests the hypothesis F=Ho=Hs for proportion of seedlings to germinate, survive, and flower.



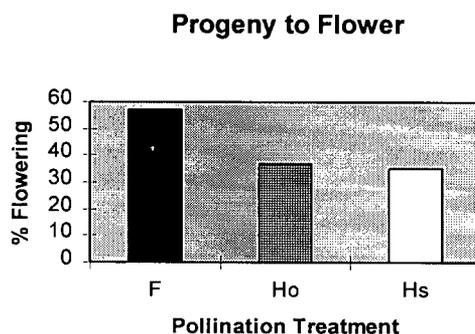
**Figure 4.1** Box plot comparing viable seed production for female parents (F), outcrossed hermaphrodites (Ho), and self-pollinated hermaphrodites (Hs) in the experimental population of *Sidalcea hendersonii*. Boxes contain the middle 50% of the data. The line dividing a box represents the median value. The T-bar represents the value of the largest observation excluding outliers, and the open circles signify outlying data points (Koopmans, 1987).



**Figure 4.2** Percent seed germination from female, outcrossed and selfed hermaphrodite parents of *Sidalcea hendersonii*.



**Figure 4.3** Percent survival of seedlings of female, outcrossed and selfed hermaphrodite parents *Sidalcea hendersonii*.



**Figure 4.4** Percent plants flowering in the first year in progeny of female, outcrossed and selfed hermaphrodite parents of *Sidalcea hendersonii*.

*Relative fitness and Inbreeding Depression:*

Calculation of the multiplicative fitness function yielded low estimates of overall fitness in all three parent groups (Table 4.4). Female parents had the highest overall fitness followed by outcrossed hermaphrodite parents, and selfed hermaphrodites had the lowest value. Relative fitness was standardized for the three parental groups where the largest overall fitness (female parents) was assigned the value of 1, and the other

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two groups were a fraction of 1. Female fitness was 82% higher on average than outcrossed hermaphrodites, and 94% higher than self-pollinated hermaphrodites. One generation of selfing in hermaphrodites reduced relative fitness three-fold. Since experimental plants were grown in a controlled greenhouse environment and no other selection against any parent group should occur, inbreeding depression may be estimated as  $\delta = 1 - \frac{w_s}{w_o}$  where  $w_o$  is the overall fitness estimate of outcrossed progeny and  $w_s$  is the overall fitness estimate of selfed progeny (after Lande and Schemske, 1985). Inbreeding depression is estimated at 0.67 ( $\delta = 1 - 0.01 / 0.03$ ). Estimates of inbreeding depression under greenhouse or growth chamber conditions are often lower than estimates under field conditions, so that this may be an underestimate.

parent	viable seed	germination	survival	flowering	Overall fitness	Relative fitness
Fo	0.54	0.58	0.98	0.57	0.17	1.00
Ho	0.32	0.27	0.91	0.37	0.03	0.18
Hs	0.13	0.23	0.83	0.35	0.01	0.06

**Table 4.4** Multiplicative fitness function (after Sakai *et al.*, 1989) for females (F), outcrossed hermaphrodites (Ho), and self-pollinated hermaphrodites (Hs) of the experimental *Sidalcea hendersonii* population. Overall fitness is the product of the proportion of viable seeds per fruit, proportion of seeds to germinate, proportion of seedlings to survive, and proportion of plants to flower in the first year.

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### *Seed weight:*

Similar to the results for natural populations, seed weight was not significantly different for female and hermaphrodite individuals in the experimental population ( $p=0.143$ ), but the seeds were generally heavier for the experimental population than for wild grown seed. The average weight for female seed was 5.45 mg ( $N=90$ ) and 5.85 mg ( $N=15$ ) for hermaphrodite seed.

## **Discussion**

### FIELD STUDY

#### *Seed measures:*

If the proportion of viable seed is averaged for all populations, the proportion of viable seed in females is 0.44 (18.80/42.9) and in hermaphrodites is 0.44 (17.70/39.80), *i.e.*  $f=1$ . Overall, no maternal effect or outcrossing advantage is evident from seed viability data gathered in the field. Fecundity differences between females and hermaphrodites have been reported to be insufficient to maintain females for many other gynodioecious species (Richards, 1986).

Seed weight is often used to estimate seed quality since larger seeds have a greater supply of resources for initial seedling growth and survivorship (Hendrix *et al.* in Ashman, 1992a). In the present analysis, however, seeds produced by females were no heavier than those of hermaphrodite plants.

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These results do not eliminate the possibility of inbreeding effects because measures of fitness in natural populations can be complicated by ecological factors. For instance, developing ovules may also abort due to insufficient pollination or limited nutrients.

The similarity in seed measures for females and hermaphrodites may also be affected by mating patterns in open pollinated populations. For instance, the protandrous flowers of hermaphrodites could favor outcrossing in hermaphrodites and increase hermaphrodite fitness. Furthermore, females could experience inbreeding depression if crossed to relatives. Biparental inbreeding in females of Hawaiian *Bidens* was as high as 25% (Sun and Ganders, 1988).

#### *Petal length and Viable seed:*

There was no consistent relationship between petal length and the proportion of viable seed in natural populations. These results do not support either hypothesis that larger petals attract more pollinators improving fertilization or that larger petals deprive the ovules of resources. In *Sidalcea oregana*, petal length did affect pollinator visitation rates (Ashman and Stanton, 1991), but was not a good predictor of pollen deposition. Ashman (1992b) suggested that increased attractiveness improved pollen removal rates thereby enhancing male fitness in hermaphrodites.

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##### EXPERIMENTAL STUDY

If a sex-related performance advantage is the result of a difference in outcrossing rates, fitness measures should be lower in self-pollinated hermaphrodites than outcrossed hermaphrodites. Alternatively, if differences in performance are a function of maternal sex effects, the fitness of both outcrossed and selfed hermaphrodites should be lower than that of females. Females and outcrossed hermaphrodites did have higher fitness values than self-pollinated hermaphrodites, which supports the outcrossing hypothesis, but females also had higher fitness than outcrossed hermaphrodites suggesting that maternal sex effects occur as well.

Other explanations for fitness differences between outcrossed hermaphrodites and females might be that outcrossed hermaphrodites could be the progeny of a self-pollinated parent, and that residual genetic load has a negative effect on the hermaphrodite's maternal capabilities. Perhaps this type of uncertainty could be avoided by repeating the crosses for a number of generations. Alternatively, the emasculation procedure could have disrupted ovule development in hermaphrodites. However, the progeny of females continued to have superior performance in later stages which should not have been affected by the emasculation.

The relative fitness of females was estimated to be 82% higher on average than outcrossed hermaphrodites, indicating that 82% of the female advantage is most likely due to some kind of maternal effect such as resource allocation differences between the sexes. However, if more resources were allocated to seeds, this was not apparent

#### Chapter 4. Inbreeding Depression and Maternal Effect

from seed weight since there was no difference between females and hermaphrodites. Hermaphrodite fitness compared to females is reduced an additional 12% when inbreeding occurs.

Inbreeding depression was estimated at 0.67 suggesting that many of the progeny from selfing do not reproduce. An estimate of 0.67 for inbreeding depression is considered to be high, although Sakai *et al.* (1989) reported inbreeding depression values ranging from 0.62-0.94 in *Schiedea salicaria*, and Schultz (1993) reported ranges of 0.96-0.98 in *Bidens sandvicensis*. Though inbreeding depression does appear to be high and differences in performance traits for outcrossed and selfed hermaphrodites are statistically significant, the values for total fitness are so minute that, perceived overall performance differences between the two may not be all that significant.

Earlier flowering of females in *S. oregana* was reported (Ashman, 1992b), but no differences in flowering time were observed in the experimental population of *S. hendersonii*.

Even though fitness values for females were higher than those for hermaphrodites, they were still surprisingly low. Negative pleiotropic effects associated with male sterility could influence yield and other fitness traits. Also, female flowers could have received defective pollen, or seed from some females may have failed to germinate because of dormancy constraints.

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### EXPERIMENTAL VS. FIELD RESULTS

Females did not appear to compensate for the loss of male function through improved seed production in natural populations, but the results for experimental crosses did reveal inherent benefits to being female. Advantages associated with outcrossing and maternal effect may appear in later life history stages in natural populations, or the environmental factors described earlier for natural populations may account for the disparate results.

Consistent with results for flower size (Chapter 3), the experimental population had much heavier seeds than natural populations, which likewise may be due to ample resources and lack of competition among the experimental plants.

### INTER-SPECIFIC COMPARISON

Similar to *Sidalcea hendersonii*, in gynodioecious *Sidalcea oregana* ssp. *spicata* in California both maternal-sex effects and inbreeding effects were observed (Ashman, 1992a). In *S. oregana*, however, the maternal-sex effects were pronounced during the juvenile stages of the life cycle while inbreeding effects were apparent primarily at the adult stage of the life cycle. For instance, the seeds of females had higher germination and produced faster growing seedlings, and outcrossed plants grew larger and produced more flowers than inbred plants. Also similar to *S. hendersonii*, Ashman's study of *S. oregana* reported that females and hermaphrodites did not differ in mean seed weight.

In the comparison population of *S. oregana* in Kamloops for the current study, large differences in viable seed production between *S. oregana* and *S. hendersonii* were detected which could be due to any of the ecological or inherent factors described earlier. The fact that the *S. oregana* plants in Kamloops produced such a high proportion of viable seed suggests that these plants are not highly inbred. This might also explain why male sterility mutations have not successfully invaded or been maintained in this population of *S. oregana*.

#### FEMALE FREQUENCY

The selfing rate of hermaphrodites has not been measured, but it could not be high enough to predict observed female frequencies using equation 4-1. If  $\delta = 0.67$  and  $f = 1$ , from results for relative seed production in natural populations, for  $p$  (frequency of females) to be greater than 0.01, hermaphrodites of *S. hendersonii* must have a high selfing rate of  $s > 0.75$ . Even if the selfing rate were 1.0, then  $p$  would only be 0.25.

Even with a liberal estimate of selfing in natural populations, these values for selfing, inbreeding depression, and relative seed production are not great enough to explain the high frequency of females in populations of *Sidalcea hendersonii*. Female frequencies of 50% in many *S. hendersonii* populations are even more perplexing (Table 4.1).

The frequency of females in natural populations is often higher than predicted by relative fecundity values and inbreeding depression in hermaphrodites suggesting that

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females may be maintained by a combination of higher fecundity, outcrossing advantage and possibly other factors (Shykoff, 1988).

#### **Conclusion**

In conclusion, while there does appear to be an inherent advantage to maternal sexual identity, and inbreeding depression was high for self-pollinated hermaphrodites in the experimental study, overall, these gains do not appear to be great enough to explain the maintenance of females at their current frequency in natural populations of *Sidalcea hendersonii*.

## Chapter 5

### Seed Predation

#### Introduction

In addition to flower morphology, female and hermaphrodite individuals of gynodioecious species can differ in phenology and biochemical composition (Delph, 1996). Insect response to this divergence of the two sexes can result in differential pollination (Chapter 3) and possibly predation.

While the ability of herbivores to discriminate among conspecific host plants has been extensively documented (*e.g.*, Mitchell, 1975; Thompson, 1985; Small *et al.*, 1989), only a few examples of sex-related predation preferences have been reported. Sex-specific leaf-feeding by insects has been observed in the dioecious species *Hippohoa rhamnoides* and *Cannabis sativa* (Gatima and Giklova cited in Bawa and Opler, 1977). Cox (1982) describes the incidental destruction of male flowers by large vertebrates seeking pollen, and staminate inflorescences of dioecious *Simarouba glauca* are considerably more likely to sustain feeding damage from moth larvae (Bawa and Opler, 1977).

Anecdotal evidence suggests that pollen/flower eating beetles preferentially attack the hermaphroditic flowers in a gynodioecious population of *Tellima grandiflora*

## Chapter 5. Seed Predation

(Wagner and Miller, 1984). Also, hermaphrodite plants in a gynodioecious population of *Iris douglasiana* experience higher levels of seed predation by moth larvae than females (Uno, 1982). However, conclusions for *Iris* were complicated by the fact that female and hermaphrodite fruits were collected at different times of the year. No well documented cases of differential predation in a gynodioecious species appear to be available.

### SEED PREDATION IN *SIDALCEA HENDERSONII*

Malvaceous plants are commonly associated with weevil seed predators of the family Curculionidae. The infamous boll weevil, *Anthonomus grandis*, parasitizes cotton crops causing at least 200 million dollars worth of damage each year (White, 1983). *Callirhoe* (Malvaceae) is a host plant for at least three weevil species: *Anthonomus fulvus*, *A. squamans*, and *Macrorhoptus estriatus* (Dorr, 1990). The weevil genus *Macrorhoptus* specializes in members of the Malvaceae for its host plants (Burke, 1973), and is frequently associated with host species of *Sidalcea*. *Macrorhoptus niger* weevils prey on the seeds of *S. malvaeflora* (Dimling, 1992), *S. campestris*, *S. virgata*, and the threatened species *S. nelsoniana* (S. Gisler, pers. comm.). Sex-related preferential feeding behavior by weevils was not reported in these *Sidalcea* studies. However, selective predation by weevils may occur in *Sidalcea hendersonii*. Hermaphrodite *S. hendersonii* plants appear to suffer more seed predation by weevil larvae than females.

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Two species of weevils parasitize the flowers of *Sidalcea hendersonii* in British Columbia, *Macrorhoptus sidalcea* Sleeper (det. Robert Anderson, Canadian Museum of Nature, Ottawa) and *Anthonomus melancholicus* Dietz (det. Horace Burke, Dept. of Entomology, Texas A & M). The two weevil species often coexist in a single host population of *Sidalcea hendersonii* and may even compete for developmental sites.

### ***Macrorhoptus sidalcea*** (Figures 5.1 and 5.3):

*Macrorhoptus sidalcea* had previously only been documented on Sea Island in Richmond, BC (Sleeper, 1957). My collections have revealed its distribution in populations of *S. hendersonii* along the east coast of Vancouver Island as well as in an additional population in Delta, BC.

Adult Curculionid beetles emerge from hibernation in late spring (Sork and Boucher, 1977), and mating pairs of *Macrorhoptus sidalcea* are observed in the new blossoms of *S. hendersonii* around June. Female weevils bore holes in immature carpels and oviposit eggs, which, in related Curculionidae, hatch in 5–7 days (*e.g.*, Sork and Boucher, 1977). The larvae complete their development in the fruit (a 5–9 seeded schizocarp) prior to seed dispersal. When a seed has been consumed, the larva tunnels through the lateral carpel wall to feed on an adjacent seed. More than one larva may complete development in the same fruit. Pupation occurs in the cavity being formed by the larva, and the adult emerges through a hole chewed in the carpel wall (Burke, 1973).

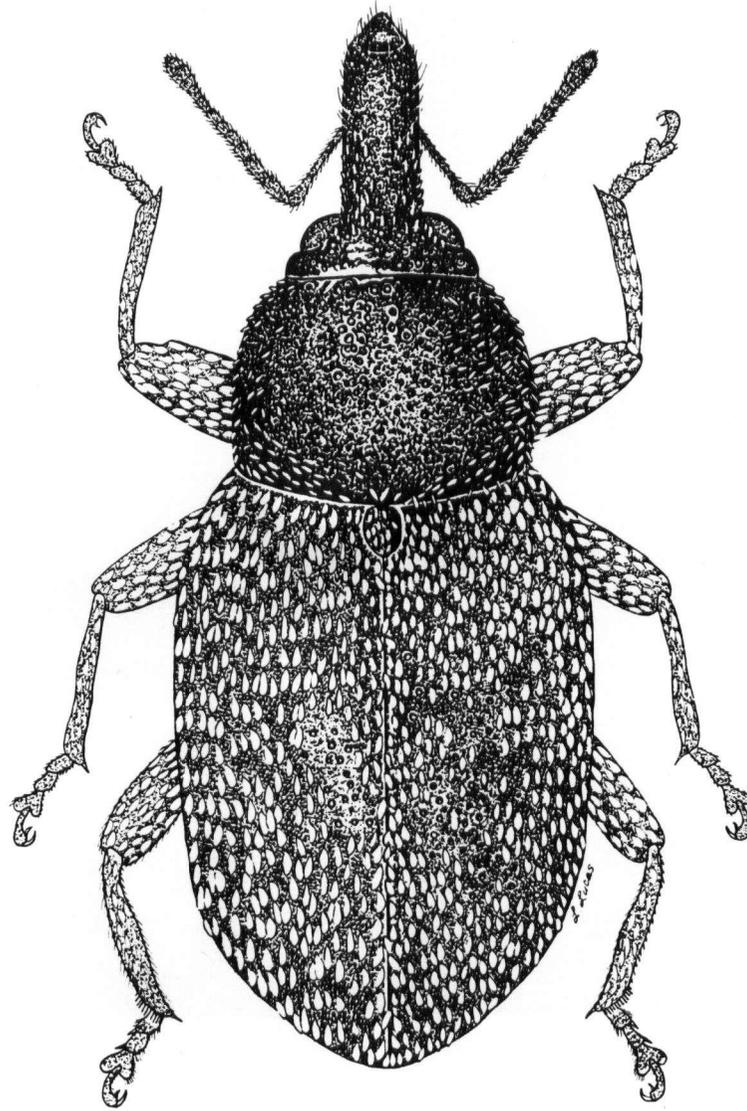
## Chapter 5. Seed Predation

Adult weevils do not appear to exploit ovules as a food source (Platt *et al.*, 1974).

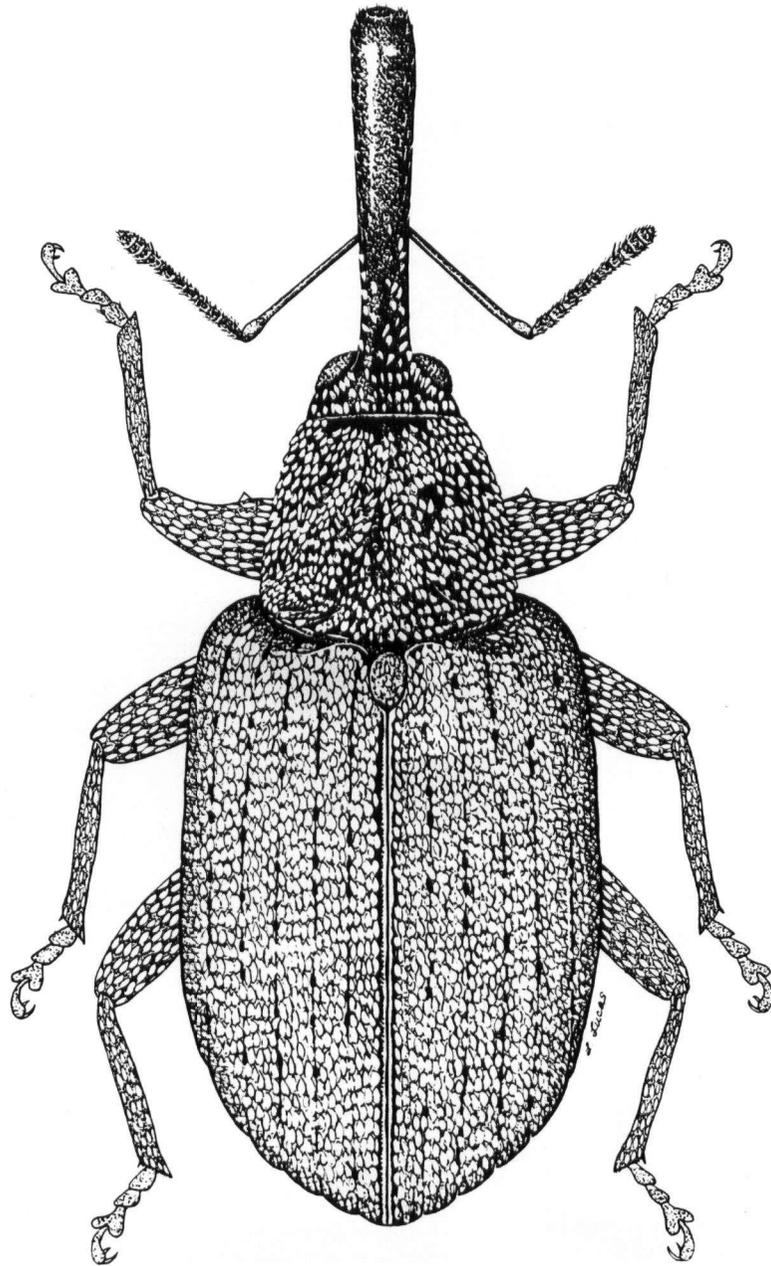
Adult *Macrorhoptus* weevils have been observed eating flower and leaf parts (S. Gisler, pers.comm.).

***Anthonomus melancholicus*** (Figures 5.2 and 5.3):

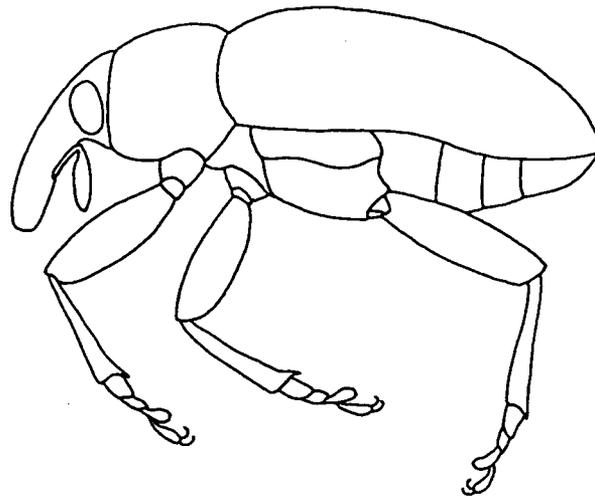
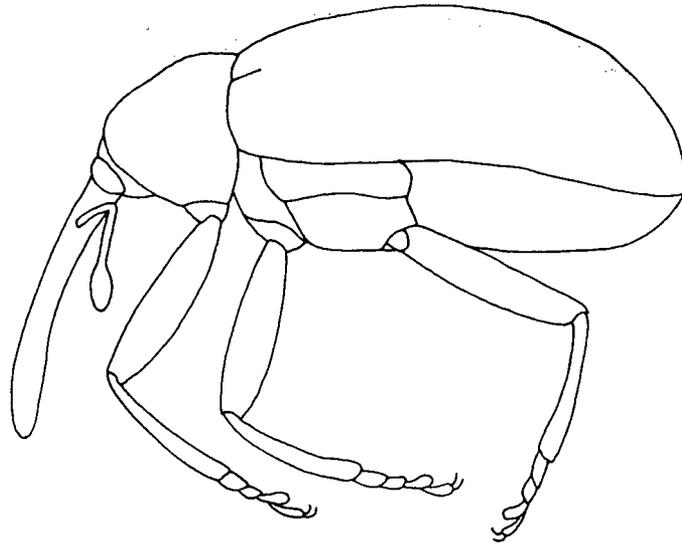
*Anthonomus melancholicus* adults appear in *Sidalcea hendersonii* populations later in the season than *Macrorhoptus sidalcea*, typically during seed-set in July and August. Like *M. sidalcea*, *A. melancholicus* weevil larvae may also use *S. hendersonii* fruit for their development. While *M. sidalcea* larvae feed only on the embryo and endosperm, creating small tunnels in the seed coat, *Anthonomus melancholicus* larvae destroy entire fruit. A related species, *Anthonomus fulvus*, has been collected in flower buds of *Callirhoe* (Dorr, 1990). The larvae of *A. fulvus* eat the mericarps of the fruit and the staminal column, pupating in the fruit. Adult *Anthonomus* weevils prefer pollen as food (H. Burke, pers. comm.). Host specialization is also common among *Anthonomus* species.



**Figure 5.1** *Macrorhoptus sidalcea*; dorsal view. Illustrated by L. Lucas (1996). Actual size approximately 2.5 mm long.



**Figure 5.2** *Anthonomus melancholicus*; dorsal view. Illustrated by L. Lucas (1996). Actual size approximately 3.2 mm long.



**Figure 5.3** *Anthonomus melancholicus* (top) and *Macrorhoptus sidalcea* (bottom); lateral view. Illustrated by L. Lucas (1996). Actual size approximately 3.2 mm long and 2.5 mm long respectively.

### **Objectives**

The objectives of this study were to determine whether weevil predators indiscriminately attack the seed of female and hermaphrodite *Sidalcea hendersonii* plants or prey selectively on one sex; identify which weevil species, if any, is a selective predator; measure the level of seed destruction and determine its relationship to female frequency.

### **Methods**

In June of 1995 and 1996, single racemes from randomly selected female and hermaphrodite individuals were assigned an identification number and labeled with an aluminum tag. The sex of each individual was recorded on a separate data sheet to eliminate bias during seed examination. The labeled racemes were collected each year in August. For each raceme, the number of fruit completely consumed and seed parasitized was recorded. Although both forms of predation represent parasitism these categories were assigned because of the apparently different feeding behaviors of the two weevil species. The completely eaten seed classification represents the feeding behavior of *Anthonomus melancholicus* larvae while the parasitized seed classification is indicative of feeding by *Macrorhoptus sidalcea*. Seeds in a fruit were considered to be "completely eaten" when nothing was left of the seed except for frass at the base of the calyx. Wholly consumed fruits were easily distinguished from aborted flowers or fruits. Aborted flowers dehisce from the stem, and aborted seeds and carpels, though

## Chapter 5. Seed Predation

small, are clearly visible at the base of the calyx. "Parasitized" seeds either actually contained larvae or had weevil exit holes in an otherwise intact seed coat. The fruit itself appeared to be complete, but individual carpels and seeds were hollowed out. Since *A. melancholicus* larvae may also be present in the fruit, instances of "parasitism" could occur for which *M. sidalcea* was not responsible. Some degree of overlap between the categories was unavoidable. To estimate the number of seeds parasitized, the first ten uneaten fruit from the base of the stem upward were sampled, and every seed was dissected.

### CALCULATIONS

- |  |
|--|
| (a) = Total fruit on raceme.<br>(b) = Number of completely eaten fruits on raceme.<br>(c) = Number of seeds in a fruit.<br>(d) = Percent of seeds completely eaten.<br>(e) = Total seeds "available" for parasitism on raceme.<br>(f) = Estimated rate of parasitism.<br>(g) = Total number of seeds parasitized on raceme.<br>(h) = Percent of seeds parasitized. |
|--|

1. Percent of seeds or fruits completely eaten was calculated by:  $d = (b/a) \times 100$ .
2. Total seeds "available" for parasitism was calculated by:  $e = c(a - b)$ .
3. Estimated rate of parasitism  $f =$  mean proportion of seeds parasitized in 10 fruits/raceme.
4. Total number of seeds parasitized was calculated by:  $g = e \times f$ .
5. Percent parasitized seeds was calculated by:  $h = (g/(a \times c)) \times 100$ .
6. Total percent seed predation (TP) was calculated by:  $TP = d + h$ .

## *Chapter 5. Seed Predation*

Student's t-test statistics were used to compare average values of female and hermaphrodite predation. A nonparametric Mann-Whitney Rank Sum Test was used when the data were not normally distributed or when the variance was unequal. Pearson product-moment correlation coefficient was used to determine the relationship between female frequency and hermaphrodite seed predation. All statistical analyses were performed with SigmaStat® v.2.0 (Jandel Scientific, 1995).

Other insect species on *Sidalcea hendersonii* were collected and identified to ensure that weevil larvae alone were responsible for the damage to seed.

## Results

Weevils were the most abundant insects on *Sidalcea hendersonii*, and no other insects were identified as seed predators (Appendix A). *Anthonomus melancholicus* was present in all of the Vancouver Island populations (Campbell River, Comox, Duncan, Port Alberni, and Sayward), but was not at Ladner in Delta, B.C. *Macrorhoptus sidalcea* was present in all of the *S. hendersonii* populations surveyed except for Duncan. Adult *M. sidalcea* weevils were observed on both sexes and more frequently on flowers while *A. melancholicus* weevils were observed more frequently on the seed capsules of hermaphrodite plants. Only three *A. melancholicus* adults were collected from female plants while 222 were collected from hermaphrodite plants. The three sightings of *Anthonomus* on females were at Duncan. No weevils were found in a comparison population of *Sidalcea oregana* at Kamloops, B.C.

### COMPLETELY CONSUMED SEED

In all Vancouver Island populations, the mean number of fruits completely eaten and the percent of seeds completely eaten was significantly higher for hermaphrodite plants than females (Table 5.1). At Ladner, females and hermaphrodites did not differ for these measures and, overall, few seeds were completely eaten. At Campbell River, more hermaphrodite seeds were completely eaten in 1995 than 1996, while at Comox and Duncan, the 1996 predation measures tended to be higher. The number of

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completely eaten seed was variable among individuals within each sex. Some hermaphrodite individuals had all of their seed eaten, while others were left untouched. Most female plants did not have any of their seed eaten, but a few individuals were severely attacked in some populations.

Population	sex	N	Mean Total # Fruits per raceme (a)	Mean # of completely eaten Fruits/raceme (b)	Mean # Seeds per fruit (c)	MEAN % COMPLETELY EATEN SEEDS/RACEME (d)	sig.
<b>1995</b>							
Campbell R.	F	40	25.5	0.51 (3.00)	7.2	2.5 (14.3)	$p < 0.001$
	H	42	22.4	16.5 (11.1)	6.7	70.7 (33.6)	
Comox	F	34	25.7	0.15 (0.50)	7.3	0.7 (2.4)	$p < 0.001$
	H	30	23.9	11.7 (9.43)	6.8	44.6 (27.8)	
Duncan	F	30	25.7	0.10 (0.40)	7.1	0.6 (2.4)	$p < 0.001$
	H	30	24.7	8.63 (8.21)	6.4	31.9 (27.4)	
Ladner	F	38	30.2	0.13 (0.53)	6.4	0.8 (3.4)	NS
	H	33	25.4	0.24 (0.56)	5.7	0.8 (1.8)	
<b>1996</b>							
Campbell R.	F	21	36.0	0.00 (0.00)	7.6	0.0 (0.0)	$p < 0.001$
	H	22	30.3	10.0 (12.4)	6.8	28.8 (30.3)	
Comox	F	27	37.5	0.26 (1.16)	7.3	1.43 (7.21)	$p < 0.001$
	H	24	37.7	19.2 (15.3)	6.9	47.1 (20.6)	
Duncan	F	30	29.7	0.00 (0.00)	7.0	0.0 (0.0)	$p < 0.001$
	H	30	19.5	8.97 (6.85)	6.4	44.0 (23.7)	
Port Alberni	F	30	15.6	0.10 (0.31)	7.6	1.2 (4.1)	$p = 0.01$
	H	28	16.5	2.89 (4.95)	7.5	15.0 (23.6)	
Sayward	F	34	23.4	0.03 (0.17)	7.3	0.23 (1.32)	$p = 0.001$
	H	36	20.0	4.06 (7.78)	6.7	19.6 (31.4)	

**Table 5.1** Completely eaten seed in wild populations of *Sidalcea hendersonii*. The standard deviation for each value is listed in parentheses. Values in columns (a) and (c) are statistically analyzed in Chapter 4. Due to rounding and a large number of zeros in the raw data, table calculations across columns may not exactly reflect the reported values from the raw data.

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PARASITIZED SEED

A statistically significant, sex-related difference in the rate of seed parasitism was observed on two occasions (Table 5.2). Females experienced higher seed parasitism than hermaphrodites in the Campbell River population in 1995, while the reverse was true in Port Alberni in 1996. At Duncan the overall level parasitism was negligible. Ladner had the highest levels of parasitism overall for both females and hermaphrodites. Levels of parasitism varied from one year to the next for both sexes; no consistent patterns were detected. Variability among individuals was high.

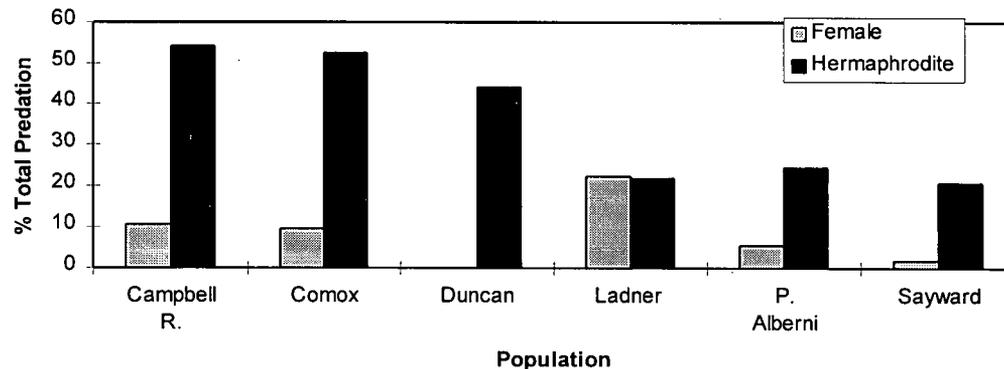
Population	sex	N	Total seeds "Available" for parasitism (e)	Est. rate of parasitism (f)	Total # seeds parasitized per raceme (g)	% PARASITIZED SEEDS PER RACEME (h)	sig.
<b>1995</b>							
Campbell R.	F	40	182.8	0.106	21.54 (30.5)	10.4 (11.2)	$p < 0.001$
	H	42	40.4	0.054	2.96 (8.20)	2.3 (7.39)	
Comox	F	34	185.4	0.084	13.80 (21.4)	8.0 (11.2)	NS
	H	30	84.3	0.046	3.95 (8.10)	2.2 (4.10)	
Ladner	F	38	194.0	0.215	45.89 (35.6)	21.5 (11.0)	NS
	H	33	144.9	0.206	34.02 (33.3)	20.4 (17.0)	
<b>1996</b>							
Campbell R.	F	21	273.4	0.084	27.20 (40.9)	8.4 (10.4)	NS
	H	22	137.5	0.076	12.74 (20.2)	6.4 (9.84)	
Comox	F	27	271.8	0.095	34.13 (60.0)	9.3 (11.7)	NS
	H	24	129.1	0.230	27.23 (35.4)	11.0 (12.4)	
Duncan	F	30	207.0	0.001	0.13 (0.69)	0.1 (0.24)	NS
	H	30	67.9	0.003	0.11 (0.4)	0.1 (0.50)	
Port Alberni	F	30	118.0	0.044	4.89 (5.98)	4.4 (5.08)	$p = 0.003$
	H	28	101.4	0.109	11.16 (11.3)	9.3 (7.51)	
Sayward	F	34	171.2	0.051	2.72 (5.20)	1.5 (2.75)	NS
	H	36	107.5	0.012	1.77 (4.04)	1.0 (2.16)	

**Table 5.2** Parasitized seed in wild populations of *Sidalcea hendersonii*. All values are population means, the standard deviation for each value is listed in parentheses. Due to rounding and a large number of zeros in the raw data, table calculations across columns may not exactly reflect the reported values from the raw data.

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### TOTAL PREDATION

Considering the percent of seed eaten and parasitized together, hermaphrodite plants have significantly higher overall seed predation than female plants ( $p < 0.01$ ) in all populations except Ladner (Figure 5.4) (Table 5.3). Relative seed survival was higher for females in all of the populations except Ladner, where seed survival was the same for females and hermaphrodites (Table 5.3).



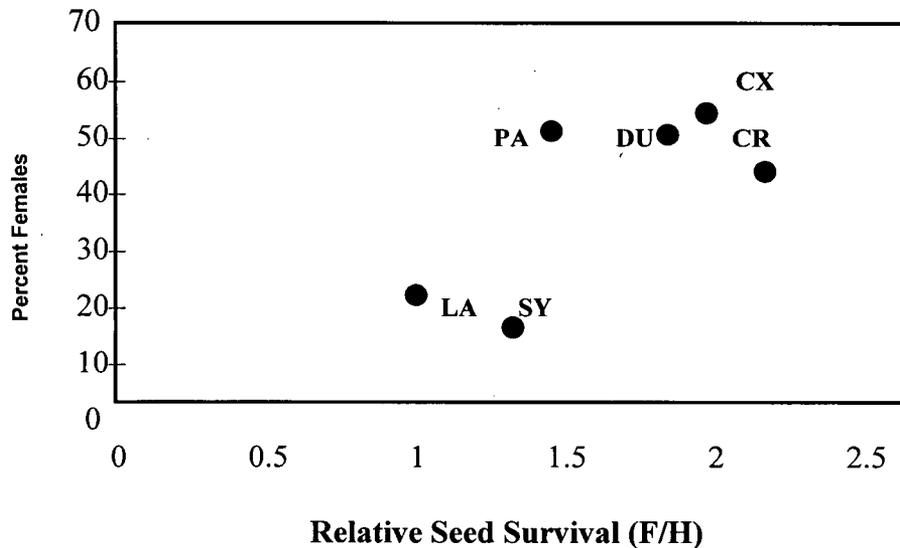
**Figure 5.4** A comparison of averages of female and hermaphrodite *Sidalcea hendersonii* plants for total percent seed predation. For those populations in which predation was measured in both 1995 and 1996, the average value of the two years is reported.

Population	sex	% TOTAL PREDATION	% SURVIVAL (1-TP)	RELATIVE SEED SURVIVAL (F/H)
<b>1995</b>				
Campbell River	F	12.9	87.1	3.2
	H	73.0	27.0	
Comox	F	8.70	91.3	1.7
	H	46.8	53.2	
Ladner	F	22.3	77.7	1
	H	21.2	78.8	
<b>1996</b>				
Campbell River	F	8.40	91.6	1.4
	H	35.2	64.8	
Comox	F	10.7	89.3	2.1
	H	58.1	41.9	
Duncan	F	0.10	99.9	1.8
	H	44.1	55.9	
Port Alberni	F	5.60	94.4	1.3
	H	24.3	75.7	
Sayward	F	1.80	98.2	1.2
	H	20.6	79.4	

**Table 5.3** Summary of seed predation and relative seed survival.

CORRELATES OF PREDATION

Female frequency in *Sidalcea hendersonii* populations was positively correlated with relative seed survival; however, the correlation was not statistically significant ( $r=0.70$ ;  $N=6$ ;  $p=0.124$ )(Figure 5.5).



**Figure 5.5** The relationship between female frequency and the relative seed survival for females and hermaphrodites in natural populations of *Sidalcea hendersonii*.

### Discussion

The extent and form of seed predation depended on which weevil species was present in the population. In populations where *Anthonomus melancholicus* was present, seed was frequently eaten entirely by the developing larvae. At Duncan, where *A. melancholicus* was the sole predator, all attacked seeds were completely eaten and few were parasitized. In populations where *Anthonomus melancholicus* and *Macrorhoptus sidalcea* coexist (Campbell River, Comox, Sayward, Port Alberni), both parasitized and completely eaten seeds were observed. At Ladner, where *M. sidalcea* occurred

## Chapter 5. Seed Predation

exclusively, essentially all of the predation was in the form of parasitism (Tables 5.1 and 5.2).

In all cases of selective predation in *S. hendersonii*, hermaphrodite plants had more of their seed completely eaten than females. Sex-related predation was revealed in estimates of completely eaten seeds, but generally was not evident from measures of parasitized seeds indicating that the more destructive *A. melancholicus* is a selective predator and not *M. sidalcea*. While *M. sidalcea* was found on both sexes, high numbers of *A. melancholicus* weevils were found almost exclusively on hermaphrodite plants further implicating *A. melancholicus*.

In other gynodioecious and dioecious species which experience differential predation (*e.g.*, Bawa and Opler, 1977; Cox, 1982; Uno, 1982; Wagner and Miller, 1984), the hermaphroditic or male flowers were always attacked more frequently than the female flowers. Female *Iris* plants were thought to escape high levels of predation since they complete flowering and their capsules mature before the onset of the predators developmental cycle (Uno, 1982). Bawa and Opler (1977) suggested a similar synchrony of male flowering and predator cycle in *Simarouba* but also investigated whether chemical defense compounds in the female flowers could cause a higher mortality in moth larvae. Two unidentified flavonoid compounds were present in the flowers of pistillate and not staminate trees, but whether these chemicals deterred larval feeding was not confirmed. The basis for weevil discrimination between the sexes was not specifically investigated for the current study, but several

plausible explanations for sex-related predation in *Sidalcea hendersonii* are outlined below.

#### HYPOTHESES FOR DISCRIMINATORY BEHAVIOR

(1) Weevils may lay their eggs in the seed of both sexes, as evidenced by equivalent levels of parasitism, but female seed may possess a chemical feeding deterrent. Or, an inhibitory chemical could discourage female *A. melancholicus* adults from laying their eggs in female seed in the first place. Chemical differences were not tested for, however, and parasitism as described in this study probably represents infestation by *M. sidalcea* rather than the selective predator *A. melancholicus*.

(2) Female *A. melancholicus* weevils discriminate between female and hermaphrodite plants specifically choosing hermaphrodite seed for oviposition. Any number of sexually dimorphic features could be used as criteria for discrimination such as differences in seed size or seed number.

(3) Competition between the two weevil species for developmental sites may necessitate host specialization. Certainly, the presence of another seed predator would change the way in which *Sidalcea hendersonii* plants are exploited. The *S. hendersonii* population at Ladner had unusually high levels of parasitism overall (21% vs. 5.3% avg. for all other populations) by *M. sidalcea* which could be due to the lack of competition from *A. melancholicus*. Direct competition between different weevil species is thought to be rare, but in some cases two species feed on the same plant at

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the same time using different parts of the plant for their development, *e.g.*, flower buds vs. seed capsules (H. Burke, pers. comm.). In the case of *S. hendersonii*, both weevils are utilizing the fruits, but their staggered life cycles reduce the degree of overlap.

The interaction between weevils and host plant may be further complicated by flowering phenology. If females flowered early in the season this might coincide with the emergence of *M. sidalcea*, while later flowering hermaphrodites would coincide with the appearance of *A. melancholicus*. However, no differences in flowering time were observed between the sexes in the experimental population (Chapter 4).

(4) Perhaps the most parsimonious explanation is simply that *Anthonomus melancholicus* weevils, which prefer to eat pollen, may select hermaphrodite flowers rather than female flowers because they are attracted to pollen and improve their reproductive efficiency by using the same plant to brood their young. If, similar to *A. fulvus*, *A. melancholicus* larvae feed on the staminal columns as well as the fruits, this could provide another incentive for selecting hermaphrodite plants over females.

Variability among individuals in predation may similarly be due to variation in flowering time, floral attractants, or host susceptibility. Differences in predation results from one year to the next may be attributed to the fact that yearly summer temperatures can be variable. The flowering season was delayed in 1996, perhaps due to cool spring temperatures, which could potentially affect levels of seed predation.

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### MATING SYSTEM AND SEX-RELATED PREDATION

Total predation is the most appropriate estimate of predation since it signifies the level of seed loss realized by the plant. Despite the fact that no patterns in sex-related parasitism were observed, total predation still differed between the sexes in the Vancouver Island populations because of the extremely high levels of completely eaten hermaphrodite seed.

Destruction of seeds by seed predators can substantially reduce the reproductive output of plants (Harper, 1977; Janzen, 1971), and a reduction in fitness of hermaphrodite plants could affect mating system dynamics. Females must produce significantly more seeds than hermaphrodites to be maintained in gynodioecious populations (Charlesworth and Ganders, 1979), and heavy predation of hermaphrodite seed could provide the necessary advantage to females of *Sidalcea hendersonii*.

The frequency of females was unusually high in several Vancouver Island populations (as high as 54%). The high frequency of females could not be completely explained by genetic control of male sterility (Chapter 2) or inbreeding depression (Chapter 4). In Chapter 4 it was demonstrated that females did not produce more seed than hermaphrodites in natural populations, and  $f = 1$  (from equation 4-1). If predation is included in the fecundity parameter, then the average value for *A. melancholicus* infested populations (CR: 0.51, CX: 0.53, DU: 0.56, PA: 0.80, SY: 0.81) is  $f = 0.64$ . With a value of  $f = 0.64$ ,  $s = 0.75$  (yields 1% females at  $\delta = 0.67$  and  $f = 1$ ), and  $\delta = 0.67$  (as described in Chapter 4), the equilibrium frequency of females is now 27 %, a

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high value which is closer to observed frequencies of females in natural populations. Weevil seed predators appear to play a significant role in the maintenance of females in these populations, and selective predation of hermaphrodite seed may also partially explain the existence of females at their current frequency.

Female frequency in *Sidalcea hendersonii* populations was positively correlated with relative seed survival (Figure 5.5). The correlation was not significant probably due to the small sample of six populations. Interestingly, in the *Sidalcea oregana* population, neither females exist nor were weevils present, while an unidentified weevil species does infest the seeds of gynodioecious *S. oregana* populations in California (T-L Ashman, pers. comm.).

### Conclusions

Sex-related predation of *Sidalcea hendersonii* seed by weevils was evident in the majority of populations surveyed. Weevil larvae, most likely of *Anthonomus melancholicus*, destroy more seeds of hermaphrodite plants than female plants significantly reducing the realized seed production of hermaphrodites. High levels of predation to hermaphrodite seed could be correlated with an unusually high frequency of females in *S. hendersonii* populations on Vancouver Island. Pest pressure appears to play a significant role in the maintenance of gynodioecy in *Sidalcea hendersonii*. This study provides the first evidence that sex-related predation may be responsible for high female frequencies in natural populations of gynodioecious species.

## Chapter 6

### General Conclusions

#### Summary

The objective of this thesis was to determine the relative importance of several factors potentially contributing to the maintenance of gynodioecy in *Sidalcea hendersonii*.

The high frequency of female plants in *S. hendersonii* populations, in combination with nuclear determination of male sterility, elevates theoretical requirements for female fitness.

Petal size had neither a positive nor negative effect on seed-set suggesting that the flower size dimorphism of *S. hendersonii* does not influence differences in seed production between the sexes.

Experimental results indicated that females have higher fitness than both cross-pollinated and self-pollinated hermaphrodites. Such an effect may be due to resource allocation differences between the sexes or other effects associated with maternal genotype. Though not tested for in this study, a pleiotropic effect of the male sterility allele could confer a fitness advantage to male steriles (Lloyd, 1974c; Ganders, 1978; Van Damme and Van Delden, 1984), or, since females are heterozygous at the male sterility locus, overdominance could be another source of female advantage. Based on

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seed-set in natural populations, however, no inherent fitness advantages for females were evident.

Inbreeding depression was determined to be high, but fitness gains for outcrossed plants were not substantial enough to explain the existence of females in natural populations at all.

Sex-related seed predation provides an additional explanation for the high frequency of females in *Sidalcea hendersonii* populations on Vancouver Island. Female plants had a reproductive edge over hermaphrodites in these populations because weevil larvae destroyed more seeds of hermaphrodite plants and substantially reduced hermaphrodite seed production.

### **Relative overall seed production**

Table 6.1 lists relative seed production for each population after both seed predation and seed abortion. At Campbell River females have three times the seed production of hermaphrodites, and Comox and Duncan, females produce almost twice the seed of hermaphrodites. But at Sayward, females had only slightly better seed production, and at Ladner and Port Alberni, hermaphrodites actually produced more viable seed than females.

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Population	% Viable Seed Females	% Viable Seed Hermaphrodites	Relative Seed Production (F/H)
Campbell River	49.4	16.9	3.0
Comox	39.8	22.4	1.8
Duncan	79.6	42.7	1.9
Ladner	29.5	37.5	0.8
Port Alberni	4.7	8.7	0.5
Sayward	41.8	32.0	1.3

**Table 6.1.** Estimate of Percent Viable Seed Production (% Viable seed = % of seed available after "total" predation  $\times$  seed production rate [*i.e.*, (average #viable seeds/fruit)/(average total # potential seeds/fruit)]).

With nuclear control of male sterility, female plants must produce at least twice as many seed as hermaphrodites to compensate for the loss of male function. While such conditions occur at Campbell River, and possibly Comox and Duncan, the advantage is still not great enough to meet the theoretical requirements for the maintenance of gynodioecy when female frequencies are as high as 50%. Low relative seed production at Sayward, Port Alberni, and Ladner presents an even greater obstacle to females. These results indicate that factors in addition to viable seed production must account for the presence of females in these populations. For instance, females may compensate for low seed production in natural populations by producing better quality offspring with higher levels of seed germination, greater survival, faster growth rates, and/or increased flower production as observed in the experimental population. Also, long term studies of these perennial plants could reveal that female plants achieve

greater lifetime seed production because they become larger or live longer (Ashman, 1992a).

An additional possibility is that founder effects or genetic drift are responsible for the high frequency of females in *S. hendersonii* populations; or, the populations may not be at equilibrium for their outcrossing rates.

### **Importance of Sex-Related Seed Predation**

In every population surveyed, except for Ladner, females would not have any seed production advantages relative to hermaphrodites if not for selective predation of hermaphrodite seed. However, the presence of female plants at Ladner, where sex-related predation did not occur, shows that females would still exist in some *S. hendersonii* populations even in the absence of differential predation. The common occurrence of gynodioecy in *Sidalcea* species suggests that evolution of gynodioecy in *Sidalcea* precedes the relationship between host and seed predators. Sex-related seed predation appears to enable females to achieve a high frequency in some populations of *S. hendersonii* and is an important factor in the maintenance of gynodioecy in others.

Although many other species of *Sidalcea* are host to weevil predators, this is the first reported case of sex-related seed predation in a *Sidalcea* species. It is possible that selective predation has not been reported for other species of *Sidalcea* because

## Chapter 6. Conclusions

they do not share the same seed predators. *Macrorhoptus sidalcea* has only been found in British Columbia, and *S. hendersonii* is its only host. *Anthonomus melancholicus* is more broadly distributed, from B.C. south to northern California, and south-east into Idaho, but the taxonomy of this species complex has not been resolved (H. Burke, pers. comm.). The relationship between *S. hendersonii* and its weevil predators could reflect a coevolutionary relationship, where host specificity is not only for sex but also for species. *Anthonomus melancholicus* and *Macrorhoptus sidalcea* or their ancestors may have accompanied *Sidalcea hendersonii* in its postglacial migration, and predators and host plant differentiated together.

Cox (1982) proposed a model for the evolution of dioecy resulting from sex-related flower destruction by vertebrate pollinators. Perhaps a model for the evolution or maintenance of gynodioecy due to sex-related seed predation could be similarly devised.

### **Evolution to Dioecy**

When gynodioecy is determined by nuclear genes, a female frequency of 50% or higher is theoretically impossible in an equilibrium population (Charlesworth and Charlesworth, 1978; Ganders, 1978). This implies that some of the populations of *S. hendersonii* are either not in equilibrium or are cryptically dioecious, *i.e.*, hermaphrodite plants essentially function as males.

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If *Sidalcea hendersonii* populations are functionally dioecious, selection may favor hermaphrodites that allocate additional resources to male function which could facilitate the evolution of a dioecious mating system. Some consider gynodioecious species with nuclear inheritance of male sterility to be unstable and that these species would tend to evolve towards dioecy (Ross, 1977; Richards, 1986; Sakai *et al.*, 1989). However, Schultz (1994) demonstrated that both nuclear and cytoplasmic-nuclear gynodioecy can evolve towards dioecy.

Gynodioecy and dioecy appear to be taxonomically limited within the Malvaceae: three genera contain gynodioecious species (*Sidalcea*, *Callirhoe*, *Cienfuegosia*), and five genera contain dioecious species (*Napaea*, *Plagianthus*, *Kydia*, *Hampea*, *Cienfuegosia*) (Dorr, 1993). Whether a dioecious mating system could eventually evolve from gynodioecy in *Sidalcea hendersonii* is difficult to predict. Nevertheless, there was no indication of female sterility in *S. hendersonii* populations since every flower examined for this study contained ovules. Furthermore, despite the high frequency of females, gynodioecy appears to persist in *Sidalcea* species with no known occurrence of dioecy.

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## Appendix A

### Population Descriptions

#### *Sidalcea hendersonii*

##### CAMPBELL RIVER

**Specific location:** Vancouver Island, B. C. Hwy. 19 north to Campbell River; east on unmarked road between First Nations Reservation Boundary and Maple St., through large parking area to tip of peninsula where several islands visible (Tyee Spit). *S. hendersonii* located on 'Nunns Island' which has a rocky outcrop at the north end and is the third island (SE) from parking area towards airport. Also, smaller population on the L-shaped peninsula which jets out from parking area.

**Population size:** 500+ individuals

**Habitat description:** sea-level; salt marsh; soil dark, organic clay; dramatic tides; dense vegetation.

##### **Associated plant species:**

*Achillea millefolium*  
*Agrostis alba*  
*Angelica lucida*  
*Aster subspicatus*  
*Carex lyngbyei*  
*Castilleja miniata*  
*Cirsium* sp.  
*Daucus carota*  
*Deschampsia cespitosa*  
*Dodecatheon pulchellum*  
*Eleocharis palustris*  
*Festuca rubra*  
*Fritillaria camschatcensis*  
*Piperia maritima*  
*Heracleum lanatum*  
*Hordeum brachyantherum*  
*Hypericum formosum*  
*Juncus balticus*

*Lathyrus palustris*  
*Lilaeopsis occidentalis*  
*Mentha arvensis*  
*Oenanthe sarmentosa*  
*Plantago macrocarpa*  
*Polygonum aviculare*  
*Potentilla pacifica*  
*Prunella vulgaris*  
*Ranunculus cymbalaria*  
*Ranunculus occidentalis*  
*Scirpus acutus*  
*Scirpus americanus*  
*Sisyrinchium idahoense*  
*Sium suave*  
*Taraxacum officinale*  
*Trifolium wormskjoldii*  
*Triglochin maritimum*  
*Vicia americana*

## Appendix A

### **Some associated invertebrates:**

[Classes] Gastropoda (snail)

[Orders] Arachnida (spider), Diptera (fly)

[Species] *Anthonomus melancholicus* (weevil), *Macrorhoptus sidalcea* (weevil), *Apis mellifera* (honey bee), *Bombus* sp. (bumble bee)

## COMOX SPIT

**Specific location:** Vancouver Island, B.C. Hwy. 19 north through Comox; *S. hendersonii* at Comox Spit. Access from nature trail starting at marina/airport off of 20<sup>th</sup> St. Another population on opposite side of spit along Comox Rd. between Sawmill and Wildlife Viewing Area.

**Population size:** 630+ individuals (both populations)

**Habitat description:** sea-level; salt marsh, dryer uplands (sawmill side); soil loam (mostly clay).

### **Associated plant species:**

*Achillea millefolium*

*Angelica geniflexa*

*Angelica lucida*

*Aster* sp.

*Carex deweyana*

*Carex lynghyei*

*Cirsium* sp.

*Epilobium ciliatum*

Fern species

*Galium aparine*

Grass species

*Hypericum formosum*

*Lathyrus sylvestris*

*Lythrum salicaria*

*Melilotus alba*

*Mentha arvensis*

*Plantago lanceolata*

*Plantago major*

*Polygonum persicaria*

*Potentilla pacifica*

*Prunella vulgaris*

*Rubus discolor*

*Rumex crispus*

*Symphoricarpos albus*

*Tanacetum vulgare*

*Taraxacum officinale*

*Typha latifolia*

Appendix A

**Some associated invertebrates:**

- [Classes] Gastropoda (snail)  
[Orders] Arachnida (spider), Diptera (fly)  
[Families] Cercopidae (spittle bug), Lathridiidae (scavenger beetle),  
Coccinellidae (lady beetle), Aphididae (aphid)  
[Species] *Anthonomus melancholicus* (weevil), *Macrorhoptus sidalcea*  
(weevil), *Philaenus leucophthalmus* (spittle bug), *Adalia*  
*bipunctata* (lady beetle), *Apis mellifera* (honey bee), *Bombus* sp.  
(bumble bee)

DUNCAN

**Specific location:** Duncan, Vancouver Island, B. C. Khenipsen Rd. (via Trunk Rd. to Tzouhalem). *S. hendersonii* grows in marsh along Khenipsen Rd.

**Population size:** 3000+ individuals

**Habitat description:** sea-level; salt marsh; soil organic loam; dense vegetation; well irrigated.

**Associated plant species:**

<i>Anaphalis margaritacea</i>	<i>Lonicera involucrata</i>
<i>Cirsium</i> sp.	<i>Plantago lanceolata</i>
<i>Chenopodium album</i>	<i>Plantago major</i>
<i>Calystegia</i> sp.	<i>Potentilla pacifica</i>
<i>Cytisus scoparius</i>	<i>Rosa nutkana</i>
<i>Epilobium ciliatum</i>	<i>Rubus discolor</i>
Grass species	<i>Rumex acetosella</i>
<i>Platanthera dilatata</i>	<i>Rumex crispus</i>
<i>Hypericum formosum</i>	<i>Scirpus</i> sp.
<i>Iris pseudacorus</i>	<i>Spiraea douglasii</i>
<i>Juncus effusus</i>	<i>Symphoricarpos albus</i>
<i>Lathyrus latifolius</i>	<i>Taraxacum officinale</i>
<i>Lathyrus sylvestris</i>	<i>Typha latifolia</i>
	<i>Vicia cracca</i>

**Some associated invertebrates:**

- [Orders] Arachnida (spider)  
[Families] Pentatomidae (stink bug)  
[Species] *Anthonomus melancholicus* (weevil); *Apis mellifera* (honey bee)

HAZELMERE GOLF COURSE

**Specific location:** New Westminster, B.C., just outside boundary of Hazelmere Golf Course.

**Population size:** < 10 individuals

**Habitat description:** uncharacteristically dry, grassy meadow; *S. hendersonii* along shallow drainage.

LADNER MARSH

**Specific location:** Delta, B.C. Ferry Rd. north past Windjammer Rd.; trail to Ladner marsh at Wildlife Viewing Tower (trailhead on left before marina).

**Population size:** > 1000 individuals.

**Habitat description:** sea-level; soil loam; large open salt marsh; *S. hendersonii* in clumps.

**Associated plant species:**

*Alisma plantago-aquatica*  
*Aster* sp.  
*Carex sitchensis*  
*Cirsium arvense*  
*Platanthera dilatata*  
*Heracleum lanatum*  
*Hypericum formosum*  
*Impatiens noli-tangere*  
*Iris pseudacorus*  
*Lathyrus palustris*  
*Lonicera involucrata*  
*Lysimachia thyrsoflora*  
*Lythrum salicaria*

*Mentha arvensis*  
*Menyanthes trifoliata*  
*Myosotis scorpioides*  
*Potentilla pacifica*  
*Rumex crispus*  
*Sagittaria latifolia*  
*Salix lasiandra*  
*Scirpus lacustris*  
*Scirpus microcarpus*  
*Sium suave*  
*Solanum dulcamara*  
*Trifolium wormskjoldii*  
*Vicia cracca*

**Some associated invertebrates:**

[Class] Gastropoda (snail)  
[Order] Diptera (fly), Acarina (mite), Thysanoptera (thrip), Heteroptera (true bug), Arachnida (spider)  
[Species] *Macrorhoptus sidalcea* (weevil), *Draeculacephala angulifera* (leafhopper), *Bombus* (bumble bee), *Philaenus* sp. (spittle bug), *Vanessa* sp. (butterfly)

*Appendix A*

PORT ALBERNI

**Specific location:** Vancouver Island, B.C. Rt. 4 towards Tofino (through Port Alberni), lft. Airport Rd. (just past bridge) to J.V. Cline Bird Sanctuary marsh.

**Population size:** ≈ 120 individuals

**Habitat description:** sea-level; salt marsh; soil pale clay; disturbed area (near lumber mill).

**Associated plant species:**

<i>Achillea millefolium</i>	<i>Mentha arvensis</i>
<i>Angelica lucida</i>	<i>Plantago lanceolata</i>
<i>Arnica mollis</i>	<i>Plantago major</i>
<i>Triteleia hyacinthina</i>	<i>Populus tremuloides</i>
<i>Carex lyngbyei</i>	<i>Potentilla pacifica</i>
<i>Cirsium</i> sp.	<i>Rosa nutkana</i>
<i>Cytisus scoparius</i>	<i>Rumex crispus</i>
<i>Galium aparine</i>	<i>Scirpus acutus</i>
Grass species	<i>Sium suave</i>
<i>Hypericum formosum</i>	<i>Taraxacum officinale</i>
<i>Juncus effusus</i>	<i>Trifolium wormskjoldii</i>
<i>Lathyrus palustris</i>	<i>Vicia cracca</i>
<i>Lonicera involucrata</i>	

**Some associated invertebrates:**

[Orders] Acarina (mite), Thysanoptera (thrip)

[Species] *Anthonomus melancholicus* (weevil), *Macrorhoptus sidalcea* (weevil), *Apis mellifera* (honey bee)

Appendix A

SAYWARD

**Specific location:** Vancouver Island, B.C. Hwy 19 north, west towards Sayward, over one-lane bridge, approx. 2 mi. to Salmon River Inn. *S. hendersonii* grows at marsh on either side of road.

**Population size:** 500+ individuals.

**Habitat description:** sea-level; soil iron-rich clay; salt marsh enclosed by conifers and dry meadows.

**Associated plant species:**

*Achillea millefolium*

*Carex* sp.

*Castilleja miniata*

*Daucus carota*

*Gaultheria shallon*

Grass species

*Juncus* sp.

*Lonicera involucrata*

*Potentilla pacifica*

*Prunella vulgaris*

*Rumex crispus*

*Trifolium wormskjoldii*

**Some associated invertebrates:**

[Orders] Acarina (mite), Thysanoptera (thrip)

[Families] Pentatomidae (stink bug), Cercopidae (spittle bug), Cicadellidae (leafhopper)

[Species] *Anthonomus melancholicus* (weevil), *Macrorhoptus sidalcea* (weevil)

Appendix A

TRIAL ISLAND

**Specific location:** South of Victoria, Vancouver Island, B.C. *S. hendersonii* on northeast side of Trial Island.

**Population size:** ≈ 8 individuals (and 30 seedlings)

**Habitat description:** > sea level; swale with no tidal exposure; soil dark loam.

**Associated plant species:**

*Allium acuminatum*

*Allium cernuum*

*Amelanchier alnifolia*

*Aster curtus*

*Brodiaea coronaria*

*Castilleja laevisecta*

*Deschampsia cespitosa*

*Dodecatheon pulchellum*

*Epilobium angustifolium*

*Festuca idahoensis*

*Fritillaria affinis*

*Grindelia integrifolia*

*Heracleum lanatum*

*Limnanthes macounii*

*Lomatium nudicale*

*Malus fusca*

*Ulex europaea*

*Plantanthera greenii*

*Populus tremuloides*

*Potentilla pacifica*

*Rosa nutkana*

*Rumex crispus*

*Salicornia virginica*

*Salix hookeriana*

*Triteleia hyacinthina*

**Some associated invertebrates:**

[Orders] Thysanoptera (thrip), Hymenoptera (wasp), Diptera (fly)

[Families] Cercopidae (spittle bug)

[Species] *Macrorhoptus sidalcea* (weevil)

\* *Sidalcea hendersonii* had previously been collected at the following locations, but no plants were located in 1995:

VICTORIA, Vancouver Island 1924

GABRIOLA ISLAND, B.C. 1912

MUSQUEM INDIAN RESERVE, Vancouver 1940.

Appendix A

*Sidalcea oregana*

KAMLOOPS

**Specific location:** North of Kamloops B.C. [Tp 21, R18, W6]. Populations near NE tributaries of Long Lake. Entrance to Batchelor Hills on SE section of hills at water tower. Right at Y just past pipeline. Left turn at 2.6km (distance from Y). At 6.5km., pass fence. First population of *S. oregana* 8.1km (from 1<sup>st</sup> Y) near pond. Second population is at 9.8 km (to left and into valley) near corral.

**Population size:** pop.1 = 37 individuals; pop.2 = >330 individuals.

**Habitat description:** 800m; swale in grassy meadow; soil glade chernozem; grazing disturbance.

**Associated plant species:**

<i>Achillea millefolium</i>	<i>Grindelia</i> sp.
<i>Calochortus macropetalus</i>	<i>Juncus balticus</i>
<i>Campanula rotundifolia</i>	<i>Poa pratensis</i>
<i>Capsella bursa-pastoris</i>	<i>Potentilla anserina</i>
<i>Centurea maculosa</i>	<i>Rosa nutkana</i>
<i>Cichorium intybus</i>	<i>Spiranthes romanzoffiana</i>
<i>Galium boreale</i>	<i>Vicia americana</i>

**Some associated invertebrates:**

[Orders] Thysanoptera (thrip), Arachnida (spider), Hymenoptera (2+ different species of wasp)  
[Families] Aphididae (aphid), Megachilidae (6+ different species of bees), Acrididae (grasshopper)  
[Species] *Bombus* sp. (bumble bee)

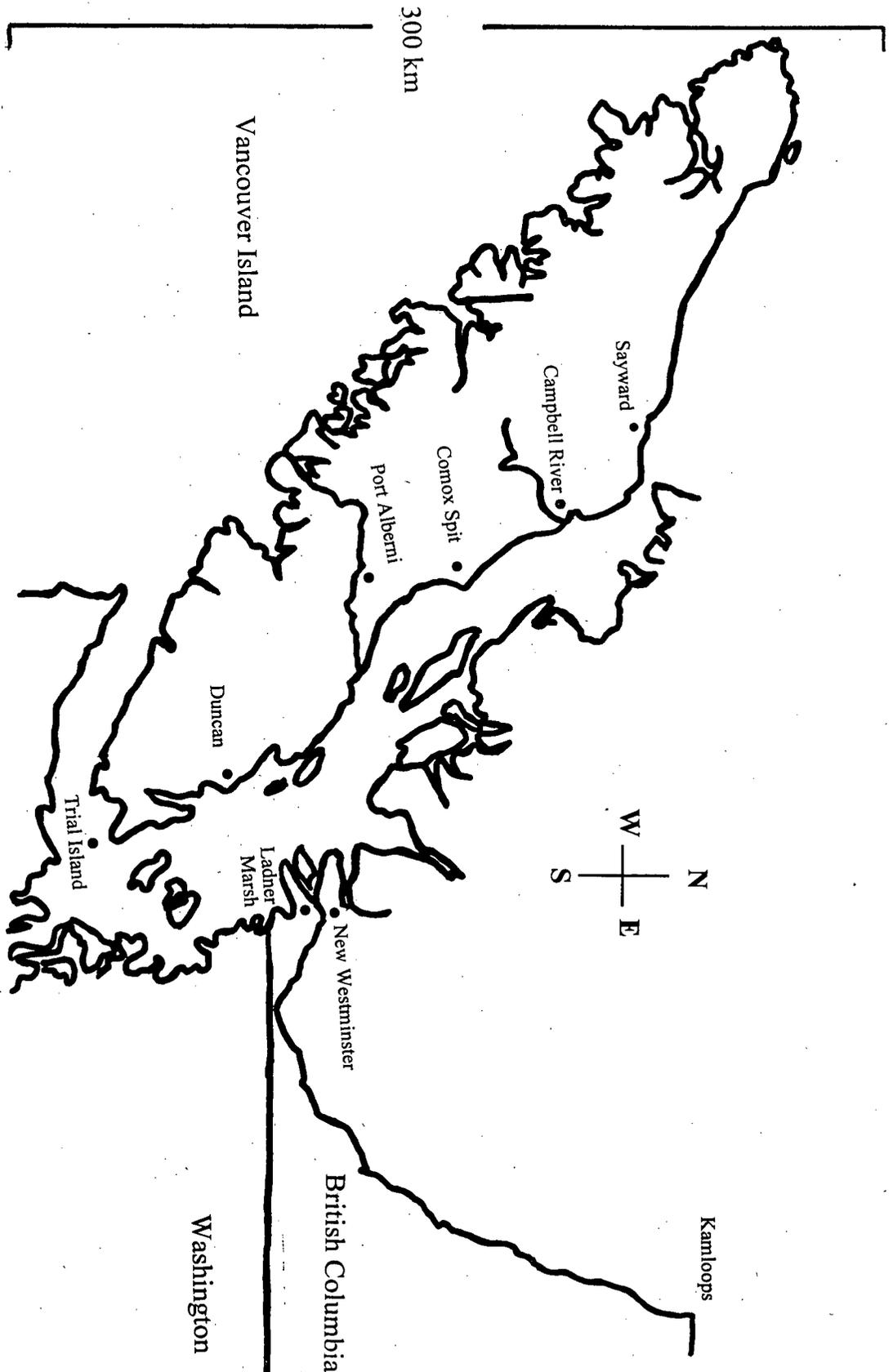


Figure A.1 *Sidalcea* populations surveyed in British Columbia (1995-1996).