## AN ABSTRACT OF THE THESIS OF

<u>Robert James Meinke</u> for the degree of <u>Doctor of Philosophy</u> in <u>Botany and Plant</u> <u>Pathology</u> presented on <u>May 8, 1992</u>. Title: <u>Systematic and Reproductive Studies of</u> <u>Mimulus (Scrophulariaceae) in the Pacific Northwest: Implications for Conservation</u> <u>Biology</u>.

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Kenton L. Chambers

The *Mimulus washingtonensis* complex is a group of morphologically similar species centered in the Pacific Northwest. All are rare, and most are under consideration for listing as endangered. Morphometric and pollination data were used in developing a revised taxonomy for the group. Five species and two varieties are recognized. *Mimulus pulsiferae* Gray is the most widespread, occurring from southern Washington to northern California. *Mimulus hymenophyllus* Meinke and *M. jungermannioides* Suksd. are cliff species endemic to river drainages in northern Oregon. *Mimulus washingtonensis* Gand. occurs in east-central Oregon and western Idaho, represented by the var. *washingtonensis* and **var. ampliatus (Grant) Meinke comb. et stat. nov.**, respectively. *Mimulus patulus* Pennell is resurrected from synonymy and differentiated from *M. washingtonensis* on the basis of morphology, distribution, and pollination biology. This autogamous species includes var. *patulus*,

occurring in the Snake River drainage, and var. *montanus* Meinke var. nov., primarily from the northern Rocky Mountains.

*Mimulus evanescens* Meinke sp. nov. is described from an extant population in Lassen County, California, and historic collections from widely scattered stations in Oregon and Idaho. The new species is morphologically intermediate between *M*. *breviflorus* Piper and *M. latidens* (Gray) Greene. Calyx and leaf morphology also suggest an affinity to *M. grayi* Grant, *M. inconspicuus* Gray, and *M. acutidens* Greene, of cismontane California. *Mimulus evanescens* should be considered critically endangered, because of its limited numbers and habitat degradation.

*Mimulus washingtonensis* is pollinated by small native bees, primarily two species of *Dialictus* (Halictidae). These bees were specific to *Mimulus* flowers but did not distinguish between flowers of *M. washingtonensis* and *M. guttatus* DC., a related species that increases with habitat disturbance. Experiments showed that mixed pollen loads diminish seed set in *M. washingtonensis*, since the thigmotropic stigma of that species closes permanently after any amount of conspecific pollen is applied. Although *M. washingtonensis* has a low pollen/ovule ratio (29.9), it is considered facultatively xenogamous based on floral morphology, stigma sensitivity, and autogamous seed set levels. Populations occurred in small, edaphically restricted patches, and they exhibited seed set reductions related to outcrossing distance. Substrate disturbance may limit reproduction in *M. washingtonensis* by changing population structure, threatening pollinators, and increasing competition for pollination by promoting growth and flowering of *M. guttatus*.

# Systematic and Reproductive Studies of *Mimulus* (Scrophulariaceae) in the Pacific Northwest: Implications for Conservation Biology

by

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Chapter 1	Introduction	1
Chapter 2	Taxonomy, distribution, and conservation status of the <i>Mimulus washingtonensis</i> complex	6
	Methods	9
	Morphometric studies Reproduction Herbarium studies	9 11 13
	Species biology	13
94. <sup>- 10</sup>	Morphological relationships Reproductive biology	13 19
	Distribution	25
	The Columbia River Gorge	28
	Taxonomic treatment	31
	Key to <i>Mimulus</i> , section <i>Paradanthus</i> in the Pacific Northwest	31
	The Mimulus washingtonensis complex Mimulus jungermannioides Mimulus washingtonensis var. washingtonensis	35 36 43
	Mimulus washingtonensis var. ampliatus Mimulus hymenophyllus Mimulus patulus var. patulus Mimulus patulus var. montanus	53 55 61 68
	Mimulus pulsiferae	71
	Taxonomic relationships	78
	Conservation considerations	81
	Distribution and habitat Reproductive biology and genetics Recommended status	81 83 85

# TABLE OF CONTENTS

Chapter 3	A new annual species of <i>Mimulus</i> (Scrophulariaceae) from th Intermountain West	ne 87
	Description of Mimulus evanescens	89
	Paratypes	92
	Taxonomic relationships	93
	Phenetic evaluation	93
	Comparisons of vegetative and floral morphology	95
	Distribution and habitat	103
	Phylogeny	104
	Conservation	107
	Key to Mimulus evanescens and allied species	108
Chapter 4	Reproductive ecology of the xerophytic ephemeral Mimulus	
	washingtonensis (Scrophulariaceae)	110
	Materials and methods	112
	Study area and greenhouse studies	112
	Floral structure	114
	Floral phenology and longevity	114
	Floral visitors	115
	Stigma sensitivity and seed set limitation	116
	Breeding system efficiency	117
	Outcrossing distance and reproductive success	119
	Results	120
	Floral structure and nectar production	120
	Floral phenology and longevity	123
	Floral visitors	126
	Stigma sensitivity and seed set limitation	128
	Breeding system efficiency	131
	Outcrossing distance and reproductive success	137
	Discussion	137
Bibliography		148

# LIST OF FIGURES

Figure		Page
2-1.	Principal components analysis of the Mimulus washingtonensis complex, based on 26 character states.	14
2-2.	Discriminant analysis of the <i>Mimulus washingtonensis</i> complex, based on plants grown under drought conditions.	17
2-3.	Breeding system experiment for species in the Mimulus washingtonensis complex.	21
2-4.	Average stigma closure speed for species in the <i>Mimulus</i> washingtonensis complex.	23
2-5.	Stigma closure time as a function of breeding system in the <i>Mimulus washingtonensis</i> complex.	24
2-6.	Range map of Mimulus jungermannioides, M. hymenophyllus, and M. pulsiferae.	26
2-7.	Range map of Mimulus washingtonensis var. washingtonensis, Mimulus washingtonensis var. ampliatus, M. patulus var. patulus, and M. patulus var. montanus.	27
2-8.	Stem pubescence of Mimulus washingtonensis.	37
2-9.	Cylindrical fruiting calyces of <i>Mimulus pulsiferae</i> , showing exserted capsule.	37
2-10.	10a. Funnelform corollas of Mimulus washingtonensis, M. jungermannioides, M. hymenophyllus, and M. patulus. 10b. Frontal view of corollas of Mimulus washingtonensis, M. jungermannioides, and M. hymenophyllus.	38
2-11.	Young Mimulus jungermannioides plant on basalt cliff.	39
2-12.	Vegetative life history of Mimulus jungermannioides.	44
2-13.	Sprouting turions of <i>Mimulus jungermannioides</i> grown in the greenhouse.	45

2-14.	Greenhouse grown plants of Mimulus washingtonensis.	46
2-15.	Habit of <i>Mimulus washingtonensis</i> in a wet spring, showing elongate peduncles and nodding flower buds.	47
2-16.	Young Mimulus washingtonensis var. washingtonensis plants growing on basalt gravel.	52
2-17.	Young plant of <i>Mimulus hymenophyllus</i> , showing characteristic long petioles, pale yellow corollas, and light sensitive fruiting pedicels.	56
2-18.	Mature plant of <i>Mimulus hymenophyllus</i> grown from seed in the greenhouse.	57
2-19.	Population of <i>Mimulus hymenophyllus</i> growing on moist basalt wall at the type locality, showing heliotropic flowers.	58
2-20.	Flowers and upper leaves of Mimulus patulus var. patulus.	62
2-21.	Mature plants of <i>Mimulus patulus</i> var. <i>patulus</i> , growing at the only known extant site.	63
2-22.	Unidentified <i>Mimulus</i> sp. that grew spontaneously in soil collected at type locality of <i>M. hymenophyllus</i> in Wallowa County, Oregon.	66
2-23.	Scanning electron micrograph of the stem of Mimulus patulus var. montanus.	70
2-24.	Mature plant of Mimulus pulsiferae growing in volcanic soil.	72
2-25.	Corollas of Mimulus pulsiferae.	73
3-1.	Habit drawing of <i>Mimulus evanescens</i> , showing details of leaf morphology, inflorescence, and relationship between pedicel and leaf blade.	90
3-2.	Principal components analysis of Mimulus evanescens, M. latidens, and M. breviflorus.	96
3-3.	Discriminant analysis of Mimulus evanescens, M. latidens, and M. breviflorus, based on 14 quantitative characters.	98

3-4.	<ul> <li>a. Close-up of fruiting calyx of Mimulus evanescens.</li> <li>b. Calyx of M. evanescens opened to show sessile capsule insertion.</li> <li>c. Calyx of M. latidens opened to show stipitate capsule insertion.</li> </ul>	101
3-5.	Mimulus evanescens growing among boulders at the type locality, along the southwest corner of Moll Reservoir in northern Lassen County, California.	105
4-1.	Lateral view of Mimulus washingtonensis flower.	121
4-2.	Scanning electron micrograph of the lower, inside floral tube of a <i>Mimulus washingtonensis</i> corolla.	122
4-3.	Scanning electron micrograph of the upper one-half of a closed <i>Mimulus washingtonensis</i> stigma.	124
4-4.	Scanning electron micrograph of a frontal view of a half-closed <i>Mimulus washingtonensis</i> stigma.	125
4-5.	Relationship between <i>Mimulus washingtonensis</i> floral age and the foraging time of principal pollinators.	129
4-6.	Relationship between <i>Mimulus washingtonensis</i> floral age and closure time for sensitive stigmas.	130
4-7.	Relationship between <i>Mimulus washingtonensis</i> floral age and stigma reopening time after tactile stimulation.	132
4-8.	Scanning electron micrograph of pollen grains imbedded in floral papillae of <i>Mimulus washingtonensis</i> .	136

# LIST OF TABLES

Table		Page
2-1.	List of morphological traits measured from plants in the <i>Mimulus</i> washingtonensis complex for use in principal components analysis.	10
2-2.	List of morphological traits measured from plants in the Mimulus washingtonensis complex for use in discriminant analysis.	12
2-3.	Amount of total variance accounted for by the first ten principal components, in a principal components analysis of morphological variation among species in the <i>Mimulus washingtonensis</i> complex.	16
2-4.	Pollen/ovule ratios for species in the Mimulus washingtonensis complex.	20
3-1.	List of morphological traits measured from <i>Mimulus</i> plants for use in principal components analysis.	94
3-2.	Amount of total variance accounted for by each principal component, in a principal components analysis of morphological variation among populations of <i>Mimulus evanescens</i> , <i>M. breviflorus</i> , and <i>M. latidens</i> .	97
3-3.	Diagnostic features of Mimulus evanescens, M. breviflorus, and M. latidens.	102
4-1.	Floral visitors observed on <i>Mimulus washingtonensis</i> along the John Day River, Grant County, Oregon.	127
4-2.	The effect of pollen quality and quantity on pollination and seed production in <i>Mimulus washingtonensis</i> .	133
4-3.	Breeding system trials for Mimulus washingtonensis.	134
4-4.	Effect of outcrossing distance on seed production in <i>Mimulus</i> washingtonensis.	138

## SYSTEMATIC AND REPRODUCTIVE STUDIES OF MIMULUS (SCROPHULARIACEAE) IN THE PACIFIC NORTHWEST: IMPLICATIONS FOR CONSERVATION BIOLOGY

## CHAPTER 1

#### INTRODUCTION

Although its roots go back centuries to the earliest naturalists, the "new" discipline of conservation biology has only recently come to the forefront as a scientific field of study. Described as mission-oriented (Soulé and Wilcox, 1980; Soulé, 1986), conservation biology incorporates pure and applied science to create a technical means for the preservation of nature. Practitioners include ecologists, systematists, geneticists, statisticians, and other scientists, who study scarce or declining species and habitats in an effort to learn more about their current status and probable future prospects. Primary goals of such research include distinguishing those taxa in need of conservation efforts, identifying causes of species rarity, evaluating genetic variation, and promoting the preservation (whenever possible) of viable populations and communities *in situ* (Soulé, 1987; Mlot, 1989; Falk and Holsinger, 1991).

Currently we are witnessing a dramatic increase in the rate of species extinctions throughout the tropical and temperate ecosystems of the world. In the United States alone, nearly 700 species have been estimated to be critically imperilled (Mlot, 1989), with hundreds more vanishing from significant portions of their historic ranges. Increasing public awareness of this impending catastrophe has begun to foster a dedication on the part of scientists, land managers, and funding agencies to focus on issues relating to biodiversity and habitat preservation. This joint commitment is a key to the success of conservation biology.

Much of the earlier literature dealing with conservation of biological resources has focussed on animal species, no doubt stemming in part from the long history of game management in Great Britain, the United States, and other industrialized nations. However, the plight and potential value of endangered plant species is becoming increasingly appreciated (Kruckeberg and Rabinowitz, 1985; Mlot, 1989; Falk and Holsinger, 1991; Guerrant, 1992). Studies are now beginning to focus on plant biology in terms of conservation questions, particularly in the areas of reproduction and genetics. Case studies are still few, however, and the number of species about which we are totally ignorant exceeds, by orders of magnitude, those for which even a minimal amount of data are available.

In Oregon and many other western states, a diversity of habitats has given rise to diverse native floras. A sizeable number of species are locally distributed, and in Oregon there are dozens of narrow endemics (Peck, 1961; Hitchcock and Cronquist, 1973; Meinke, 1982). Although only three Oregon species are listed as threatened or endangered by the federal government (U.S. Fish and Wildlife Service, 1991), and 19 by the state (Oregon Department of Agriculture, 1989), it is estimated that as many as 150-200 species may be legitimately in need of protection (Oregon Natural Heritage Program, 1991; Meinke, unpublished). This estimate is based on very limited information, because even the geographic ranges of many of these species are poorly known. Consequently, most of the studies that have been completed on rare plants in Oregon have focussed on problems of distribution and abundance, and comparatively little research has been conducted on their biology.

Systematics is perhaps the most fundamental consideration in conservation biology, since we cannot protect an organism unless we are aware of its existence. In the tropics, many species of plants and animals are being extirpated even before they can be described in the literature. New tropical plants are being reported in virtually every issue of the American periodicals *Brittonia*, *Systematic Botany*, and *Annals of the Missouri Botanical Garden*, and many others are described in foreign journals. Novel taxa are still occasionally located in temperate regions as well, and several have been discovered in Oregon during the last decade. New plant species are often discovered in the tropics through basic field inventory, while in the temperate regions they are commonly uncovered during revisionary studies. Such studies may also be important in reevaluating previously described taxa, particularly in light of current biological information on the group.

The research presented in this thesis focuses on the taxonomy and reproductive biology of a group of relatively rare herbaceous species in the genus *Mimulus* (Scrophulariaceae). Commonly known as monkeyflowers, *Mimulus* species are found throughout most of the old and new worlds, but primarily in the western United States. The genus is large, and includes many endemic species in California and Oregon (Munz, 1959; Peck, 1961). A significant number of these are now candidates for listing as threatened or endangered under state and federal laws. The species studied here are affiliated with *M. washingtonensis*, perhaps the best known of a group of mostly local endemics geographically centered in northeastern Oregon. The taxonomy and distribution of this complex was poorly understood, and the species were inadequately circumscribed in existing literature (Grant, 1924; Cronquist, 1959; Peck, 1961). Moreover, no information was available on the pollination requirements of any of the species, a matter of concern because pesticide spraying is not uncommon in some of the areas inhabited by these taxa.

In Chapter 2, the species of the *Mimulus washingtonensis* complex are taxonomically revised on the basis of morphological analyses, chromosome number, geographic distribution, and habitat. Taxonomic decisions were derived from studies of preserved herbarium materials as well as living cultures established in the Oregon State University greenhouses. A review of possible evolutionary relationships is offered, as a basis for future studies of the *M. washingtonensis* complex and its allies. Aspects of taxonomy, distribution, and habitat, and their relevance to conservation of the species are discussed.

Chapter 3 provides a description and illustration of a new species, *Mimulus evanescens*, from the northern Great Basin of California, Oregon, and Idaho. Morphometric analyses show that the new species is intermediate to *M. breviflorus*, of the Intermountain Region, and *M. latidens* of the California Central Valley. Grant (1924) believed *M. breviflorus* was closely allied with the *M. washingtonensis* complex, but it may be more closely related to *M. latidens* and other low elevation species of cismontane California. *Mimulus evanescens* is known from only a single extant location, in northeastern California, and has only been collected eight times over the last century. The new species may be confined to low riparian areas, in portions of the Great Basin that are now mostly rangeland for sheep and cattle. The native vegetation in these ecosystems is substantially altered by domestic grazing, with the associated habitats among the most endangered in western North America.

The reproductive ecology of *Mimulus washingtonensis* is reviewed in Chapter 4. Observations were made concerning floral structure and nectar production, phenology, insect pollinators, stigma sensitivity, and seed set limitations. Experimental studies of breeding system efficiency were also conducted, and an investigation into genetic relatedness among neighboring subpopulations was completed.

#### **CHAPTER 2**

## TAXONOMY, DISTRIBUTION, AND CONSERVATION STATUS OF THE MIMULUS WASHINGTONENSIS COMPLEX

*Mimulus* (Scrophulariaceae) is a predominantly western North American genus of annual and perennial herbs, currently believed to comprise 100 (Holmgren, 1984) to 150 (Munz, 1959) species. The majority of taxa occur in and west of the Sierra Nevada of California (Grant, 1924; Pennell, 1951; Munz, 1959), although the genus is found worldwide except for Europe and Antarctica. Many of the species are conspicuous and showy flowered, and are frequently prominent elements of native spring floras.

In the latest monograph of the genus, Grant (1924) subdivided *Mimulus* into 10 sections based on morphological similarities. The section *Diplacus* is today usually considered to constitute a separate genus, primarily because of its woody habit. Otherwise, most current floristic treatments in the United States still follow the species alliances proposed by Grant (1924), with her sections generally corresponding to easily recognized, evidently natural groupings. Genetic studies by Vickery (1969) have confirmed the existence of crossing barriers between several of these groups, although evolutionary relationships within much of the genus are still not well understood.

Even though *Mimulus* was reasonably well defined at the sectional level, there were a number of small species complexes that Grant (1924) found difficult to place. These were organized into the section *Paradanthus*, a problematic and probably paraphyletic group (Argue, 1980, 1986). The 40-50 species presently referred to the section are worldwide in distribution, but primarily occur in California and Oregon. The traits used to recognize *Paradanthus* were a prismatic calyx with equal or subequal teeth, funnelform corollas, and a central placental column that separates near the apex or not at all. Grant (1924) believed the additional characteristics that distinguished species groups within *Paradanthus* did not warrant separate taxonomic status, and she elected to align all species into a single section for the sake of convenience. She conceded that elements of *Paradanthus* were not necessarily closely related and that their taxonomic affiliation had limited phylogenetic implications.

Paradanthus is represented in the Pacific Northwest primarily by two yellowflowered species assemblages, one centered around *Mimulus moschatus* Dougl. in Lindl. and the other around *M. washingtonensis* Gand. The former group includes the widespread species *M. floribundus* Dougl. in Lindl., and both *M. floribundus* and *M. moschatus* are geographically and morphologically linked to several less common taxa occurring in California. *Mimulus moschatus* and *M. floribundus* occur throughout western North America and are represented by many geographic races. The *M. washingtonensis* complex, as defined by Grant (1924), consisted of *M. washingtonensis*, *M. pulsiferae* Gray, and *M. ampliatus* Grant, three locally distributed annuals from eastern Oregon, Idaho, and northern California. Diagnostic traits for this group included moderately to strongly bilabiate flowers, firmly adherent placentae, cylindrical fruiting calyces, long-pedicelled flowers, viscid pubescence, and petiolate leaves. Several of these characters were also shared by members of the *M. moschatus* complex, and Grant (1924) implied a close relationship between the two species groups.

Recently, taxonomic interest in the section *Paradanthus* has been generated by the discovery of several new endemic species in California and Oregon (Meinke, 1983; Heckard and Shevock, 1985; Heckard and Bacigalupi, 1986). Although these species show apparent similarities to particular described taxa, their affiliations within the section are somewhat doubtful. To understand the phylogeny of *Paradanthus*, it is important to study initially the individual species groups proposed by Grant (1924) at the level of the whole organism. The information gathered can then be used to construct hypotheses of relationship that may be tested using molecular or cladistic methods. Biological data that are combined with morphometric studies are often valuable in taxonomic assessments of indeterminate species groups. The synthesis of such information may be particularly meaningful in the evaluation of ephemeral genera such as *Mimulus*, whose species are more apt to be influenced by environmental factors and hence are often phenetically difficult to resolve.

The present study examines the morphology, ecology, and distribution of the *Mimulus washingtonensis* complex, including those species known to Grant (1924) as well as subsequently published taxa. These include *M. washingtonensis*, *M. pulsiferae*, *M. ampliatus*, *M. hymenophyllus* Meinke, *M. patulus* Pennell, and *M. jungermannioides* Suksdorf. Morphological, reproductive, phytogeographic, and

8

environmental attributes were examined for each taxon, in an attempt to circumscribe the group and provide insights into evolutionary relationships within the complex and the genus. The biology and habitat requirements of the species were also evaluated in terms of their conservation needs, inasmuch as all are uncommon and most are local endemics. Each species, with the exception of M. *pulsiferae*, is under consideration for listing as threatened or endangered under state or federal laws.

## METHODS

*Morphometric studies.* Seeds were collected from across the range of the species and used in establishing greenhouse populations for morphological evaluations. *Mimulus ampliatus* was not cultivated due to the lack of a seed source for this extremely rare taxon. A minimum of 60 plants of each of the remaining species were maintained in greenhouse cultures during 1986 and 1987. These were grown in soil collected from field population sites, which was kept moist throughout the growing period but not fertilized. Cultivated plants flowered and developed normally under a spring and summer photoperiod. Morphological relationships between the taxa were examined using a principle components analysis (PCA), employing 26 character states (Table 2-1) commonly used in *Mimulus* taxonomy. These were measured primarily from fresh material, if possible, but supplemented with herbarium specimens to include as much geographic variability as possible.

**Table 2-1.** List of morphological traits measured from plants in the Mimulus washingtonensis complex for use in principle components analysis. Dimensions are in millimeters.

 (1) Habit (prostrate versus erect). (2) Duration (perennial versus annual). (3) Presence or absence of a basal rosette. (4) Petiole length of cauline leaf (third node).
 (5) Leaf blade width. (6) Leaf blade length. (7) Stem pubescence 1-2-celled or multicelled. (8) Pedicel length. (9) Pedicel orientation (horizontal, horizontal arcuate, straight ascending, or ascending arcuate). (10) Calyx width in fruit. (11) Calyx length in fruit. (12) Calyx teeth shape in fruit (deltoid versus roundedmucronate). (13) Calyx tube shape in fruit (cylindrical, campanulate, or urceolate).
 (14) Corolla length. (15) Corolla limb width. (16) Ratio of corolla tube length to limb length. (17) Depth of inner petal sinuses (lower corolla lip). (18) Presence or absence of lower corolla palate. (19) Presence or absence of white patches on corolla. (20) One or two flowers at a node (second flowering node). (21) Length of stigma lobes. (22) Style length. (23) Style included in tube, throat, or excluded. (24) Style glabrous versus hispid. (25) Capsule length. (26) Capsule width. Fifteen plants were evaluated per species, except for *M. patulus*. Thirty examples of this species were included, to take into account an undescribed variant detected during examination of herbarium collections.

Mimulus patulus, which is recognized taxonomically in this study, has been considered a small-flowered extreme of M. washingtonensis (Cronquist, 1959). A review of herbarium material showed that members of section Paradanthus, in general, have been difficult to distinguish when plants are diminished in size due to environmental factors. To investigate this problem, plants were grown under a drought regime in the greenhouse from April through June, where soil was allowed to remain dry for several days between waterings after seedlings were established. Ten plants each of M. washingtonensis, M. pulsiferae, M. hymenophyllus, M. jungermannioides, and M. patulus were then compared morphologically. Also included in the study were stressed plants of M. floribundus and M. guttatus DC., species which often co-occur with members of the M. washingtonensis complex and which are occasionally confused with them. Sampled plants were harvested 3-4 weeks after flowering commenced and were measured for nine traits (Table 2-2) that are readily apparent on dwarfed individuals and frequently used in Mimulus keys. A discriminant analysis was performed to evaluate dissimilarities between samples.

**Reproduction.** A pollen/ovule ratio was estimated for each species grown in the greenhouse, following the methods of Cruden (1977). A reproductive assessment of the *Mimulus washingtonensis* complex was also conducted. Species were compared with respect to (1) autofertility in the absence of pollinators, both in

**Table 2-2.** List of morphological traits measured from plants in the Mimulus washingtonensis complex for use in discriminant analysis. Analysis was performed on drought-stressed plants, and employed simple characters commonly used in keys to Mimulus.

(1) Plant height. (2) Leaf blade broad (cordate to ovate) versus narrow (lanceolate to linear). (3) Pedicel length. (4) Calyx width in flower. (5) Calyx length in flower. (6) Corolla length. (7) Corolla limb width. (8) Corolla strongly or weakly bilabiate. (9) Style length.

the field (using caged plants) and greenhouse; and (2) seed set resulting from open pollination in the field. Many species of *Mimulus* possess thigmotropic stigmas, which may close rapidly in response to stimulation by pollinators. A comparison was made of the times required for stigma closure for each species in the *Mimulus washingtonensis* complex (except *M. ampliatus*). Seed germination requirements and other life cycle aspects for the species were also reviewed, based on field observations and an earlier study (Meinke, unpublished).

Herbarium studies. Specimens from the following herbaria were examined to gather data used in the taxonomic treatment of the *Mimulus washingtonensis* complex: BRY, DS, US, ORE, OSC, WILLU, M, NY, WS, WTU, RM, UC, JEPS, CAS, ID, IDF, UTC, P, and CU. Morphological measurements were gathered to augment greenhouse studies, and distributional data were collected to prepare range maps. Since the species under study are mostly rare, field trips were taken during the spring and summers of 1985-1990 to supplement existing herbarium collections. Efforts were made to locate additional populations, and observations were recorded concerning habitat preferences. Chromosome counts were obtained for selected populations, using meiotic squashes stained in acetocarmine.

#### SPECIES BIOLOGY

Morphological relationships. The results of the PCA are presented in Fig.2-1. The first principal component accounted for 82.4% of the variation in the data



First Principal Component (82.4%)

Fig. 2-1. Principal components analysis of the Mimulus washingtonensis complex, based on 26 character states (see Table 2-1). Scatter diagram generated by plotting the first two factors of variation, depicting populations of *M. hymenophyllus* (1), *M. jungermannioides* (2), two variants of *M. patulus* (3 and 4), *M. ampliatus* (5), *M. washingtonensis* (6), and *M. pulsiferae* (7). See text for discussion.

set, the second for only 6.6%, with the remaining components negligible (Table 2-3). Characters that weighted heavily on the first axis were overall corolla length, corolla limb width, and style length. Traits weighing heavily on the second axis were calyx length, capsule length, and pedicel length. The analysis clearly demonstrates phenetic differences between five of the six species evaluated. However, *Mimulus washingtonensis* and *M. ampliatus* overlapped considerably based on the traits used in the analysis. The two variants of *M. patulus* were also very similar morphologically. *Mimulus jungermannioides* and *M. hymenophyllus* were comparable as well, which was not unexpected considering that both are largeflowered species ecologically adapted to cliffs.

Plants that were drought-stressed in the greenhouse were stunted after 11 weeks of growth, but all flowered and set seed. There was a decrease in phenetic dissimilarity between stressed plants based on discriminant analysis as compared with the PCA, demonstrated by the discriminant analysis (Fig. 2-2). Reductions in plant size and floral morphology associated with environmental stress masked morphological differences between the species that are more apparent under normal conditions. *Mimulus guttatus*, a distantly related species in section *Simiolus*, has occasionally been mistaken for *M. pulsiferae* and other taxa. Normally very distinct because of an irregular, inflated calyx, large bilabiate corolla, and sessile upper leaves, depauperate individuals of *M. guttatus* can be difficult for non-specialists to distinguish (Fig. 2-2).

Component Number	Percent of Variance	Cumulative Percentage
	00.41	00.41
1	82.41	82.41
2	6.63	89.04
3	4.45	93.49
4	2.70	96.20
5	.78	96.98
6	.72	97.70
7	.62	98.32
8	.42	98.74
9	.33	99.07
10	.18	99.25

**Table 2-3.** Amount of total variance accounted for by the first ten principalcomponents, in a principal components analysis of morphological variation amongspecies in the Mimulus washingtonensis complex.



First Discriminant Function

Fig. 2-2. Discriminant analysis of the *Mimulus washingtonensis* complex, based on plants grown under drought conditions. Traits used in this analysis are listed in Table 2-2. Scatter diagram generated by plotting canonical variables, showing relationships of *M. washingtonensis* (1), *M. jungermannioides* (2), *M. hymenophyllus* (3), *M. patulus* (4), and *M. pulsiferae* (5). Included for comparison were *Mimulus floribundus* (6) and *M. guttatus* (7). See text for discussion.

Mimulus floribundus exhibits an equally close morphological association with members of the *M. washingtonensis* complex. Although *M. washingtonensis* is widely reported to occur in Washington (Cronquist, 1959; Peck, 1961), modern collections supposedly representing the species from that state have all proven to be M. floribundus. Fig. 2-2 illustrates that the two species, easily separable when not dwarfed, can appear very similar under drought conditions when only traditional key characters are considered. In particular, the qualitative traits often used in keys, such as the degree of corolla irregularity (Cronquist, 1959), the shape of the calva in fruit (Grant, 1924), and general pubescence features (Peck, 1961), are more subject to interpretation and are of minimal value in distinguishing dwarfed specimens. The result has been a pattern of misidentification, in which a substantial number of reports of rare species such as M. washingtonensis and M. pulsiferae have been based on common taxa, usually M. floribundus or M. guttatus. In the field, conditions such as shallow substrate and excessive competition were observed to similarly modify plant morphology.

Cronquist (1959) relegated *Mimulus patulus* to synonymy under *M*. *washingtonensis*, but the morphometric studies here demonstrate the distinctness of the two taxa (Fig. 2-1). Plants grown in a common greenhouse environment retained the differences observed in the field and on herbarium sheets. Even when under stress, *M. patulus* and *M. washingtonensis* were among the least similar taxa (Fig. 2-2).

**Reproductive Biology.** Pollen/ovule ratios are summarized in Table 2-4. Autogamous and open-pollinated seed sets for members of the Mimulus washingtonensis complex are compared in Fig. 2-3. All species are genetically selfcompatible, but only *M. patulus* produced as many seeds from autogamy as from open field pollination. The extremely low pollen/ovule (P/O) ratio for this species is consistent with those of other highly inbred angiosperms (Cruden, 1977). This was a significant departure from the apparently outcrossed breeding system of M. washingtonensis, which produced far fewer seeds by self-pollination than did M. patulus (Student-Newman-Keuls Test; P < 0.01). The P/O ratios of M. washingtonensis and the other three species (Table 2-4) suggest facultative autogamy based on Cruden's (1977) estimates. However, the low estimates of P/O ratio are a result of the extremely high number of ovules produced by most *Mimulus* species. and they are therefore not comparable to most of the species surveyed by Cruden (1977). The results here are comparable to the P/O ratios obtained for the M. guttatus complex (Ritland and Ritland, 1989) and suggest facultatively xenogamous breeding systems, where selfing is possible to varying degrees but outcrossing may be more common. For each of the species in Table 2-4, excluding *M. patulus*, open-pollinated seeds sets were significantly higher than autogamous seed sets (Student-Newman-Keuls Test; P < 0.05). Differences between autogamy in the field and greenhouse were universally non-significant, implying that all species except M. patulus may be potentially pollinator limited. Mimulus hymenophyllus

**Table 2-4.** Pollen/ovule ratios for species in the Mimulus washingtonensis complex. Quantities are averages of eight flowers per species,  $\pm$  one standard deviation. Samples were taken from greenhouse populations. Means (by column) followed by different superscripts are significantly different (Student-Newman-Keuls Test, P < 0.05).

Species	Pollen/Ovule Ratio	Ovule Number
Mimulus washingtonensis	29.9±4.1ª	523±77ª
Mimulus jungermannioides	27.6±4.5 <sup>a</sup>	489±61*
Mimulus hymenophyllus	48.8±5.9 <sup>b</sup>	146±31 <sup>b</sup>
Mimulus pulsiferae	15.6±1.9°	$301 \pm 52^{\circ}$
Mimulus patulus	$4.1 \pm 0.5^{d}$	253±44°
	,	



Mimulus Species Sampled

Fig. 2-3. Breeding system experiment for species in the *Mimulus* washingtonensis complex. Treatments were (1) self-pollination in the field with plants caged to exclude potential pollinators, (2) self-pollination in a pollinator-free greenhouse, and (3) open pollination of plants in a field population. Labels on the x axis are as follows: Miwa (*M. washington-ensis*); Miju (*M. jungermannioides*); Mihy (*M. hymenophyllus*); Mipu (*M. pulsiferae*); and Mipa (*M. patulus*).

produced the fewest seeds of any species, and had the highest P/O ratio, presumably due to having the fewest ovules.

There were differences among species with respect to the time required for stigmas to close after tactile stimulation (Fig. 2-4). A positive correlation existed between the rate of autogamy for a species and stigma closure time (Fig. 2-5). Of significance is the contrast between stigma reactions of *Mimulus washingtonensis* and *M. patulus*.

Field observations showed that several small native bees in the families Halictidae and Megachilidae were the primary visitors to flowers of *Mimulus washingtonensis*, *M. hymenophyllus*, and *M. jungermannioides*. Native bees also visited *M. pulsiferae*, but the major visitor recorded for this species was a longtongued fly (*Oligodranes* sp.) in the family Bombyliidae. No insects were recorded foraging on *M. patulus* flowers.

The life cycle of *Mimulus jungermannioides* is perennial. *Mimulus patulus*, *M. washingtonensis*, and *M. pulsiferae* are all winter annuals and possess seeds that require chilling prior to germination (Meinke, unpublished). Seeds of *M. jungermannioides* and *M. hymenophyllus* (also annual) are not dormant and germinate readily when capsules dehisce. Those of *M. hymenophyllus*, however, may become dormant if not germinated within a few weeks of dispersal. After this, they germinate more readily if exposed to a period of cold temperature. None of the species appear to germinate preferentially in dark or light.



Fig. 2-4. Average stigma closure speed for species in the *Mimulus* washingtonensis complex. Stigma lobes were touched once with a small brush on the first and third days after flowers opened, with the time required for closure then measured (N = 25 flowers per species per day). *Mimulus patulus* stigmas were mostly unresponsive, resulting in fewer measurements (N = 9for Day 1, and N = 5 for day 3). Closure speeds for **Miwa**, **Miju**, and **Mihy** were statistically equivalent, while those for **Mipu** and **Mipa** differed significantly with each other and the other three species (Student-Newman-Keuls Test; P < 0.05). Labels on the x axis are as follows: Miwa (*M.* washingtonensis); Miju (*M. jungermannioides*); Mihy (*M. hymenophyllus*); Mipu (*M. pulsiferae*); and Mipa (*M. patulus*).



Fig. 2-5. Stigma closure time as a function of breeding system in the *Mimulus washingtonensis* complex. A strong positive correlation existed between self-pollination and the amount of time a stigma takes to close after mechanical stimulation. Closure times are from Day 1 in Fig. 2-4, and seed set data are from caged plants in Fig. 2-3.
## **DISTRIBUTION**

The geographic range of the *Mimulus washingtonensis* complex is illustrated in Figs. 2-6 and 2-7. *Mimulus pulsiferae* (Fig. 2-6) has the widest distribution, occurring from extreme southern Washington to north-central California. This species grows along the east slope of the Cascade Mountains from Washington to central Oregon, then shifts to the west slope as it continues south into the Sierra Nevada. Although *M. pulsiferae* is widespread it is also sporadic, especially in the northern portion of its range. This is the only species in the complex distributed at least partially west of the Cascade-Sierran crest.

The remaining species all occur east of the Cascades, and some of these are very local. *Mimulus hymenophyllus* has been collected from a single canyon near the Snake River in eastern Wallowa County, Oregon. *Mimulus jungermannioides* (Fig. 2-6) is endemic to bluffs along the Columbia River and its major tributaries in northern-central Oregon. Occurrences of this species are isolated and limited to a few canyon walls. *Mimulus patulus* var. *patulus* (Fig. 2-7) is known primarily from historic collections, mostly in the vicinity of the Wallowa Mountains and the Snake River Canyon of northeast Oregon and adjacent Washington and Idaho. *Mimulus patulus* var. *montanus* (described herein) is more widespread, occurring from northeast Oregon to northwestern Wyoming (Fig. 2-7). It is the only taxon in the complex extending to the Rocky Mountains. *Mimulus washingtonensis* var. *washingtonensis* is apparently restricted to the John Day River drainage in the east-



Fig. 2-6. Range map of *Mimulus jungermannioides*, *M. hymenophyllus*, and *M. pulsiferae*. Symbols may represent one or more collections.



Fig. 2-7. Range map of Mimulus washingtonensis var. washingtonensis, M. washingtonensis var. ampliatus, M. patulus var. patulus, and M. patulus var. montanus. Symbols may represent one or more collections.

central portion of the state (Fig. 2-7), occurring on rock shelves and gravelly slopes. Historic collections from a locality along the Washington side of the Columbia River (see discussion below) are of dubious origin. *Mimulus washingtonensis* var. *ampliatus* (combination proposed herein) is endemic to west-central Idaho (fig. 2-7), immediately east of the Snake River. It is known from a single extant population.

All of the species except *Mimulus pulsiferae* and *M. patulus* var. *montanus* are endemic to basalt substrates. *Mimulus pulsiferae* occurs on cinders or basaltic soils in Oregon, Washington, and northeastern California, but frequents granitic and other surfaces in the Sierra Nevada. Substrates for all species are moist in early spring, but typically dry out by early summer. Little is known of the substrate requirements of *M. patulus* var. *montanus*.

The Columbia River Gorge. A factor to consider in the distribution of the Mimulus washingtonensis complex is a series of collections made by Wilhelm Suksdorf in the late nineteenth and early twentieth centuries. These were purportedly taken mostly from the Washington side of the Columbia River Gorge, near the town of Bingen (Fig. 2-6), and are distributed in several major herbaria (WS, GH, MO, and DS, among others). They include the type collections of both *M. jungermannioides* and *M. washingtonensis*. If the collection information provided by Suksdorf is accurate, then the distributions of *M. washingtonensis* and *M. patulus* would have to be amended to include southern Washington. However, there is evidence to suggest that much of Suksdorf's data locational cannot be taken literally. First, it is remarkable that Mimulus washingtonensis, M.

jungermannioides, and M. patulus, three species with distinct distributions elsewhere, would co-occur along the Columbia River near Bingen as implied by Suksdorf's collections. In addition to these species, M. breviflorus and M. floribundus were also reported from there. This unique admixture could have resulted if seeds were washed down the Columbia River from its tributaries, with the species then establishing as waifs. Some of the Suksdorf collections were labelled as having been taken from the "moist sandy banks of the Columbia," and at least one collection was listed for the Hood River area on the Oregon side of the Columbia. However, these localities are farther west and generally more mesic than any sites known for the species today. Heckard (1960) doubted locational data for Phacelia collections by Suksdorf, based on geographic and morphological study. In his treatment of *Phacelia heterophylla*, he pointed out that the type specimen of *P*. leucophylla var. suksdorfii, "although reported to have been collected in the Columbia River Gorge in the vicinity of Bingen," was much more likely to have been collected farther east on the arid Columbia Plateau.

Suksdorf also reported unusual collection dates for several *Mimulus* species, including *Mimulus washingtonensis* and *M. patulus*. Many collections were labelled as growing and flowering during the late fall, which is otherwise unprecedented for any of the species in question. In particular, it would have been very unlikely for plants of *M. washingtonensis* and *M. patulus* to have been in early flower at this time. Greenhouse studies (Meinke, unpublished) have shown that these species require cold stratification for germination, virtually assuring a spring blooming period in nature. Even with mild temperatures and continual moisture, greenhouse plants seldom live longer than five or six months. The plants on many of Suksdorf's collections appeared less than three months old, and some were only in bud. The germination requirements and the dates of these collections therefore appear contradictory. Another interesting point is that the *M. washingtonensis* collections usually had plants with full capsules, an indication of insect mediated pollination (Fig. 2-3). This seems improbable in October and November.

It may be that Suksdorf obtained seeds of these species during collecting trips outside the gorge area, and grew them together near his home along the Columbia River. They could have then become adventive along the river, or perhaps they were reintroduced repeatedly. A number of his labels bear wording to the effect that specimens were collected from his garden. Moreover, some of Suksdorf's collections are of plants with unusual morphological attributes. This could have been an environmental response to an atypical habitat or perhaps a result of hybridization between species that were normally allopatric. For example, M. serotinus Suksd., a species considered synonymous with M. floribundus by Grant (1924), exhibits characteristics of *M. washingtonensis*, *M. patulus*, and *M.* floribundus. It was recorded many times by Suksdorf, but only near Bingen and never by anyone after his death in 1932. Without living material, it is difficult to judge the relationship of this problematic taxon. In fact, most of the Mimulus species reported by Suksdorf near Bingen have never been relocated there. The original sites may now be inundated, as the collections were made before

impoundment of the lower Columbia. It is important, from an historical perspective, to be aware of these early collections. However, there are enough inconsistencies associated with them to warrant caution from a biogeographic perspective.

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## TAXONOMIC TREATMENT

The following key places *Mimulus washingtonensis* and its allies in a morphological context within the section *Paradanthus* in the Pacific Northwest. It is important to note that depauperate or immature specimens of most species can be very difficult to identify. The key is followed by species descriptions and lists of representative specimens.

## Key to Mimulus, Section Paradanthus in the Pacific Northwest.

- Plants conspicuously glandular-hairy; corollas less than 3.0(-3.5) cm long; of various habitats, frequently east of the Cascade Range
  - 2 Plants glandular-pilose or -villous (at least on the newer growth), the length and cell number of stem and calyx hairs clearly not uniform, most of these ranging from 2-5 mm long and 3-7 celled (excluding the terminal gland); plants perennial (except *M. floribundus*)

- 3' Inflorescence clearly racemose; habitat various

  - 4' Flowers (13-)15-30 mm long; perennials
    - - 5' Corolla strongly bilabiate, the lobes evidently unequal; calyx teeth broad, 1-2 mm long; plants developing from scaly, subterranean buds (turions); cliffs near the Columbia, Deschutes, John Day, and Umatilla Rivers in north-central Oregon...... M. jungermannioides Suksd.
- 2' Plants glandular-puberulent, the length and cell number of stem and calyx hairs essentially uniform, all less than 1.5 mm long and 1-(2)-

celled (excluding the terminal gland); annual species (rarely overwintering for a second year in *M. hymenophyllus*)

- 6' Calyx regular, the lobes all equal and possessing acerose,
  eglandular cilia along the margins; east of the Cascade Range
  or, if west, then not on rock outcrops or cliffs (go to lead 7)
- 7 Flowers (13-)15-30 mm long; corolla conspicuously bilabiate, with white palate ridges exposed on the inner throat

  - 8' Length of cauline petioles less than the blades; corolla 1.5-3 times longer than the calyx; stigmas flabelliform; capsules oblong to lanceolate; erect annuals, not hanging from cliffs
    - Plants copiously puberulent; style hispid-pubescent; north central Oregon in the John Day River drainage......
       M. washingtonensis Gand. var. washingtonensis

- 9' Plants sparsely pubescent; style glabrous; western Idaho.....
  ..... M. washingtonensis Gand. var. ampliatus (Grant) Meinke
- 7' Flowers less than 13(-15) mm long; corolla weakly irregular to regular, never bilabiate, palate ridges absent

10 Cauline leaves (at least those on upper stems) sessile

- 11 Leaf blades linear to narrowly oblanceolate; petals notched at the apex; fruiting calyx open-cylindric...... *M. suksdorfii* Gray
- 10' Cauline leaves distinctly petioled, blades various
  - 12 Blades of the cauline leaves cordate to broadly lance-ovate, strongly denticulate, narrowing abruptly to petioles that usually equal or exceed the blades
    - 13 Stem hairs strictly glandular; fruiting pedicels erect and straight; from west of the Rocky Mountains, centered in northeast Oregon...... M. patulus Penn. var. patulus
    - 13' Stems minutely hispid, with apiculate, non-glandular hairs scattered among the glandular ones; fruiting pedicels often arcuate; in and east of the Rocky Mountains, from Idaho and Montana south to northwest Wyoming..... M. patulus Penn. var. montanus Meinke

- 12' Blades of the cauline leaves lanceolate to oblong-linear, weakly denticulate or entire, gradually narrowing to petioles about one-third to one-fifth their length

The Mimulus washingtonensis complex. The members of the Mimulus washingtonensis complex have been allied in this study because of morphological and geographical similarities. They comprise a group of mostly endemic species centered in northeastern Oregon, which share a chromosome number of n = 16. The species are also ecologically similar, occurring almost exclusively on basalt rocks or basaltic gravels or pumice. Generally, they occur in moist microsites within otherwise xeric environments. These species differ from other groups in

*Paradanthus* by combining the following traits: (1) short, one- or two-celled leaf and stem hairs (except in *M. jungermannioides*), which make the pubescence appear to be of even length to the naked eye (Fig. 2-8); (2) placentae that remain adherent, even in fruit; (3) cylindrical fruiting calyces (Fig. 2-9) with short, equal teeth; (4) yellow, funnelform corollas (Fig. 2-10a); (5) mostly broad, petioled leaves; and (6) a preference for basalt substrates. *Mimulus washingtonensis, M. jungermannioides*, and *M. hymenophyllus* each possess a large, bilabiate corolla. The amount of petal fusion differs in the three species and is contrasted in the following descriptions. Frontal views of the corollas of these species are shown in Fig 2-10b for reference.

Mimulus jungermannioides Suksdorf (Fig. 2-11)—Deut. Bot. Monatsschr. 18:154, 1900.—TYPE: USA, Washington, Klickitat Co., on steep, overhanging.

damp cliffs near Bingen, August-November, 1892, Suksdorf 1470 (holotype: MO!; isotypes, F, NY, UC!, WTU!).

Perennial herb on vertical cliffs, prostrate and drooping with age, roots and base of main stem often reddish-orange, stem  $\pm$  brittle, branching near the base, 0.5-3.8(-6.0) dm long, copiously slimy-villous with multicelled hairs, many often exceeding 1.2 mm in length, the pubescence appearing uneven to the naked eye, plants propagating by means of overwintering turions, these appearing in late summer and fall on the ends of filiform stolons up to 8 dm long, the stolons and young leaves with short, one or two celled glandular hairs, these appearing equal in



Figs. 2-8 (top) and 2-9 (bottom). Fig. 2-8 (scale = 2.5X). Stem pubescence of Mimulus washingtonensis. Depicted are the short, even-lengthed glandular hairs common to all the species of the *M. washingtonensis* complex except (in part) *M. jungermannioides*. Fig. 2-9 (scale = 3.5X). Cylindrical fruiting calyces of *M. pulsiferae*, showing exserted capsule (at right).



Figs. 2-10a (top) and 2-10b (bottom). Fig. 2-10a (scale = 1.2X). Funnelform corollas of (from left to right) Mimulus washingtonensis, M. jungermannioides, M. hymenophyllus, and M. patulus. Fig. 2-10b (scale = 1.5X). Frontal view of corollas (from left to right) of M. washingtonensis, M. jungermannioides, and M. hymenophyllus. Petal sinus depth differs between the species.



Fig. 2-11. (scale = 0.6X). Young *Mimulus jungermannioides* plant on basalt cliff (late May, Deschutes County, Oregon).

length to the naked eye; *leaves* numerous, cauline, dark to light green, occasionally anthocyanic beneath, blades thickish except in shaded populations, broadly lanceolate to cordate, acute, 1.0-3.5(-4.3) cm long, 0.8-2.5 cm wide, sharply and  $\pm$ irregularly denticulate, rarely subentire, broadly triangular to subcordate at the base, petioles (2-)4-18(-28) mm long; pedicels  $\pm$  ascending, less than or exceeding the blades, 10-35(-45) mm long in flower, slightly longer in fruit; flower buds  $\pm$  erect, but rarely nodding; calyx green, glandular pubescent with multicelled hairs, tubular, 4.5-8.5 mm long and 2.2-4.9 mm wide at anthesis, becoming urceolate in fruit, up to 11.0 mm long and 6 mm wide, teeth equal or sometimes bifid, 0.8-1.5 mm long, 0.6-1.3 mm wide, deltoid to rounded mucronate in flower and fruit, ciliate; corolla funnelform, strongly bilabiate with a prominent lower palate, (12-)15-22(-26) mm long, yellow with scattered red dots and two white patches on the lower lip, puberulent externally with thickened yellow hairs on the inner lower lip, ventrally pubescent within, the tube slightly exserted, the throat moderately flaring, the petal lobes short and spreading, with moderately deep sinuses; stamens glabrous, included; style glabrous, scarcely included to slightly exserted and exceeding the stamens, stigma lips lanceolate, laciniate; *capsule* lanceolate, included in the calyx tube at maturity, 5.0-9.2 mm long and 2.5-3.8 mm wide, the placentae firmly adherent; seeds ovoid to oblong, 0.3-0.5 mm long; chromosome number n = 16.

Representative specimens. USA, Oregon, Gilliam Co., Condon, 30 July 1931, Jones 28832 (DS, UC); along cliffs near Columbia River and Interstate 84, 12 km east of the John Day River, 27 May 1985, *Meinke 3000* (OSC, to be distributed). Hood River Co., sandy bottoms at Hood River, 20 June 1886, *Henderson 761* (US). Sherman Co., Grants, 27 June 1886, *Suksdorf s.n.* (PH); along south side of Interstate 84, on cliffs near John Day Dam rest area, 11 June or Riverh
1985, *Meinke 2999* (OSC, to be distributed). Umatilla Co., Reith, 30 June 1978, *Dwan 25* (OSC); 9.6 miles west of Pendleton, on cliffs above Umatilla River, 15 June 1985, *Meinke 3008* (OSC). Wasco Co., Bakeoven Creek, 27 May 1933, *Peck 17310* (CAS, PH, UC, WTU) and 10 June 1985 *Meinke 2995* (OSC, to be distributed); Sherar's Bridge, 19 June 1951, *Ripley and Barneby 10834* (CAS, WILLU) and 10 June 1985 *Meinke 2997* (OSC, to be distributed);
ca. 3 km downstream from Dant, along the Deschutes River, May 1984, *Halvorsen 174* (OSC). Wheeler Co., along John Day River, 12 km downriver from Jefferson

Co. line, 11 May 1986, *Meinke 3095* (OSC).

Mimulus jungermannioides is exceptional in the M. washingtonensis complex by combining multicelled, mostly villous pubescence with the shorter type characteristic of the other species. Stems, older leaves, and calyces on most plants appear softly hirsute, sometimes prominently so at the base, while young leaves and stolons have the short, even hairs found in the annual taxa. Mimulus jungermannioides also differs by having calyces that become more or less urceolate in fruit, trapping seeds within as capsules disperse. For a rare species, M. jungermannioides is unexpectedly polymorphic among populations, possibly a result of vegetative reproduction combined with a fragmented distribution pattern (see discussion below).

*Mimulus jungermannioides* is similar to *M. moschatus* and *M. floribundus* with respect to pubescence, fruiting calyx, and flower size. It is distinguished in the field by the bilabiate corolla, style length (not exceeding the throat in the latter two species), and life history (see below).

**Distribution and habitat.** *Mimulus jungermannioides* occurs sporadically along the Columbia River and in canyons along or near the lower Deschutes, John Day, and Umatilla Rivers (Fig. 2-6). Collectively, existing populations are believed to have fewer than 2,000 plants. The type collection from near Bingen, Washington, may have been a chance introduction at that location (see earlier discussion of *Mimulus* distributions in the Columbia River Gorge). Although the type label indicates the collecting site as "steep or overhanging damp cliffs," Suksdorf indicates in an accompanying packet that at least some of the specimens came from the riverbank (e.g., the Columbia), collected on 11 November 1896. The species is not known to have been relocated in Washington (J. Gamon, Washington Department of Natural Resources, personal communication).

*Mimulus jungermannioides* is a basalt cliff endemic, growing from fissures in vertical walls. Plants rarely occur at the base of cliffs after rockslides but are not believed to persist. Populations have been observed on north, east, and west exposures, occasionally sheltered by overhangs. The altitudinal range for the

species extends to 1150 m but is much lower in and near the Columbia River Gorge. Associate species include *Penstemon richardsonii*, *Clematis ligusticifolia*, *Eriogonum compositum*, *Festuca idahoensis*, *Poa cusickii*, and *Rhus toxicodendron*.

Phenology and life history. *Mimulus jungermannioides* flowers primarily from late April through early July, although sporadic blooming may continue into August. The perennial life cycle of the species is noteworthy and unique (Fig. 2-12). Plants react to a declining photoperiod by developing long, thread-like stolons, which usually appear by late August, depending on cliff exposure. The stolons become negatively phototropic at the tips, and creep into crevices in the cliffs to produce scaly, starch-rich turions up to 1.5 cm long. These dormant, highly condensed shoots overwinter after parent ramets wither, then grow rapidly the following spring (Fig. 2-13). Despite producing many flowers, field observations suggest that most plants in nature originate asexually. This is based on a nondestructive census of stem bases, which checked for the characteristic dark scales that remain after turions bolt. A scattered sampling of 100 separate ramets from three populations showed at least 89% to be of vegetative origin.

Mimulus washingtonensis Gand. var. washingtonensis (Figs. 2-14, 2-15).—Bull.
Soc. Bot. France IV. 19:281, 1919.—TYPE: USA, Washington, Klickitat
Co., low sandy banks of the Columbia, Bingen, October-November, 1885,
Suksdorf 560 (holotype: LY; isotypes: CAS!, CU!, DS!, GH, MO!, ORE!,



Fig. 2-12. Vegetative life history of *Mimulus jungermannioides*. Ramets are initiated in early spring as winter dormant turions bolt (A). Flowering and fruiting occurs in spring and early summer, followed by late summer production of stolons. Stolon tips are light sensitive, and enter cracks in the cliff. Turions subsequently develop at the ends of stolons, and overwinter in soil at the base of the crevices.



**Fig. 2-13.** (scale = 2X). Sprouting turions of *Mimulus jungermannioides* grown in the greenhouse. Decussate bracts at the base of the young stem persist as dark, shrivelled scales in mature ramets.



Fig. 2-14. (scale = 1.2X). Greenhouse grown plants of Mimulus washingtonensis.



**Fig. 2-15.** (scale = 0.6X). Habit of *Mimulus washingtonensis* in a wet spring, showing elongate peduncles and nodding flower buds. Leaves are often narrower and with shorter petioles under more xeric conditions (Wheeler County, Oregon).

OSC!, PH!, UC!, US!, WS!, WTU!). Numerous sheets were distributed under *Suksdorf 560*, which included dates of "September-November, 1893-1904" and "September, November, 1883," in addition to that given above. These collections collectively display a wide range of morphology, with many specimens possibly representing hybrid forms of undetermined parentage.

Annual herb, copiously glandular-puberulent, the hairs short and appearing of even length to the naked eye, less than 0.8 mm long, mostly one or two celled, moist to the touch; stems slender, erect, one to few arising from near the base, sparingly to occasionally highly branched above, (4-)6-25 cm tall, with elongated internodes; *leaves* mostly cauline, olive green above and often anthocyanic ventrally, the blades broadly ovate to more often lanceolate, often narrowly so, acute, margins entire or denticulate, 4-16(-23) mm long, 2-11 (-16) mm wide, leaf bases cuneate, petioles 2-14 mm long; *pedicels* slender, straight and ascending to horizontally spreading and  $\pm$  arcuate; 1.5-4.9(-6.2) cm long, much exceeding the leaves; flower buds nodding; calyx green at anthesis, glandular-puberulent, often becoming anthocyanic with age, tubular in flower and fruit, 5.0-8.0(-9.0) mm long, 1.4-3.7 mm wide, the teeth triangular, acute, 0.8-1.2 mm long, ciliate on the margins; corolla strongly bilabiate with a well-developed lower palate, funnelform, yellow, with scattered reddish dots on the lower lip flanked by two oblong white patches, (14-)17-28 mm long, the tube narrow and  $\pm$  exserted, the limb 10-19 mm wide,

petal sinuses shallow, lower palate prominently bearded with yellow, clavate hairs extending into the throat, corolla otherwise glabrous; *stamens* barely included, about equal with the style, glabrous; *style* minutely hispid, the stigma usually slightly exserted, the lobes flabelliform, shallowly laciniate-margined; *capsule* included, lanceolate, 4.5-8.5 mm long, 2.0-4.5 mm wide, the placentae adherent to the apex; *seeds* ovoid or oblong, brown, ca. 0.2-0.5 mm long; *chromosome number* n = 16.

Representative specimens. USA, Oregon, Grant Co., John Day River (South Fork), 8 miles south of Dayville, 22 May 1953, *Cronquist 6995* (CAS, DS, *Feerk* ID, MO, NY, OSC, UC, WTU); <sup>2</sup>Squaw Creek and Kimberly, 29 April - 4 May <sup>2</sup> 1925, *Henderson 5177* (DS, MO, ORE); <sup>3</sup>12 km south of Dayville, 28 May 1985, *Feerk Meinke 3002* (OSC); Ritter, along the Middle Fork of the John Day River, 30 May <sup>2</sup> 1985, *Meinke 3017* (OSC). Hood River Co., Hood River area, 21 August 1887, *– Dickson and Drake s.n.* (NY). Wasco Co., John Day River at Clarno, 3 July 1921, <sup>2</sup> *Peck 10880* (WILLU). Wheeler Co., 10 miles northwest of Mitchell, 21 June <sup>2</sup> 1953, *Cronquist 7231* (NY, UC, WTU); 5.6 miles south of Twickenham, 25 May, <sup>2</sup> 1978, *Halse 1798* (OSC) *and* 30 May 1987, *Meinke 3290*; slopes 3 km east of Service Creek, above John Day River, 8 June 1985, *Meinke 2993* (OSC, to be distributed). Umatilla Co., north fork of the John Day River, 4 km west of Highway 95, 29 May 1985, *Meinke 3015* (OSC). Mimulus washingtonensis var. washingtonensis is the most xerophytic of the taxa considered here. Plants of this species generally devote more energy to reproductive as opposed to vegetative tissue (Fig. 2-14), and they often produce showy floral displays in wet years. Leaves, particularly under droughty conditions, may be small and inconspicuous. The flabelliform stigmas and nodding floral buds of this and the following taxon are unique in the *M. washingtonensis* complex.

This taxon has been primarily confused with *Mimulus floribundus*, especially in Washington. The latter species is readily distinguished by a weakly bilabiate corolla, multi-celled, uneven vegetative pubescence, and urceolate fruiting calyces. Identifications of small, pressed specimens of *M. washingtonensis* and *M. floribundus* can be unreliable.

**Distribution and habitat.** *Mimulus washingtonensis* var. *washingtonensis* is believed to be restricted to the John Day River drainage of eastern Oregon (Fig. 2-7). The only authentic collections of this species not reported from this range are those by Suksdorf, from the Washington side of the Columbia River Gorge, and at a site near Hood River, Oregon. The rationale for questioning the origin of these collections was discussed earlier. Several searches over the last decade have failed to locate any populations of this species in Washington, and none have been recorded from Bingen since the late 1920's. Contemporary reports of *Mimulus washingtonensis* from Washington state are based on misidentifications of *M*.

floribundus, M. moschatus, M. alsinoides, and depauperate specimens of M. guttatus.

Mimulus washingtonensis var. washingtonensis occurs in shallow basalt gravels (Fig. 2-16), with plants congregating in narrow channels, along open slopes, or on rocky shelves near seeps. The elevational range is between 950-1250 m, excluding historic sites in the Columbia River Gorge which are estimated to have occurred at less than 50 m. The early reports of the species by Suksdorf, along the banks of the Columbia River, have perpetuated the mistaken belief that *M*. washingtonensis chiefly occurs at low elevations in sandy soils (Grant, 1924; Cronquist, 1959). Associate species recorded along the John Day River include Astragalus diaphanus, Mimulus guttatus, Penstemon deustus, Phacelia heterophylla, Allium parvum, and Streptanthus cordatus.

Phenology and life history. *Mimulus washingtonensis* is a winter annual, germinating in February or March and flowering in May and June. The species apparently develops seed banks, due to initial seed dormancy, which provide for locally large populations in favorable years. *Mimulus washingtonensis* co-occurs at several sites with *M. guttatus* and may compete with this species for pollinators. An interesting component of the soil biota that is frequently found with *M. washingtonensis* is the cyanobacterium *Nostoc commune*, an organism able to fix atmospheric nitrogen. Soils analyses (C. Wright, unpublished data) have shown that



Fig. 2-16. (scale = 0.3X). Young Mimulus washingtonensis var. washingtonensis plants growing on basalt gravel (late May, Grant County, Oregon).

the basalt substrates inhabited by *M. washingtonensis* can be very low in soil nitrogen.

Mimulus washingtonensis Gand. var. ampliatus (Grant) Meinke, stat. and comb.
nov.—Mimulus ampliatus Grant, Ann. Missouri Bot. Gard. 11:99-388,
1924.—TYPE: USA, Idaho, Nez Perce Co., about Lake Waha, 2000-3500 ft,
27 June 1896, Heller and Heller 3330 (holotype: MO!; isotypes: CU!,
JEPS!, RM!, UC!, WTU!).

Morphologically allied to *Mimulus washingtonensis* var. *washingtonensis*, differing as follows: Weakly puberulent to occasionally glabrescent annual; *leaves* mostly broadly ovate below to somewhat lanceolate above, often coarsely denticulate, (0.6-)0.8-2.7 cm long and up to 1.9 cm wide; *corolla* broadly funnelform, the tube scarcely exserted, the throat ampliate and often appearing ventricose in well developed specimens; *style* glabrous; *chromosome number* unknown.

Representative specimens. USA, Idaho, Idaho Co., east of the Snake River (T26N R1W Sec. 1), 4800 feet, 3 July 1991, *Stein s.n.* (OSC). Lewis Co., Lewyer Canyon, 3 miles north of Ferdinand, 28 May 1940, *Christ 10983* (NY). Nez Perce Co., Lake Waha, 23 June 1890, *Henderson 2675a* (CU, WS); spring banks, ridge above Lake Waha, 12 June 1944 *Christ 14222* (ID, NY, OSC, WS). *Mimulus washingtonensis* var. *ampliatus* is separated from all other taxa within its range by the large, bilabiate corolla, the regular calyx, and the stem and leaf pubescence traits. The morphological differences between var. *ampliatus* and var. *washingtonensis* are consistent but minor, and their distributions are completely separate (Fig. 2-7). These factors support the recognition of the two taxa as infraspecific geographic segregates.

**Distribution and habitat.** *Mimulus washingtonensis* var. *ampliatus* is apparently limited to Idaho, Lewis, and Nez Perce Counties in west-central Idaho (Fig. 2-7), on foothills east of the Snake River. Most collections are known from the vicinity of Lake Waha, southeast of Lewiston, although the most recent collection here is from 1944. This variety was rediscovered in 1991 in Idaho County, at what is presently the only extant location known.

Early labels from Lake Waha collections indicate that *Mimulus washingtonensis* var. *ampliatus* grew in moist sites on basalt outcroppings. The extant population in Idaho County was located in a grazed field on shallow, rocky soil, associated with *Ventenata dubia*. Elevations of collection sites range from ca. 1000-1450 m.

Phenology and life history. *Mimulus washingtonensis* var. *ampliatus* is an annual with large corollas generally similar to those of var. *washingtonensis*. There

are no data available concerning pollination, seed production, or germination requirements for this rare taxon.

Mimulus hymenophyllus Meinke (Figs. 2-17, 2-18, 2-19).—Madroño 30:147-152, 1983.—TYPE: USA, Oregon, Wallowa Co., Horse Creek, ca. 12 km south of the Imnaha River, 1075 m, 21 July 1980, Meinke and Kennison 2656 (holotype: OSC!; isotypes: ID!, NY!, ORE!, UC!, US!, WS!, WTU!).

Annual to rarely biennial herb, growing in masses on cliffs, prostrate to suberect, often hanging, roots yellow to pale orange, stems one to several from the base, fragile, 0.5-3.2 dm long with elongate internodes, sparingly branched throughout, glandular-puberulent with slimy-viscid transparent hairs less than 0.8 mm long, these mostly one- or two-celled and appearing of even length to the naked eye; *leaves* scattered, prominent, cauline and basal, mostly pale green but occasionally anthocyanic beneath, blades thin, broadly lanceolate to ovate, acute, 1.0-3.5(-4.5) cm long, 0.6-3.2 cm wide, denticulate to rarely entire, broadly triangular to cordate at the base, petioles usually 1.5-3.0 times longer than the leaf blades; *pedicels* reflexed or more often ascending, 0.5-1.8 times the petiole length but seldom exceeding the blades, becoming secund in fruit; *calyx* green, weakly glandular, broadly tubular in flower, (2.5-)3.5-5.5(-6.0) mm long at anthesis, campanulate and accrescent in fruit, up to 7.0 mm long and 5 mm wide in fruit, teeth equal, 0.5-1.2 mm long and  $\pm$  deltoid in flower, becoming rounded and mucronate in fruit, ciliate;



**Fig. 2-17.** (scale = 0.7X). Young plant of *Mimulus hymenophyllus*, showing characteristic long petioles, pale yellow corollas, and light sensitive fruiting pedicels (extending away from the stem, above the tip of the thumb).



Fig. 2-18. (scale = 0.4X). Mature plant of *Mimulus hymenophyllus* grown from seed in the greenhouse. Well-watered plants such as this may rarely overwinter in a semi-dormant state if placed outside, and briefly flower the following spring. The species is believed to typically behave as an annual.



Fig. 2-19. (scale = 0.25X). Population of *Mimulus hymenophyllus* growing on moist basalt wall at the type locality, showing heliotropic flowers (early June, Wallowa County, Oregon).

*corolla* narrowly funnelform, moderately bilabiate with a weak lower palate and  $\pm$  open throat, (15-)18-23(-28) mm long, light yellow with scattered red dots and two white patches on the lower lip, puberulent externally with thickened yellow hairs on the inner lower lip, ventrally pubescent within, the tube exserted, the throat moderately flaring, the petal lobes short and spreading, with deep sinuses; *stamens* glabrous, included in the upper corolla throat; *style* glabrous or with a few hairs, included, exceeding the stamens, stigma lips lanceolate, fringed; *capsule* globose to broadly lanceolate, barely included in the calyx tube at maturity, 3.0-6.0 mm long and 2.5-5.7 mm wide, the placentae firmly adherent; *seeds* ovoid to oblong, blunt, longitudinally wrinkled, 0.65-0.90 mm long; *chromosome number* n = 16.

Representative specimens. USA, Oregon, Wallowa Co., along Horse Creek (T2N R48E Sec. 36), 900 m, 20 June 1979, *Leary and Leary 3312* (OSC); ca. 14 km south of the Imnaha River, east slope of Horse Creek, 1150 m, 26 June 1979, *Meinke, Leary, and Bafus 2365* (OSC); Horse Creek area (T1N R49E Sec. 7), 3500 feet, 2 June 1980, *Krantz 210* (OSC); ca. 9 km south of the Imnaha River, cliffs above Horse Creek Trail, 9 June 1987, *Meinke 3321* (OSC, to be distributed).

Mimulus hymenophyllus is the other rock wall endemic in the M. washingtonensis complex. The large, light yellow flowers, elongate petioles, and secund fruiting pedicels are distinctive (Figs. 2-17). Mimulus hymenophyllus has filmy, watery herbage that becomes very thin and papery when pressed. Although initially believed to be a perennial (Meinke, 1983), the species was shown by subsequent study to be annual or rarely a weak biennial (Fig. 2-18). *Mimulus hymenophyllus* has the largest seeds of any species in the complex (up to 0.9 mm long) and possesses the largest pollen grains ( $\sim 35 \ \mu m$  diameter) as well (Argue, 1986).

**Distribution and habitat.** *Mimulus hymenophyllus* is known with certainty only from Horse Creek Canyon, in Wallowa County, Oregon (Fig. 2-6), although unvouchered sightings are reported for cliffs along Cow Creek, a nearby parallel drainage (Meinke, 1983). Two collections from just outside these areas may be of this species, one from near the mouth of the Imnaha River in Oregon (*Henderson, Wellner, and Bingham 1358* [ID]) and the other taken near Pittsburg Landing on the Idaho side of the Snake River (*Mattson and Bishoff s.n.* [IDF]). Additional material is needed before any conclusions can be reached concerning these sites.

Mimulus hymenophyllus is restricted to moist basalt walls, generally with a western exposure (Fig. 2-19). Along Horse Creek, populations are scattered on cliffs above the east bank, mostly in areas of diffuse sunlight. Elevations here range from 850-1300 m. The hills above the cliffs are dominated by native bunchgrasses (Agropyron spicatum and Festuca idahoensis), while the cliffs and the area below are included in a mixed coniferous zone (Pinus ponderosa, Pseudotsuga menziesii, Abies grandis, and Holodiscus discolor). Sedum leibergii, Penstemon triphyllus, P. wilcoxii, Glossopetalon nevadense, and Cystopteris fragilis are common associates of M. hymenophyllus on the basalt.
Phenology and life history. *Mimulus hymenophyllus* produces flowers from late April into July, rarely as late as September depending on moisture availability and exposure. Plants often grow in masses on the cliff walls where flowers are heliotropic at first, but ultimately develop negatively phototropic pedicels that grow away from light after pollination (Figs. 2-17, 2-19). Capsules are thus oriented for seed dispersal into cliff crevices. Since *M. hymenophyllus* is dependent on sexual reproduction, light-sensitive pedicels are viewed as an adaptation to continued survival in a vertical habitat.

Mimulus patulus Penn. var. patulus (Figs. 2-20, 2-21).—Proc. Acad. Nat. Sci.
Philadelphia 99:162, 1947.—TYPE: USA, Washington, Whitman Co.,
Waiwawai, May 1887, Elmer 752 (holotype: PH!; isotypes: MO!, RM!, US!, WS!).

Annual herb, glandular-puberulent, the hairs short and appearing of even length to the naked eye, less than 0.6 mm long, mostly one- or two-celled; *stems* very slender, erect, usually simple, rarely branched above, (3-)5-15(-24) cm tall, with  $\pm$  elongated internodes; *leaves* mostly cauline, few, green above and often anthocyanic beneath, the blades ovate or rarely lanceolate, acute, margins  $\pm$  entire or weakly denticulate, 4-12(-17) mm long, 3-10(-14) mm wide, leaf bases cuneate, petioles (5-) 8-25 mm long; *pedicels* slender, ascending, 1.0-2.5(-3.8) cm long, exceeding the leaves at the upper nodes; *calyx* green at anthesis, occasionally



Fig. 2-20. (scale = 1.8X). Flowers and upper leaves of *Mimulus patulus* var. *patulus*. Corollas drop shortly after anthesis, and anthers dehisce in the bud. Plants were grown from seed in the greenhouse.



Fig. 2-21. (scale = 0.3X). Mature plants of *Mimulus patulus* var. patulus, growing at the only known extant site. Plants grew at the base of basalt walls and boulders, with *M. guttatus* (early June, Wallowa County, Oregon).

anthocyanic in fruit, glandular-puberulent, tubular in flower and fruit, 4.8-7.2 mm long, 1.2-3.3 mm wide, the teeth deltoid, acute or mucronate, 0.7-1.1 mm long, ciliate on the margins; *corolla* funnelform but scarcely bilabiate, sometimes appearing half closed at anthesis, lacking a well-developed lower palate, yellow, occasionally with a few brownish-red specks on the lower lip, 6-10(-12) mm long, the tube narrow and included, the limb 3-5 mm wide, lower lip sparsely bearded with yellow, clavate hairs extending into the throat, or glabrous; *stamens* included, equal with the style, glabrous, usually opening in the bud; *style* glabrous, 2-5 mm long, the stigma included, the lobes lanceolate,  $\pm$  laciniate-margined; *capsule* included, broadly lanceolate, 3.5-5.5 mm long, 1.6-3.0 mm wide, the placentae adherent to the apex; *seeds* tiny, ovoid, brownish, ca. 0.2-0.3 mm long; *chromosome number* ca. n = 16.

Representative specimens. USA, Idaho, Nez Perce Co., near Lewiston, 19 June 1894, *Henderson 2676* (RM, WS). Oregon, Baker Co., Robinette 20 May 1950, *Peck 26000* (CAS) and *Peck 28082* (WILLU); Wallowa Co., Imnaha Canyon, 3 miles above Imnaha, 4 July 1933, *Peck 17494* (CAS, DS, NY, WILLU); Buckhorn Springs, 25 June 1934, *Peck 18282* (DS, NY); south side of Cactus Mountain, 2 June 1979, *Leary 3114* (OSC); base of rock wall along Oregon Highway 3, 0.4 km south of Washington state line, 25 May 1986, *Meinke 3163* (OSC). Washington, Asotin Co., near the Grande Ronde River, south of Asotin, 27 May 1949, *Baker 5999* (NY). Whitman Co., along the banks of the Snake River, at Almota, September 1897, Piper 2791 (WS). "Washington Territory," 1883, Brandegee 1014 (PH).

This species is the basis for Cronquist's (1959) report of *Mimulus washingtonensis* from southeast Washington. *Mimulus patulus* is distinctive in having long-petioled, ovate leaves, and a small, weakly bilabiate corolla that lacks a conspicuous lower palate. Floral differences between this species and the largeflowered *M. washingtonensis* are pronounced (see Fig. 2-10a) and remain evident in greenhouse grown plants. The shorter, even pubescence and tubular fruiting calyx of *M. patulus* serve to separate it from *M. floribundus*.

An unusual culture of *Mimulus*, originating from the type locality for *M*. *hymenophyllus*, bears mention. The plants appeared spontaneously in the greenhouse in early August, in soil collected the previous June for use in cultivating *M. hymenophyllus*. The flowers were tiny (< 4 mm long) and closed at anthesis, the leaves were large (15-25 mm long), broadly ovate, and short-petioled, and the internodes compact (Fig. 2-22). The plants were closest to *M. patulus* in floral morphology, but came from a *M. hymenophyllus* site. It is possible that inadvertently collected seeds of either species were stimulated to germinate following soil disturbance and watering, with the late summer photoperiod then inducing an unusual growth form. However, purposely cultivated plants of *M. patulus* and *M. hymenophyllus* were also growing in the greenhouse at the same time, and these



Fig. 2-22. (scale = 0.7X). Unidentified *Mimulus* sp. that grew spontaneously in soil collected at type locality of *M. hymenophyllus* in Wallowa County, Oregon. The plants were cleistogamous, but had features of *M. patulus* and *M. hymenophyllus*. See text for discussion.

appeared normal in every respect. The enigmatic plants were never observed in the field, and their relationships remain unclear.

**Distribution and habitat.** *Mimulus patulus* has been reported from springs and rocky riverbanks in the Snake River drainage of northeastern Oregon, extreme southeastern Washington, and immediately adjacent Idaho (Fig. 2-7). Peck (1961) reported the species from Lake County, in southeast Oregon, based on specimens of *M. floribundus. Mimulus patulus* ranges from ca. 1300 m, along the rim of lower Hell's Canyon in Oregon, down to ca. 250 m along the Snake River in Idaho. There is evidence that *M. patulus* prefers basalt substrates, based on field observations (Fig. 2-21). Associate species include *Tonella floribunda, Astragalus arthuri, Mimulus guttatus, Bromus tectorum*, and *Ribes* spp.

Phenology and life history. Examinations of floral biology showed that *Mimulus patulus* is a highly autogamous species (Fig. 2-3). Flowers open only after anthers have dehisced, and stigmas are generally pollinated before anthesis (Fig. 2-20). Seed set is consistently high in this species, but outcrossing is probably very limited. *Mimulus patulus* is a winter annual, with innate seed dormancy broken by cold temperatures (Meinke, unpublished). Germination occurs in late winter or early spring, with plants flowering from May to early July.

Mimulus patulus Penn. var. montanus Meinke, var. nov.—TYPE: USA, Idaho, Benewah Co., forks of the St Mary's River, Coeur d'Alene Mountains, 4 July 1895, Leiberg 1167 (holotype: US; isotypes: MO, RM).

Herba annua nana, 3-8(-12) cm alta; *caule* glanduloso-pubescenti et minute hispida; *foliis* ellipticis vel ovatis, denticulatis, lamina 4-12 mm longa, petiolo lamina plerumque longiore; *pedicellis* tenuibus,  $\pm$  ascendentibus, curvati; *corolla* flava, 4-8 mm longa, calyce 1.5-2.0 plo longiore, tubo inserto; *stylo* glabro, incluso; *capsula* inclusa.

Morphologically allied to *Mimulus patulus* var. *patulus*, differing as follows: Plants annual, 3-8(-12) cm tall; *stems* glandular-puberulent as well as minutely hispid; *fruiting pedicels* elongate and horizontal to  $\pm$  ascending, often arcuatespreading; *corolla* 4-8 mm long, tube inserted; *chromosome number* unknown.

Representative specimens. CANADA, Alberta, Waterton National Park, 11 July 1948, Underhill s.n. (PH). USA, Idaho, Benewah Co., at Santa, 7 June 1927, Epling, Wyckoff, and Offerd s.n. (PH). Bonner Co., 2 miles east of Thame Ferry, along Pend Oreille River, 18 June 1944, Christ 14247 (NY). Idaho Co., Selway Falls, 20 miles east of Lowell, 3 June 1949, Christ 18261 (ID, WS); above junction of Meadow Creek and Selway River, 3 June 1954, McMullen 1545 (ID). Shoshone Co., 2 miles below Bullion Pass, 15 July 1955, Baker 13477a (ID). Montana, Flathead Co., MacDonald Lake, 7 August 1893, *R. Williams 343* (US, RM); Going-to-the-Sun, Glacier National Park, 20 July 1932, *McLaughlin 2537* (PH). Oregon, Wallowa Co., Buckhorn Springs (on herbarium sheet with var. *patulus*), 25 June 1934, *Peck 18282* (WILLU, NY); Wallowa Mountains, 2 July 1963, *Mason 6188* (OSC). Wyoming, Park Co., 0.5 mile east of Pahaska along Highway 14, 13 July 1981, *Evert 3136* (RM). Teton Co., Leigh Lake, Grand Teton National Park, 2 August 1932, *L. Williams 992* (RM); mouth, North Fork Teton Canyon, 25 June 1956, *Anderson 366* (RM, UC).

*Mimulus patulus* var. *montanus* is an upper elevation phase of the species, which differs mainly in possessing minute, eglandular hairs on the herbage (Fig. 2-23) that are mostly mixed in to some degree with short glandular puberulence. Specimens of the new variety also commonly have smaller flowers and horizontally spreading, arcuate fruiting pedicels, which are best seen in well developed plants. The differences are minor but correlate with a geographic range largely allopatric with that of var. *patulus*. The two taxa overlap only in the Wallowa Mountains of northeast Oregon. In western Colorado, along the east slope of the Rocky Mountains, *Mimulus* populations occur that appear to have characteristics of *M*. *patulus* var. *montanus* and *M. floribundus*. Observations of the latter species have shown it to be extremely polymorphic throughout its range, and further study is needed to determine the relationship of these taxa.



**Fig. 2-23.** *(see sidebar for scale).* Scanning electron micrograph of the stem of *Mimulus patulus* var. *montanus.* The minutely hispid, eglandular pubescence is diagnostic, and is usually mixed with glandular puberulence.

**Distribution and habitat.** *Mimulus patulus* var. *montanus* extends from the Wallowa Mountains of extreme northeastern Oregon to the Rocky Mountains of Alberta, Montana, and northern Wyoming (Fig. 2-7). Little is known concerning the habitat for this variety, although herbarium labels report moist, rocky sites above 1200 m, often near streams or rivers.

Phenology and life history. Herbarium data show *Mimulus patulus* var. *montanus* to be in bloom from June through early August. No information is available concerning life history aspects, although morphology suggests an autogamous breeding system comparable to var. *patulus*.

Mimulus pulsiferae Gray (Figs. 2-24, 2-25).—Proc. Am. Acad. Arts 11:98, 1876. —TYPE: USA, California, Indian Valley, 1873, Ames 21 (holotype: GH!).

Annual herb, glandular-puberulent, the hairs short and appearing of an even length to the naked eye, less than 0.8 mm long, mostly one- or two-celled, moist to the touch; *stems* slender, erect, one to few arising from the base, simple or sparingly branched above, (3-)5-12(-18) cm tall, the first and sometimes second node after the rosette often more elongated than the rest; *leaves* basal and cauline, a small rosette present on juvenile plants that typically persists in adults, blades rarely anthocyanic beneath, ovate to usually oblanceolate, often narrowly so, acute to  $\pm$  obtuse, margins entire or with a few coarse teeth, 3-14(-23) mm long, 2-9(-15) mm



**Fig. 2-24.** (*scale* = 1.6X). Mature plant of *Mimulus pulsiferae* growing in volcanic soil. The basal rosette is partially obscured in this photograph (early June, Jefferson County, Oregon).



Fig. 2-25. (scale = 3X). Corollas of *Mimulus pulsiferae*. Corollas are weakly bilabiate to nearly regular. These were photographed in mid-morning after corolla lobes had fully opened (Jefferson County, Oregon).

wide, leaf bases cuneate, rarely subsessile at upper nodes, usually with ascending petioles 2-9 mm long; *pedicels* slender, horizontally spreading and arcuate in fruit, 1.4-3.8 cm long, exceeding the leaves; *calyx* green at anthesis, glandular-puberulent, tubular in flower and fruit, 5.0-7.5(-8.5) mm long, 1.5-3.9 mm wide, to 9.5 mm long in fruit, becoming accrescent and glabrescent with age, the teeth triangular, acute, 0.9-1.1 mm long, ciliate on the margins; *corolla* weakly bilabiate, funnelform, closing at night, lower palate not extended, yellow, with reddish dots on the lower lip (5-)7-13(-15) mm long, the tube  $\pm$  exserted, the limb 4-9 mm wide, lower palate bearded with yellow, clavate hairs extending ventrally into the throat, corolla otherwise glabrous; *stamens* included, about even with the style, glabrous; *style* glabrous, 3-8(-10) mm long, stigma lobes lanceolate, scarcely fringed; *capsule* nearly equal to or longer than the calyx, narrowly oblong, 4-11 mm long, 3-4 mm wide, the placentae adherent to the apex; *seeds* ovoid or broadly oblong, brownish, ca. 0.4-0.5 mm long; *chromosome number* n = 16.

Representative specimens. USA, California, Butte Co., near Cohasset, 12 April 1915, *Heller 11805* (CAS, CU, DS, NY, OSC, PH); Jonesville, 6 July 1931, *Copeland 687* (DS, NY, ORE, RM, US). Eldorado Co., Welber Creek, east of Camino, 21 May 1962, *Bacigalupi and Heckard 8572* (JEPS). Lake Co., between Kelseyville and Lower Lake, 5 May 1934, *Baker 7632* (CAS). Lassen Co., 12 miles north of Fall River Mills, 26 May 1940, *Hitchcock 6611* (NY, WTU, UTC); Westwood, 14 June 1943, *Ripley and Barneby 5735* (PH). Modoc Co., Egg Lake,

10 June 1980, Heckard 5228 and Bacigalupi (JEPS). Napa Co., La Jota Plateau, 8 May 1893, Jepson 59m (JEPS). Nevada Co., Bear Valley, 21 July 1898, Jepson 57m (JEPS); Grass Valley, 28 May 1933, Rose 33206 (PH, RM, WTU); Hell's halfacre, west of Nevada City, 6 May 1976, McNeal 1906 (BRY). Placer Co., Shirttail Creek, Tahoe National Forest, 20 July 1952, Stebbins 3881 (JEPS). Plumas Co., 5-6 miles east of Chester, 10 June 1940, Pennell 25787 (PH). Shasta Co., Goose Valley, 11 July 1912, Eastwood 756 (DS, NY, PH); west of Buckhorn Lodge, Route 299, 7 June 1940, Pennell 25707 (JEPS, NY, PH, US); Hatchet Mountain, 10 June 1940, Rose 40486 (PH); Mount Shasta, 5 July 1940, Cooke 15242 (OSC, PH). Siskiyou Co., Sisson, 10 June 1897, Brown 322 (PH); Metcalf's Ranch, base of Mt. Eddy, 20 June 1919, Heller 13271 (NY, PH); Mount Eddy, 17 July 1921, Heller 13573 (WTU). Solano Co., Ridge Road, 2.4 miles north of Mix Road (Vaca Mountains), 25 may 1979, Willoughby 4273 (JEPS); Tehama Co., Battle Ground Meadows, 23 June 1925, Grinnell s.n. (JEPS). Trinity Co., 0.5 mile north of Wildwood, 12 June 1951, Bacigalupi 3372 (JEPS). Tuolumne Co., Twain Harte Post Office, 3 June 1944, Alexander and Kellogg 3624 (DS, JEPS, PH). Oregon, Crook Co., Grizzly Butte, 18 June 1894, Leiberg 275 (ORE). Deschutes Co., 1 mile north of Sisters, 1 July 1985, Magee and Meinke 3006 (OSC). Douglas Co., Cougar Bluffs, 6 June 1979, Fosback s.n. (OSC); Jefferson Co., Camp Sherman, 26 June 1949, Peck 25879 (WILLU) and 4 July 1982, Chambers 5001 (OSC). Jackson Co. 4 miles west of Prospect, 26 May 1924, Sherwood 910 (PH, WILLU); (labelled as Douglas Co.), 20 miles west of Crater Lake near Abbott Butte, 22 June

1972, Mitchell 103 (RM). Klamath Co., near head of Denny Creek, 3 July 1927, Applegate 5152 (DS, WILLU). Lane Co., Castle Rock, 15 June 1991, Dimling s.n.
(OSC). Washington, Klickitat Co., Falcon Valley, June 1885, Suksdorf 793 (DS, JEPS, US, WS); Trout Lake, 26 July 1886, Suksdorf s.n. (G).

*Mimulus pulsiferae* is usually easy to recognize, even when immature, due to the small flowers, weakly bilabiate flowers (Fig. 2-25), and elongated early internodes. The small basal rosette is also diagnostic, although this is often shrivelled and sometimes crumbled or broken off on herbarium sheets. In addition, no other species has capsules that equal or exceed the length of the calyx (Fig. 2-9). The corollas of *M. pulsiferae* are somewhat nyctitropic and are often partially closed at night and in the early part of the day. This is unique in the *M. washingtonensis* complex and not reported elsewhere in the genus.

Within its range, *Mimulus pulsiferae* may be confused with *M. floribundus* and *M. alsinoides*. The latter species has a flaring, bilabiate corolla (usually with a large red blotch), an irregular calyx with elongated, more or less fused lower teeth, and dilated petioles. *Mimulus floribundus* has a corolla similar to *M. pulsiferae*, but is differentiated by multicellular pubescence, urceolate fruiting calyces, and (usually) strongly denticulate leaf blades.

Specimens of what may be *Mimulus pulsiferae* were collected in June, 1883, near Hyampom in Trinity Co., California (*Rattan s.n.*; DS 400963, 49215, 49216

and CAS 414279), in the outer Coast Range. It is unique in having more or less inflated fruiting calyces and distinctly purplish corollas.

**Distribution and habitat.** Along the east base of the Cascade Mountains *Mimulus pulsiferae* ranges from extreme southern Washington to southern Oregon (Fig. 2-6). On the west slope of the Cascades the species occurs from Lane County, Oregon to California, where it extends to the central Sierra Nevada and occasionally the northern Coast Range. The elevational range for the species is ca. 300-1500 m in Oregon and Washington, rarely extending to 2500 m in California. *Mimulus pulsiferae* typically grows in damp depressions or on vernally moist, exposed slopes, usually in or near coniferous forest. Associate species include *Githopsis specularioides, Linum digynum, Mimulus moschatus, Eriogonum compositum, Antennaria neglecta, Penstemon* spp., and *Trichostema oblongum*.

Phenology and life history. *Mimulus pulsiferae* is an annual that germinates in the winter, forming a weak basal rosette. The species is unusual in the *M*. *washingtonensis* complex in possessing flowers that produce an appreciable quantity of nectar, which often evaporates by afternoon. In Oregon, this attracts a species of long-tongued beefly (*Oligodranes* sp.) as the apparent primary pollinator, which usually visits flowers in the morning shortly after corolla lobes begin to open. All other species in the *M*. *washingtonensis* complex appear to be visited primarily or exclusively by various pollen-gathering Hymenoptera. *Mimulus pulsiferae* plants are relatively efficient self-pollinators (Fig. 2-3), but field observations revealed numerous insect visits to flowers on sunny days. The species blooms from late April into July, with phenology for any given year or site dependant on soil moisture and elevation.

## **TAXONOMIC RELATIONSHIPS**

A systematic study based solely on morphology, reproductive biology, and habitat cannot provide definitive information on the phylogeny of a group of species. It can, however, contribute valuable information that may be used to produce a classification for general taxonomic use. Data at the level of the whole organism are also important to the development of evolutionary hypotheses, which are often the basis for taxonomic studies involving molecular methods.

The species of the *Mimulus washingtonensis* complex appear closely related based on morphology, chromosome number, distribution, and habitat. However, it is by no means certain that the species represent a monophyletic group. *Mimulus jungermannioides* has been included in this study primarily because of its long style, short pubescence on the stolons, and large, bilabiate corolla that closely resembles that of *M. washingtonensis*. This species is otherwise similar to *M. floribundus* and *M. moschatus* by having urceolate fruiting calyces and villous, multicelled pubescence. The life cycle of *M. jungermannioides* is strikingly similar to that of *M. primuloides* (Douglas, 1981). Although found in montane fens rather than on

cliffs, *M. primuloides* also produces summer stolons that develop subterranean, overwintering propagules. No other species in the genus have comparable life histories. In speculating on relationships, it seems more likely that the pubescence traits and calyx shape of *M. jungermannioides* arose independently rather than its complex vegetative life cycle (Fig. 2-13). The former traits may also have been acquired through hybridization, or it may be that *M. jungermannioides* is a relict progenitor of *M. floribundus* and *M. moschatus*. Conversely, *M. jungermannioides* could be a species derived from *M. primuloides* in relatively recent times. During the last glacial periods, *M. primuloides* may have occupied the lower valleys in central Oregon, away from the mountains. Populations could have ultimately become stranded at seeps and springs along cliffs, as the climate became xeric after the last ice sheets retreated. *Mimulus primuloides* was not included with the species in this study because of its cobwebby leaf pubescence, large, nearly regular corolla, scape-like inflorescence, and habitat dissimilarity.

Mimulus pulsiferae also has suspected affinities outside the M.
washingtonensis complex. The species is macromorphologically allied with M.
arenarius, and the two taxa apparently co-exist along a narrow zone in the central
Sierra Nevada. Grant (1924) remarked on the apparent relationship of these species,
and preliminary observations of herbarium material suggest that they may hybridize.
Both species possess a basal rosette, an unusual character not seen elsewhere in the
M. washingtonensis complex. However, they differ in corolla, calyx, and

pubescence features, as well as pollen exine sculpturing (Argue, 1986). Based on these traits, *M. arenarius* appears most closely allied to *M. floribundus*.

*Mimulus hymenophyllus* is an unusual annual species with no obvious relationships. It has been included within the *Mimulus washingtonensis* complex because of the corolla morphology, pubescence type, and chromosome number. Initially, *M. hymenophyllus* was considered most closely related to *M. jungermannioides* (Meinke, 1983), because both species are cliff endemics with similar gross morphology. That proposed relationship seems less likely now based on the broad differences in life history, where *M. jungermannioides* has been shown to be a stoloniferous perennial. The combination of calyx morphology, leaf form, seed size, and seed dispersal mechanism are unique among potential relatives in section *Paradanthus*. Recent studies by Argue (1986) on pollen and seed characters support the distinction of *M. hymenophyllus* from other members of the *M. washingtonensis* and *M. moschatus* alliances.

Mimulus washingtonensis and M. patulus may be the most closely related species of the complex. They differ mostly in leaf morphology and corolla size and shape. Although the two taxa were considered conspecific by Cronquist (1959), greenhouse studies unequivocally demonstrated the differences between them based on corolla morphology and breeding system. *Mimulus washingtonensis* and M. *patulus* have also been differentiated on the basis of seed and pollen microsculpture (Argue, 1986). *Mimulus patulus* may be a selfing derivative of Mimulus *washingtonensis*, arising from var. *ampliatus* (based on distribution and habitat) or

80

var. washingtonensis (based on pubescence). Mimulus washingtonensis var. ampliatus is morphologically closest to M. washingtonensis var. washingtonensis, but their habitats and distribution are dissimilar. Mimulus patulus var. montanus is problematic. While appearing most similar to var. patulus, it may intergrade with M. floribundus in the Rocky Mountains south of Wyoming. The latter species is extremely variable and not well understood, particularly outside of California.

### CONSERVATION CONSIDERATIONS

Distribution and habitat. Rarity and restricted distributions may be signs of endangerment even if species are not actively threatened by land use activities. All of the species in the *Mimulus washingtonensis* complex are rare, and many are very locally distributed. Two taxa, *M. pulsiferae* and *M. patulus* var. montanus, are relatively widespread geographically but are not common. *Mimulus pulsiferae* may be the least jeopardized, as it has been collected a number of times in the southern end of its range in the Sierra Nevada. However, in the Pacific Northwest the species is infrequently recorded and is considered to be in need of monitoring (Oregon Natural Heritage Program, 1991). Natural resource agencies should be made aware of the few localities of *M. pulsiferae* known for public lands and should protect them if possible. Although not an endangered species per se, *M. pulsiferae* populations in Oregon and especially Washington represent a scarce element of the native flora. They typically occur in or near marketable timber zones, and may be impacted negatively by logging activities. *Mimulus patulus* var. *montanus* is also distributed over a relatively broad area, but most of the collections are decades old. Additional inventory for this variety is needed.

Of the remaining species considered here, *Mimulus patulus* var. *patulus* and *M. washingtonensis* var. *ampliatus* are the rarest. Both taxa are currently known from only a single population, each of these reported to have fewer than 150 plants. The two populations are located in unprotected sites, i.e., along a narrow roadside for *M. patulus* and in a heavily grazed pasture for *M. washingtonensis* var. *ampliatus*. Several historic sites for *M. patulus* are now under water along impounded portions of the Snake River. Other known collection sites have been searched for the species, without success. *Mimulus washingtonensis* var. *ampliatus* was thought to be extinct until its rediscovery in 1991 by Forest Service botanists. Hillsides around Lake Waha in Idaho's Nez Perce County, where most historic collections were gathered, is now largely developed for summer homes. The area is difficult to gain access to, due to the private land holdings.

Mimulus jungermannioides, M. washingtonensis var. washingtonensis, and M. hymenophyllus are narrowly distributed endemics. The latter species occurs in a single narrow drainage, extending intermittently for only a few km. However, it occurs in very remote country and is naturally protected on the cliffs it inhabits. Mimulus jungermannioides also occurs exclusively on cliffs, but many of the known sites are easily approached and are located along busy highways or near popular recreation sites. Road building has probably destroyed populations of this species in the Columbia River Gorge and possibly elsewhere. Lowering of the water table, caused by repeated years of low rainfall and possibly agriculture drawoff, may eliminate some of the seeps that now support populations. *Mimulus washingtonensis* var. *washingtonensis* occurs in or near riparian areas, on thin soils subject to disturbance by livestock. Pools that accumulate near seeps in the basalt bedrock attract cattle, which in turn may trample fragile substrates supporting *M*. *washingtonensis* populations.

*Reproductive biology and genetics.* The pollination biology and seed germination requirements of rare species can be important considerations in determining suitable management and recovery programs. Preliminary work conducted during this study showed that some of the species in the *Mimulus washingtonensis* complex are highly self-pollinated, while others may be more apt to outcross. An earlier study (Meinke, unpublished) revealed variation in seed germination strategies within the complex as well. Both aspects of biology would be important to consider should species or populations become extinct and reintroduction be contemplated.

Based on the results of the breeding experiments, the taxa studied here apparently benefit from insect mediated pollination. The only possible exception was *Mimulus patulus*, which seemed chiefly autogamous. If attempts were made to reintroduce a species in the field, enough seeds would need to be placed to promote populations large enough to attract potential pollinators. Although all the species are capable of autogamy, seed set is increased and reproductive vigor presumably enhanced by outbreeding. The long term prospects for populations would thus be enhanced. Artificial seed banking should include accessions from as many populations as possible to ensure genetic variability at reestablishment sites. Time of reintroduction is probably also important. Seeds of species with innate dormancy should be sown in the late fall, to ensure exposure to dormancy-breaking cold temperatures while minimizing pre-germination seed pool attrition. Another approach would be to germinate seeds in the lab or coldframe and then reestablish populations using seedlings. However, transplanting such tiny plants is often difficult.

The reproductive biology of *Mimulus jungermannioides* demands special consideration. Although individual plants of the species often produce thousands of seeds over a field season, few plants are apparently established via sexual reproduction. Most are initiated vegetatively by overwintering turions. Plants readily produce turions in cultivation, and this would provide a sure and easy method to reestablish clones in the field. Since populations of *M. jungermannioides* are mostly clonal, the introduction of turions from one population into another could also provide an immediate boost of genetic variability. However, since seed set does not appear limited in *M. jungermannioides* and sexual establishment is rare, there seems little value in this. A deleterious effect of such gene mixing might be to swamp local genetic differences. Populations are largely isolated, and their clonal nature may be responsible for relatively rapid incorporations of genetic novelties that

have arisen through drift or mutation. Localized patterns of morphological differentiation have been observed among populations of *M. jungermannioides*.

Recommended status. Populations of Mimulus pulsiferae are small and scattered, especially in Oregon and Washington, but the species as a whole is apparently not jeopardized. If agencies are willing it should be possible to manage for the continued existence of this species, even in timbered areas of the Pacific Northwest, with minimal conflict.

Based on available data on distribution and habitat, both Mimulus washingtonensis var. ampliatus and M. patulus var. patulus should be considered critically endangered. Continued inventory of likely habitat is needed to understand the current distribution and biology of these taxa. Too little information is available on M. patulus var. montanus to make any recommendation. Herbarium studies suggest that Mimulus washingtonensis var. washingtonensis has always been scarce, even before domestic grazing became widespread in eastern Oregon. The habitat changes effected by grazing may now be exacerbating the vulnerability of the species by fostering introduced weeds, which compete with M. washingtonensis for scarce moisture and pollinators. Mimulus jungermannioides is not threatened by grazing but could lose populations to road building or drought. Moreover, asexual reproduction may have resulted in selection for unique gene combinations at the various disjunct locations for this species. As a result, the destruction of individual populations may be more of a genetic loss than might be expected for exclusively sexual species. Both M. jungermannioides and M. washingtonensis var.

washingtonensis should be protected as threatened species, with emphasis in future studies placed on genetic studies (for *M. jungermannioides*) and disturbance ecology (for *M. washingtonensis*).

*Mimulus hymenophyllus* is very local, but there are no obvious extant threats to this species other than limited numbers and potential losses due to drought. However, because of its scarcity, and a lack of information concerning pollination biology in nature, it is prudent to maintain the species as a candidate for protection under state and federal regulations. Further studies are needed to determine if the species requires specific insect pollinators and if these may be vulnerable to any current or anticipated land management activities. If additional populations cannot be located, *M. hymenophyllus* may eventually warrant a threatened listing based on distributional considerations alone.

#### CHAPTER 3

# A NEW ANNUAL SPECIES OF *MIMULUS* (SCROPHULARIACEAE) FROM THE INTERMOUNTAIN WEST

*Mimulus breviflorus* is a diminutive, self-pollinating annual occurring primarily east of the Sierra Nevada and Cascade Mountains in the northwestern United States and adjacent British Columbia. Little is known concerning the evolutionary relationships of this species. In the only comprehensive monograph of the genus, Grant (1924) placed *M. breviflorus* in section *Paradanthus*, an assemblage of small, problematic species groups that are considered difficult to align taxonomically and probably paraphyletic (Argue, 1980). Indeed, in a proposed phylogenetic chart Grant (1924) affiliated the yellow-flowered *M. breviflorus* with the *M. moschatus* alliance, while in the text of her paper she associated the species with members of the *M. inconspicuus* group, particularly the white to pinkishflowered *M. latidens*.

The proposed relationship between *Mimulus breviflorus* and *M. latidens* is largely based on shared features of the corolla and calyx. Both species possess short, inconspicuous corollas and strongly plicate, chartaceous fruiting calyces that are inflated with age. Although inflated calyces are also described for some members of the *M. moschatus* complex (Grant, 1924; Munz, 1959; Holmgren, 1984), the consistently reduced, essentially regular flowers of *M. breviflorus* and *M. latidens* are unlike any species in that group. The calyx morphology and texture of the two species is also different, being singularly reminiscent of *M. inconspicuus*  and its suspected relatives (Grant, 1924), and the general habit of *M. breviflorus* and *M. latidens* is more comparable to this group than to any other.

Despite the clear floral similarities, *Mimulus breviflorus* and *M. latidens* are quite distinct with respect to geography and habitat. *Mimulus breviflorus* is a basin and range species, principally occurring in well-drained, rocky environments near rain pools and ephemeral streamsides, often at middle and upper elevations. It has rarely been recorded south of extreme northeastern California, and only then above 2000 m. *Mimulus latidens* occurs on mostly on poorly drained flats or slopes subject to vernal inundation, primarily below 800 m. The species is virtually endemic to California, extending from the Central Valley to northern Baja California. The apparent uncertainty by Grant (1924) over the taxonomic placement of *M. breviflorus* may have stemmed in part from the distribution of the species, which overlaps extensively with several members of her *M. moschatus* complex but is allopatric with *M. latidens* and the *M. inconspicuus* group, all of which are restricted to cismontane California.

During the present study several unusual herbarium collections identified as *Mimulus breviflorus* were located, originating from several scattered stations across the upper Great Basin and its northern periphery. The collections were mostly several decades old, the most recent dating from 1958. Despite the localities and the evidently yellow flowers, the plants were similar to *M. latidens* in many respects. An unexpected opportunity to observe a population of this entity in the field was presented in 1990, during a chance visit to a small reservoir in northern

Lassen County, California. The unique and consistent combination of features noted in the collections, including the bright yellow corollas, were even more conspicuous in the living plants, prompting a re-evaluation of their relationship with *M*. *breviflorus* and *M. latidens*. The anomalous populations are here considered to represent a new species, which is described, illustrated and contrasted morphologically and geographically with potentially allied taxa.

### DESCRIPTION OF MIMULUS EVANESCENS

Mimulus evanescens Meinke, sp. nov. (Fig. 3-1).-TYPE: USA, California,

Lassen Co., 20.5 km east of Adin, beyond north side of Ash Valley Rd., ca. 0.1 km east of the Lassen National Forest boundary, in broken boulders and heavy gravel adjacent to Moll Reservoir, T38N R10E NW<sup>1</sup>/4 SW<sup>1</sup>/4 Sect. 25, ca. 1500 m, 27 June 1990, *Meinke and Kaye 5900* (holotype, OSC; isotypes, NY, UC, MO, UTC).

Herbae annuae, puberulentes,  $\pm$  viscido-villosae; *caulis* tenuis, erectis, (6-)10-25 cm altis, internodiis elongatis; *foliis* late ovatis vel lanceolatis, lamina integerrima vel parce denticulata, acuta, 1.0-3.8 cm longa, 0.7-2.9 cm lata, 3(-5) nervis, base lata, sessili vel subsessili; *pedicel* foliis brevioribus, tenuibus, ascendentibus; *calyce* in statu florifero 3.5-6.5 mm longo, 1.5-3.5 mm lato, in statu fructifero late urceolato, 7.0-11.0 mm longo, 5.0-8.5 mm lato, valde glabro,



Fig. 3-1. Habit drawing of *Mimulus evanescens* Meinke, showing details of leaf morphology, inflorescence, and relationship between pedicel and leaf blade length. Illustration prepared from the type collection and photographs from the type locality.

dentibus ciliati, late triangularibus,  $\pm$  subaequalibus, acutis; *corolla* flava, brevi, 4.0-9.5 mm longa, calyce ca. 1.5 plo longiore, tubo incluso, lobis  $\pm$  aequalibus, patulis, erectis; *staminibus* stylo aequalibus, inclusis, glabris; *stylo* glabro, 3.0-7.8 mm longo, labiis stigmatis laciniatis, subaequalibus; *capsula* inclusa, subglobosa, 4.8-9.0 mm longa, sessili vel substipitata; *seminibus* late oblongis, ca. 0.3-0.6 mm longis.

Annual, + succulent herb, glandular-puberulent throughout (except the calyces), the hairs short and appearing of even length to the naked eye, mostly onecelled (excluding the gland), moist or slimy to the touch; stems slender, erect to slightly decumbent in robust individuals, simple or branched from near the base, often sparingly branched above as well, (6-)10-25 cm tall, with elongated internodes; *leaves* acute, broadly ovate to somewhat lanceolate, 1.0-3.8 cm long, 0.7-2.9 mm wide, evenly distributed, unreduced at the upper nodes, not forming a rosette, the lower ones abruptly petiolate or subsessile, petioles 1-3 mm long, leaves broadly sessile above, blades with 3(-5) primary veins, the margins entire or more commonly denticulate; *pedicels* slender, 8-18 mm long, ascending in flower and fruit, shorter than the leaves in fruit or rarely nearly as long; inflorescence racemose, flowers axillary; calyx green at anthesis, becoming stramineous and anthocyanic along the angles with age, tubular-campanulate in flower, 3.5-6.5 mm long, 1.5-3.5 mm wide, accrescent and broadly urceolate to oval in fruit, 7.0-11.0 mm long, 5.0-8.5 mm wide, the tube chartaceous and glabrous, the orifice

narrowing and becoming somewhat oblique, the angles strongly plicate, the teeth broadly triangular, acute, 0.8-1.6 mm in fruit, ciliate on the margins, barely unequal, the uppermost lobe occasionally appearing slightly longer in some flowers; *corolla* short and with inconspicuous lobes, essentially regular, clear yellow or occasionally with a few tiny brownish dots in the throat, 4.0-9.5 mm long, the tube included or barely exserted, the limb exceeding the calyx by 2-3 mm, lobes short and subequal, erect, entire, glabrous externally, bearded internally with a few  $\pm$  clavate hairs extending in a line from the lower palate into the floral tube; *stamens* included, about equal with the style, glabrous, whitish; *style* glabrous, included, 3.0-7.8 mm long, stigma lips equal or subequal, shallowly laciniate-margined; *capsule* included, subglobose, 4.8-9.0 mm long, extending to ca. 1.0-2.5 mm below the sinuses of the calyx teeth, sessile, or rarely with an abbreviated stipe up to ca. 0.5 mm long, the placentae adherent to the apex; *seeds* ovoid or broadly oblong, brownish, ca. 0.3-0.6 mm long.

The specific epithet is in reference to the vanishing riparian and streamside habitats within Great Basin native range, and the elimination or reduction of many species dependent on these ecosystems.

Paratypes. USA, California, Lassen Co., 10 miles south of Ravendale, 9
June 1940, Pennell 25763 (P); 4.8 miles south of Madeline, 17 June 1958, Raven
and Solbrig 13298 (JEPS); Modoc Co., along Willow Creek, June 1894, Austin s.n.
(UC). Idaho, Owhyee Co., meadow, 3 miles south of Riddle, 1 July 1949,

Holmgren and Holmgren 7973 (CAS, UC, WS, WTU). Oregon, Crook Co.,
Grizzly Butte, 18 June 1894, Leiberg 275 (NY, ORE, US); Gilliam Co., forks of
Cottonwood Canyon, 6 June 1894, Leiberg 156 (NY, ORE, P, US); Grant Co.,
Ochoco National Forest, Graylock Butte, 6 July 1912, Ingram s.n. (RM); Harney
Co., dry watercourse near Frenchglen, 26 June 1942, Peck 21389 (CAS, NY, P,
UC, WILLU).

### TAXONOMIC RELATIONSHIPS

*Phenetic evaluation.* To investigate the phenetic relationships of the new species, a data set was compiled by scoring eighteen vegetative and reproductive character states (Table 3-1) from 114 *Mimulus* collections. Measurements were taken from 15 populations each of *M. latidens* and *M. breviflorus*, and eight populations of *M. evanescens*. Three plants were measured per collection to provide population averages for each quantitative trait, which were used in the data matrices for the analyses below. Sample populations for *M. latidens* and *M. breviflorus* were selected from herbarium collections representing the geographic range of each species. All current and historical sites for *M. evanescens* were utilized.

The data set was used to investigate morphological relationships between *Mimulus evanescens*, *M. latidens*, and *M. breviflorus* via a principal components analysis (PCA). Clustering relationships of sample populations were compared along the first two axes of variation, and graphically displayed. As a second

**Table 3-1.** List of morphological traits measured from Mimulus plants for use in principal components analysis. Thirty-eight study populations were sampled, including 15 each for M. latidens and M. breviflorus, and 8 for M. evanescens. An average measurement was derived for each trait (from 3 samples per population) for use in the analyses.

(1) Presence or absence of a basal rosette. (2) Length of initial stem leaf. (3) Width of initial stem leaf. (4) Length of upper cauline leaf. (5) Width of upper cauline leaf. (6) Base of cauline leaf (sessile versus distinctly petiolate). (7) Peduncle length (in fruit). (8) Calyx length (in fruit). (9) Calyx width (in fruit). (10) Length of calyx teeth (in fruit). (11) Overall corolla length. (12) Corolla color (yellow versus rose, whitish, or ochroleucous). (13) Length of lower corolla lip. (14) Width of lower corolla lip. (15) Length of capsule. (16) Width of capsule. (17) Capsule insertion (base sessile versus distinctly stipitate). (18) Stem and leaf pubesence (clearly glandular-puberulent versus glabrous or subglabrous).

measure of overall dissimilarity, canonical discriminant analysis was performed on the same populations, using only the quantitative characters from Table 3-1. On herbarium specimens the resolution of certain qualitative traits, such as flower color and degree of pubescence, is occasionally open to interpretation, particularly if specimens were poorly preserved after collection. The use of discriminant analysis was an attempt to see if the elimination of diagnostic qualitative traits would result in a weaker phenetic relationship than that depicted by PCA. The multivariate statistical package in STATGRAPHICS (v. 4.0) was used in the two analyses.

As displayed by PCA, the *Mimulus* populations formed three, well-defined clusters conforming to *M. evanescens*, *M. latidens*, and *M. breviflorus* (Fig. 3-2). The first two principle components accounted for 88% of the total variance (Table 3-2), indicating that the PCA plot (Fig. 3-2) is a good gauge of the overall differences among the species. Discriminant analysis, using only quantitative characters, resulted in a similar pattern, although *M. evanescens* clustered somewhat closer to *M. latidens* in the resulting graph (Fig. 3-3). In both analyses, *M. evanescens* is clearly intermediate to *M. latidens* and *M. breviflorus*, although the new species appears to show a slight affinity towards *M. latidens* when qualitative traits are not considered.

Comparisons of vegetative and floral morphology. Upon first inspection, Mimulus evanescens appears to be an exceptionally robust version of M. breviflorus. The yellow, virtually regular corollas, mostly nonstipitate capsules, and short-



First Principal Component (73.9%)

Fig. 3-2. Principal components analysis of *Mimulus evanescens*, *M. latidens*, and *M. breviflorus*. Scatter-diagram generated by plotting the first two factors of variation, showing populations of *M. evanescens* (triangles), *M. latidens* (open circles), and *M. breviflorus* (closed circles). See text for discussion.
Component Number	Percent of Variance	Cumulative Percentage	
1	73.88	73.88	
2	14.18	88.06	
3	3.75	91.81	
4	2.41	94.22	
5	2.12	96.34	
6	1.22	97.56	
7	.57	98.13	
8	.46	98.59	
9	.39	98.98	
10	.29	99.27	
11	.21	99.48	
12	.17	99.65	
13	.12	99.78	
14	.11	99.89	
15	.06	99.95	
16	.05	100.00	

**Table 3-2.** Amount of total variance accounted for by each principal component, in a principal components analysis of morphological variation among populations of Mimulus evanescens, M. breviflorus, and M. latidens.



First Discriminant Function

Fig. 3-3. Discriminant analysis of *Mimulus evanescens*, *M. latidens*, and *M. breviflorus*, based on 14 quantitative characters (see Table 3-1). Scatter-diagram generated by plotting canonical variables, showing relationships of *M. evanescens* (triangles), *M. latidens* (open circles), and *M. breviflorus* (closed circles) populations. The elimination of qualitative morphological characters, included in the principal components analysis (see Fig. 3-2), resulted in a closer relationship between *M. evanescens* and *M. latidens*.

puberulent foliage and stems are traits that are essentially identical in the two species. Since *M. evanescens* also develops papery, inflated fruiting calyces, which is the most prominent character in most specimens of *M. breviflorus*, it is understandable that the identity of the new species has been obscured. The oversized habit of *M. evanescens* is striking, however, and all of the floral and vegetative characteristics are larger, on average, than in *M. breviflorus*. The significance of this proportional difference was evident when contrasting the only known extant population of *M. evanescens* (at the type locality) with greenhousegrown plants of *M. breviflorus*. Although many plants of the new species suffered from insect predation in the field, and appeared underdeveloped, undamaged individuals in moist microsites commonly grew to 2 dm or more. Conversely, the *M. breviflorus* plants cultivated in the greenhouse (originating from three distinct populations in eastern Oregon) never exceeded 12 cm in height. Rather than growing taller with age, they tended to branch out and become unusually floriferous. Although some of the herbarium specimens of *M. evanescens* are not particularly large, it is suspected that this is due to moisture limitation rather than genetic potential, based on observations of living plants.

In addition to the overall size disparity, there are other features that readily separate *Mimulus evanescens* from *M. breviflorus*. Most evident are the leaves, which are ovate to broadly lanceolate in the new species as compared to rhombicovate or narrowly lanceolate in *M. breviflorus*. Moreover, only the lowest leaves of *M. evanescens* are petiolate (Fig. 3-1), and these abruptly so, while leaf blades of *M. breviflorus* narrow gradually into slender, evident petioles at all nodes. In fruit, the pedicels of *M. breviflorus* generally exceed or at least equal the leaf blades. Those of the new species are virtually always shorter, and in some instances the leaf blade exceeds the calyx as well. Finally, the fruiting calyx of *M. evanescens* is much more plicate (Fig. 3-4a), and typically exceeds the length of the mature capsule by 1.5-3.0 mm (Fig. 3-4b). In *M. breviflorus* ripe capsules are approximately the same length as the calyx.

The overall dimensions of *Mimulus evanescens*, as well as the strongly angled fruiting calyx and broad, sessile leaves, are traits that also suggest a strong relationship with *M. latidens*. In fact, *M. evanescens* was shown to be closer to *M. latidens* in one of the numerical analyses (see Fig. 3-3). There are substantial differences between these taxa, however, including flower color, pedicel length in relation to leaf length, stem pubescence, capsule insertion (Figs. 3-4b,c), and the presence of a basal rosette in *M. latidens*. Dissimilarities among the three species are summarized in Table 3-3.

Other small-flowered annuals that might be confused with *Mimulus* evanescens are primarily in the *M. moschatus* complex. These include *M.* floribundus, *M. patulus*, and *M. pulsiferae*. Of these, only *M. floribundus* is characterized as having an inflated fruiting calyx (Grant, 1924), which can be distinguished by multicellular pubescence and narrow, lanceolate teeth. These three species are further differentiated from *M. evanescens* by distinctly petiolate upper leaves and bilabiate corollas. Depauperate annual forms of *M. guttatus* are also to



Fig. 3-4. a. Close-up of fruiting calyx of *Mimulus evanescens*. b. Calyx of *M. evanescens* opened to show sessile capsule insertion. c. Calyx of *M. latidens* opened to show stipitate capsule insertion.

Character	M. evanescens	M. breviflorus	M. latidens
Plant height	(6-)10-25 cm	3-10(-14) cm	10-26 cm
Pubescence	Glandular-puberulent	Glandular-puberulent	Subglabrous
Basal rosette	No	No	Yes
Leaf base	Petiolate at base, sessile above	Petiolate throughout	Petiolate at base, sessile above
Leaf blade shape	Ovate to lanceolate	Elliptic-lanceolate	Broadly ovate
Cauline leaf length	1.0-3.8 cm	0.4-1.7 cm	0.8-3.2 cm
width	0.7-2.9 cm	0.2-0.5(-0.8) cm	0.5-1.7 cm
Pedicels	0.8-1.8 cm long, < the blades	0.5-1.9 cm long, > the blades	1.0-3.3 cm long, > the blades
Fruiting calyx length	7-11 mm	4-8 mm	9-12 mm
width	5.0-8.5 mm	3.0-4.5 mm	6-8 mm
Corolla color	Yellow	Yellow	Whitish, shaded rose or yellow
Corolla length	4.0-9.5 mm	3.5-5.5 mm	9.0-11.5 mm
Capsule insertion	± sessile	$\pm$ sessile	clearly stipitate
Capsule length	4.8-9.0 mm, clearly inserted	4.5-8.0 mm, about equalling calyx	6.0-9.0 mm, clearly inserted
Distribution	Great Basin and vicinity	Great Basin and vicinity	Cismontane California
Elevation	~ 1200-1700 m	300-2900 m	~ < 800 m

Table 3-3. Diagnostic features of Mimulus evanescens, M. breviflorus, and M. latidens.

be expected in moist sites within the range of *M. evanescens*. This common yellowflowered species can be separated by petiolate leaves and strongly zygomorphic corollas. Although the calyces of *M. guttatus* are also markedly inflated, they are distinctly irregular and oriented horizontally in fruit. *Mimulus suksdorfii* is the only other annual monkeyflower in the Pacific Northwest with features comparable to *M. evanescens*. Rarely exceeding 6 cm in height, this compact, freely branched species is further recognized by obtuse, linear-oblong leaves, a cylindrical fruiting calyx, and emarginate corolla lobes.

Distribution and habitat. Mimulus evanescens is distributed widely along the northwestern edge of the Great Basin, ranging from southwest Idaho west through eastern Oregon and south into northeastern California, at elevations from ca. 1200-1700 m. *Mimulus breviflorus* is more widespread and considerably more common. Although inconspicuous even when in bloom, it has been recorded from numerous collections, located throughout much of the northwestern United States east of the Sierra Nevada and Cascade ranges. Outlying populations are known from southern British Columbia and south (rarely) in the mountains to near Lake Tahoe. *Mimulus breviflorus* has a broader elevational range than *M. evanescens*, occurring from roughly 300-2900 m, although usually encountered over 1500 m.

*Mimulus latidens* is essentially a California endemic, distributed below 800 m from the northern Central Valley south to San Diego. The most southerly populations are known from northern Baja California, while four historic collections

from southwestern Oregon originally identified as *M. breviflorus* represent the northern range limits. A recently discovered Great Basin population of *M. latidens*, occurring at ca. 1700 m in Lake County, Oregon (Shelly, 1986), is considered noteworthy as it is the single locality where the range of this species overlaps either *M. evanescens* or *M. breviflorus*. This area is in the flyway for various waterfowl species migrating northeast from central California.

The habitat of *Mimulus evanescens* can only be evaluated from the type locality, adjacent to Moll Reservoir in Lassen County. The plants here were scattered among large rock fragments and alongside small boulders, in well-drained gravelly soil that had apparently been inundated earlier in the spring (Fig. 3-5). This population was observed in 1990 and 1991. Associate species at this site included *Mimulus floribundus*, *Porterella carnosula*, *Collinsia grandiflora*, *Downingia* sp., *Mimetanthe pilosa*, *Heterocodon rariflorum*, *Poa bulbosa*, *Marsilea vestita*, *Bromus* spp., and *Machaerocarpus californicus*. The other sites for *M*. *evanescens* are known only through herbarium labels, most of which provide little, if any, significant habitat information. Indications are that these sites generally resemble the type locality, consisting mostly of rocky streambanks or drying watercourses. *Mimulus breviflorus* occurs in comparable microsites, also frequenting wet, rocky sites that often dry out by late spring or early summer.

**Phylogeny.** Morphologically, *Mimulus evanescens* appears most closely related to *M. breviflorus* and *M. latidens*, and exhibits characteristics of both taxa.



Fig. 3-5. *Mimulus evanescens* growing among boulders at the type locality, along the southwest corner of Moll Reservoir in northern Lassen County, California.

*Mimulus latidens*, in turn, seems to also have a strong affinity to three species comprising the *M. inconspicuus* complex in California (see key below), based primarily on flower color, stipitate capsules, calyx morphology, leaf shape, and glabrous habit. Aside from general vegetative and floral similarities, the inflated, plicate fruiting calyx is the principal trait linking these six species together. Whether or not this feature implies a monophyletic group is open to debate, however, since inflated calyces have evidently arisen independently in *Mimulus* on more than one occasion. Nonetheless, the shape and texture of the calyces of these species do appear distinctive.

The recognition of *Mimulus evanescens* allows for a re-evaluation of the relationship of *M. breviflorus* with the rest of the genus. The morphology of *M. evanescens*, transitional between *M. breviflorus* and *M. latidens*, suggests that the new species could have arisen through hybridization. However, this seems improbable considering the current geographical and ecological separation of the putative parents, and the fact that *M. breviflorus* is highly autogamous. A more likely scenario postulates *M. evanescens* as a descendant of *M. latidens*, with concurrent or subsequent development from it of the smaller-flowered *M. breviflorus*, presumably under selective pressure promoted by the inherent droughty conditions of the Great Basin. *Mimulus breviflorus* is ubiquitous and commonly collected, while *M. evanescens* is apparently rare and widely scattered, providing circumstantial support for this idea. The discovery of the anomalous *M. latidens* 

a method by which this relationship might have developed. If genotypes of *M*. *latidens* capable of survival outside of California's Central Valley were transported to the Great Basin by migrating ducks or geese, the means and opportunity for adaptive radiation would have existed. This phylogenetic hypothesis could be tested using molecular techniques.

*Conservation.* Virtually nothing is known concerning the biology and abundance of *Mimulus evanescens*. It is not encouraging, however, that only nine collections of this monkeyflower are known to exist, with only two of these recorded since 1958 and these from the same general location. The distribution of the species is apparently limited to damp or wet sites within open rangeland, mostly in areas with a long history of intensive grazing by cattle and sheep.

Mimulus evanescens may never have been a common species and this, coupled with its occurrence in a largely unprotected habitat, may be contributing to the overall rarity of the species. As an initial step, *M. evanescens* should be added to federal and state lists of candidate endangered species, which comprise those taxa under formal consideration for legal protection on public lands. Although known from Idaho, Oregon, and California, it is expected that northern Nevada is also within the historic range of the species. Placing the species on candidate lists would bring *M. evanescens* to the attention of land managers and possibly result in specific inventory for new populations. While it is expected that additional field work may turn up further localities for *M. evanescens*, the new species should be considered extremely vulnerable for the present.

### **KEY TO MIMULUS EVANESCENS AND ALLIED SPECIES**

- 1 Plants evidently glandular-puberulent on the stems and leaves; corollas wholly yellow, 3.5-9.0 mm long; capsules sessile; species east of the Sierra Nevada and Cascade Ranges, mostly in the Pacific Northwest
- 1' Plants glabrous on the stems and leaves, or minutely puberulent; corollas predominantly rose-purplish, white, or ochroleucous; capsules short-stipitate; species from west of Sierra Nevada and southern Cascade ranges, primarily in California

  - 3' Calyx teeth obtuse to apiculate, appearing truncate in fruit, less than 0.7 mm long; anthers ciliate; style longer than the ovary
    - 4 Corolla 8-11 mm long, scarcely exceeding the calyx tube .....

- 4' Corolla 13-17 mm long, clearly exserted from the calyx tube
  - 5 Pedicels longer than the leaves; calyx glabrous ...... M. acutidens
  - 5' Pedicels shorter than the leaves; calyx glandular-hairy ...... M. grayi

#### **CHAPTER 4**

## REPRODUCTIVE ECOLOGY OF THE XEROPHYTIC EPHEMERAL MIMULUS WASHINGTONENSIS (SCROPHULARIACEAE)

The monkeyflower genus *Mimulus* is a taxonomically and biologically complex taxon comprised primarily of showy-flowered annual and perennial herbs. Although cosmopolitan in distribution (Grant, 1924), the majority of species are restricted to western North America, with roughly 90 occurring in California and Oregon (Cronquist, 1959; Munz, 1959; Peck, 1961). In the last twenty years, several *Mimulus* species have been the subject of extensive experimental work on genecological variation, gene flow, and crossing barriers, primarily using cultivated populations. These investigations have largely focussed on *M. lewisii* and *M. cardinalis* (Hiesey et al., 1971), and more recently on members of the polymorphic *M. guttatus* species complex (Vickery, 1978; Griffiths et al., 1981; Waser et al., 1982; Ritland and Ganders, 1987; Ritland, 1989; Ritland and Ritland, 1989). Little is known about the biology of other *Mimulus* species, many of which are common and among the most conspicuous elements of the western herbaceous flora.

Only a few studies have looked in detail at natural pollination biology in the monkeyflowers. Böcher and Philipp (1985) investigated reproduction in *Mimulus repens* in New Zealand, and Harris (1979) observed insect-mediated pollination of *M. guttatus* in the Wasatch Mountains of Utah. Dole (1990) studied the significance of autogamy and corolla abscission in *M. guttatus*, a typically outcrossed species (Ritland and Ganders, 1987). *Mimulus* species are of general interest because of

their sensitive, bilabiate stigma lobes that close, usually in a matter of seconds, after receiving tactile stimulation. The ability of stigmas to react thigmonastically to pollinator contact is considered an adaptation that promotes outcrossing in facultatively xenogamous species (Darwin, 1876; Miyoshi, 1891; Newcombe, 1922, 1924; Proctor and Yeo, 1972). The phenomenon, however, has received minimal attention in the recent pollination literature, despite sporadic occurrence in several families of the Scrophulariales.

The present paper describes the pollination ecology of *Mimulus washingtonensis* Gand., a genetically self-compatible, spring flowering annual that germinates in late winter. Plants produce one or more erect stems up to three dm tall, each developing several to many bright yellow flowers 20-25 mm long. Corollas are characteristically bilabiate, with a broad limb and extended lower lip. The lower palate is prominently bearded with thickened hairs, which extend down the corolla tube under the anthers. Stamens are slightly inserted, with anthers aligned along the crest of the corolla directly behind the stigma. Capsules are capable of producing hundreds of small seeds, which are dormant when dispersed and must overwinter prior to germination.

Despite the specific epithet, *Mimulus washingtonensis* is believed to be endemic to the arid John Day River drainage of east-central Oregon. Populations are known from perhaps twenty scattered localities in or near riparian zones, with most of these apparently threatened by the impacts of domestic grazing. All known sites are restricted to nitrogen-poor basaltic gravels, and typically grow in dense patches with few, if any, immediate associates. Patches range in size from a few dozen to several thousand individuals, and the plants attract numerous insects during their brief blooming period in May or June.

*Mimulus washingtonensis* is an appropriate subject for an investigation of pollination biology in *Mimulus* because its floral size and morphology are representative of the larger flowered species that predominate the genus. Moreover, a greater understanding of the reproductive requirements of this rare species may prove valuable in efforts to conserve it and other endemic monkeyflowers in the Pacific Northwest. The purpose of this study was to examine the reproductive ecology of *M. washingtonensis* in relation to pollination mechanisms, pollinator behavior, and fecundity. This was accomplished by (1) observing floral morphology and potential pollinators; (2) studying the role of stigma sensitivity in pollination and seed set; (3) evaluating breeding system efficiency through a series of experimental pollinations; and (4) determining the potential for outcrossing distance to influence mating success.

### MATERIALS AND METHODS

#### Study area and greenhouse studies. Field studies of Mimulus

washingtonensis were conducted in northern Grant County, Oregon (T7S R29E Sect. 2; elevation 1200-1300 m), along an open, south-facing slope located 50-200 m above the John Day River. Local vegetation consists of a *Pinus ponderosa*-

Pseudotsuga menziesii forest interspersed with open slopes of bunchgrass-steppe, the latter characterized by species of Ribes, Amelanchier, Penstemon, Festuca, and Agropyron. Dispersed within the steppe community are horizontal outcrops of basaltic scoria and bare bedrock. These sites often remain moist through the spring from subsurface seepage, and annually they support several patches of M. washingtonensis. Mimulus guttatus was the only species that routinely grew with or adjacent to M. washingtonensis on the basalt substrates, although several others, including Allium parvum, Gayophytum diffusum, Streptanthus cordatus, and Astragalus diaphanus, were occasional associates. Riparian species, primarily Alnus sinuata, Salix exigua, Scirpus nevadensis, and Mimulus guttatus, distinguished nearby swales and sites along the river.

Seedlings of *Mimulus washingtonensis* were collected from the study site to establish greenhouse populations used in experimental pollinations. The greenhouse was kept free of insect pests and unwanted potential pollinators and the temperature maintained between 17 and 30 °C. Plants were grown in a mixture of vermiculite and basalt topsoil collected from the field (1:3), and provided with a single application of a general purpose organic fertilizer five to six weeks after estimated germination. To simulate natural conditions, potting soil was watered twice a week (but not saturated) and inoculated with colonies of *Nostoc commune*, a nitrogenfixing cyanobacterium that frequently associates with *M. washingtonensis* in the field. *Floral structure and nectar production.* Observations of floral morphology were made from wild-collected and cultivated plants. Scanning electron microscopy (SEM) was used to document important morphological features of the stigma and corolla. Tissue samples examined by SEM were fixed in FAA for three hours, then moved through solutions of increasing concentrations of acetone in water and trichlorotrifluroethane (TF) in acetone, 15 minutes per change. From absolute TF, samples were critical point dried and coated with ca. 200 A° of 60:40 Au/Pd alloy in a vacuum evaporator prior to examination.

Calibrated micropipettes were used to measure the volume of floral nectar. Eight field populations, in addition to the one at the main study site, were sampled to assess the potential for variability in nectar production over geographic range. Fifteen flowers (on separate plants) were examined from each locality, over two or three days. Flowers were sampled in the early morning prior to pollinator activity. Greenhouse grown plants were also checked, to determine if nectar accumulation occurs in the absence of pollinators.

Floral phenology and longevity. Buds and open flowers were observed in the greenhouse to determine the sequence of anther dehiscence and stigma receptivity. Observations were recorded hourly for eight flowers, with stigmas considered receptive when lobes opened. To confirm that open lobes are an indication of stigma receptivity, pollen was placed on unpollinated stigmas of 1) unopened buds; and 2) flowers open for 96-120 hours. After hand pollination, corollas and androecia were removed to eliminate the chance of subsequent autogamy. Flowers were then allowed to mature and inspected for seed development. To investigate the effect of pollination on floral life span, ten handpollinated, emasculated flowers open for 1-3 hours were compared with emasculated, unpollinated flowers on the same plants. A mean floral age (hours from initial corolla expansion to abscission) was recorded for each group. Pairwise *t*-tests were used to verify significant differences between seed sets in the two hand pollination experiments.

*Floral visitors.* Approximately 100 hours were spent during the months of May and June observing floral visitors to *Mimulus washingtonensis*. A census of all floral visits was made during 27-31 May, 6-10 June, and again from 20-23 June, between ca. 0900 and 1600 hours each day. Observation time was divided equally between four stations at the study site. Pollinator visits were tallied by species, with the duration of selected visits measured with a digital timer. The relationship between floral age (in days) and foraging time on previously unvisited flowers was evaluated for primary pollinators. Plants were caged and then systematically opened to allow exposure of different aged flowers to pollinators. Data were evaluated by correlation analysis and linear regression. The quantity and species composition of pollen loads carried by predominant floral visitors were estimated by microscopic visual examination with a hemocytometer. Pollen grains adhering to captured insects were removed for examination after rinsing in 10% ethanol.

Stigma sensitivity and seed set limitation. Mimulus washingtonensis plants in the greenhouse were studied to determine if a correlation existed between floral age and stigma lobe sensitivity. Forty flowers from separate plants were tagged, and emasculated as corollas opened in the morning. The lower, unpollinated stigma lobes of each were then briefly stimulated with a camel hair brush. A digital timer was used to measure the time required for complete stigmatic closure, as well as the time necessary for stigmas to reopen after selected stimulations. This was repeated over five days (between 1000 and 1400 hours) until corollas senesced. Observations of pollinators contacting stigmas were made at the study site.

Sensitive stigmas may limit reproduction if lobes remain closed after a single pollen application. The potential for this was investigated by comparing seed sets among a series of greenhouse crosses that varied in terms of the pollen quality and quantity used in pollination. *Mimulus washingtonensis* plants used in the experiment were transplanted from a single patch in nature. Flowers had corollas and androecia removed prior to pollinations to avoid stigmatic contamination. Open stigmas were given one of four pollen applications, as follows: 1) a large dose of conspecific pollen (2500-3000 grains); 2) a small conspecific dose (40-50 grains); 3) a mixed dose of 2500-3000 grains consisting of ca. equivalent parts pollen from *Mimulus washingtonensis* and *M. guttatus*, a suspected competitor for pollination at the study site having a comparable floral morphology; and 4) a large dose of *M. guttatus* pollen (2500-3000 grains). *Mimulus guttatus* plants were grown from seed in the greenhouse. The size of the pollen grains of this species closely approximates those

of *M. washingtonensis*, but can be distinguished by their irregularly synaperturate, often spiraperturate exines (pollen Type I of Argue, 1980). Pollen grains of *M. washingtonensis* are tricolporate, with longitudinal endoaperturate slits (pollen Type II). After pollination, 10 flowers from each pollen application treatment were allowed to mature fruit without additional manipulation. Two additional sets of 10 flowers per treatment had stigmas reapplied with pollen after 2-3 hours, with all flowers receiving a large conspecific dose (2500-3000 grains). In one set, closed stigma lobes were gently separated (if necessary) with fine-tipped forceps prior to the supplemental pollination. In the other set, pollen was merely applied to the outside of the lower stigmatic lobe, approximating insect pollination of a recently visited flower. Mean seed sets were then compared for significant differences by Duncan's Multiple Range Test.

Breeding system efficiency. Experiments were conducted to compare the seed set of Mimulus washingtonensis under different potential breeding conditions. Thirty flowers from ten or more plants were used in each of the following tests, with seeds counted 3-5 days before expected capsule dehiscence. To check for agamospermy, flowers of cultivated plants were emasculated in bud and left to mature in the greenhouse. The level of seed set after autogamous self-pollination was determined for flowers grown under field conditions and in the greenhouse. Wild plants growing on open slopes were covered with fine-meshed cages that excluded insect pollinators but otherwise permitted exposure to the elements, while

greenhouse plants were allowed to develop seeds free of disturbance. Mean seed sets were also recorded for flowers that had been manually selfed (in the field and greenhouse), with 2500-3000 pollen grains applied per stigma. To estimate outcrossing levels, seed sets were measured for open-pollinated flowers that had been emasculated as corollas expanded, and open-pollinated, unmanipulated controls. All field pollination trials were conducted on plants occurring within a single patch of ca. 1200 plants. Seed sets were compared for significant differences by a Duncan's Multiple Range Test.

Eight flowers, each from a separate greenhouse plant, were used to estimate a mean pollen/ovule ratio for the species (Cruden, 1977). The contents of one unopened anther per flower were macerated in a solution of cotton blue in lactophenol. The pollen/ovule ratio was calculated by estimating the total number of grains in the eight anthers (using a hemocytometer), multiplying this figure by the number of stamens per flower (four), and then dividing by the total number of ovules produced.

Dole (1990) demonstrated that delayed selfing of unpollinated *Mimulus* guttatus flowers may occur, when residual pollen is transferred from anthers to stigma lobes as corollas abscise. Due to similarities in floral morphology, it was it is possible or likely that the same mechanism may also promote autogamy in *M*. washingtonensis. In addition, a second means of delayed self-pollination in *M*. washingtonensis was speculated to occur, when pollen becomes trapped in the dense pubescence lining the base of the corolla after falling from open anthers. Selfing

could result if the stigma lobes brush this area when corollas abscise. To evaluate the relative importance of floral papillae in promoting self-pollination in M. washingtonensis, nearly senescent flowers from greenhouse plants were examined for the presence of pollen in the floral tube. Sections of upper corolla tube containing the papillae (not including the external palate) were excised and washed in an aniline blue solution to reveal pollen grains. In addition, an experiment was designed to test the relationship between corolla pubescence, corolla abscission, and delayed self-pollination. Seed sets were compared between (1) flowers with newly dehisced anthers that were induced to drop corollas only 8-10 hours after anthesis, by gently pinching the receptacle and then exposing the plants to breeze from a greenhouse fan; (2) flowers with the lower, pubescent portion of the corolla tube and throat cut away when flowers opened, just prior to anther dehiscence; and (3) unmanipulated controls (these were the autogamously pollinated greenhouse flowers from the breeding system trials previously described). Corolla dehiscence in (2) and (3) was allowed to occur naturally, in a wind-sheltered area of the greenhouse.

Outcrossing distance and reproductive success. To investigate the effect of outcrossing distance on seed set in Mimulus washingtonensis, an experiment was conducted at the study site using three distinct patches, or subpopulations, designated as A, B, and C. These were oriented horizontally across a 20-30° incline, with the margin of patch A 28 m from B, and B 39 m from C. No other subpopulations of M. washingtonensis occurred within 140 m. In early May, 20 pairs of juvenile

plants per patch were transplanted to the greenhouse. Paired individuals grew as nearest neighbors in the field ( $\sim$  5-20 cm apart), and were potted together after transplantation. Ten pairs were collected from the upper end of each patch, and ten from 3-5 m downslope, along the lower periphery. Beginning in July, plants were 1) cross-pollinated with their nearest neighbor; 2) cross-pollinated with plants that had grown at the opposite end of the same patch in nature; and 3) reciprocally crossed with plants from the other patches. Ten flowers, each from a different plant, were used per treatment. These were emasculated prior to pollination, with pollen doses estimated at between 2500 and 3000 grains. Seed sets were calculated from nearly mature, indehisced capsules, with treatment means compared by a Duncan's Multiple Range Test.

#### RESULTS

# Floral structure and nectar production. Flowers of Mimulus washingtonensis are borne in loose racemes and oriented horizontally, with the extended lower lip of the corolla functioning as a pollinator landing platform (Fig. 4-1). Here, a central groove is flanked by two lateral palate ridges, each covered with a dense tuft of hairs up to 1.5 mm long. These extend into the corolla and are replaced by progressively smaller papillae (Fig. 4-2), which carpet the ventral surface to a point 2-4 mm beyond the anthers. The two pairs of stamens are unequally inserted in the tube, along the corolla roof, with the anthers separated by



Fig. 4-1. Lateral view of *Mimulus washingtonensis* flower. Note elongate pedicel, protruding lower corolla lip, and exserted stigma lobes situated just above pollinator landing area. Stigma lobes are open, indicating an unpollinated condition. (Scale = 1.5X)



**Fig. 4-2.** Scanning electron micrograph of the lower, inside floral tube of a *Mimulus* washingtonensis corolla. Tens of thousands of clavate papillae are distributed from just inside the corolla orifice to approximately one-third of the way down the tube, occurring directly under the two pairs of anthers.

2-3 mm. The style terminates in an unevenly bilobed, laciniate stigma (Fig. 4-3) situated on the same plane as the anthers. Stigma lobes are slightly exserted from the corolla (Fig. 4-1) and prominently bearded on the receptive inner surface with glandular trichomes (Fig. 4-4). At anthesis, they are spread ca. 150-180°, widening to 220-240° as flowers age. The slightly longer lower lobe ultimately curls back in unpollinated flowers, towards the longer pair of stamens, but does not contact the anthers.

Flowers of *Mimulus washingtonensis* are virtually nectarless. Trace amounts of fluid ranging from 0.01-0.05  $\mu$ l, were measured in the corolla tubes of ca. 10% of the flowers sampled. There were no evident differences in nectar production between field and greenhouse populations, populations from different geographic areas, or samples taken at different times of the day.

Floral phenology and longevity. The timing of pollen release and the initial opening of stigma lobes differed considerably. Anther sacs dehisced 4-8 hours after corollas opened, while stigmatic lobes first separated in the bud 12-24 hours prior to anthesis. *Mimulus washingtonensis* flowers are therefore briefly protogynous prior to becoming cosexual. Hand pollinations confirm that fertilization can occur whenever stigmatic lobes are reflexed, as there was no significant difference in mean seed set associated with stigma age (t = 1.20, P = 0.25, n = 10). Fertilization was found to significantly decrease floral longevity, with corollas of unpollinated



Fig. 4-3. Scanning electron micrograph of the upper one-half of a closed *Mimulus* washingtonensis stigma. This is a dorsal view, showing the slightly shorter upper stigmatic lobe pressed against the lower lobe. Note pectinate margins, particularly of the lower stigma lobe, which aids in the removal of pollen from the bodies of insect visitors as the stigma closes upon contact.



**Fig. 4-4.** Scanning electron micrograph of a frontal view of a half-closed *Mimulus washingtonensis* stigma. Note long, glandular trichomes lining the inner surface of the stigmatic lobes, which brush pollen from pollinators as the stigmas close.

flowers surviving an average of 55.4 hours longer than those of pollinated flowers (40.9 hours versus 96.3 hours, respectively; t = 11.46,  $P \ll 0.001$ , n = 10).

Floral visitors. Fourteen insect species were recorded visiting flowers of *Mimulus washingtonensis*, with eleven carrying at least some *M. washingtonensis* pollen (Table 4-1). The commonly observed seed beetle *Acanthoscelides aureolos* is an unlikely pollinator because of its small size (0.5-1.5 mm), tiny pollen loads (usually < 10 grains), and habit of foraging on the floor of the corolla, well below the stigma. The other insect species are large enough to effect pollination, although species of *Bombus*, *Osmia*, and *Oligodranes* were infrequent, with many plant species represented in the pollen-loads.

The most common floral visitors were two unidentified species of *Dialictus* (Halictidae), small (3-4 mm long) native bees that jointly accounted for ca. 89% of all recorded visits (excluding Coleoptera). Pollen loads, located on the thorax and abdomen, were comprised of 98% *Mimulus* pollen, indicating that during the field studies these insects were apparent facultative specialists on *Mimulus*. Microscopic examinations showed that about 58% of this pollen was from *M. washingtonensis* and ca. 40% from *M. guttatus* (n = 12). Although *M. guttatus* infrequently grew within *M. washingtonensis* patches, the species often occurred as close as 2-5 m away (along moist edges of the basalt outcrops), and was common near the river less than 100 m distant. In areas of sympatry, tracking of pollinators revealed no apparent discrimination in flower selection, suggesting that the bees were

**Table 4-1.** Floral visitors observed on Mimulus washingtonensis along the John Day River, Grant County, Oregon. Observations were made over ca. 100 hours, during peak blooming for the species in May and June. Data in the third and fourth columns represent means and standard deviations, followed parenthetically by ranges. For the third column, all visits recorded in column two were used to generate timing data, except for the Dialictus spp. where n = 240 for the two species together. The pollinator length of visit data were gathered under normal field conditions, and combine visits to previously pollinated and unpollinated flowers of all ages (refer to Fig. 4-5 for information on Dialictus spp. behavior on unpollinated flowers only). Column four refers to pollen grains of M. washingtonensis carried by floral visitors, estimated using a hemocytometer.

Floral Visitors	No. of visits observed	Length of Visit (seconds)	Pollen Carried (grains)
Coleoptera			
Bruchidae Acanthoscelides aureolos	145	NA'	$4.5 \pm 3.8 \ (0-28, \ n = 20)$
Diptera			
Bombyliidae Oligodranes cincturus	23	3.2±2.3 (1-7)	$17.7 \pm 8.6 \ (0-58, \ n = 8)$
<u>Syrphidae</u> Chrysotoxum ventricosum	14	1.4±0.9 (1-4)	0 (n = 6)
Hymenoptera			
Apidae			
Bombus fervidus	6	1.2±0.7 (1-3)	$56.5 \pm 35.7 (15-114, n = 4)$
B. flavifrons	2	1.0 (1)	not sampled
B. huntii	7	1.4±0.6 (1-3)	112 (112, $n = 1$ )
Halictidae			
Dialictus spp. (2)	657	5.8±6.7 (1-33)	$584 \pm 571.1$ (6-1558, n = 25)
Evlaeus sp.	4	1.9±0.7 (1-5)	0 (n = 2)
<u>Megachilidae</u> Ashmeadiella			
spp. (2)	7	$2.4 \pm 1.1$ (1-5)	17 (17, n = 1)
Hoplitis albifrons Megachiles	3	2.7±1.3 (1-4)	66 (66, $n = 1$ )

<sup>1</sup> Duration of visits for this beetle were not timed because they appeared to be sheltering in flowers as opposed to actively foraging. Estimated durations ranged from several minutes to overnight.

specializing on the *Mimulus* floral morphology and color but on neither species in particular. *Dialictus* individuals approach *Mimulus* flowers head on, landing on or near the hairs of the lower corolla lip. The bees then climb up, grasping the style just behind the stigma and enter the flower upside down. In so doing, the bees brush pollen from their undersurface across the stigma lobes as they manipulate the anthers. They remain inverted while inside the corolla, usually turning around prior to exiting the flower. The time *Dialictus* individuals spent foraging was negatively correlated with floral age for unpollinated flowers (Fig. 4-5).

Stigma sensitivity and seed set limitation. The stigmas of Mimulus washingtonensis react thigmonastically to mechanical stimulation and closed rapidly when contacted by floral visitors. There was a strong correlation between stigma reaction time and floral age, with lobes closing completely in an average of 1.9 seconds on the first day after anthesis (Fig. 4-6). By the fourth day, time for complete closure averaged 16.5 seconds. By the sixth day, when most unpollinated flowers abscise, stigma lobes were largely insensitive or showed incomplete closure. When contacted by pollinators, the lower stigmatic lobe moves forward and, aided by its pectinate edge and ciliate inner surface, appears to comb pollen from the body of foraging insects. As the lower lobe folds upwards it overlaps the smaller upper lobe, with the closed stigma ending up pressed against the roof of the corolla. When left unpollinated, *M. washingtonensis* stigma lobes always reopened after



**Fig. 4-5.** Relationship between *Mimulus washingtonensis* floral age and the foraging time of principal pollinators. The data here depict foraging by *Dialictus* (Halictidae) bees, which comprised the vast majority of visits to *M. washingtonensis* flowers during the study. There was a significant negative correlation between the two variables (r = -.87, P  $\leq 0.001$ ), indicating that as flowers age they become less attractive to pollinators. The equation for the linear regression is y = 13.35-2.65x. Descriptive statistics for daily visitation times (in seconds) are as follows, with mean and standard deviation followed parenthetically by the range: Day 1. 12.65  $\pm 4.93$  (5.6-24.4, n = 20); Day 2. 7.40  $\pm 3.63$  (2.3-15.6, n = 20); Day 3. 2.94  $\pm 2.56$  (0.8-10.0, n = 15); Day 4. 1.36  $\pm 0.90$  (0.5-3.6, n = 14); Day 5. 1.02  $\pm 0.85$  (0.2-2.8, n = 10); Day 6. 1.08  $\pm 0.75$  (0.2-2.1, n = 6).



**Fig. 4-6.** Relationship between *Minulus washingtonensis* floral age and closure time for sensitive stigmas. There was a significant positive correlation between the two variables  $(r = .91, P \ll 0.001)$ , indicating that as unpollinated flowers age their stigmas become less sensitive to tactile stimulation. By the third or fourth day, the time required for stigmatic closure exceeds the time an insect spends foraging on the flower (see Fig. 4-5), increasing the potential for self-pollination as insects exit the corolla. The equation for the linear regression is y = -4.72 + 5.29x. Descriptive statistics for daily closure times (in seconds) are as follows, with mean and standard deviation followed parenthetically by the range: *Day 1*.  $1.94 \pm 0.76$  (0.6-4.1, n = 125); *Day 2*.  $4.35 \pm 1.84$  (1.2-9.1, n = 125); *Day 3*.  $10.14 \pm 4.02$  (3.8-19.8, n = 100); *Day 4*.  $16.48 \pm 4.11$  (8.3-25.0, n = 75); *Day 5*.  $22.74 \pm 5.03$  (11.7-37.0, n = 75).

stimulation, with the amount of time required for reopening increasing with floral age (Fig. 4-7).

*Mimulus washingtonensis* stigmas remained completely or partially closed if pollinated with conspecific pollen, but reopened if pure applications of *M. guttatus* pollen were applied. Repollination of flowers with closed stigmas (Table 4-2) did not result in significant increases in mean seed set over that of controls. However, manually reopening and then repollinating stigmas of flowers where initial pollen applications were low resulted in a significant increase. Moreover, flowers pollinated with a mixed species load also showed an increase in seed set if stigma lobes were reopened prior to repollination. A t-test showed the difference between these means (178.6 versus 274.8) to be nearly significant (t = 2.04, P = 0.0506). When only foreign pollen (i.e., *M. guttatus*) was initially applied, stigmas did not remain closed, and controls did not develop seed. If *M. washingtonensis* pollen was added to stigmas previously pollinated with *M. guttatus* pollen, the resulting seed set was significantly lower than in repollinated flowers where no foreign pollen had been previously applied.

**Breeding system efficiency.** Breeding system experiments are summarized in Table 4-3. Flowers tested for agamospermy failed to set any seed. Results of the other tests reveal considerable variation in seed production within and between treatments. Open-pollinated flowers developed significantly more seed than flowers which were autogamously or manually self-pollinated in the field or greenhouse.



Fig. 4-7. Relationship between *Mimulus washingtonensis* floral age and stigma reopening time after tactile stimulation. There was a significant positive correlation between the variables (r = .74, P  $\leq 0.001$ ), a further indication of a reduction in stigma sensitivity with age (also see Fig. 4-6). The equation for the linear regression is  $y = 9.99 \pm 4.96x$ . Descriptive statistics for daily reopening times (in minutes) are as follows, with mean and standard deviation followed parenthetically by the range: Day 1. 12.70  $\pm$  5.37 (5-25, n = 20); Day 2. 21.95  $\pm$  6.05 (7-34, n = 20); Day 3. 27.95  $\pm$  4.88 (15-35, n = 20); Day 4. 28.88  $\pm$  4.22 (18-36, n = 20); Day 5. 33.97  $\pm$  7.09 (16-48, n = 20).
**Table 4-2.** The effect of pollen quality and quantity on pollination and seed production in Mimulus washingtonensis. Three sets of emasculated flowers were manually given one of four initial pollen applications (all outcrossed). Pollinated stigmas remained closed after pollination with any amount of M. washingtonensis pollen, but reopened if only M. guttatus pollen was applied. Control flowers (the first set, in data column 1) were allowed to mature without additional pollination. The second set of flowers (data column 2) was repollinated 2-3 hours after the initial application, by separating the closed stigma lobes and applying 2500-3000 grains of M. washingtonensis pollen. The third set (data column 3) was similarly repollinated, but without any manipulation of the stigmas. The data represent mean seed set per treatment, standard deviation, and range (n = 10 flowers for each combination). Means followed by different superscripts (a, b, or c) are significantly different at the 5% level (Duncan's Multiple Range Test).

	Seed Sets		
Initial Pollen Applications	Control	Supplemental Pollination (1) (reopened stigma)	Supplemental Pollination (2) (closed stigma)
Large dose of pure <i>M. washingtonensis</i> pollen (2500-3000 grains)	270.6 <sup>a</sup>	291.1 <sup>a</sup>	276.8°
	± 99.7	± 82.3	± 82.3
	(112-390)	(140-401)	(117-367)
Small dose of pure <i>M. washingtonensis</i> pollen (40-50 grains)	14.9°	264.7 <sup>a</sup>	15.0°
	± 7.6	± 80.4	± 8.1
	(4-31)	(117-341)	(5-32)
Large dose of mixed pollen, ca. equal parts	178.6 <sup>ª,6</sup>	274.8 <sup>a</sup>	163.5 <sup>a,b</sup>
<i>M. washingtonensis</i> and <i>M. guttatus</i> (2500-	± 115.7	± 94.1	± 85.1
3000 grains combined)	(56-365)	(99-398)	(56-302)
Large dose of pure <i>M. guttatus</i> pollen (2500-3000 grains)	0.0	*	108.8 <sup>b,c</sup> ** ± 13.7 (0-44)

\*/ Not applicable, since application of pure *M*. guttatus pollen did not result in permanent stigmatic closure after pollination.

\*\*/ Stigmas of these flowers were not closed after intial pollination, but are included in this column because they were not manually reopened prior to repollination.

Seed Set **Breeding System** 0.0 Agamospermy Autogamy 23.3<sup>a</sup> ± 32.1 (0-119) Field 15.6° ± 25.0 (0-112) Greenhouse Manual self-pollination 125.7<sup>b</sup> ± 64.3 (29-274) Field 121.6<sup>b</sup> ± 68.9 (17-231) Greenhouse Open pollination (flowers emasculated at anthesis)  $172.4^{\circ} \pm 91.5$  (41-339) 166.6° ± 79.6 (46-313) Open pollination (controls)

**Table 4-3.** Breeding system trials for Mimulus washingtonensis. Data are means followed by standard deviation and range (n = 30 for all tests). Means followed by different superscripts are significantly different at the 5% level (Duncan's Multiple Range Test).

Autogamously selfed flowers produced low seed sets, and quantities did not differ significantly between the field and greenhouse. Seed sets of manually self-pollinated flowers exhibited more variability, but were significantly higher than any of the autogamous treatments. There was no significant difference in seed production between emasculated and unmanipulated open-pollinated flowers, implying that insects are the primary agent in pollen transfer.

The pollen/ovule ratio for *M. washingtonensis* was determined to be 29.9  $\pm$  4.1 (SD) for a sample of eight flowers.

As unpollinated flowers age, pollen grains fall from open anthers and lodge among the clavate papillae lining the corolla tube below (Fig. 4-8). When the corolla finally detaches, usually on the fifth or sixth day after opening, it may hang on the style up to several hours before dropping. During this time the style is often bent in such a way that the stigma lobes may brush the floral papillae. The presence of these tiny hairs, in combination with older, less sensitive stigmas, was important to delayed self-pollination in *M. washingtonensis*. Flowers with corollas where the lower tube had been cut away averaged only  $1.1 \pm 2.5$  seeds per capsule. Where flowers were induced to drop corollas prematurely when stigmas were still highly sensitive, seed set was also meager, averaging  $3.8 \pm 4.1$ . This was significantly lower than controls ( $15.6 \pm 25.0$ ; t = 2.553, P = 0.013), which were allowed to age naturally without manipulation.



**Fig. 4-8.** Scanning electron micrograph of pollen grains imbedded in floral papillae of *Mimulus washingtonensis*. Pollen grains fall from opened anthers inserted above papillae in the corolla tube, and then may be swept up by open stigma lobes as corollas of unpollinated flowers abscise. This mechanism facilitates delayed self-pollination in the event of outcrossing failure.

Outcrossing distance and reproductive success. Within subpopulations of Mimulus washingtonensis there was no evidence of any optimal outcrossing distance (Table 4-4). The amount of seed produced by plants cross-pollinated with nearest neighbors was statistically equivalent to that developed from crosses between more distant plants from the same patch. There was, however, a general tendency for higher levels of seed set to result in M. washingtonensis from within- as opposed to between-patch crosses. In one combination, where plants of subpopulations A and B were crossbred, seed sets were significantly lower than for any of the within-patch crosses. Seed production was lowest when subpopulation B served as pollen donor.

## DISCUSSION

Flowers of *Mimulus washingtonensis* promote outcrossing early in development, but are genetically and mechanically capable of selfing if insect pollinators are not forthcoming. These observations are similar to those reported for other members of the genus (Ezell, 1971; Kiang, 1972; Vickery, 1978; Harris, 1979; Böcher and Philipp, 1985; Dole, 1990) and only a few species are believed to be obligately self-pollinating (Grant, 1924; Kiang, 1973; Ritland and Ritland, 1989; Meinke, in prep.). In addition to the current research, few studies have focused on natural pollination by insects of *Mimulus* (Kiang, 1972; Harris, 1979; Böcher and Philipp, 1985). For those species studied, pollination by smaller solitary or semisocial bees predominates, with visits by bumblebees (*Bombus*) and other large **Table 4-4.** Effect of outcrossing distance on seed production in Mimulus washingtonensis. Three distinct patches (subpopulations) were selected for within- and between-patch crossing experiments. The three subpopulations occurred at the same level across a common slope, with subpopulation A located 28 meters from B, and B 39 meters from C. Data indicate mean seed sets and standard deviation, followed parenthetically by the range (n = 10 for all combinations). For the reciprocal between-patch crosses, a  $\Im$  sign indicates pollen donor. Means followed by different superscripts are significantly different at the 5% level (Duncan's Multiple Range Test).

Pollination Treatment	Subpopulation A	Subpopulation <b>B</b>	Subpopulation C
Crossed with nearest within-patch neighbor	279.9° ± 32.2 (212-326)	258.8 <sup>a,b</sup> ± 45.8 (198-314)	275.9° ± 40.5 (211-345)
Crossed with plant from opposite end of the same patch	285.5° ± 64.7 (190-388)	265.8 <sup>a,b</sup> ± 56.2 (189-345)	296.9 <sup>a</sup> ± 46.3 (235-378)
Crossed with plant from A	_	(Að) 214.1° <u>+</u> 55.5 (145-312)	(Að) 230.2 <sup>b,c</sup> ± 51.4 (154-311)
Crossed with plant from <i>B</i>	(B♂) 151.8 <sup>d</sup> ± 46.0 (89-234)	_	(Bさ) 230.7 <sup>b,e</sup> ± 48.9 (165-311)
Crossed with plant from C	(Cð) 273.8 <sup>ª,b</sup> ± 38.4 (178-314)	(Cඊ) 287.7° <u>+</u> 30.3 (233-345)	_

Hymenoptera comparatively rare and of short duration. Bumblebee visits averaged about a second for *M. washingtonensis* (Table 4-1) and *M. guttatus* (Harris, 1979), and may be largely ineffectual in terms of significant pollen transfer. Since these insects often gather pollen incidentally, while probing for nectar (Faegri and van der Pijl, 1971; Heinrich, 1975, 1976), Harris (1979) suggests that brief, erratic visits to *M. guttatus* merely reflect the efforts of newly emerged nectar foragers that had not yet established floral preferences. Field observations of *M. washingtonensis* during the present study are not inconsistent with this hypothesis.

Structurally, flowers of bee-pollinated *Mimulus* species appear best suited for nototribic pollination, where pollen is deposited on the back of the insect as it enters the corolla, usually in search of nectar. This is the most prevalent mode of pollen transfer in the Scrophulariaceae and related families (Macior, 1974; Faegri and van der Pijl, 1979). Functionally, however, pollination in *M. washingtonensis* and morphologically allied species, such as *M. guttatus* (Harris 1979), is often sternotribic, where small, mostly pollen-gathering bees enter flowers upside down and ventrally contact the anthers and stigmas. Similar mechanisms are reported for certain species in the distantly related genera *Pedicularis* and *Antirrhinum* (Macior, 1970, 1974).

In *Pedicularis*, the evolutionary transition from nectariferous to nectarless breeding systems appears to be correlated, at the species level, with a shift towards sternotribic pollination (Macior, 1970). Such a transition may also be occurring in *Mimulus*. Harris (1979) has convincingly demonstrated a lack of nectar production

in populations of M. guttatus from northern Utah, but this condition is apparently not ubiquitous over the range of the species. Nectar-producing populations of M. guttatus are known from Oregon and California, particularly in coastal localities, where pollinators were primarily bumblebees and occasional hummingbirds (A. Moldenke, pers. comm.). Kiang (1972) similarly reports Bombus as a major pollinator of M. guttatus in the Sierra Nevada of California, and describes visitation patterns suggestive of nectar foraging. Considerable ecological and genetic variation between local races of M. guttatus has been documented (Vickery, 1978), and it is possible this species will select for nectar production at the population level, conceivably in response to the presence or absence of certain pollinator classes. If pollen-foraging bees are long established in an area and are efficient pollinators of Mimulus, energy expenditures for nectar may be unnecessary. However, if populations occur where these insects are rare or absent, as in certain montane and coastal areas, nectar production may be a requisite investment if adequate levels of seed set are to be maintained. Mimulus washingtonensis fits the former scenario, occurring in habitats with halictid-dominated bee faunas.

In self-compatible *Mimulus* species, with floral morphologies that appear adapted for outcrossing, autogamy has been thought to result from incidental contact of anthers and stigmas after anthesis, either before or during corolla abscission (Grant, 1924; Dole, 1990). The current study demonstrates an additional mechanism that facilitates selfing in the event insect mediated pollination fails, wherein pollen grains that fall among the minute, clavate hairs lining the tube and throat (Figs. 4-2, 4-8) are swept up by open stigmatic lobes as corollas wither and drop. Thus, even though anthers may shrivel and lose most of their pollen within three to five days, unvisited flowers can retain corollas for a longer period and still have the potential for delayed pollination and limited seed set.

The retention of older, unpollinated flowers by *Mimulus washingtonensis* may serve an advertising function. Presumably all undamaged flowers, regardless of age, function equally in drawing potential pollinators to a patch. However, once pollinators have arrived older flowers appear less likely to develop xenogamously fertilized seed, based on the relationship between foraging time and floral age (Fig. 4-5). This suggests that maintaining older flowers is valuable in promoting outcrossing at the patch level, since single plants rarely have more than three or four flowers open at a given time.

The outcrossing advantage possessed by the younger flowers is also related to protogyny and the action of the thigmotropic stigmas. Although *M. washingtonensis* has a relatively short female phase, it tends to coincide with initial pollinator activity on sunny days, suggesting that considerable outcrossing occurs at this stage. If a flower begins to release pollen before it is pollinated, as is especially likely to occur on windy or overcast days, insect-mediated selfing of younger flowers is precluded by the rapid closing of the stigma lobes. As flowers age, the time required for stigmatic closure increases (Fig. 4-6). Concurrently, pollinator foraging time decreases (Fig. 4-5), consistent with a gradual depletion of available pollen in the anther sacs. Eventually, as the time required for stigmas to close overtakes foraging

time (in three to four days), the chance of insect-mediated self-fertilization increases.

The adaptive significance most often attributed to sensitive stigmas is their presumed role in promoting cross-pollination (Darwin, 1876; Newcombe, 1922, 1924; Meeuse, 1961; Proctor and Yeo, 1972), where sudden stigma closure reduces the potential for selfing as insects crawl about in flowers of self-compatible species. It has also been suggested that the lobes aid in brushing pollen grains from visiting insects (Meeuse, 1961) and, when closed, provide a protective chamber for pollen germination (Brown, 1913; Newcombe, 1922, 1924).

While the potential for sensitive stigmas to enhance fecundity is apparently high, particularly where inbreeding depression may be a problem, the system is not without its drawbacks. This is especially true for species such as *Mimulus repens* (Böcher and Philipp, 1985) and *M. washingtonensis*, where stigmas are apt to remain permanently closed after initial pollination even if only a few pollen grains are applied (Table 4-2). Under these circumstances, any limitations to pollination resulting from insect behavior are intensified, since flowers are usually dependent on a single pollinator visit to effect seed set. If it is assumed that the quantity of pollen carried by pollinators varies over time, then this should be reflected by seed production if pollen loads are routinely below the level required for maximum fertilization. For example, the amount of seed set by an individual *M. washingtonensis* flower should relate to the developmental stage of the flowers previously sampled by its pollinator. Pollinations mostly preceded by visits to female phase blossoms, or persistent older flowers with little remaining pollen, could be expected to yield fewer seeds than those preceded by visits to flowers with recently dehisced anthers. This may explain much of the variance recorded among seed sets of open-pollinated flowers in *M. washingtonensis* (Table 4-3).

The fact that Mimulus washingtonensis is potentially pollen-limited in nature is clearly demonstrated in Tables 4-2 and 4-3, where open-pollinated flowers produced far fewer seeds than flowers which received large pollen applications by Another mechanism that may lower reproductive success in the species is the hand. inhibition of fertilization through interspecific pollen transfer (Table 4-2). This is because stigmas will remain closed even if conspecific pollen is mixed with foreign pollen. All floral visitors of M. washingtonensis collected during this study carried mixed pollen loads. The most frequent visitors (Dialictus spp.) carried roughly 98% Mimulus pollen, but often as a mixture of grains from M. washingtonensis and the similarly flowered M. guttatus that grew nearby. This suggests that even though the two Dialictus species exhibited high fidelity for Mimulus flowers, they may potentially decrease fecundity, at least in *M. washingtonensis*, by not discriminating between the species in areas of sympatry. Such floral constancy would presumably enhance pollination efficiency in sites where M. washingtonensis and M. guttatus do not coexist. Finally, permanent closure of stigmas after a single pollinator visit potentially restricts the number of male parents in a seed family, reducing the genetic variability of a clutch.

A comparison of *Mimulus washingtonensis* flowers with those of three close relatives in eastern Oregon reveal some interesting relationships. Two of these

species, M. jungermannioides and M. hymenophyllus, also occur in highly localized patches and possess comparatively large, impressive corollas (Meinke, 1983). *Mimulus floribundus*, a common species that is more continuously distributed, has much smaller flowers (5-12 mm). All of these taxa are basically nectarless, genetically self-compatible, and predominantly pollinated by the same suite of small bees, mostly species of Osmia and Dialictus. Field studies of the four species (Meinke, unpubl.) revealed similar pollinator visitation rates as well as nonsignificant between-species differences in numbers of seed produced. Corolla size is evidently not correlated with reproductive success in these species, yet three out of the four species produce large, energetically costly flowers. It may be that the conspicuous flowers of *M. washingtonensis* and the other showy species function as lures for unconditioned nectar feeders, such as bumblebees, whose flight patterns would be more likely to effect long-distance outcrosses than the local foraging of solitary bees. Retention of large flowers may also simply reflect selection for increased heterozygosity through increased outbreeding, since a reduction in corolla size in self-compatible lineages in *Mimulus* frequently results in increased autogamy due to the reorientation of floral organs (Grant, 1924; Kiang, 1973; Vickery, 1978). It should be emphasized that unless habitat quality varies significantly in time, selection for heterozygosity and increased fitness through cross-fertilization is unlikely, in and of itself, to maintain large flowers. Mimulus washingtonensis, M. jungermannioides, and M. hymenophyllus are all restricted to narrow relatively stable, basaltic habitats (Meinke, 1983).

Another factor that may limit the evolution of increased selfing is inbreeding depression (Wright, 1977; Schemske, 1983). Data from Tables 4-3 and 4-4 show that seed production from self-pollinations in Mimulus washingtonensis were consistently reduced compared to those resulting from between-patch outcrosses. This suggests that smaller flowered phenotypes, at least in M. washingtonensis, may suffer a relative fitness disadvantage due to the greater fecundity of larger-flowered morphs that would be more apt to outcross and produce more seed. The edaphic specializations of *M. washingtonensis* and the other the large flowered species, responsible for the patchy distributions and local seed dispersal, further enforce outbreeding by limiting immigration of selfing genes that might neutralize inbreeding depression. Mimulus floribundus, on the other hand is widespread and not substrate specific, often occurring extensively in stream beds or other habitats where hydrochory is facilitated. This suggests that gene flow in this species, possibly in combination with reduced genetic load, has influenced the evolution of a smaller flowered, yet still potentially xenogamous, breeding system.

Information gained by this study, on various aspects of pollination and reproduction, should be helpful in developing a conservation strategy for *Mimulus washingtonensis*. Artificial seed banking, particularly for annuals, is often the only effective means of repopulating rare species that become depleted in the wild (Guerrant, 1992). Any attempt to reintroduce a facultative outcrosser such as *M. washingtonensis* requires an adequate seed reserve to ensure that sufficient genetic diversity is present in new populations. The data here show that inbreeding

depression is a potential hazard for the species. This, coupled with inefficient autogamy (Table 4-3), could hamper long term prospects for small patches. Furthermore, data in Table 4-4 suggest that limited outbreeding depression may also occur in *M. washingtonensis*. Even when patches of the species are in close proximity (i.e., within 30-40 m), outbreeding between them may be rare. The apparent reliance on small bee pollination may confine gene flow to the extent that limited breeding barriers develop over rather short distances. As a result, it seems especially important for seed used in reestablishing *M. washingtonensis* to consist of similar genotypes, preferably originating from the same subpopulation.

The sensitive stigma of *Mimulus washingtonensis* is valuable to the species in promoting cross-fertilization, but it may also limit seed set when pollinated with mixed pollen loads. This problem intensifies when potentially competing species, such as *M. guttatus*, increase in number. Although *M. guttatus* is a native species, it is often weedy and will increase with disturbance as long as substrates remain moist. Disturbances associated with cattle grazing, for example, often leads to conditions that allow this species to proliferate. Table 4-2 demonstrates that reproduction in *M. washingtonensis* is limited when *M. guttatus* contaminates pollen loads. Populations of *M. washingtonensis* were noted during this study where co-occurring *M. guttatus* plants outnumbered the former species ten to one. Although the mixed pollination experiment in Table 4-2 used approximately half and half *M. washingtonensis/M. guttatus* pollen, it is possible that combined pollen loads highly biased towards *M. guttatus* could still cause permanent closure of *M.* 

washingtonensis stigmas. Mimulus washingtonensis seed set in combined populations may thus be proportional to the density of *M. guttatus* plants, assuming that pollinators fail to discriminate between the species at all localities where these species are sympatric.

The survival of *Mimulus washingtonensis* is evidently strongly keyed to habitat integrity. Disruption of the thin basalt substrate that supports this rare endemic species is expected to limit reproduction by interfering with population structure, upsetting nutrient balances, threatening pollinators, and increasing competition for pollination. Reductions in seed set in turn limits the input to soil seed banks, a critical aspect in the life history of xerophytic annuals. This study underscores the necessity of detailed investigation in understanding the conservation biology of many of our rare species.

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