EXPERIMENTAL HYBRIDIZATION BETWEEN MOUNTAIN AND COASTAL FORMS OF *SIDA FALLAX* WALP., AND BETWEEN *S. FALLAX* AND *S. RHOMBIFOLIA* L. (MALVACEAE)

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY
OF HAWAIʻI IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF

MASTER OF SCIENCE

IN

BOTANY
(ECOLOGY, EVOLUTION, AND CONSERVATION BIOLOGY)

MAY 2005

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ACKNOWLEDGEMENTS

I would like to thank my thesis committee members, Dr. Curt Daehler, Dr. Gerald Carr, and Dr. Clifford Morden for their guidance and support throughout the project. I am also grateful for the support and help provided for this project by my family and friends for working in the greenhouse, collecting the data, and editing my writing.

ABSTRACT

Natural hybridization has been a significant evolutionary process in the development of the Hawaiian flora. In this research, I documented the outcome of experimental hybridization in *Sida* species in order to determine the degree of crossability and the morphological characteristics and fitness of hybrids. I examined hybridization between two morphologically distinct mountain and coastal forms of indigenous *Sida fallax* (Malvaceae), and between these and the alien species *S. rhombifolia*. This study could not confirm any hybridization between *S. fallax* and *S. rhombifolia*. In contrast, pollination experiments indicated that beach and mountain ecotypes of *S. fallax* were highly crossable, and the hybrids were viable. Investigation of morphological variation among reciprocal hybrids between beach and mountain ecotypes of *S. fallax* indicated that hybrids do not express uniformly intermediate characteristics. Out of 17 morphological traits examined in hybrids, four traits were intermediate, three traits were dominant (inherited from one parent), one trait was extreme relative to both parents. Nine traits did not statistically differ among beach, mountain and hybrid plants. The overall growth, flowering, and survival rate of hybrids, under well-watered and drought treatments, as well as pollen stainability, suggest that the reciprocal hybrids between beach and mountain *S. fallax* are equally fit relative to the parental ecotype. These morphological analyses and fitness comparisons of divergent ecotypes of *S. fallax* and their hybrids provide a baseline study on the heritability of morphological characters while also raising questions about the mechanisms that have maintained divergent forms of *S. fallax* in the Hawaiian Islands.

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Introduction

The role of hybridization in evolution

Hybridization as an important evolutionary process has long been recognized by plant biologists (Allen 1949, Anderson 1949, Heiser 1949, Stebbins 1950, Baker 1951, Anderson and Stebbins 1954, Gillett 1972b, Heiser 1973, Stace 1975, Grant 1981, Ganders 1989, Kaneshiro 1990, Harrison 1993b, Arnold and Hodges 1995, Rieseberg 1995, Arnold 1997, Briggs and Walters 1997, Arnold *et al.* 1999, Ellstrand and Schiernbeck 2000). Numerous evolutionarily important outcomes can result from hybridization. Hybridization can lead to increases in genetic diversity, origination of new adaptations, transfer of adaptations, formation of new reproductive barriers, formation of a stable hybrid zone, formation of new species, fusing of taxa, or extinction of one of the hybridizing species (Anderson 1949, Stebbins 1950, Stace 1975, Rieseberg and Ellstrand 1993, Arnold 1997, Rieseberg and Carney 1998, Arnold *et al.* 1999, Carney *et al.* 2000). The outcome of hybridization depends on countless factors; including the level of reproductive isolation between species, habitat preferences, life history characters, the fertility and viability of hybrids, and habitat disturbance through natural or human causes (Carney *et al.* 2000). Various investigations have focused on different aspects of hybridization, including hybrid morphology (McNaughton and Harper 1960, Benson *et al.* 1967, Wilson 1992, Rieseberg and Ellstrand 1993, Valverde *et al.* 1996, Albert *et al.* 1997, McDade 1997, Runyeon-Lager and Prentice 2000), artificial hybridization (Mayer 1991a, Motley and Carr 1998, Weller *et al.* 2001), reproductive biology (Ellstrand and

Elam 1993, Schierup and Christiansen 1996, Fischer and Matthies 1997, Fishman and Willis 2001, Weller *et al.* 2001, Wendt *et al.* 2001), isozymes, allozyme, and more recently DNA analyses (Standley 1990, Cruzan *et al.* 1993, Kron *et al.* 1993, Rieseberg and Ellstrand 1993, Lack 1995, Smith *et al.* 1996, Gallagher *et al.* 1997, Hollingsworth *et al.* 1999, Yang *et al.* 2000, Alice *et al.* 2001, Baldwin *et al.* 2001, Caraway *et al.* 2001, Faure *et al.* 2002).

Definition of hybridization

The term "hybrid" has been applied to a wide range of phenomena. A hybrid could be the product of any natural or artificial mating or crossing: between two varieties; between different genotypes belonging to the same population; between different species; or the products of grafting between specifically or genetically different stocks and scions (Stebbins 1950, Stace 1975, Grant 1981, Harrison 1990, Arnold 1997). Some terminological restriction is necessary in order to discuss natural and artificial hybridization usefully from an evolutionary point of view (Stebbins 1950, Stace 1975, Grant 1981, Harrison 1990, Arnold 1997). The term used by this review is adapted from Harrison (1990): hybrids are crosses between "individuals from two populations, or groups of populations, which are distinguishable on the basis of one or more heritable characters." This definition includes both hybridizations that occur in natural and experimental settings. One major advantage of this definition is that it does not depend on the acceptance of any particular species concept (Harrison 1993a, b).

Hybridization and native Hawaiian plants

Gillett (1972b) stated that natural hybridization has been a significant evolutionary process in the development of the Hawaiian flora, listing *Bidens, Scaevola, Pipturus,* and *Gouldia* (classified as *Hedyotis* today) as examples. Morphological variation associated with natural and artificial hybridization of the Hawaiian silversword alliance, *Argyroxiphium, Dubautia,* and *Wilkesia,* are particularly well documented (Gillett 1972a, Carr 1978, Carr and Kyhos 1981, Carr 1985, 1986, Mayer 1991a, Carr 1995, Carr *et al.* 1996, Caraway 1997, Carr *et al.* 1999, Caraway *et al.* 2001). Carr (1985, 1995) reported 35 different naturally occurring hybrid combinations in the silversword alliance, five involving different genera. Other well-studied examples of hybridization in the Hawaiian flora are *Cyrtandra* (Gesneriaceae) (Smith *et al.* 1996), *Scaevola* (Goodeniaceae) (Gillett 1966), *Lipochaeta* (Compositae) (Rabakonandrianina 1980, Rabakonandrianina and Carr 1981), *Lobelioideae* (Campanulacae) (Carlquist 1980, Lammers 1988b, 1988a), *Labordia* (Loganiaceae) (Motley and Carr 1998), *Rubus* (Rosaseae) (Randell and Morden 1999, Randell 2000), *Schiedea* and *Alisinidendron* (Caryphyllaceae) (Weller *et al.* 2001), and *Wikstroemi*a (Thymelaeaceae) (Mayer 1991a, b).

Studies suggest that the frequency of hybridization may be higher in insular floras than in continental floras (Rattenbury 1962, Gillett 1966, Gillett 1972b, Raven 1973, Carr and Kyhos 1981, Carr 1995). Ellstrand et al. (1996) reported hybridization occurs in 37 of 216 (16%) native Hawaiian genera, although hybrids are uncommon in many of these genera. Ellstrand et al. (1996) conducted a survey that revealed an unequal distribution of spontaneous hybridization in five geographic regions (British Isles, Scandinavia, the

Great Plains and Intermountain West of North America, and Hawaiian Islands). The Hawaiian Islands scored the lowest frequency of hybridization among five regions. The authors noted that this may be because of the lack of information, since the tropical floras have received less biosystematic attention than the temperate floras. The lack of information quantifying hybridization, introgression, and speciation is still noted in regard to insular floras such as the Hawaiian Islands (Gillett 1972b, Ellstrand *et al.* 1996, Arnold 1997, Caraway 1997, Caraway *et al.* 2001).

In the research proposed here, I will document the outcome of experimental hybridization in *Sida.* I will focus on hybridization between two morphologically distinct mountain and coastal forms of indigenous *Sida fallax* (Malvaceae), and between these and the alien species *Sida rhombifolia*. Careful investigation of morphology, the reproductive biology, and relative fitness of hybrids is necessary in order to assess the evolutionary consequences and ecological implications of hybridization (Arnold and Hodges 1995, Campbell *et al.* 1997, Leebens-Mack and Milligan 1998, Rieseberg and Carney 1998, Arnold *et al.* 1999, Arnold *et al.* 2001). The purpose of this research on *Sida* is to observe hybrid morphology, expression of traits, fertility and fitness, and to assess possible barriers that may exist between populations or species. This study of *Sida* will provide an additional example of hybridization between distinctive ecotypes within species, as well as between introduced and native species of the Hawaiian flora.

Physical and ecological background

The Hawaiian archipelago is an extensive band of 132 islands, reefs, and atolls extending nearly 2,600 km along a southeast to northwest line in the middle of the Pacific Ocean from the youngest island of Hawaii (154°40’ W, 18°54’ N) to Kure, the oldest atoll (178°75' W, 28°15' N) (Wagner 1991). The great diversity of habitats and climatic variation found on the eight principal Hawaiian Islands is well known (Carlquist 1980, Wagner *et al.* 1999). The Hawaiian Islands are nearly 3,000 km from the nearest continent, and comprise the most isolated archipelago of comparable size (16,642 km^2) and topographic diversity (sea level to 4,205m) on the globe (Loop and Mueller-Dombois 1989). The age of the oldest parts of the high Hawaiian Islands is estimated as 11.3 million years (MacDonald and Abbot 1970).

The present native flora of Hawaii has been estimated to consist of a little over 1,700 species and varieties; it developed from about 272 original arrivals that became successfully established (Fosberg 1948, Wagner 1991, Wagner *et al.* 1999). The most important feature that affects evolution of the Hawaiian flora is its geographic isolation. Evolution in its diverse environments has proceeded in nearly total isolation, resulting in a high degree of endemism in the flora and fauna (Carlquist 1980, Athens 1997, Wagner *et al.* 1999). Mayr (1942) emphasized that variation can accumulate only when a large and long-term geographical barrier prevents or drastically limits migration between the differentiating population and the parental population. The isolation of the Hawaiian island chain from other land masses, the isolation between the separate islands in the chain, the equable but variable climate, the topography which led to isolation of small populations in deep valleys or separate mountain peaks, and isolation brought about by lava flows and formation of kipukas are all factors that have permitted evolution to occur at an especially rapid rate (Lamoureux 1973). The extreme vulnerability of island biota and ecosystems to alien invaders also has long been recognized (Darwin 1859, Fosberg 1963, Gillispie 1997, Daehler and Carino 2001).

Sida fallax Walp.

The genus *Sida* has 125-150 species primarily of tropical and subtropical regions of the world (Bates 1999). In Hawaii, the genus is represented by one indigenous and six naturalized (introduced) species (Bates 1999), and probably more species are present in cultivation. *Sida fallax* Walp. is the most widespread, variable taxon of Malvaceae in the Hawaiian Islands (Bates 1999). This prostrate to erect perennial shrub is indigenous from China to the Pacific islands including Hawaii (Bates 1999). The calyx tube (6-9 mm long) is strongly ribbed and the five parted petals (8-15 mm long) are yellow to orange-yellow, sometimes having dark maroon at the base (Bates 1999). The flowers are often solitary, or 2-7 may occur together at nodes and near branch tips (Bates 1999). The chromosome number is $2n = 28$ (Bates 1999).

Sida fallax is found on Midway Atoll, Nihoa, and all the main Hawaiian Islands, occurring in coastal shrubland communities (near sea level to 300 m elevation), lowland dry communities (15-600 m elevation), and montane communities (500 to 2,700 m elevation) (Wagner *et al.* 1999). Two morphologically distinct ecotypes (beach and mountain) are recognized (Stephens 2000). The coastal type is prostrate or a sub-shrub up to 1.2 m tall found on arid exposed headlands and other coastal sites (Figure 1.1). The mountain type is an erect tree-like shrub found in montane and mesic communities (Figure 1.2). A variety of intermediate sizes and habitats are also recognized (Bates 1999).

Figure 1.1. The beach ecotype *Sida fallax*. Makapu‘u, Hawaii.

In the coastal shrubland community, the environment is harsh, with highly seasonal precipitation, large temperature fluctuations, intense solar radiation, and annual rainfall often less than 500 mm (Wagner *et al.* 1999). In this habitat, *Sida fallax* forms prostrate mats on sand dunes and poorly consolidated volcanic soils in near-shore environments with high exposure to salt-laden winds (Wagner *et al.* 1999). In the dry-mesic montane community where Sida fallax also occures, the climate is seasonal with drought conditions during the summer and annual rainfall typically 500 to 1,200 mm (Wagner *et al.* 1999). In this habitat, an erect ecotype of *S. fallax* predominates (Wagner *et al.* 1999). Both ecotypes are capable of withstanding summer-drought conditions, under which few other species, native or alien, can survive (Wagner *et al.* 1999). *Sida fallax* can even persist in seasonal pastures (Wagner *et al.* 1999). Morphological variations within and between populations of *S. fallax* including variation in stature, pubescence, leaf size and shape, and character of the inflorescence are recognized (Stephens 2000).

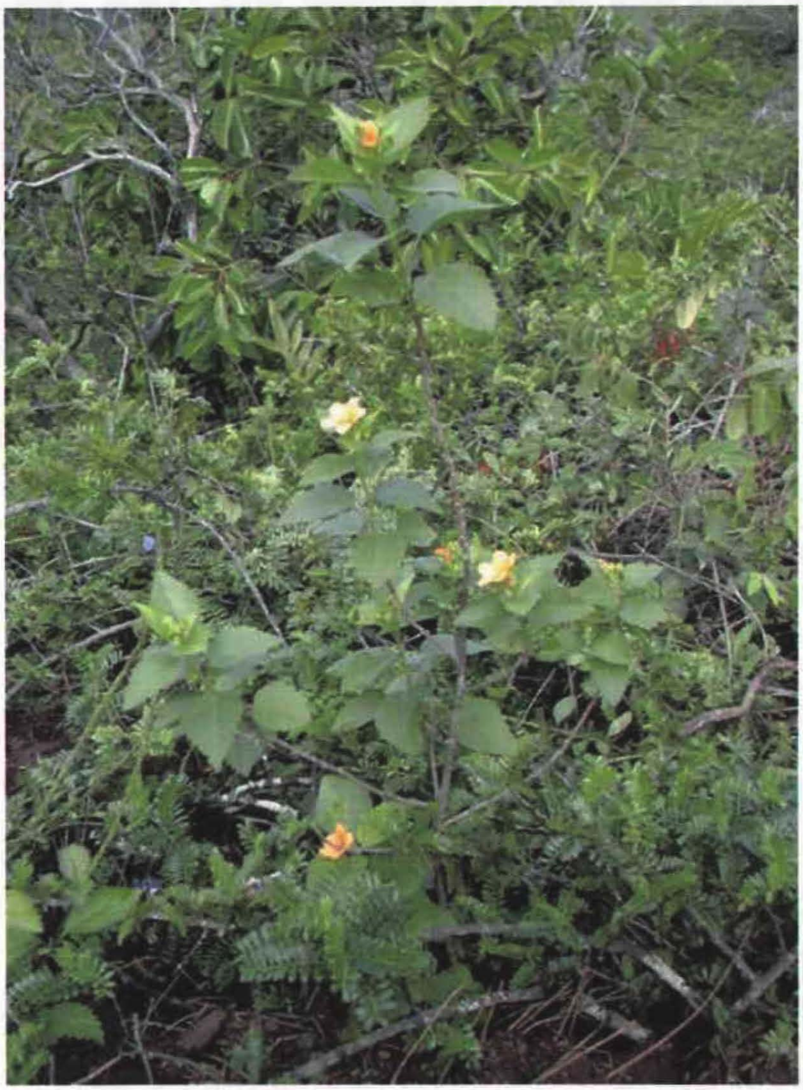


Figure 1.2. The mountain ecotype *Sida fallax*. Hawai'iloa Ridge, Hawaii.

The Hawaiian name for *Sida fallax* is 'ilima. Hawaiians also recognized and named four different forms of 'ilima. 'Ilima-lei or royal 'ilima, is a large shrub cultivated for the yellow to rich orange flowers most often used for lei (Neal 1965, Kepler 1983). A single lei requires about 500-600 flowers (Neal 1965, Kepler 1983). ʻIlima blossoms were so highly cherished that it was one of the few non-food and non-fiber plants cultivated by ancient Hawaiians (Kepler 1983). ʻIima-ku-kula is a wild tall form with irregular flowers less often used for lei, and having woody stems which could be used for building or baskets (Neal 1965, Kepler 1983). ʻIlima-ku-kahakai or ʻilima papa (flat ʻilima) is a low form creeping on rocks (Neal 1965, Kepler 1983). ʻlima-koli-kukui is a cultivated form with reddish brown flowers (Neal 1965, Merlin 1977).

Hawaiians used flowers and roots of ʻilima to cure general debility. The juice of pressed flowers was given to children, and pregnant women sometimes ate the flowers until the time of childbirth (Merlin 1977). Other *Sida* species are also known to have medicinal uses. Pope (1968) noted that one or two *Sida* species are listed as medicinal plants in the National Standard Dispensatory, 1905. *Sida cordifolia* L. is used in folk medicine for the treatment of inflammation of the oral mucosa, blenorrhea, asthmatic bronchitis and nasal congestion in Brazil (Franzotti *et al.* 2000). Franzotti et al. (2000) confirmed its low toxicity and presence of analgesic and anti-inflammatory effects. A comprehensive description of Hawaiian ethnobotanical uses of ʻilima is presented by Stephens (2000).

Photosynthesis, leaf spectral characteristics and water relations of the coastal and mountain populations of *Sida fallax* in Hawaii have been studied and discussed in detail by Stephens (2000). The coastal and mountain plants have two types of trichomes: stellate trichomes and stalked grandular trichomes (Stephens 2000). Stephens (2000) recognized that leaves sampled from the beach populations are more pubescent than the mountain populations. Based on leaf reflectance and pubescence of top and bottom surface, leaf mass per unit area and internode length, Stephens (2000) concluded that the pubescence of *S. fallax* leaves may increase the reflectance of solar radiation but this does not detrimentally affect photosynthesis. Stephens (2000) also detected that coastal and mountain plants in the field undergo changes in tissue elasticity and make osmotic adjustments, possibly in response to seasonal water defects.

Bates (1999) noted that the range of morphological and ecological variability encompassed by *S. fallax* may deserve recognition as infraspecific taxa. Classification of many groups within the Hawaiian flora is difficult due to complex morphological variation that suggests the occurrence of natural hybridization (Gillett 1972b).

***Sida rhombifolia* L.**

Sida rhombifolia L. is a polymorphic pantropical weed (Bates 1999). In Hawaii, it is naturalized in disturbed areas from coastal sites and uplands to 1,240 m elevation on all of the main islands (Bates 1999). Hillebrand (1888) reported *S. rhombifolia* as being possibly indigenous. *Sida rhombifolia* has rhombic to elliptic leaves with serrate margins near the tip and entire margins toward base (Bates 1999). Flowers are solitary in the leaf axils and have a strongly ribbed calyx and yellow to yellowish orange corolla (Bates 1999) (Figure 1.3). *Sida rhombifolia* is used medicinally throughout the tropics of both hemispheres (Caius 1986). The species also is known to produce calcium oxalate crystals in leaves as defense against herbivores (Molano-Flores 2001). Chromosome numbers are $2n$ = 14, 16, 18, 28, and 36 (Bates 1999).

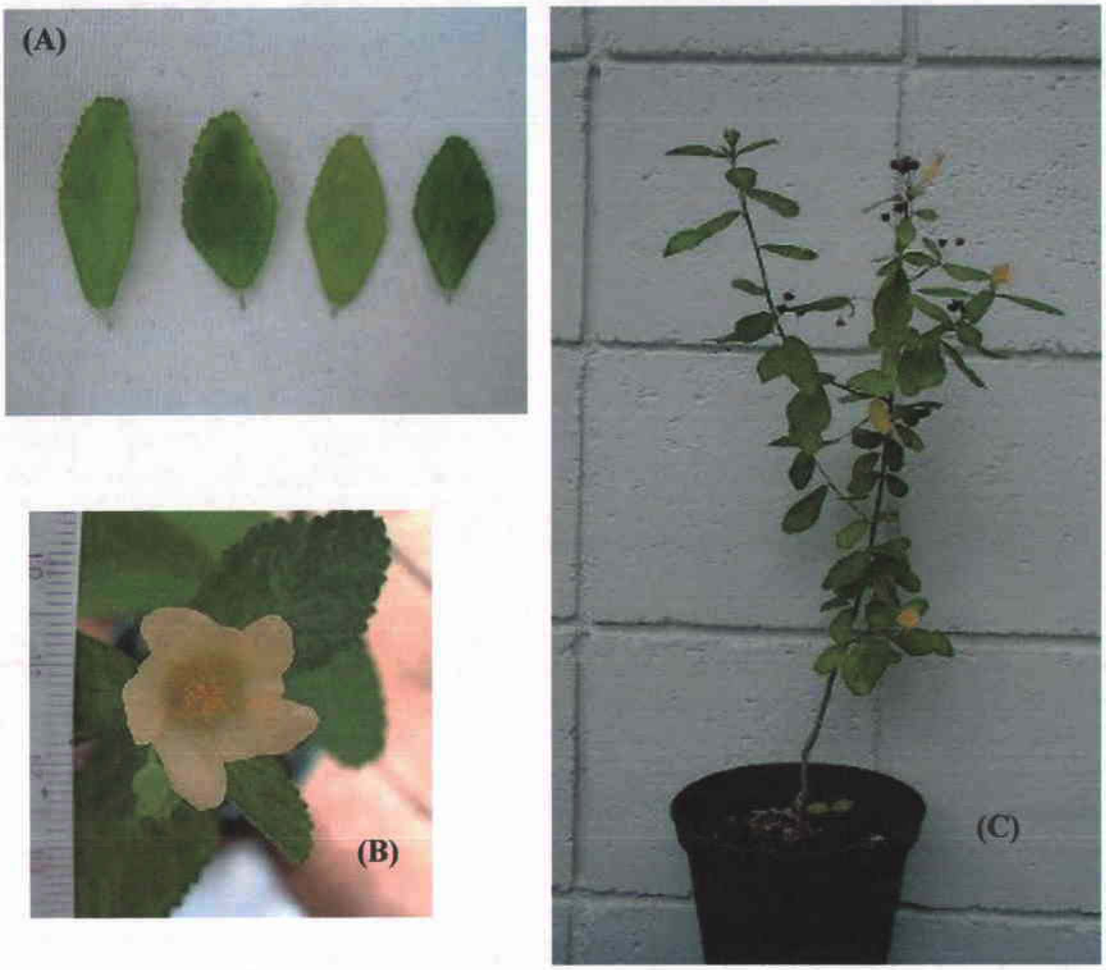


Figure 1.3. Typical leaves (A), a flower (B) and a habit (C) of *Sida rhombifolia.*

In the Northern Territory of Australia, *Sida* species, including *Sida rhombifolia,* have strong, well developed tap roots to from nearly monospecific stands of plants up to 1 m high (Forno *et al.* 1992). The plant is listed in the noxious weed list for Australian States and Territories prepared by the National Weeds Strategy Executive committee, and its entry into the region is prohibited or regulated under the Plant Diseases Act 1974 (Wills and Keighery 1994, Wills and Robinson 1994). *Sida rhombifolia* is also considered invasive under certain conditions in the Southern United States, from Virginia to Florida and Texas.(Harvill *et al.* 1977, Wunderlin *et al.* 1996).

Morphological variations within the species over the wide ecological range

Gillett (1972b) noted that the diversity of the Hawaiian flora is conspicuously limited at the generic level, but this is largely offset by the existence of an unusually broad range of complex morphological diversity at the species level. Many genera, both large and small, are characterized by remarkable specific and trans-specific variation (Gillett 1972b, Wagner *et al.* 1999). Although many genera are treated as monotypic, they may in fact, comprise several taxa (Wagner *et al.* 1999). Wagner *et al.*(1999) listed 23 native Hawaiian plant species that appear to have unusually complex variation patterns (Table 1.1). These polymorphic species have evolved in response to the diversity of habitats and the geological recency of the main islands (Wagner *et al.* 1999). *Myoporum sandwicense* and *Sophora chrysophylla* are additional species with complex patterns of variation (Mueller-Dombois 1975). These variable species often have evolved distinctive ecotypes and intermediate individuals may be found at ecotones, such as the margin between a bog and a rain forest (Carlquist 1980).

Morphological character expressions of progeny of polymorphic native Hawaiian species collected from different populations have been investigated for *Metrosideros polymorpha* (Kitayama *et al.* 1997), *Acacia koa* (Sun *et al.* 1996, Daehler *et al.* 1999), and *Sida fallax* (Stephens 2000) in field and common garden studies. Results revealed that the many of extreme phenotypic traits in local populations were genetically determined instead of environmentally induced.

Hybridization between divergent forms

Carlquist (1980) suggested that occasional hybridization and adaptation of hybrid products to local habitats may explain the wide range of morphological variations among species that occupy every sort of ecological opportunity from moderately dry forest to bog in the Hawaiian Islands. Carlquist (1980) hypothesized that various forms of the species have evolved by way of adaptation to various distinctive sites in the islands, and that intermediate types formed by hybridization occupy intermediate areas. The same scenario could account for morphological variation over the wide range of environments

Table 1.1. Taxa with unusually complex variation patterns (Wagner *et al.* 1999).

Acacia koa
Chamaesyce celastroides
Chamaesyce multiformis
Coprosma ochracea/C. pubens
Cyrtandra hawaiensis
Gnaphalium sandwicensium
Hedyotis schlechtendahliana
Hedyotis terminalis
Hibiscus kokio
Labordia hirtella
Lipochaeta rockii
Lysimachia hillebrandii/L. remyi
Metrosideros polymorpha
Myrsine lessertiana
Pelea elliptica complex
Peperomia cookiana
Phyllostegia ambigua
Plantago pachyphylla
Portulaca villosa
Sida fallax
Styphelia tameiameia
Vaccinum spp.
Wikstromia oahuensis

observed in *Sida fallax*. Bates (1999) reported that the naturalized pantropical species, *Sida cordifolia*, which grows at low elevation near coasts, has long been confused with the prostrate coastal populations of the indigenous *Sida fallax* complex. Carlquist (1980) also suggested that *Sida cordifolia* hybridizes naturally with *Sida fallax*. However, there is no study that has investigated hybridization among *Sida* species in Hawaii.

An example of hybridization in *Sida* has been presented by Dawar et al. (1994, 1996). In Pakistan, the genus *Sida* is represented by 12 species and infraspecific taxa (Abedin 1979). One of the variable species, *Sida ovata*, was thought to be a single taxon that later diverged into two distinct species, *Sida ovata* and *S. tiagii* (Bhandari 1978, 1990). Hybridization between *Sida ovata* and *S. tiagii* was reported based on morphology, chemistry, cytology (Dawar *et al.* 1996) and breeding studies (Dawar *et al.* 1994). *Sida ovata* and *Sida tiagii* are distinguished by calyx length, schizocarp morphology, habit, and flower colors (Dawar *et al.* 1996). Hybridization is common between these two sympatrically occurring species (Dawar *et al.* 1994, 1996).

The example of hybridization in *Sida* species in Pakistan suggests that hybridization in this genus may not be uncommon. Thus, investigation of the relationship among beach and mountain forms of *Sida fallax* and other *Sida* species in Hawaii may reveal the distinguishable infraspecific groups and hybrids between species.

Plant hybridization and invasion

A major focus of hybridization studies has been to examine natural hybrid zones as a means to understand divergent evolution and other evolutionary phenomena (Arnold *et al.* 2001). However, more recent hybridization studies have focused on the question of how hybridization affects communities, and the ecology and conservation of ecosystems

(Levin, 1996; Whitham, 1999; Allendorf, 2001). For example, hybridization may speed up the extinction of rare species (Levin *et al.* 1996) and promote invasiveness of introduced species (Weber and D'Antonio 1999, Ellstrand and Schiernbeck 2000).

Ellstrand and Schierenbeck (2000) stated that hybridization may result in critical evolutionary changes that enhance the invasiveness of hybrid lineages. Not all hybridization leads to increased fitness or adaptive evolution (Arnold 1997). However, Ellstrand and Schiernbeck (2000) noted that generation of novel genotypes, increase in genetic variation, and fixed heterosis promoted by hybridization may catalyze the evolution of invasiveness. Human activities may open new hybrid zones that might be better suited for hybrids than for their parent population (Ellstrand and Schiernbeck, 2000). Ellstrand and Schiernbeck (2000) also hypothesized that hybridization, even among well differentiated populations, ought to leave relatively high levels of within-population polymorphism. *Echium plantagineum*, a noxious weed of Australia (Burdon and Brown 1986) and *Bromus tectorum*, an introduced weed of North America (Novak and Mack 1993) are two examples of invasions followed by hybridization between well differentiated populations that resulted in populations with more genetic diversity than their parent populations.

The impact of introduced invasive plants has been recognized as a threat to native species (Wallace 1889, Elton 1958, Daehler and Strong 1997, Daehler and Carino 1998, Daehler 1999, Daehler and Carino 2001). Thus, the threat of hybridization and introgression between native and introduced plant has attracted attention in the context of conservation of native plants (Levin *et al.* 1996, Daehler and Carino 1998, 2001). Daehler and Strong (1997) used RAPDs to detect hybridization between *Spartina* *alternifora* and native *S. foliosa* (California cordgrass) that co-occur in the San Francisco Bay, California. A greenhouse study found that the F_1 hybrids had low self-fertility but high fertility when back-crossed with pollen of the native, *S. foliosa* (Daehler and Strong, 1997). Morphology of the hybrids was intermediate between parental species (Daehler and Strong 1997). Field observation suggested that naturally occurring hybrids were recruiting more rapidly than the native *S. foliosa* (Daehler and Strong 1997).

Daehler and Carino (2001) reviewed the literature on the threat of alien pollen, hybrid fitness, and frequency of native-alien hybridization involving plants in the British Islands. It was concluded that nearly 7.5 % of the 892 aliens that have congeneric native species have hybridized with the natives and that 6% of the 712 native species have hybridized with an introduced congener (Daehler and Carino 2001).

Daehler and Carino (2001) also proposed a list of potential native-alien hybrids in the Hawaiian Islands. The list revealed that a total of 59 genera including 176 endemic flowering plant species in the Hawaiian Islands are potentially threatened with hybridization by alien congeners (Daehler and Carino 2001). An additional 27 genera with 82 endemic species are also at risk when the non-naturalized alien horticultural plants are considered. Following the British Isle example (6% natives have formed hybrids), one might expect about 15 [(176 + 82) x 0.06 = 15.48] endemic species to hybridize with alien species in the Hawaiian Islands (Daehler and Carino 2001).

A well documented case of hybridization between introduced and native species in Hawaii was reported by Randell (2000). Randell (2000) studied hybridization between naturalized and endemic *Rubus* (Rosaceae) species in Hawai'i. Many species in this genus are thought to have arisen during the Pleistocene Era as a result of hybridization and apomixis (Wagner *et al.* 1999). The *Rubus* study reported that the native species *R. hawaiiensis* hybridizes naturally with the introduced species, *R. rosifolius*, a common weed in disturbed mesic to wet forests in Hawaii (Wagner *et al.* 1999, Randell 2000). The naturally occurring hybrids were morphologically and genetically intermediate to the parent species (Randell 2000). These hybrids appeared to be sterile, and hybridization was occurring in an asymmetrical manner with *R. rosifolius* acting as the maternal parent for sampled hybrids (Randell 2000). Thus, Randell (2000) concluded the genetic assimilation of *R. hawaiensis* by *R. rosifolius* would be an unlikely event if the sterile individuals sampled are representative of the entire hybrid population. The study called for further investigation of hybridization-invasion mechanisms, reciprocal crosses, and hybrid fitness to assess the threat to native *R. hawaiiensis* from competition with introduced *R. rosifolius* and hybrid populations (Randell 2000).

Hybridization, restoration and conservation

A recent study of native plant community restoration by Hufford and Mazer (2003) raised new questions concerning the consequence of sampling protocols and of intraspecific hybridization between locally adapted and transplanted populations. When there are rare populations remaining at a restoration site, translocation of conspecific populations with compatible and synchronous mating systems can result in intraspecific hybridization (Hufford and Mazer 2003). Consequences of intraspecific hybridization might include heterosis and/or outbreeding depression that may determine the success or the failure of the ecological restoration (Hufford and Mazer 2003). Additional research of hybridization between genetically distinct populations of the same species is needed to determine the potential risks of introducing foreign genotypes into locally adapted populations.

Sida fallax is abundant in a wide range of Pacific environments. Unlike many native species in Hawaii, it is not threatened or endangered. However, Kirch (1982) has estimated that by late in the period of prehistoric Polynesian occupation (A. D. 1600), 80 % of all of the lands in Hawaii below about 1500 feet in elevation had been extensively altered by the human inhabitants. After the arrival of Europeans, even more natural habitat was lost, and the rate of the human disturbances and the loss of the natural habitats continue to increase today. Therefore, hybridization studies focused on the common native plants may be instructive for developing information and ideas for future conservation of native species.

Coastal and mountain populations of *Sida fallax*, and *S. rhombifolia* are usually isolated by allopatry. However, I expect these *Sida* species to readily hybridize in the experimental setting. A detailed investigation of hybridization in *Sida* will contribute to an understanding of the variation and divergent evolution of the Hawaiian species and provide a means to assess the role of hybridization in community dynamics.

Thesis Proposal

Statement of purpose

The purpose of this study is to investigate the possible outcomes of hybridization between morphologically distinctive ecotypes of indigenous *Sida fallax*, and between *S. fallax* and introduced *S. rhombifolia*. Assessment of infra- and inter-specific hybrids involving *S. fallax* may provide additional information to characterize the nature and implications of hybridization in the Hawaiian Islands.

In the proposed study, I will attempt to experimentally produce hybrids between mountain and coastal populations of *Sida fallax*, and also hybrids between *S. fallax* and *S. rhombifolia*. Artificial crossability, self incompatibility or incompatibility between two divergent forms of *S. fallax*, and between *S. fallax* and *S. rhombifolia* will be assessed. The morphological characteristics of parent sets and hybrids will be documented and compared. The basic mode of inheritance for distinct traits (dominant, recessive or additive) will be determined by comparing hybrids and parents. The pollen stainablility of hybrids and parents will be evaluated and compared. The seed germination rate, growth rate, flowering rate, and survival rate of hybrids and parents will be compared.

Hypotheses

Given the variation in morphology of *Sida fallax* the following hypotheses are proposed:

Hypothesis 1: Artificially produced hybrids between mountain and coastal ecotypes of *Sida fallax* and between *S. fallax* and *S. rhombifolia* will exhibit morphology intermediate to the parent forms. Comparisons of morphological characteristics such as flower color, petal shape, mericarp color, awn length, leaf shape, leaf margin, and habit in ecotype hybrids and the parental ecotypes will reveal heritable phenotypic traits.

Hypothesis 2: Artificially produced hybrids between mountain and coastal ecotypes of *Sida fallax* and between *S. fallax* and *S. rhombifolia* will be less fit than progeny derived from either ecotype of *Sida fallax*. Hybrids will show lower pollen stainability, lower seed production, lower seed mass and number of mericarps per fruit, lower germination velocity, slower growth rate, lower flowering rate and lower survival rate than the control crosses grown under the same conditions. Hybrids will also have lower survival than the control crosses when grown under stressful (drought) conditions.

Materials and Methods

Seed collections

Experiments will be conducted at the Pope Laboratory greenhouse, on the campus of the University of Hawaii. The original plants used to obtain seeds for this study consisted of a subset of plants used by Stevens (2000). The plants were grown from seeds collected from six different sites on the island of Oahu: at Wa‘ahila Ridge (elevation of 366 m, annual rainfall of 2000 mm), Hawaii Loa Ridge trail (elevation of 330 m, annual rainfall of 1250 mm), Kuaokala Forest Reserve (elevation of 266 m, annual rainfall of 900 mm), Makapuu beach park (near sea level, annual rainfall of 800 mm), Kaena Point Natural Area Reserve (near sea level, annual rainfall of 800 mm), and at Sandy Beach Park (near sea level, annual rainfall 800 mm). Rainfall data for these sites are from Giambelluca et al. (1996). Plants from the mountain populations were observed to have upright growth form with nearly glabrous leaves, and plants from the coastal populations were prostrate with pubescent leaves (Stephens 2000). Seeds of *Sida rhombifolia* were also collected at Kuaokola (Stephens 2000). Seeds from these populations of *Sida* were germinated and grown in two-gallon pots in Sunshine soil medium (J. G. Smith & Company, Illinois) at the University greenhouse during 1998 and 1999 (Stephens 2000). Among these plants, subsets of seven beach and five mountain plants of *S. fallax,* will be used to produce hybrids between these divergent forms. Four mature *S. rhombifolia* plants will be used to produce hybrid crosses between *S. fallax* and *S. rhombifolia.*

Experimental crosses

Reciprocal hybrid crosses between mountain (M) and coastal or beach ecotypes (B) of *Sida fallax* will be accomplished in the greenhouse by hand pollination. In addition, controlled crosses between individuals from the same ecotype (MxM and BxB: the first letter indicates maternal parent and the second letter indicates paternal parent) will be done. Attempts to produce interspecific hybrids will be made by reciprocally hand pollinating *S. fallax* (B and M) and *S. rhombifolia* (A) in the greenhouse. The progeny from all intra-specific crosses will be used as control groups to compare with the progeny from inter-specific hybrid crosses (MxA, BxA, AxM, and AxB). The rate of successful fruit production per pollination attempt will be used to assess the artificial crossability of each type of cross.

Morphology and fitness measurements

All fruits produced from the experimental crosses will be collected and the number of mericarps per fruit, mass (g) of mericarps per fruit, color of the mericarps, and awn length (mm) will be measured. The color of the mericarps will be ranked as black, brown and tan. One mericap per fruit will be randomly selected to measure awn lengths. Awn length will be measured to the nearest 0.01 mm using a Brown & Shape digital caliper (Model 599-571-4). All of the different individual parent combinations produced will be selected for germination. Before planting, all seeds will be removed from the mericarps and the seed coats will be nicked to enhance germination. Each seed will be planted in a 5x5 inch pot with potting soil mix. All seeds will be planted at the same time and randomly positioned on the greenhouse benches. All pots will receive water equally to keep the soil moist.

The morphology of the inter- and intra-specific hybrids, controls and parent populations will be observed by measuring the petal shape, petal color, flower diameter, leaf shape, leaf area, leaf margin, and habit. Flowers from all parental individuals and hybrids will be photographed using a digital camera with a size reference next to the flower. These images will be used to compare the diameter of the flower, shape and color of the petals, and presence or absence of the dark spot at the base of the petals among the specific parents and their progeny in order to observe the heritibility of these traits. Three leaves will be collected from each parent, hybrid, and control. Leaves will be collected from the third to fourth node proximal to the apex of the second or third lateral branch proximal to the main stem apex. Leaves from hybrids and controls will be collected after the first flower production to insure the maturity of the plants. Leaf shape will be scored as rhombic (diamond shape), ovate (egg-shaped), cordate (heart-shaped), and reniform (kidney-shaped). Leaf margin will also be scored as serrate (sharp edged) or crenate (rounded). The habit will be scored as upright, prostrate or intermediate by observing the mature plants. Comparisons of morphological characteristics in parent populations, hybrids and controls will be used to assess heritability of phenotypic traits.

Fitness studies

The reproductive success, seed germinability, seed germination rate, pollen stainability, growth rate, and survival in the drought treatment will be used to determine fitness. The reproductive success will be measured by the number of mericarps per fruit and the mass of mericarps per fruit. Seed germinability will be determined by the fraction of the seeds that germinated. Seed germination rate will be calculated using the modified seed germination index of germination velocity suggested by Khan and Rizvi (1994). The Seed germination velocity = $\Sigma\ G/t$, where G is percentage of seed germination at 2 days intervals, and t is total germination period. The protocol used by Mayer (1991a) will be modified to evaluate the pollen stainability as an estimation of pollen viability. The ratio of stained/unstained pollen grains in aniline blue in lactophenol will be recorded for a minimum of 100 pollen grains per sample (Hauser and Morrison 1964). The growth rate will be calculated by measuring the main shoot length and the number of leaves produced in each two weeks period. The number of days it takes a plant to produce its first flower after germinating will be recorded to mark the plant's maturity and the flowering rate.

Drought treatments

Environmental stress severely restricts the distribution and productivity of plants. In particular, salinity and drought are two major constraints that limit agricultural production world-wide (Boyer 1982). Salinity and drought are also commonly experienced by *Sida fallax* in nature. Plants have evolved various protective mechanisms that allow them to acclimate to unfavorable environments for continued survival and growth. Stephens (2000) conducted salt spray treatments in the greenhouse using mountain and coastal ecotypes of *S. fallax*. Under the salinity treatments, both mountain and coastal ecotypes showed seasonal plastic responses in leaf area, leaf mass per unit area and leaf reflectance (Stephens 2000).

The objective of the drought treatments is to identify differences in growth of the total stem length and leaf number, the date of the first flower production, and the survival over the period of time under the treatments. The study will show the degrees of drought tolerance among hybrids and parent populations. Data from the drought treatment will allow us to compare whether fitness of the hybrids relative to the parents are dependent or independent of environment.

All crosses derived from artificial pollinations will be subjected to either drought or well-watered conditions. Drought treatment will start two weeks after transplanting to minimize the effect of the transplant shock. For each control and hybrid type, plants will be divided into two watering groups, the well-watered control and the drought. The well-watered group will be watered almost every day to keep the soil moist and the drought group will be watered every two or three days. Water treatments will be applied for four months. If a plant dies during the treatment, the number of the days it survived will be recorded.

Chapter 2

Reproductive Biology and Hybridization of Mountain and Beach Ecotypes of *Sida fallax* Walp. (Malvaceae)

Introduction

Mating systems are one of the most important factors determining genetic variability in plant species (Hamrick *et al.* 1979, Kearns and Inouye 1993, Borba *et al.* 2001). Careful examination of mating systems may reveal the probability of hybrid formation between closely related taxa (Carney *et al.* 1994). Understanding how plant mating systems function (e.g., selfing, outcrossing, mixed mating, apomixes) is fundamental for determining their influence on the process of reticulate evolution (i.e., evolution that results from natural hybridization between divergent lineages) (Kearns and Inouye 1993, Carney *et al.* 1994, Dawar *et al.* 1994).

Studies suggest that the frequency of hybridization is considered to be even higher in insular floras than in continental floras (Rattenbury 1962, Gillett 1972b, Raven 1973, Carr and Kyhos 1981, Carr 1995). Gillett (1972b) stated that natural hybridization has been a significant evolutionary process in the development of the Hawaiian flora, listing *Bidens, Scaevola, Pipturus,* and *Gouldia* as examples. The lack of information quantifying hybridization, introgression, and speciation is still noted in regard to insular floras such as Hawaiian Islands (Gillett 1972b, Ellstrand *et al.* 1996, Arnold 1997, Caraway *et al.* 2001).

Sida fallax Walp. is the most widespread and variable taxon of Malvaceae in the Hawaiian Islands (Bates 1999). It is widespread on Pacific islands including Midway Atoll, Nihoa, and all the main Hawaiian Islands, where it occurs in coastal shrubland communities and montane communities. Two extreme ecotypes (mountain and beach) are recognized corresponding to beach and mountain populations (Stephens 2000). The coastal type is a prostrate scrambler or subshrub up to 1.2 m tall, found on arid exposed headlands and other coastal sites. The mountain type is an erect tree-like shrub found in montane and mesic communities (Bates 1999). Reiseberg (2000) pointed out that based on computer simulation studies, hybrid speciation is consistently favored by niche divergence and spatial isolation. Hybridization between the two extreme forms of *S. fallax* is unknown since they are usually geographically isolated. However, putative intermediate forms are recognized in limited sites on Oahu (Koko head, Koko Crator, Diamond Head and Ewa) (personal observation; Bates 1999). The scaricity of these intermediate forms may be due to the urbanization of the intermediate habitat.

A need for biosystematic analysis of possible infraspecific taxa of *Sida fallax* has been recognized by Bates (1999). However, only one recent study of *Sida fallax* ecotypes has been conducted (Stephens 2000). Information on the possible existence of breeding barriers between two forms of *S. fallax* would be helpful to evaluate the mode of evolution of these forms.

In this thesis, I document the outcome of experimental hybridization between two morphologically distinct mountain and coastal forms of indigenous *Sida fallax* (Malvaceae). The purpose of the research is to assess the potential for hybridization and gene flow between the two ecotypes of *S. falla*. This study also provides additional information about the breeding system, seed characters and germination of *S. fallax* and the experimental hybrids.

Materials and Methods

Source of study plants

The experimental studies reported here were conducted at the Pope Laboratory greenhouse, on the campus of the University of Hawaii. The original plants used to obtain seeds for this study were a subset of plants used by Stevens (2000). The plants were grown from seeds collected from six different sites on the island of Oahu. The locations, elevations and annual rainfalls of the seed collection sites are summarized in Table 2.1 and Figure 2.1.

Plants from the mountain populations (elevation higher than 266 m) were observed to have an upright growth form with nearly glabrous leaves, and plants from the coastal populations (near sea level) were prostrate with pubescent leaves (Stephens 2000). Seeds from all six collection sites were germinated and grown in two-gallon pots in Sunshine soil medium (J.G. Smith & Company, Illinois) in the greenhouse during 1998 and1999 (Stephens 2000). A total of 11 mature beach and 10 mature mountain plants of *S. fallax* from 6 populations (Table 2.1) were used to produce the hybrids between these divergent forms.

Experimental pollination

A total of eight combinations of hand pollinations were carried out to test crossability and to produce seeds in the greenhouse (Table 2.2). Intra-specific pollination involving the mountain (M) and beach (B) forms of *Sida fallax* were performed reciprocally (MxB and BxM: the female parent is listed first). Crosses were also made between individuals of the same *S. fallax* ecotype (MxM and BxB) to serve as ecotype standards. Self-pollination of individuals in each ecotype (Mself and Bself) was also done.

Pollination was carried out from 1999 to 2001. A sharp-tipped forceps was used to collect mature anthers from a pollen donor and transfer pollen to the stigmas of a maternal parent. *Sida fallax* flowers year-round and individual flowers open for a single day. The available flowers were pollinated at various times of the day in order to obtain seeds from as many combinations of crosses as possible. All pollinated flowers were monitored for two to three weeks, and the success or failure in seed production was recorded.

All data were averaged for the individual parent or the individual hybrid combination. Hybrid data were averaged again according to the common mother plant. Finally, these values were averaged to compare the different plant types or hybrid types using ANOVA or a student t-test.

Breeding system of *Sida fallax*

Observations of the cultivated plants suggested that *Sida fallax* is self-incompatible. This simplified cross pollinations for *S. fallax*, because the flowers did not need to be emasculated. Self-compatibility was assessed in order to justify results from artificial pollinations between *S. fallax* individuals without emasculation.

Table 2.1. Collection sites of original maternal plants. Rainfall data of these sites is from Giambelluca et al. (1996).

Collection Site	Elevation (m)	Annual Rainfall (mm)
Mountain *S. fallax* (M)		
Wa‘ahila Ridge	366	2000
Hawai‘iloa Ridge trail	366	1250
Kuaokala Forest Reserve	266	900
Beach *S. fallax* (B)		
Makapu‘u beach park	near sea level	800
Ka‘ena Point Natural Area Reserve	near sea level	800
Sandy Beach Park	near sea level	800

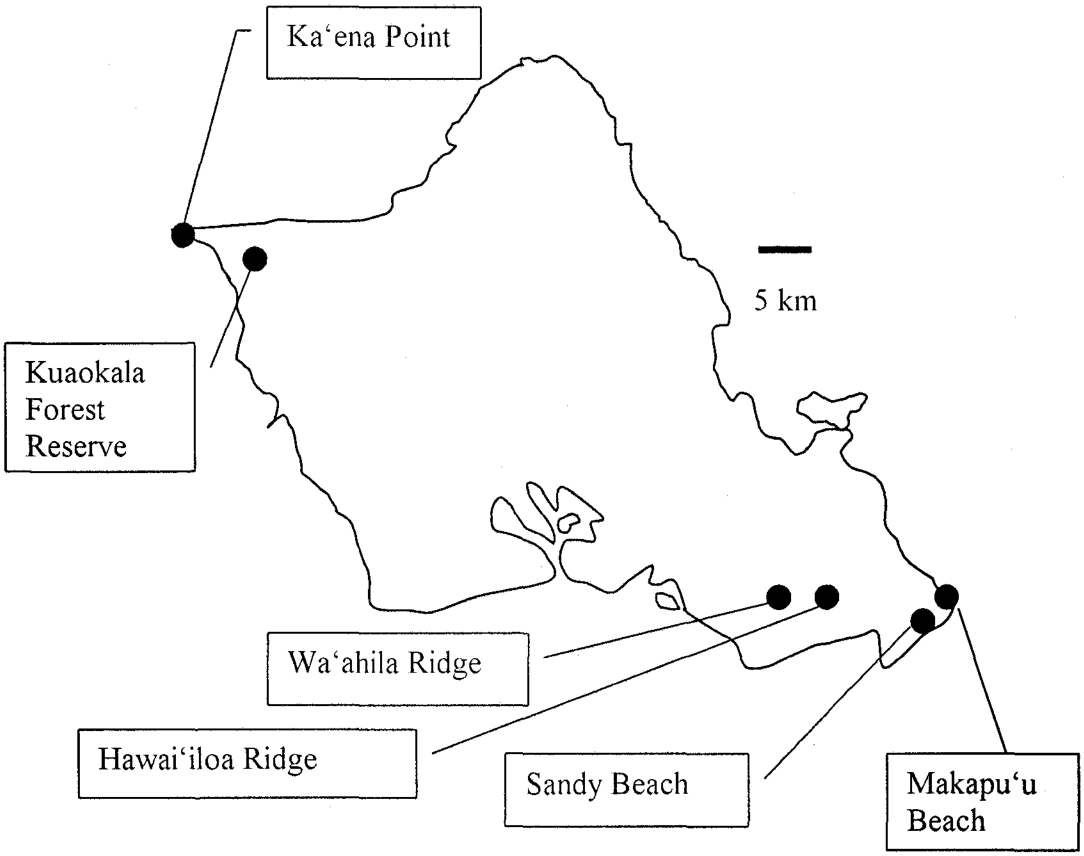


Figure 2.1. Collection sites of original plants.

Flower bagging and self-pollination experiments

To assess self-pollination in *Sida fallax*, two flowers per individual from 16 *S. fallax* plants (each of nine beach and seven mountain plants) were isolated in fine nylon mesh bags. Each flower bud for testing was enclosed in a nylon mesh bag for 1-2 days before opening, to ensure isolation from insects that were occasionally observed in the greenhouse. Two to three weeks after flowering, the success or failure of seed production was recorded.

In addition to the bagged selfing experiment, ten flowers from each of 16 *Sida fallax* plants were manually self-pollinated by dusting self pollen on the stigma without mesh bagging. An additional 10 flowers from these individual plants were also observed without any manipulation (controls).

Artificial crossability

Pollination success was determined two or three weeks after pollination by harvesting the shizocarp (fruit) which contains the mericarps (seeds). When a shizocarp was empty (all mericarps appeared to be aborted), the pollination was considered a failure. When the shizocarp contained any number of healthy looking mericarps, the pollination was considered successful. The number of pollination successes divided by the total number of pollination attempts is reported as the % crossability for each type of cross.

Seed (mericarp) fertility and characteristics

Both fertile (good) and sterile (aborted) seeds (mericarps) were counted for each category of fruit set. Determining the fertility or sterility of seeds was not difficult, as sterile ones usually appeared to be smaller in size and had an obviously wrinkled mericarp. The number of fertile mericarps (seeds) per fruit, the number of aborted mericarps (seeds) per fruit, and the mass of both fertile and sterile mericarps per fruit were obtained. The length of the awn of the mericarp was measured to the nearest 0.01 mm using digital calipers, and averaged, from three mericarps per fruit. Mericarp color was recorded as black, brown or tan.

Germination velocity

Non-aborted seeds were tested for germination in August, 2001. Before planting, all seeds were stripped from the mericarps and nicked to enhance germination. Nicking of seeds was done with a sharp razor under a dissecting microscope. Seeds were submerged in solidified 2% water agar in covered, clear-plastic Petri dishes. The dishes were randomly positioned on lab benches in an air-conditioned room (74° F), under natural light. The dishes were monitored every day for two months, and dates of germination were recorded. The agar medium was replaced as needed to prevent desiccation. The germination rate was observed for 60 days, and the germinated seeds were counted at 2-day intervals (30 observations). The germination rate was determined using a modification of Timson's index of germination velocity (Timson 1965, Khan and Rizvi 1994).

$$\text{Germination velocity} = (\Sigma\, G)/t$$

(where G is the percentage of seeds germinated at any given 2-day interval, and t is the total germination period).

The maximum values possible using this index with the data was 50 (=3000/60: if 100 % germination occurred from the first observation, $\Sigma\, G$ = 100 % x 30 observations =3000, divided by *t* total germination period = 60 days). A higher index value means a higher germination rate.

Table 2.2. Results of flower bagging and self-pollination experiments. Selfing rates sharing the same letter do not differ significantly (P>0.05). B = Beach and M = mountain *S. fallax* ecotypes.

	Number of maternal plants	Number of maternal plants with seeds	Number of flowers pollinated	Number of flowers producing seeds	Selfing rate (%)
Bagged, unmanipulated					
B	9	0	18	0	0a
M	7	0	14	0	0a
Unbagged, Self-pollinated					
B	9	4	90	14	15a
M	7	2	70	10	14.2a
Unbagged, unmanipulated					
B	9	3	90	6	6.6a
M	7	1	70	2	2.8a

Results

Flower bagging and self-pollination experiments

Nine beach and seven mountain *Sida fallax* individuals were observed for ability to self pollinate (Table 2.2). All bagged flowers of *S. fallax* plants failed to produce any seeds.

The mean success of self-fertilization (selfing rate) following artificial self-pollination of the beach plants (B-self) was 15 % and of the mountain plant (M-self), 14.2 % (Table 2.2). The mean seed set of the unmanipulated, unbagged beach and mountain plants was 6.6 % and 2.8 %, respectively (Table 2.2). Four out of nine beach plants, and two out of seven mountain plants, produced some seed when self-pollinated (Table 2.2). However, unbagged flowers of *S. fallax* produced mostly aborted seeds whether artificially selfed or left undisturbed in the greenhouse.

Artificial crossability

All artificial reciprocal crosses produced seeds (Table 2.3). Crosses with the mountain *S. fallax* mother yielded the highest mean % crossabilty (MxB = 95.5 %) and the crosses with the beach *S. fallax* mother yielded the lowest mean % pollination success (BxM =71.0 %) among the four intraspecific *S. fallax* crosses (Table 2.3). However, differences among the mean % pollination success within and between beach and mountain ecotypes of *S. fallax* (BxM, MxB, BxB, MxM), were not significant (*p*=0.078).

Table 2.3. Seed production as a result of pollination within and between beach and mountain *Sida fallax* ecotypes. Mean crossability rates sharing the same letter do not differ significantly (*p*>0.05). B = Beach and M = mountain *S. fallax* ecotypes. SD = Standard Deviation.

	Number of Maternal plants	Number of unique crossing combinations *	Total number of flowers pollinated	Mean crossability (%)	Mean crossability (%) SD
MxM	10	35	77	91.2a	15.2
BxM	9	41	137	71.0a	27.8
BxB	10	50	125	82.9a	20.2
MxB	10	40	128	95.5a	13.3

*Crosses made from different maternal plant –paternal plant combinations.

Seed measurements

Controls

The mean number of fertile seeds/fruit set for *S. fallax* was 6.6 (BxB) and 5.2 (MxM). Mean seed mass/fruit (including the mericarps), seed (mericarp) color, and awn length were significantly different between BxB and MxM. The beach *S. fallax* produce higher mean seed mass (mean BxB = 0.02 g/mericarp) than the mountain *S. fallax* (mean MxM = 0.013 g/mericarp) (Table 2.4). The beach *S. fallax* tended to produce mericarps of lighter color and with shorter awns (mean BxB = 0.5 mm) compared to the mountain *S. fallax* (mean MxM = 2.14 mm) (Table 2.4). As expected, mericarp color and awn length of fruits generated by the experimental treatments were similar to those features observed in non-experimental plants of the corresponding maternal taxon.

Seed set following self-pollination

Only four beach and two mountain individuals of *S. fallax* yielded seeds when

Table 2.4. Seed parameter means. Mericarp color was coded as 1 = black, 2 = brown, 3 = light brown. Means for a given character sharing the same letter do not differ significantly ($P>0.05$). B = Beach and M = mountain *S. fallax* ecotypes.

Cross	Number of maternal plants	Number of flowers	Normal seeds/fruit	Aborted seeds/fruit	Seed mass/fruit (mg)	Mericarp Color	Awn (mm)
Within ecotypes							
BxB	10	98	5.6a	0.6a	0.02b	2.5a	0.5a
MxM	7	68	5.2a	0.9a	0.013a	1.7b	2.1b
Selfs							
B self	4	25	1.2c	4.7c	0.004c	2.3a	0.5a
M self	2	12	3.1c	4.9c	0.008c	1.7b	1.9b
Between ecotypes							
BxM	10	140	4.6a	1.4a	0.015a	2.4a	0.4a
MxB	7	152	5.1a	1.2a	0.014a	1.5b	2.2b

artificially self pollinated. Both B-self and M-self yielded significantly lower mean numbers of seeds/fruit set, mean seed mass/fruit set, and higher mean numbers of aborted seeds/fruit set than the controls (BxB and MxM) (Table 2.4).

Hybrid between beach and mountain Sida fallax

The mean number of seeds (mericarps)/fruit and the mean number of aborted seeds (mericarps)/fruit among the reciprocal hybrids and controls were not significantly different (Table 2.4). The mean seed (mericarp) mass/fruit was not significantly different between the reciprocal hybrids (BxM and MxB); however, the control cross of the beach *Sida fallax* (BxB) produced significantly higher seed mass/fruit than the hybrids (Table 2.4).

Germination velocity

Seeds derived from self pollination and crossing within ecotypes

The germination velocity among beach and mountain *S. fallax* (BxB and MxM) and selfs (B-self and M-self) did not significantly differ (Table 2.5). The average germination velocity of MxM (18.97) and M-self (18.80) were noticeably slower than BxB (25.39) and B-self (24.10) (Table 2.5).

Table 2.5. Mean germination velocity (GV) of *Sida* seeds derived from various pollination treatments. Means sharing the same letter do not differ significantly (P>0.05). B = Beach and M = mountain *S. fallax* ecotypes. The maximum GV for these data using Timson's Index is 50.

Cross	# of Maternal plants	N of seed set	GV Minimum	GV Maximum	Mean GV	Standard Dev
Within ecotypes						
BxB	5	36	7.667	42.667	25.397a	15.328
MxM	6	40	8.258	27.406	18.973a	6.307
Selfs						
B-self	4	9	0	47.356	24.109a	16.856
M-self	2	10	13.020	24.583	18.802a	8.2176
Between ecotypes						
BxM	7	32	0	43.074	29.449a	15.511
MxB	6	38	14.985	46.667	25.82a	11.803

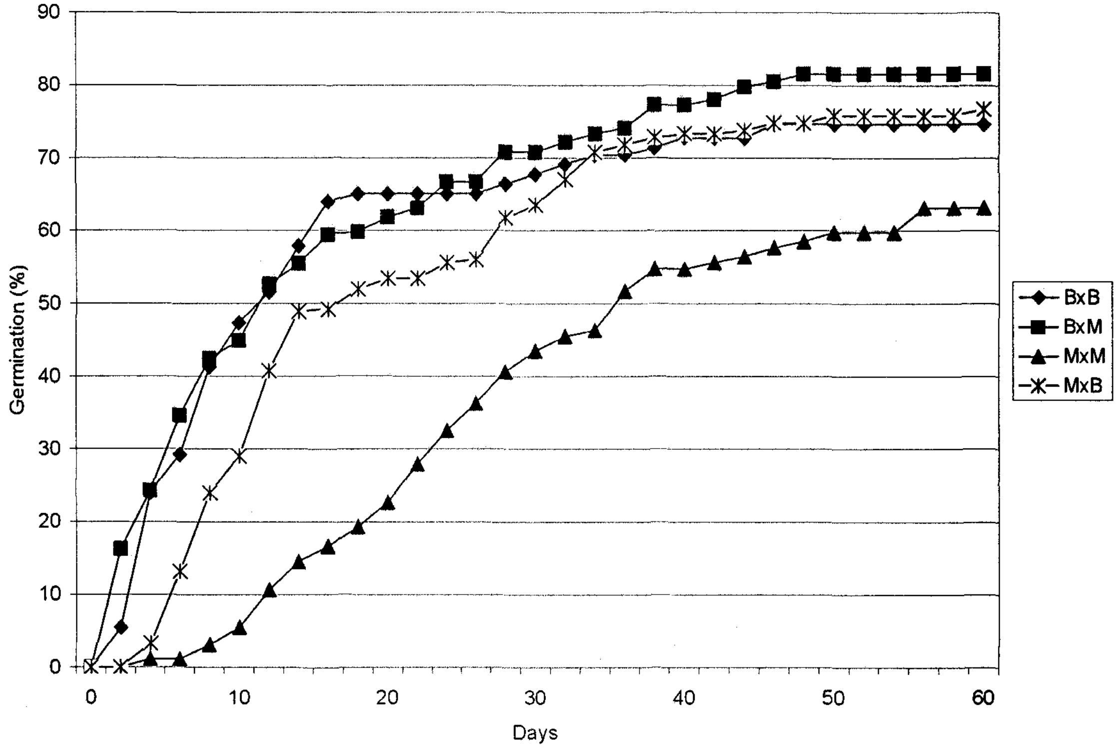


Figure 2.2. Seed Germination (%) for seeds produced by crossing between beach (B) and mountain (M) forms of *Sida fallax* and seeds from crosses within the two ecotypes.

Seeds derived from cross pollination of beach and mountain S. fallax

Figure 2.2 shows the germination responses over the 60-day observation period of hybrids between beach and mountain *S. fallax*. Seeds resulting from crosses within the mountain ecotypes (MxM) were slower to germinate and had lower germination (%) compared to BxB progeny and hybrids between ecotypes at the end of the 60-day observation period (Figure 2.2). However, there were no significant differences among the mean germination velocities of the hybrids (BxM = 29.44, MxB = 25.82) and control crosses (BxB = 25.39 and MxM = 18.97) (Table 2.5, Figure 2.2).

Discussion

Breeding system of *Sida fallax*

Many flowers of *Sida fallax* observed in the green house did not produce fruits. Thus, seed production in *S. fallax* was expected to require some form of externally mediated pollen transfer either within or between individuals. Results from crossing experiments suggest that *S. fallax* is mainly be an outcrossing species since seed production is greatly enhanced by cross-pollination. Most individuals produced no seeds at all following self-pollination; however, some individuals may have had limited self-compatibility. But in all cases where apparent selfed seeds were observed, the flowers had not been bagged, the possibility remains that outcrossing had occurred in these cases as the result of occasional pollinators entering the greenhouse. When flowers were bagged (pollinator exclusion), no seeds developed in the selfing treatment. If selfing does occasionally occur, the smaller sample size of bagged flowers observed (n=2/individual) may be the reason for not detecting selfed seed set in the bagged flowers.

The mean number of aborted seeds /fruit of selfs (B-self = 4.65 and M-self = 4.85) was significantly different from the corresponding maternal control (BxB = 0.60 and MxM = 0.9) (Table 2.5). However, results from germination velocity of selfs (B-self and M-self) were not significantly different from controls (BxB and MxM) (Table 2.6). These results suggest that good seeds produced by self (B-self and M-self) may have been the result of stray cross pollinations in the greenhouse. Further morphological observations of plants resulting from self and cross-pollination treatments also supported this conclusion (discussed in Chapter 3 and 5).

Features of breeding systems, such as self-compatibility and self-incompatibility, may not be absolute (Kearns and Inouye 1993, Borba *et al.* 2001). Even within populations, individuals can exhibit differences in the amount of outcrossing or selfing (Brown *et al.* 1985). Some individuals of typically self-incompatible species may occasionally set fruit when self-pollinated (Schlessman *et al.* 1990). De Nettancourt (1977) suggested that self-fertilization may occur in 'self-incompatible' species under certain conditions such as high temperatures, in situations where pollination of ripe stigmas is long delayed, or at the end of the flowering season. Temperatures in the Pope Laboratory greenhouse where the experiments were conducted often gets high during summer seasons (>86° F) and might have facilitated the few putative cases of selfing.

Artificial crossability, seed measurements and germination velocity

Hybrids between beach and mountain Sida fallax

Grant (1981) distinguished between the formation of a hybrid race and that of a hybrid species. In a hybrid race, some kind of isolation (internal or external) still has to be achieved for divergence to continue to the species level. Grant (1981) stated that such isolation could be completed internally via recombinational speciation or externally through geographic or ecological isolation. Beach and mountain forms of *Sida fallax* are geographically isolated and are morphologically divergent. I suspected that the distinct forms may have developed some degree of genetic isolation. However, crossing experiments did not detect any sign of hybrid barriers between two forms of *S. fallax*.

Other fitness measures also revealed that hybridization between two forms of *Sida fallax* does not seems to have deleterious effects, as there were no significant differences in number of seeds/fruit, number of aborted seeds/fruit, and seed mass/fruit among the cross pollinated plants and controls. Mericarp color and awn length in fruits resulting from the pollination treatment were similar to the maternal parent characteristics. This makes sense since the mericarp develops from the ovular tissues and usually has the genetical characters of the mother plant (Boesewinkel and Bouman 1995).

Individuals from beach and mountain ecotypes of *Sida fallax* exhibited variations in seed mass, mericarp color, awn length and germination velocity. Many species exhibit a discrete, rather than continuous variation in seed mass and seed morphology, producing two or more seed morphs that may differ in germination requirements, germination rate, and dispersal, as shown for species of *Atriplex* (Chenopodiaceae) (Osmond *et al.* 1980), and for *Bidens pilosa* (Asteraceae) (Forsyth and Brown 1982). It is usually assumed that diversified germination, which results in varied germination in space and time, is particularly important in unpredictable environments (Cohen 1968).

Sida fallax flowers all year round; yet, wild populations of *S. fallax* seem to bloom more abundantly during the spring and early summer months (Pope, 1986). Therefore, *S. fallax* may possess some degree of dormancy to prevent germination during the dry summer season. Although all seeds had been nicked in an effort to overcome dormancy, it is interesting that many seeds still showed delayed germination. Dormancy plays an important part in preventing seeds from germinating at a time that would be unfavorable for growth and establishment (Fenner and Kitajima 1999). Although the differences were not significant, the mountain *S. fallax* (MxM) had the lowest mean germination velocity (Figure 2.15). Rapid germination in the coastal plants seeds may increase the chance of survival by quickly responding to rapid changes in soil moisture in the dry beach habitats compared to the mountain habitats.

Conclusions

Sida fallax was found to be primarily self-incompatibe. There is a possibility that a few plants were capable of producing self-seeds at a reduced rate following deliberate application of self pollen to the stigmas; however, in the cases were this was observed, the flowers had not been bagged, and there is a possibility that the few seeds that developed were due to stray insects entering the greenhouse and effecting cross-pollination. Beach plants tended to have lighter colored mericarps with shorter awns, compared to mountain plants. Seeds of the beach plants also tended to have slower germination rates. The two ecotypes of *S. fallax* were highly crossable in the artificial pollination experiment. The seed characters (seed number and mass/fruit) and germination velocity of intraspecific hybrids were similar to controls derived from intra-ecotype crosses.

Chapter 3

Morphological Assessment and Pollen Stainability of Hybrids Between Mountain and Coastal Ecotypes of *Sida fallax* Walp. (Malvaceae)

Introduction

Many animals and plants exhibit extensive morphological variation across their geographical range (Hughes *et al.* 2001). Sometimes the variation occurs within the same geographic region. Such distinct genotypes (or populations) that result from adaptation to local environmental conditions are called ecotypes (Hufford and Mazer 2003). Ecotypes of the same species are frequently capable of interbreeding. If these sympatric forms are discrete, then the term 'polymorphism' is applied (Hughes *et al.* 2001). Many studies have examined the evolutionary significance of polymorphisms and ecotypes within species. Classic examples of populations with geographic variation reviewed by Ricklefs (2001) are; differentiation in populations of the yarrow, *Achillea millefolium* (Clausen *et al.* 1948), the Japanese field cricket *Teleogryllus* (Masaki 1967), the land snail, *Cepaea nemoralis* (Jones *et al.* 1977), the saddleback tamarins (*Saguinus fuscicollis*) in the upper reaches of the Rio Jurua in the Western Amazon basin of Brazil (Peres *et al.* 1996).

The Hawaiian flora has been recognized for morphological variability of a continuous nature (Fosberg 1948, Gillett 1972b, Carlquist 1974, Mayer 1991b, Wagner *et al.* 1999). Some extreme examples of morphological variability in Hawaiian flora are *Metrosideros polymorpha* (Myrtaceae) (Mueller-Dombois 1975, Mueller-Dombois *et al.* 1981, Kitayama *et al.* 1997), *Cyrtandra* (Gesneriaceae) (Smith *et al.* 1996, Carr 1998), *Acacia koa* (Fabaceae) (Daehler *et al.* 1999), and *Wikstromia* (Thymelaeaceae) (Mayer

1991a, 1991b). Mechanisms that may promote this kind of variation include hybridization and adaptive radiation (Carlquist 1974, Carlquist 1980).

The current Hawaiian angiosperm flora is thought to have arisen from 270 to 282 successful colonization events (Fosberg 1948, Wagner 1991). The diversity of habitats, island environments and the geological recency of the main islands provide a large array of opportunities for evolutionary differentiation (Wagner *et al.* 1999). Wagner *et al.* (1999) listed 25 native Hawaiian species as polymophic, and suggested that detailed studies may support formal recognition of additional taxa. *Sida fallax* is one of these polymorphic species.

Sida fallax Walp. (Malvaceae) is widespread on Pacific islands including Midway Atoll, Nihoa, and all the main Hawaiian Islands where it occurs in coastal shrubland communities (sea level to 300 m elevation), lowland dry communities (15-600 m elevation), and montane communities (500 to 2,700 m elevation) (Gagne and Cuddihy 1999). Morphological variation exists within and between populations, especially in stature, pubescence, leaf size and shape, and floral characters (Bates 1999). Two extreme ecotypes are recognized between the beach (coastal) and mountain populations (Stephens 2000). The coastal type is a prostrate scrambler or subshrub up to 0.2 – 1.2 m tall, found on arid exposed headlands and other coastal sites. The mountain type is an erect tree-like shrub up to 2.5 m tall found in montane and mesic communities (Bates 1999).

Hybridization between these two extreme forms of *S. fallax* is unlikely since they are usually geographically isolated. However, putative intermediate forms are recognized in limited sites in Oahu (Koko head, Koko Crator, Diamond Head and Ewa) (Yorkston, personal observation). The uncommonness of these intermediate forms may be due to the urbanization of intermediate habitat. Despite some apparently limited intergradations, the pattern of morphological and ecological variability of *Sida fallax* suggest that infraspecific taxa could be recognized (Bates, 1999).

Intermediacy of hybrid morphology

Traditionally, the expression of morphological, chemical, and physical traits in hybrid taxa was expected to be intermediate between parents because hybrids combine alleles from their parental species and most phenotypic traits are thought to be associated with many additive genes (Arnold and Hodges 1995, Rieseberg 1995, Arnold *et al.* 2001, Rosenthal *et al.* 2002). However, recent reviews of hybrid research from natural populations and manipulative experiments, and found that the expression of traits in hybrids shows a diversity of patterns (Arnold and Hodges 1995, Arnold 1997, Arnold *et al.* 2001, Johnston *et al.* 2001). Expression of traits in hybrids may be additive (intermediate), dominant (similar to one of parent), extreme (greater or less than either parent), or absent (completely missing) relative to the parents (Rieseberg and Ellstrand 1993, Fritz *et al.* 1994, Orians *et al.* 1999). Understanding heritability of ecologically important traits such as body size, fertility, and disease resistance is important because the degree and type of heritability of these traits determines the response to selection and could be a key to understanding how natural populations evolve and how they can be best managed in conservation programs (Snustad *et al.* 1997, Keller *et al.* 2001).

The specific purpose of this study is to provide a detailed assessment of morphological differences among experimentally produced putative hybrids between beach and mountain forms of *Sida fallax* as well as controlled crosses within each ecotype. Careful investigation of morphological characteristics of putative hybrids is necessary in order to evaluate credibility of the cross pollinations and to assess the evolutionary consequences and ecological implications of hybridization (Arnold and Hodges 1995, Campbell *et al.* 1997, Rieseberg and Carney 1998, Arnold *et al.* 1999, Arnold *et al.* 2001, Rosenthal *et al.* 2002). Pollen stainability of the resulting putative crosses was also assessed to determine if the experimental hybrids expressed differences relative to non-hybrids offspring derived from the same parents. This study of *S. fallax* documents another case of hybridization in the Hawaiian flora in addition to the many other cases already recognized (Gillett 1972b, Rabakonandrianina 1980, Rabakonandrianina and Carr 1981, Carr 1987, Mayer 1991a, Carr 1995, Smith *et al.* 1996, Caraway 1997, Motley and Carr 1998, Daehler *et al.* 1999, Randell and Morden 1999, Randell 2000, Caraway *et al.* 2001, Daehler and Carino 2001, Weller *et al.* 2001).

Materials and Methods

Plant materials

Experiments were conducted at the Pope Laboratory greenhouse, at the University of Hawaii. The original plants used to generate hybrids and non-hybrid seeds were a subset of the plants used by Stevens (2000). These plants were grown from seeds collected from six different sites on the island of Oahu. The locations, elevations and annual rainfalls of the seed collection sites are summarized in Table 3.1 and Figure 3.1.

Plants from the mountain populations (elevation higher than 266m) had an upright growth form with nearly glabrous leaves, and plants from the coastal populations (near sea level) were prostrate with pubescent leaves (Stephens 2000). Seeds from all six collection sites were germinated and grown in two-gallon pots in Sunshine soil medium (J. G. Smith & Company, Illinois) in the greenhouse during 1998 and1999 (Stephens

2000). A total of 11 mature beach and 10 mature mountain plants of *Sida fallax* from 6 populations (Table 3.1) were used to produce putative hybrids between and within these divergent forms.

Table 3.1. Collection sites of original maternal plants. Rainfall data of these sites is from Giambelluca et al. (1996).

Collection Site	Elevation (m)	Annual Rainfall (mm)
Mountain *S. fallax* (M)		
Wa'ahila Ridge	366	2000
Hawai'iloa Ridge trail	330	1250
Kuaokala Forest Reserve	266	900
Beach *S. fallax* (B)		
Makapu'u beach park	near sea level	800
Ka'ena Point Natural Area Reserve	near sea level	800
Sandy Beach Park	near sea level	800

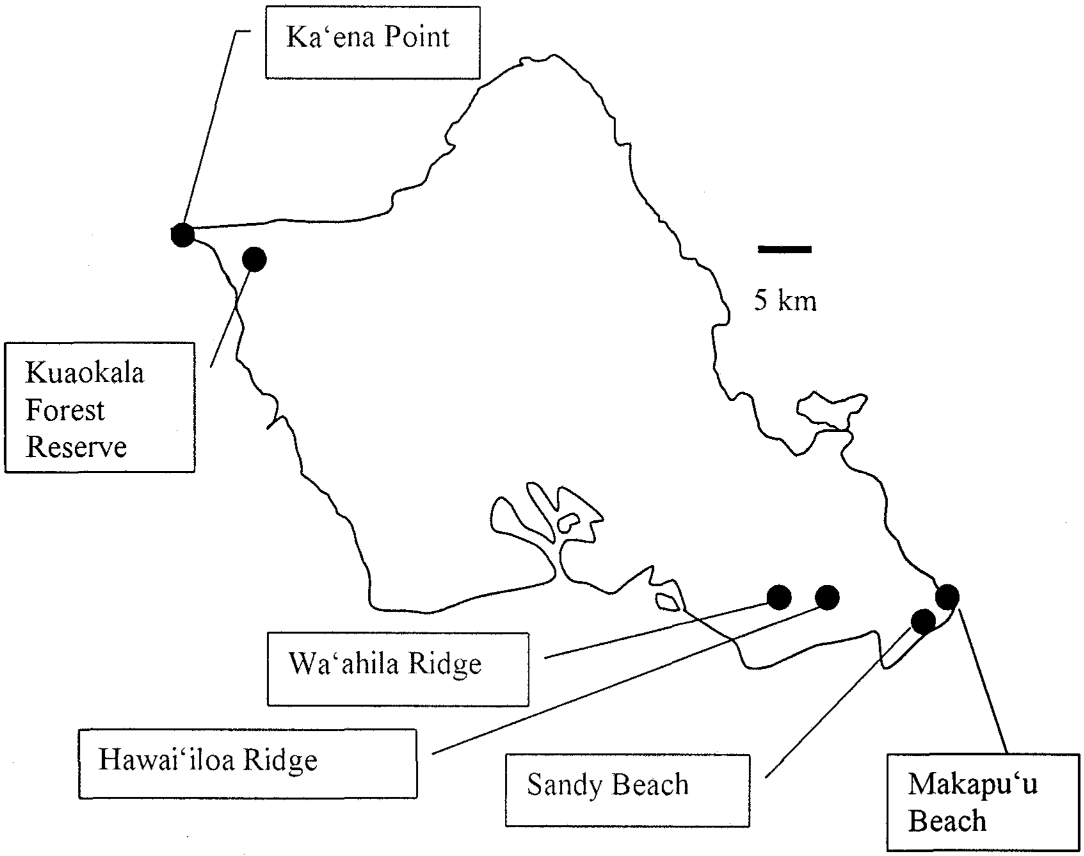


Figure 3.1. Collection sites of original plants.

Experimental pollination

Two divergent forms (M indicates the mountain form and B indicates the beach form) of *Sida fallax* were reciprocally crossed (MxB and BxM: the female parent is listed first). In addition, individuals within each ecotype were cross-pollinated to perpetuate the parental forms (hereafter refered to as "controls") to serve as a basis of comparison with the results of cross pollinations between ecotypes (Table 3.2).

Table 3.2. *Sida fallax* putative cross pollination matrix.

	Paternal plant	
Maternal plant	M	B
Mountain *S.fallax* (M)	MxM	MxB
Beach *S. fallax* (B)	BxM	BxB

Pollinations were carried out from 1999 to 2001. A sharp-tipped forceps was used to collect mature anthers from a pollen donor and dust pollen on the stigmas of a maternal parent. The flowers were pollinated at various times of the day in order to obtain seeds from as many combinations of crosses as possible. All pollinated flowers were monitored for two to three weeks, and eventually any seeds produced were collected for germination. Results from crossing experiments (Chapter 2) suggested that *Sida fallax* is mainly an outcrossing self-incompatible species that requires pollen a pollen vector, and may also exhibit at least partial self-incompatibility. Few seeds were formed following selfing, and seed production was greatly enhanced by cross-pollination. Thus, *S. fallax* flowers in the crossing treatments were not emasculated. It was assumed that any products of self-pollination that might appear among the experimental progeny would be detected by morphological analysis.

Good seeds that were not obviously aborted were planted in August 2001. Each seed was stripped from the mericarp and nicked before planting in order to enhance germination. Nicking of seeds was done by making a small incision in the seed coat with a sharp single-edged razor blade under the dissecting microscope. Seeds were submerged in solidified 2% water agar in covered, clear plastic Petri dishes. The dishes were randomly positioned on lab benches in an air-conditioned room (74° F) under sun light. The dishes were monitored every day for two months and dates of germination (= first emergence of the radicle) were recorded. Within a week after germination (2-7 days), each seedling was transplanted from the water agar to a 12.7x12.7 cm pot with Big R potting soil mix (Cascade Forest Product, Inc. California), and randomly located on the Pope Laboratory greenhouse benches. All plants used for this study were well watered to keep the soil moist.

Morphological traits

The morphology of putative hybrids between beach and mountain forms and control plants was assessed using 17 characters (Table 3.3). All measurements were taken from flower and leaf samples collected from hybrid and control plants between 2001 and 2002 after first flowering. At least one flower and two leaves were sampled per individual plant. Leaves were collected from the third and fourth node proximal to the tip of the main (longest) stem.

Table 3.3. Traits assessed in the morphological analysis of *Sida fallax*.

Character
Leaf width (cm)
Leaf blade length (cm)
Width/length ratio
Leaf base Lobe (cm)
Leaf pubescence top (# of trichomes/mm)
Leaf pubescence bottom (# of trichomes/mm)
Leaf margin (1=sharp serrate, 0 = crenate)
Petiole length (cm)
Flower diameter (mm)
Petal length long (mm)
Petal length short (mm)
Petal lobe tatio (short/long) (mm)
Petal width (mm)
Style length (mm)
Pedicel length (cm)
Petal color (1=pale yellow, 2=medium yellow, 3=dark yellow)
Dark spot on flower (0=absent,1=light, 2=medium, 3=dark)

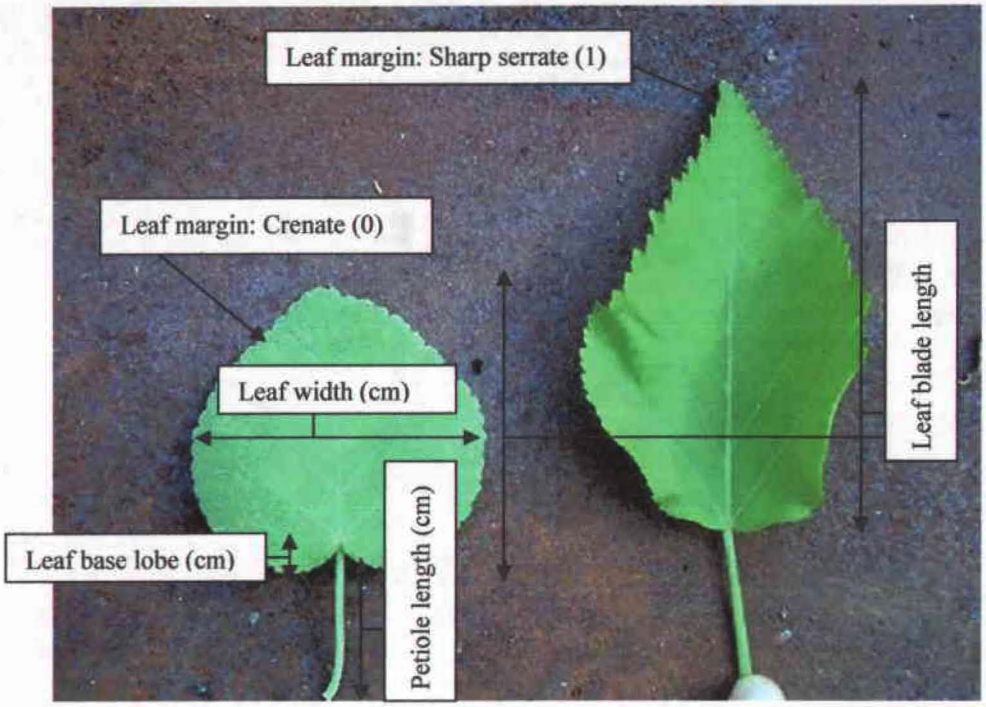


Figure 3.2. Leaves of selected *Sida fallax* from the study. Leaf width and length, petiole, and leaf base lobe were measured in cm and leaf margin was assigned between sharp serrate (1) to round (0).

Eight leaf characters were assessed to compare morphology of hybrids and controls (width, blade length, width and length ratio, leaf base lobe length, top and bottom surface pubescences, leaf margin, and petiole length) (Table 3.3). Leaf width was measured at widest part of the leaf blade and blade length was measured from the apex to the bottom of the base (Figure 3.2). The leaf width:length ratio was obtained to assess leaf shapes. Leaf base lobe length was determined by measuring the length of the sinus at the base of the blade (Figure 3.2). Leaf pubescence on top and bottom surfaces was assessed by counting the number of trichomes/mm profiled on a thin transection of the

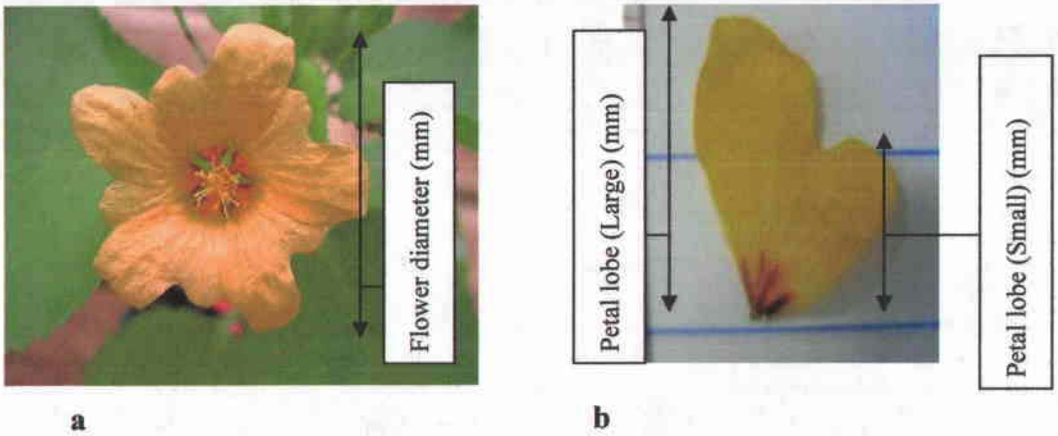


Figure 3.3. A flower (a) and a petal (b) of selected *Sida fallax* from the study. The flower diameter was measured at the widest part of the fully opened flower (mm) (a). The petal lobe was measured for the largest and the shortest parts to assess the depth of the petal lobe (mm) (b). The petal width was the measured at the widest part (mm) (b).

leaf (near the center avoiding the midrib area) under the dissecting microscope at 24 x magnification. Leaf margin was assigned to sharp serrate (1) or crenate (0) (Figure 3.2).

Nine flower characters were assessed to compare the morphology of putative hybrids and controls (flower diameter, large petal length, small petal lobe length, petal lobe ratio, petal width, style branch length, pedicel length, petal color and presence of a dark spot at base of the flower) (Table 3.3). Flower diameter was measured at the widest part of the fully opened flower (Figure 3.3 a). Petals were separated from the base of the flower to measure the length and width (Figure 4.3 b). Length of the asymmetrically 2-lobed petal was measured from the base to the tip for the large lobe and the small lobe (Figure 4.3 b). The petal lobe ratio was assessed by dividing the large petal lobe length by the small petal lobe length. Style branch length was measured to the nearest 0.01 mm from the tip of a stigma to the distal end of the staminal column by using a digital caliper. Petal color was assigned to one of three categories (1=pale yellow, 2=medium yellow, 3=dark yellow). Presence or absence of the maroon spot at base of the flower was recorded in four categories (0=absent, 1=light, 2=medium, 3=dark).

The data were analyzed using SYSAT 9 (SSPS., Inc., Chicago). All measurements were averaged at the level of the individual plant or individual hybrid combination, and then averaged again based on the common maternal plant to analyze variation among different hybrid combinations. Finally, these values were averaged to compare hybrids versus beach or mountain ecotypes in an ANOVA and a student t-test as needed.

Digital images

Flowers from most parental plants, putative hybrids, and controls were photographed by digital camera with a size reference next to the flower. These images were used to help trace inheritance of floral traits from parental plants to putative hybrids and controls.

Voucher specimens

Voucher specimens were prepared for three mountain (# 1-3) and three beach plants (# 4-6) that were used as parents. In addition, representative vouchers were prepared for three plants in each putative hybrid category (BxM and MxB) (# 19-21 and 13-15) and in each control category (BxB and MxM) (# 26-28 and 11, 12 and 17). The vouchers are deposited at the primary research herbarium located in the University of Hawaii (HAW).

Pollen stainability

The percentage of stainable pollen was calculated to estimate the pollen viability of the two ecotypes of *Sida fallax* (BxB and MxM) and their reciprocal hybrids (BxM, MxB) using a solution of aniline blue in lactophenol (see (Hauser and Morrison 1964, Stanley and Linskens 1974) and following the protocol suggested by Kearns and Inouye (1993). Pollen was collected and stained as individual plants flowered in the morning and afternoon. Pollen from all parental plants and reciprocal hybrids were compared. Between one and five flowers were sampled from each specimen, depending on availability. Pollen viability can be affected by environmental factors such as temperature, humidity and the specific time and date of the collection. Therefore, it would be ideal to collect pollen at the same time and day, in order to compare the viability of different plants. However, this was not always possible, and I assumed the conditions were relatively stable in the greenhouse during the period of sample collections.

Pollen grains were stained for at least 24 hours before determining stainability. Observations of stainability were made at the maximum magnification (32X) under a dissecting microscope. The percentage of stained pollen grains ($n = 100$) was recorded. Only uniformly well-stained grains of normal size were scored as "stained".

Results

Morphological traits

There was a wide range of variation in the leaf shape between and within the ecotypes of *Sida fallax* (Figure 3.4). The mountain plants tended to have spade-shaped leaves with a weakly cordate base and the beach plant leaves tended to be more circular shaped with a deeper cordate base Table). Leaf base lobe length, leaf pubescence on top and bottom surfaces and pedicel length differed significantly ($p > 0.05$) among the beach, mountain and hybrid *Sida fallax* (Table 3.4).

Leaf base lobe was much deeper and more defined in the beach plants (mean BxB = 0.53 cm) compared to mountain plants (mean MxM = 0.06 cm), and hybrids were intermediate (mean BxM = 0.35 cm and MxB = 0.43 cm) (Table 3.4). The beach plant leaves were more pubescent (mean BxB top = 9.3/mm; bottom = 13.3/mm) than those of mountain plants (mean MxM top = 0.4/mm; bottom = 2.4/mm), and hybrids were intermediate (mean BxM top = 3.4/mm, bottom = 7.2/mm; mean MxB top = 3.4/mm, bottom = 8.7) (Table 3.4).

A typical leaf of the mountain plant had a sharply serrate margin (mean MxM value = 1) and the typical beach plant leaf had a crenate margin (mean BxB value = 0.24). The leaf blade length tended to be longer for the mountain plant (mean MxM = 5.56 cm) than the beach plant (mean BxB = 4.05 cm) (Figure 3.4). Leaf length and leaf margin of hybrid plants were similar to the mountain plants than the beach plants (Figure 3.4).

Out of 10 flower characters assessed, the pedicel length was the only one that significantly differed ($p < 0.05$) among the beach, mountain and hybrid forms (Table 3.4). The mean pedicel length of the beach form (3.7 cm) exceeded that of the mountain form (1.8 cm) and the hybrids were intermediate (BxM = 3.3 cm, MxB = 2.94 cm) (Table 3.4). The mean petal width of the mountain form (10.42 mm) was significantly narrower ($p < 0.05$) than the beach (15.4 mm) and hybrid forms (MxB = 12.5 mm, MxB = 13.4 mm) (Table 3.4).

Table 3.4. Mean values for each of 17 traits of putative hybrids between beach (B) and mountain (M) forms of *Sida fallax* and controls grown together in a greenhouse. All measurements were averaged at the level of the individual plants or individual hybrid combination, and then averaged again based on the common maternal plant. For each character, means sharing the same letter do not differ significantly ($P>0.05$).

Cross type	BxB		BxM		MxB		MxM	
Character	Mean	Standard Dev	Mean	Standard Dev	Mean	Standard Dev	Mean	Standard Dev
Leaf width (cm)	4.12a	0.96	4.42a	1.07	4.66a	1.20	4.98a	1.29
Leaf blade length (cm)	4.05a	1.06	4.86b	1.13	5.19b	1.29	5.56b	1.03
Leaf width/length ratio	1.03a	0.12	0.92a	0.11	0.90a	0.11	0.91a	0.26
Leaf blade base lobe length (cm)	0.54a	0.17	0.36b	0.14	0.43b	0.27	0.06c	0.099
Upper leaf surface (# of tricomes/mm)	9.3a	3.6	3.4b	3.6	3.4b	2.9	0.4c	0.512
Lower leaf surface (# of tricomes/mm)	13.3a	4.6	7.2b	4.3	8.7b	3.6	2.4c	0.727
Leaf margin (0=crenate, 1=sharp serrate)	0.24a	0.52	0.85b	0.36	0.91b	0.28	1.00b	0.00
Petiole length (cm)	2.35a	0.83	2.15a	0.93	2.20a	0.80	1.95a	0.63
Flower diameter (mm)	25.2a	7.3	27.5a	6.2	28.1a	4.2	21.7a	7.20
Petal length large lobe (mm)	15.3a	2.6	15.1a	3.1	15.8a	2.5	13.7a	3.5
Petal length small lobe (mm)	11.7a	2.5	10.3a	2.4	11.1a	1.8	9.5a	2.5
Petal lobe ratio (large:small)	1.3a	1.3	1.4a	2.1	1.4a	2.3	1.4a	2.6
Petal width (mm)	15.4a	3.5	12.5a	3.6	13.4a	2.3	10.4b	2.8
Style branch length (mm)	3.7a	0.8	3.3a	0.7	3.5a	0.6	3.1a	0.4
Pedicel length (cm)	3.68a	0.66	3.30b	0.54	2.94b	0.69	1.80c	0.75
Petal color (1=pale yellow, 2=medium yellow, 3=dark yellow)	1.71a	0.76	1.62a	0.65	1.86a	0.72	2.22a	0.44
Dark spot of flower (0=absent, 1=light, 2=medium, 3=dark)	1.29a	0.49	1.77a	0.83	2.25b	0.60	1.56a	0.73

The dark spot in the center of the flower was significantly more pronounced ($p < 0.05$) in MxB hybrids (mean dark spot value = 2.25) than in the beach (mean dark spot value = 1.28), mountain (mean dark spot value = 1.56) and BxM hybrid (mean dark spot value = 1.77) plants (Table 3.4).

Flower diameter and petal length, petal lobe length, style branch length, and petal color did not show significant difference among the ecotypes or hybrids (Table 3.4). The average values suggested that the mountain (mean MxM = 1.54 mm) and hybrid (mean BxM = 1.04 mm and MxB = 1.37 mm) plants tend to have deeply lobed petals compared to the beach plants (mean BxB = 0.44 mm) (Table 3.4). The average values also suggest that the mountain plants (mean MxM = 3.07 mm) have shorter styles than the beach plants (mean BxB = 3.70 mm); hybrid plants were intermediate (mean BxM = 3.33 mm, mean MxB = 3.46 mm) (Table 3.4).

The expression of morphological traits in hybrids is summarized in Table 3.5. Beach and mountain *Sida fallax* were statistically distinct ($p > 0.05$) in 7 out of the 17 assessed characters. Hybrids did not differ from parents for 9 characters. Hybrids expressed 4 intermediate and 3 dominant traits out of 17 characters studied. None of the traits scored in the parents was completely lacking in the hybrids; one of the traits was expressed in an extreme fashion in hybrids.

Digital images

Selected images of leaves (Figure 3.4), habits (Figure 3.5), and seeds (Figure 3.6) show typical morphological characters of beach, mountain and hybrid *Sida fallax*. Figure 3.7 shows the flower of the maternal, paternal, and F_1 plants in the same row to allow identification of the heritable characters. The dark spot in the center of the flower appears to be an extreme character for MxB (Table 3.4). The patterns of expression between parent and offspring of other characters such as flower size, petal shape, flower color, and filament length were not clearly resolved (Table 3.4, Figure 3.7).

Table 3.5. Summary of morphological trait expression in hybrids between beach and mountain *Sida fallax*. Categories of expression were: same, intermediate, dominance (similar to one of parent), extreme (greater of less than either parent) and absent (completely missing) relative to parents. B or M indicates parent types and X indicates both BxM and MxB.

Character	Parents distinct?	Same	Intermed.	Dominant	Extreme
Leaf Width (cm)		X			
Leaf blade length (cm)	Yes			M	
Leaf width/length ratio		X			
Leaf blade base lobe length (cm)	Yes		X		
Upper leaf surface (# of tricomes/mm)	Yes		X		
Lower leaf surface (# of tricomes/mm)	Yes		X		
Leaf margin (0=soft crenate, 1=sharp serrate)	Yes			M	
Petiole length (cm)		X			
Flower diameter (mm)		X			
Petal length large lobe (mm)		X			
Petal length small lobe (mm)		X			
Petal lobe length ratio (mm)		X			
Petal width (mm)	Yes			B	
Style branch length (mm)		X			
Pedicel length (cm)	Yes	X			
Petal color (1=pale yellow, 2=medium yellow, 3=dark yellow)			X		
Dark spot of flower (0=absent, 1=light, 2=medium, 3=dark)		(BxM)			MxB
Total	7	9	4	3	1

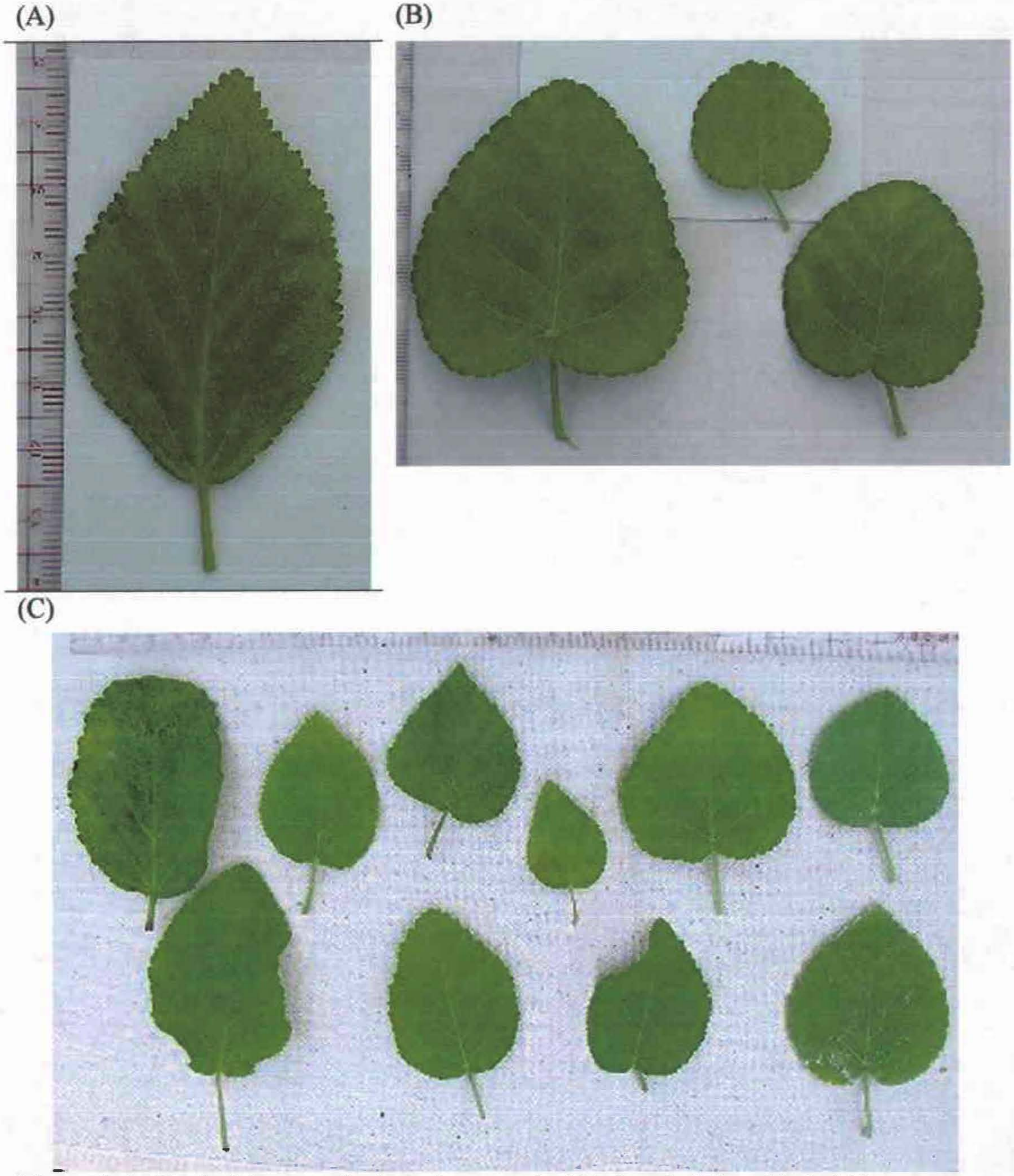


Figure 3.4. Typical leaves of selected mountain (A), beach (B) and hybrid between beach and mountain forms (C) of *Sida fallax*. Note that the mountain leaf has a sharp serrate margin and the beach leaves have a smooth round margin. The leaves of the beach form tend to have more pronounced basal lobes. Hybrid leaves show a lot of variation (C).

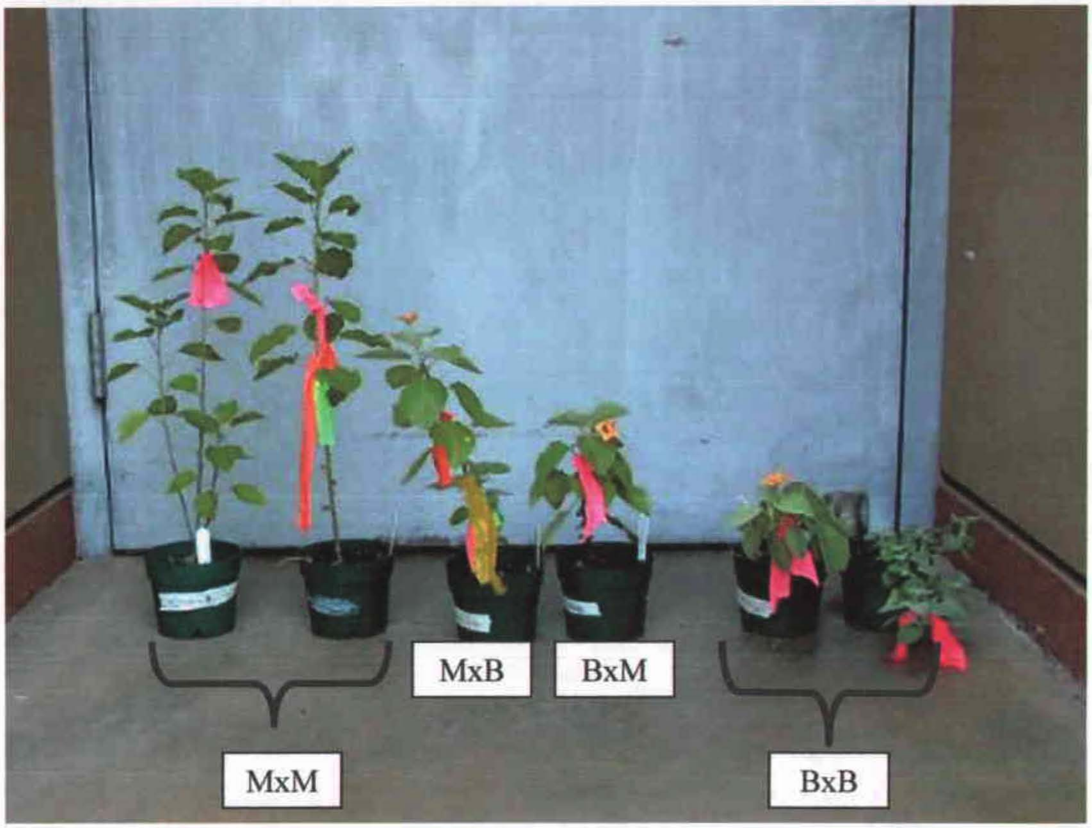


Figure 3.5. Habits of selected mountain (left), hybrids (center) and beach (right) *Sida fallax*. Note that hybrids show intermediate habit. These plants were germinated at the same time and grown under the same condition for seven months.

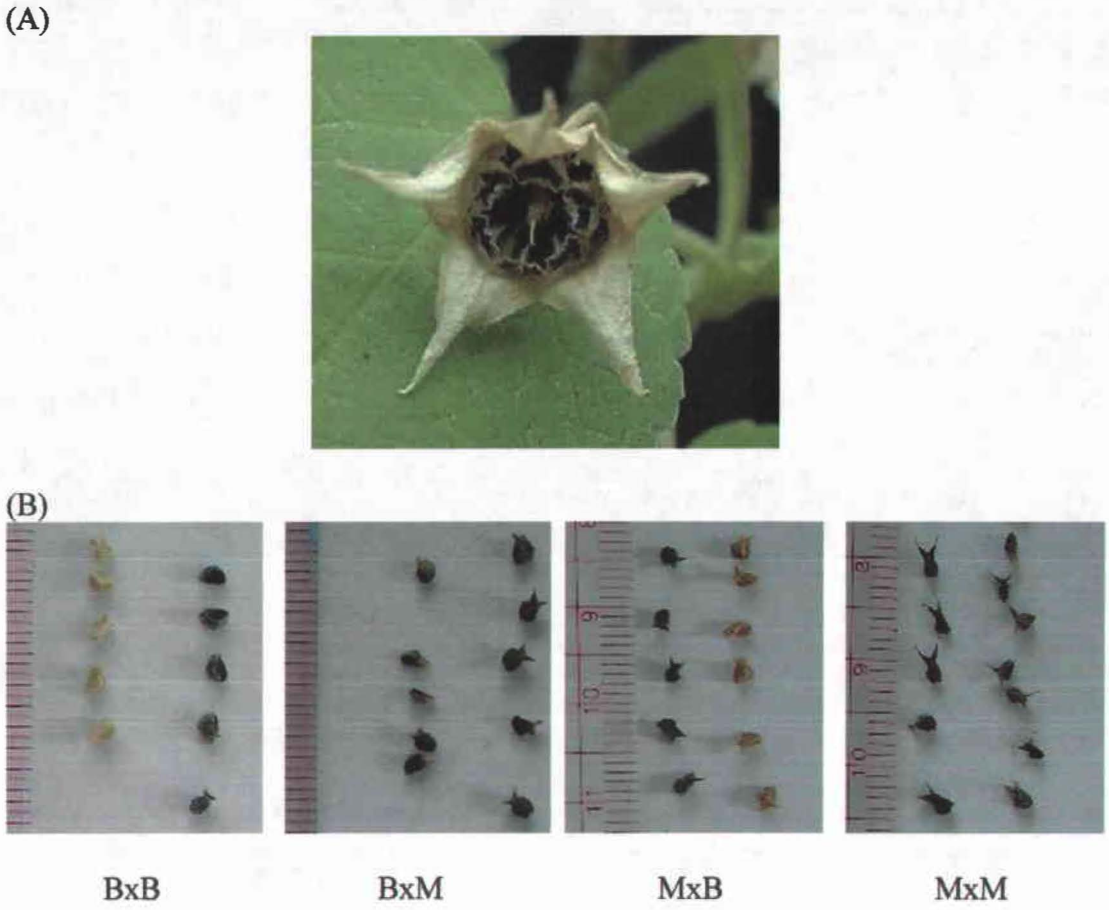


Figure 3.6. *Sida fallax* seeds (mericarps) in the fruit (schizocarp) (A) and selected seeds of *Sida fallax* (beach, mountain and hybrid) (B). Note the variation in seed shape, awn length and color.

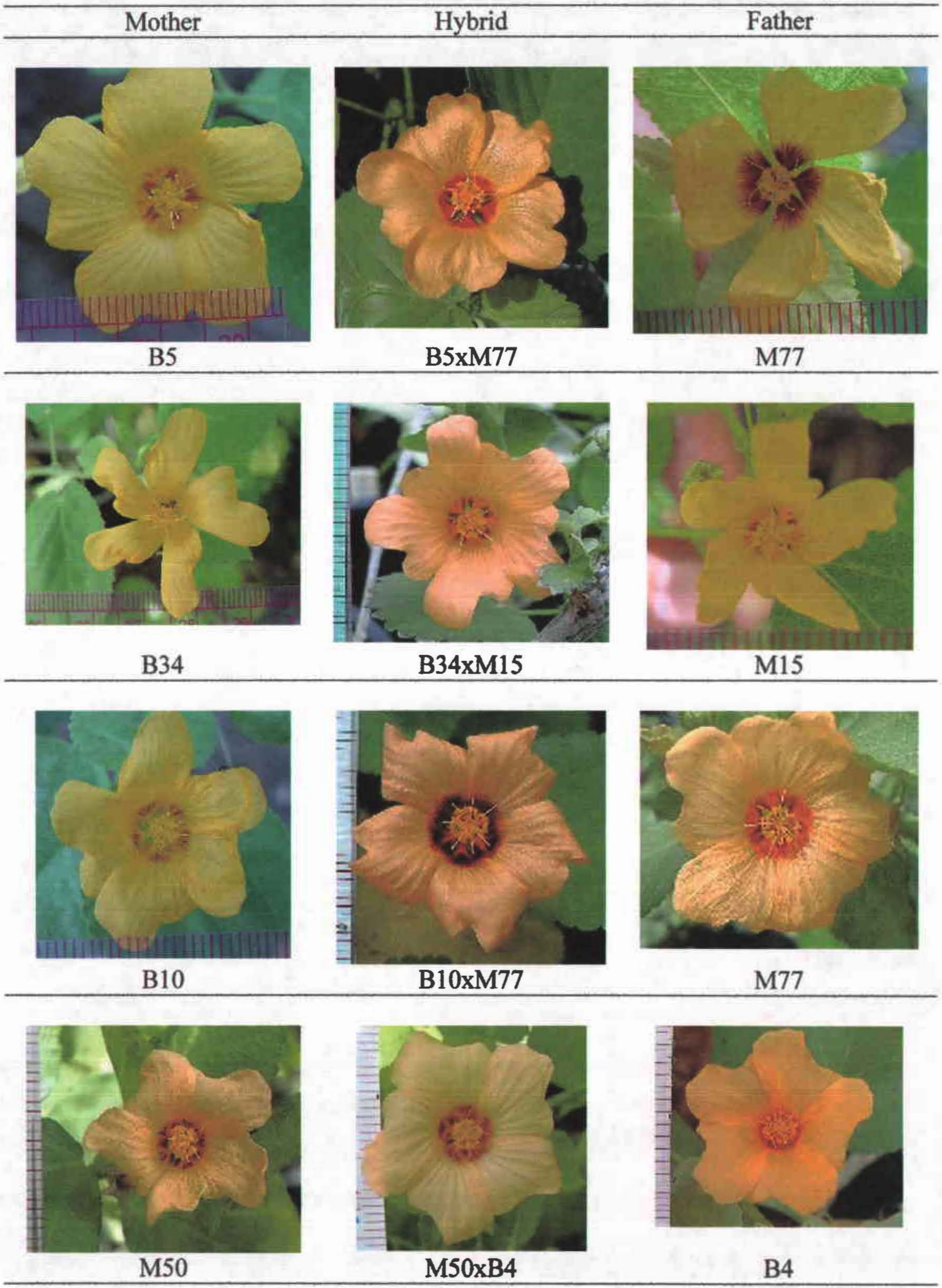


Figure 3.7. Selected flowers of *Sida fallax*. Left; maternal parent; right; paternal parent; center, F_1 hybrid.

Mother	Hybrid	Father
		
B4	B4xB34	B34
		
M13	M13xM50	M50
		
B33	B33xB6	B6
		
M38	M38xB5	B5

Figure 3.7. Continued.

Pollen stainability

Progeny of all crossing categories displayed near or above 90% mean pollen stainablity (Table 3.6). Mean pollen stainability of MxM progeny was the lowest among the crossing categories (87.4 %) and BxB was the highest (98.3 %). There were no significant differences in mean pollen stainablity among the crossing categories. One individual MxB putative hybrid had a pollen stainability of only 12.5 %.

Table 3.6. Mean pollen stainabilility of *Sida fallax* parental plants (B and M), and crosses (BxB MxM, BxM and MxB). Mean stainability was averaged per individual plants and per pollination treatment. A minimum of 100 grains per flower was counted.

Pollination Treatment	Mean ± s.d. (%)	Minimum and maximum values (%)	N of individuals sampled
BxB	98.3 ± 1.8	94.1-100	14
MxM	87.4 ± 14.7	77-100	10
BxM	98.0 ± 2.5	92-100	10
MxB	93.8 ± 16.5	12.5-100	30
Parental plants			
B	94.7 ± 6.8	82.6-97.8	8
M	98.5 ±1.1	97.3-100	4

Discussion

Morphological traits

Morphologial variability was high within beach and mountain forms of *Sida fallax* and in the hybrids between them (Table 3.4). Out of 17 traits assessed in this study, 9 traits were the same for beach and mountain types, while 4 traits differed between beach and mountain types and were intermediate in the hybrids. Three of traits (leaf length, leaf margin, petal width) seemed to be dominant characters based on hybrid mophology. The dark spot of the flower was the only extreme trait expressed in hybrids. The color of the dark spot of MxB was significantly darker than both parents. These results indicate that the putative reciprocal hybrids were indeed successful.

Although it has long been recognized that hybrids sometimes exhibit extreme or novel characters, these have often been considered to be rather rare and unimportant from an evolutionary perspective (Stebbins 1974). However, Rieseberg and Ellstrand (1993) compiled a list of 46 studies that report morphological character expression in hybrids and found that over 10 % of the characters measured in first generation hybrids were extreme. The study indicated that even higher proportions of extreme characters are displayed by later generation hybrids (30.6 %) and hybrid taxa (16.0 %) (Rieseberg and Ellstrand 1993). Furthermore, 64 % of the first generation hybrids, 89 % of the later generation hybrids, and all of the hybrid taxa exhibited extreme or novel characters (Rieseberg and Ellstrand 1993). The high frequency of extreme characters in hybrids supports the view of hybridization as a source of variation upon which selection can act (Rieseberg and Carney 1998). These studies also revealed that F_1 hybrids expressed a high proportion of parental (45.2 %) and intermediate (44.7 %) morphological characters (Rieseberg and Carney 1998). In *Sida fallax*, F_1 hybrids were also shown to be a mosaic of both parental and intermediate, plus one extreme morphological character rather than just intermediate ones.

An earlier investigation of morphological and physiological characters in beach and mountain *Sida fallax* (Stephens 2000) compared herbarium specimens from Pacific Islands at the Bishop Museum. Stephens (2000) studied the collections from Eastern Polynesia (Australes, Marqueses, and Societies), Micronesia (Wake Islands, Marshall Islands, Gilbert Islands, Northern Mariana Islands, and Caroline Islands), Asia (China), and Northern Polynesia (Line Islands, Phoenix Island, Howland Island, Enderbury Island, Fanning Island, Gardner Island, Christmas Island, Starbuck Island, Sidney Island, and Hull Island). Stephens (2000) reported that morphological variations such as prostrate and upright growth forms (beach and mountain), size of the leaves, shape of the leaves, and degree of pubescence seen in *S. fallax* in the Hawaiian Islands are consistent with those of other Pacific collections. *Sida fallax* is the most widespread and variable taxon of Malvaceae in the Hawaiian Islands (Wagner *et al.* 1999). The great variation in morphology observed between and within beach and mountain *S. fallax* is most likely due to the hybridization and introgression between ecotypes. The hybridization between ecologically diverse forms may contribute to the successful distribution of *S. fallax* in a diversity of ecological habitats in the Pacific Islands and China (Bentham 1861, Wagner *et al.* 1999).

Beach and mountain forms are statistically distinct in 7 out of 17 morphological traits (Table 3.4 and 3.5). The breeding study of *Sida fallax* (Chapter 2) suggested that there are no barriers to crossing between the divergent forms. However, the divergent beach and mountain ecotypes are more common than the intermediate forms in the field (personal observations). This indicates there may be ecological barriers existing between beach and mountain ecotypes. One possible barrier may be the physical distance between the two ecotypes. Beach and mountain populations of *Sida fallax* may be geographically isolated by distance. The other possible barrier could be a lack of intermediate habitat due to human disturbances. Most of the current natural habitats of the beach and mountain forms of *S. fallax* are clearly separated. The relative rarity of the intermediate forms in nature could reflect the fact that intermediate habitats suitable for hybrids are already in short supply, perhaps due to human habitat disturbance and/or occupied by other competitive weedy introduced species. The lack of a potential contact zone may enforce divergence of the beach and mountain ecotypes. Quantitative investigations of *S. fallax* morphology in the currently limited intermediate habitats are needed to reveal the extent of hybridization of these forms in nature.

Sida fallax ecotypes may be still in the process of diverging and, given enough time without the intermediate habitats, may differentiate further. For example, *Sida ovata* occurring in Pakistan, was once treated as a single taxon and reported to have white and yellow flowering forms (Parker 1918, Jafri 1966). *Sida ovata* recently has been distinguished as *S. ovata* Forsssk and *S. tiagii* Bhandari (= *S. pakistanica* Abedin) (Bhandari 1978). *Sida ovata* has an erect habit; its flowers are white, and the shizocarp is incompletely enclosed by the calyx. *Sida tiagii* has a semi-erect habit, yellow flowers, a shizocarp completely encosed by the calyx, and mericarps that are reticulate over the entire surface. *Sida ovata* is distributed in drier part of Africa, Arabia, Iran, Pakistan and India, whereas *S. tiagii* is restricted to Socotra, Pakistan and India (Dawar *et al.* 1996). Furthermore, hybridization between these two species is reported to display some of the intermediate characteristics of the parent species (Dawar *et al.* 1994, 1996). Dawar *et al.* (1994) noted that hybridization between these species may be restricted due to ecological and genetic factors. In Hawaii, there is a form of *Sida fallax* called Black Coral 'Ilima, collected from one locality on Kauai (Taylor 1998, Tswei 2001). Black Coral 'Ilima seems to have diverged from the standard mountain type (Taylor 1998, Tswei 2001). It has large blossoms and dark stems with flowers roughly twice the size of regular *Sida fallax* (Figure 3.8). No quantitative studies have investigated the physical characteristics and distribution of Black Coral 'Ilima.

The genus *Sida* consists of about 150 species distributed in the tropical and subtropical regions of the world (Abedin 1979, Wagner *et al.* 1999). The genus is taxonomically poorly known, with groupings based on unreliable morphological traits such as number of seeds per mericarp, presence of epicalyx, presence of endoglossum, and arborescent habit (Bates 1968). In Hawaii, *Sida cordifolia* has long been misapplied to the considerably different, prostrate, coastal entity in the *Sida fallax* complex with cordate leaves (Wagner *et al.* 1999). In Hong Kong, China, *S. fallax* was noted possibly as a variety of *S. rhombifolia* (Bentham 1861). Aguilar et al. (2003) obtained sequences for 58 species of Malvaceae, including *Sida,* based on the internal transcribed spacers of nuclear ribosomal DNA (ITS1 + ITS2 nrDNA) in an attempt to clarify phylogenetic relationship and classification. Unfortunately, this study did not include *Sida fallax.*

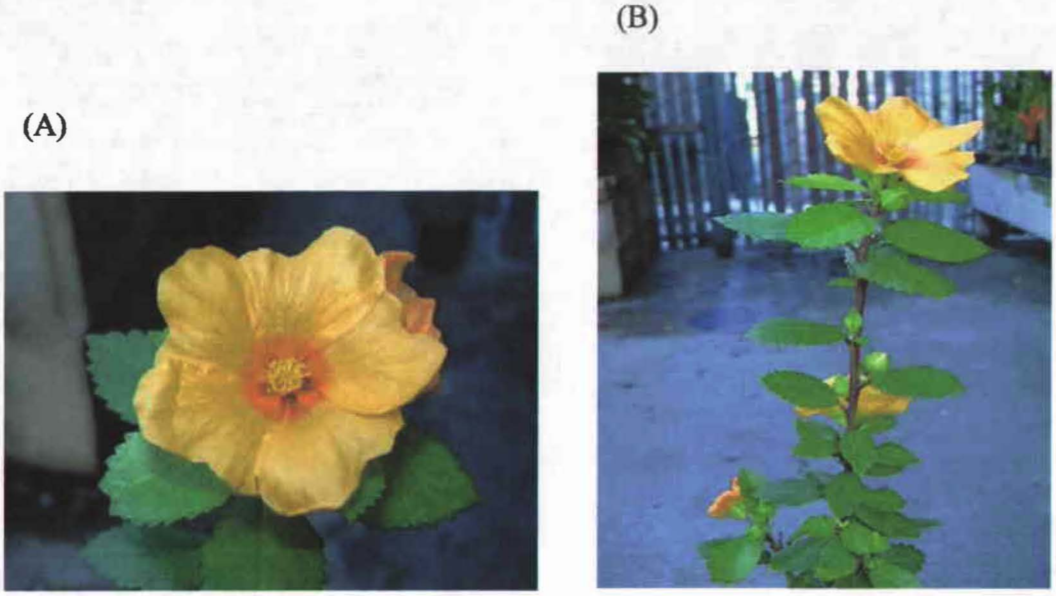


Figure3.8. A selected cultivated flower of Black Coral 'Ilima. Its showy flowers are often more than 3 cm in diameter (A). The Black Coral 'Ilima gets its name from the stiff black stems which look like black coral (B).

Maskas and Cruzan (2000) used chloroplast DNA (cpDNA) variation and nested clade phylogeographic analyses to infer the historical processes that have contributed to the high level of morphological and ecological diversification present in a group of herbaceous perennials (the *Piriqueta caroliniana* complex, Turneraceae) in North America and the Bahamas. Maskas and Cruzan (2000) pointed out that the recent theoretical advances have provided the necessary foundations for the phylogenetic and geographic information that have allowed questions concerned with patterns of genetic variation above the population and below the species level to be addressed (Slatkin and Maddison 1989, 1990, Templeton *et al.* 1995). Their study integrated information on morphological and ecological variation within plant species with historical patterns of dispersal and migration to provide insights into the ecological and evolutionary processes responsible for their diversification (Maskas and Cruzan 2000). Integration of methods such as chloroplast DNA (cpDNA) and nested clade phylogeographic analyses in the study of *Sida fallax* may help to provide an understanding of the historical processes responsible for determining the distribution of genetic diversity in contemporary populations of this complex species.

Pollen stainability

Numerous crossing studies have been performed to estimate strength of postmating reproductive barriers in both closely and distantly related plants species (Heiser 1949, Stebbins 1950, Grant 1981, Levin 1993, Rieseberg and Carney 1998). Common postmating barriers include hybrid weakness or inviability, hybrid sterility and hybrid breakdown, in which first generation (F_1) hybrids are robust and fertile, but later generation hybrids are weak or inviable (Rieseberg and Carney 1998). Extensive variability in viability and fertility has also been observed within and between hybrid generations from interspecific and intraspecific crosses (Rieseberg and Carney 1998). Grant (1964) noted that variability levels tend to be greatest in F_2 and first back-cross (BC_1) generations.

Pollen stainablity in *Sida fallax* parental plants (B and M) and progeny derived from these crosses (BxB, MxM and BxM and MxB) were equally high (range between 87-99%). In this study, F_2 and back-cross generations were not investigated fully but preliminary data revealed relatively high viability among BC_1 progeny (>90% pollen stainability, n = 12 plants). These results suggest that the two ecotypes of *S. fallax* do not have strong post mating barriers.

Conclusions

The morphological data presented here for *Sida fallax* provides a baseline to understand heritability of morphological characters and to reveal potentially useful characteristics for making taxonomic determinations within this complex species. The Hawaiian Islands provide a showcase for the process of evolutionary divergence and hybridization, as demonstrated by the dramatic examples from plants and animals (Craddock 2000). Incorporation of genetic analyses with knowledge of the morphological and ecological divergence of natural populations of *Sida fallax* on other Hawaiian Islands and Pacific regions would be worth persuing as a means to gain insight into the complexities of hybridization and evolutionary processes.

Chapter 4

Growth, Flowering and Survival Rates of Hybrids Between Mountain and Coastal Forms of *Sida fallax* Walp. (Malvaceae)

Introduction

Zones of hybridization may occur when divergent populations come into contact and interbreed in nature (Arnold, 1997; Campbell, 2001). Several conceptual frameworks or models have been presented to predict the outcome of natural hybridization episodes (Table 4.1). Arnold (1997) reviewed numerous studies that estimated the fitness of hybrid and parental genotypes or classes and found that hybrid individuals often possess equivalent or higher fitness relative to their progenitors.

Environmental conditions can alter the expression of particular traits such that relative performance of hybrids may vary across environments (Arnold 1997, Wang *et al.* 1998, Orians *et al.* 1999, Arnold *et al.* 2001). Therefore, the observation of hybrid performance under various growing conditions is crucial to assess whether hybrid genotypes may be viable in natural habitats. A few reciprocal transplant studies have been conducted in natural hybrid zones (Levin and Schmidt 1985, Emms and Arnold 1997, Wang *et al.* 1998, Campbell and Waser 2001), but no studies of hybrid fitness have

Table 4.1. Conceptual models of the natural hybridization outcome.

Hybrids are functionally impotent (Darwin 1859)
Hybridization is an evolutionary dead end (Mayr, 1942)
Hybridization as speciation processes (Dobzhansky, 1973)
Hybrids may be more fit than their parents in certain environment (Endler 1977, Moor 1977, Wang *et al.* 1997)
Hybrids zones can be stable through a balance between selection against hybrids and gene flow between the parent populations (Barton, 1979)
Hybrids are unfit in the parental habitats (Howard, 1982; Harrison, 1986)
Hybrid individuals often possess equal or higher fitness relative to their progenitors (Arnold, 1997)

been done that involve native Hawaiian plant species.

Sida fallax Walp. (Malvaceae) is a widespread species indigenous from China to Pacific Islands, including Midway Atoll, Nihoa, and all the main Hawaiian Islands. It occurs in coastal shrubland communities (to 300 m elevation), lowland dry communities (15-600 m elevation), and montane communities (500 to 2,700 m elevation) (Gagne and Cuddihy 1999). Morphological variation exists within and between populations, especially in stature, pubescence, leaf size and shape, and nature of the inflorescence (Bates 1999). Two extreme ecotypes, (beach and mountain) are recognized (Stephens, 2000). The coastal type is a prostrate subshrub up to 1.2 m tall, found on arid, exposed headlands and other coastal sites. The mountain type is an erect, tree-like shrub found in montane and mesic communities (Stephens 2000). The extistence of some *S. fallax* plants with apparent intermediate morphology was mentioned by Bates (1999) but no formal analyses of these specimens were conducted.

Hybridization between the two ecotypes of *S. fallax* has not been documented. The ecotypes are usually geographically isolated with inhospitable urban or agricultural lands between them. However, occasional intermediate forms can be seen in limited sites on Oahu (Diamond head, Ewa, Koko head, Koko Crator, Makapu'u, Personal Observation).

The present study documents the outcome of experimental hybridization between the morphologically distinct mountain and coastal forms of *Sida fallax*. The resulting hybrids were grown in a greenhouse and a drought treatment was applied to determine if the experimental hybrids expressed differences in fitness (growth rate, flowering rate, and survival rate) relative to non-hybrid offspring derived from the same parents.

Materials and Methods

Plant materials

The data were collected in the Pope Laboratory greenhouse, on the campus of the University of Hawaii. The original plants used to obtain hybrid and non-hybrid seeds were a subset of the plants used by Stevens (2000). These original plants were grown from seeds collected from six different sites on the island of Oahu. The location, elevation and annual rainfall at each seed collection site are summarized in Table 4.2 and Figure 4.1.

Plants from the mountain populations (elevation higher than 266 m) were observed to have an upright growth form with nearly glabrous leaves; plants from the coastal populations (near sea level) were prostrate with pubescent leaves (Stephens 2000). Seeds from all six collection sites were germinated and grown in two-gallon pots in Sunshine soil medium (J.G. Smith & Company, Illinois) in the greenhouse during 1998 and 1999 (Stephens 2000). A total of 11 mature beach and 10 mature mountain plants of *Sida fallax* from 6 populations (Table 4.2) were used for cross pollination of these divergent forms.

Table 4.2. Collection sites of original maternal plants. Rainfall data of these sites is from Giambelluca et al. (1996).

Collection Site	Elevation (m)	Annual Rainfall (mm)
Mountain *S. fallax* (M)		
Wa'ahila Ridge	366	2000
Hawai'iloa Ridge trail	330	1250
Kuaokala Forest Reserve	266	900
Beach *S. fallax* (B)		
Makapu'u beach park	near sea level	800
Ka'ena Point Natural Area Reserve	near sea level	800
Sandy Beach Park	near sea level	800

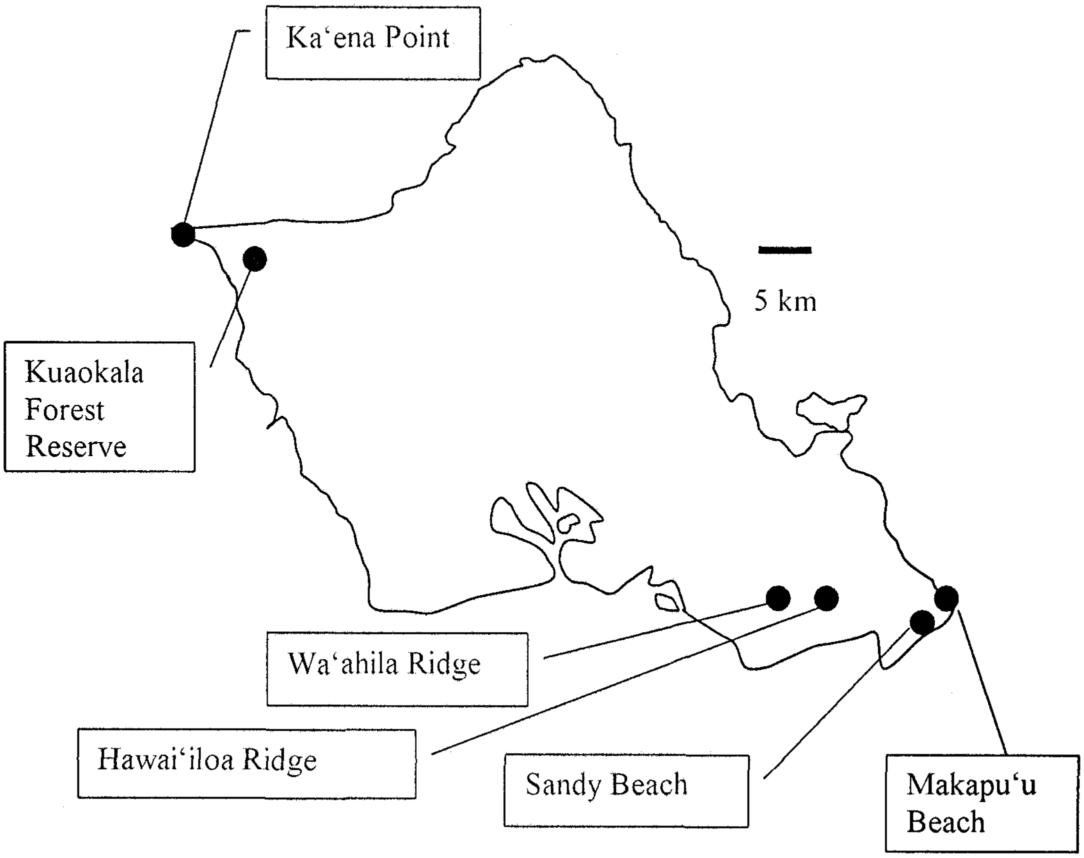


Figure 4.1. Collection sites of original plants.

Hybrids and control crosses

Two divergent forms (M = mountain form and B = beach form) of *Sida fallax* were reciprocally crossed by hand pollination in the green house (Table 4.3). Following standard convention, the female parent is listed first.

Pollination was carried out from 1999 to 2001. A sharp-tipped forceps was used to collect mature anthers from a pollen donor, dusting pollen on the stigmas of a maternal parent. The flowers were pollinated at various times of the day in order to obtain seeds from as many combinations of crosses as possible. All pollinated flowers were monitored for two to three weeks, and eventually any seeds produced were collected for germination.

Good seeds that were not obviously aborted were planted in August 2001. Each seed was stripped from the mericarp and nicked before planting in order to enhance germination. Nicking of seeds was done by creating a small incision in the seed coat with a sharp single-edge razor blade under the dissecting microscope. Seeds were submerged in 2% water agar in covered, clear plastic Petri dishes. The dishes were randomly positioned on lab benches in an air-conditioned room (74° F) under sunlight. The dishes were monitored every day for two months and dates of germination (first emergence of the radicle) were recorded.

Within a week after germination (2-7days), seedlings were transplanted from the

Table 4.3. *Sida* cross pollination matrix.

	Paternal parent	
Maternal parent	M	B
Mountain *S. fallax* (M)	MxM	MxB
Beach *S. fallax* (B)	BxM	BxB

water agar to a 12.7x12.7 cm pot with Big R potting soil mix (Cascade forest products, Inc., Novato, CA), and randomly located on benches in the Pope Laboratory greenhouse (University of Hawaii, Manoa). Initially, all seedlings were well watered for three weeks to minimize transplant shock. However, most of seedlings did not increase in stem length and leaf number after the transplanting until the soil quality was improved in February 2002. All pots were carefully replaced with a manually blended soil mix (4 potting soil: 4 peat moss:1 compost: 1 perlite) recommended for native Hawaiian plants (Culliney and Koebele 1999) in order to hold moisture, reduce soil temperature and enhance further growth of the seedlings. All pots were equally well watered for three weeks to keep the soil moist to reduce the second transplant shock.

Drought treatments

Individual seedlings derived from each maternal plant of each cross-pollination category were randomly assigned to either of two levels of drought conditions: well-watered to keep the soil moist and drought (watered only when plants showed visible signs of wilting). Usually, the well-watered pots were watered everyday, and the drought pots were watered every 2 to 3 days. All pots were randomly positioned and relocated monthly.

Leaf and stem growth rate, flowering rate and survival

Growth rate (leaf number and stem length), flowering rate, and survival rate were compared among different types of crosses under both drought and well-watered treatments to test for differences in the ability to tolerate water stress.

Ten observations (week 1, 2, 3 and 4; and month 2, 3, 4, 5, 6, and 7) were made to determine main stem length and leaf numbers of individual plants. Plants were carefully monitored every day to record the date of the first flower production. The flowering time was calculated as the number of days from the germination date to first flowering. At the end of 7 months, the proportion of plants flowering and surviving among each cross type and between the two water treatments was determined. The data were analyzed using SYSTAT 9 (SSPS, Inc., Chicago).

Results

Leaf and stem growth

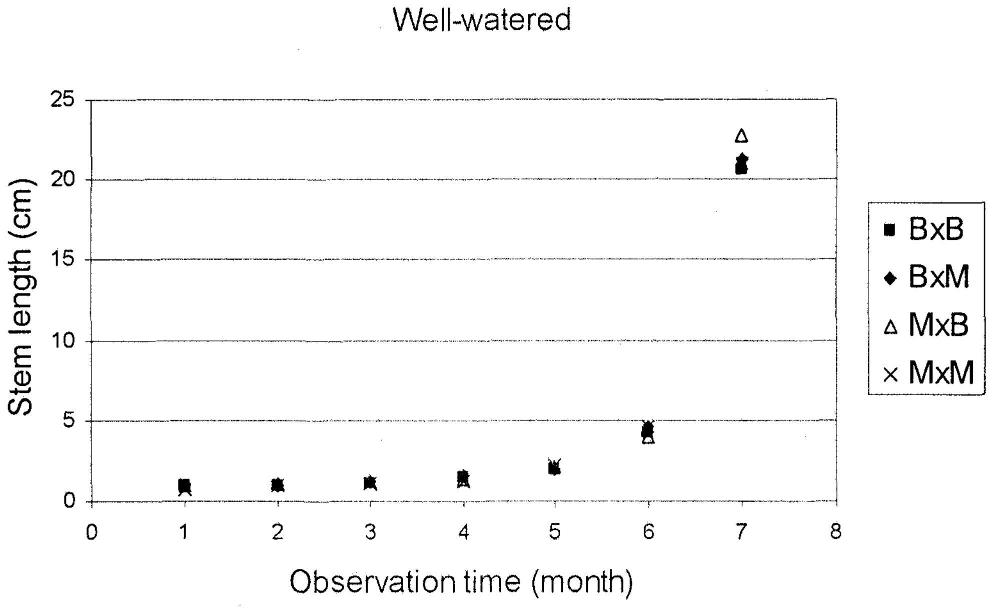
The stems and leaves of all plants grew slowly for the first six months and quickly increased between six and seven months for both well-watered and drought treatments (Figure 4.2 and 4.3). There were no significant differences between controls (BxB amd MxM) and hybrids (BxM and MxB) in mean stem length and mean total leaf number, regardless of the drought treatments at any observation period. The mean stem length among hybrids ranged from 19-25 cm (Figure 4.2), and the mean total leaf number ranged from 9-13 (Figure 4.3) at the seventh month observation.

Flowering rate

Some progeny derived from each of the crossing combinations flowered, with 23-27 % of the plants under the well-watered condition, but only 6.2-18.7 % of plants under the drought condition flowered within 10 months from germination (Table 4.4). The flowering rate was significantly lower under the drought treatment than the well-watered treatment for progeny produced from all crosses, except BxB (Table 4.4 and Figure 4.4). When plants were well-watered, the mean flowering rate was not significantly different among progeny of the different crossing categories (Figure 4.4). The mean age of the plants at first flowering ranged from 236-253 days depending on the cross type, but, there were no significant differences in the average age at flowering between treatments nor among the crossing categories (Table 4.4).

Survival rate

The survival rate (%) among progeny form all crossing categories of *Sida fallax* did not differ significantly for any observation period regardless of the drought treatment (Figure 4.5). Survival ranged from 45.0-74.3 % under the well-watered conditions and 31.2-52.8 % under the drought treatment. Survival of MxM progeny were was highest under the well-watered and lowest under the drought treatment. However, the difference was not significant.



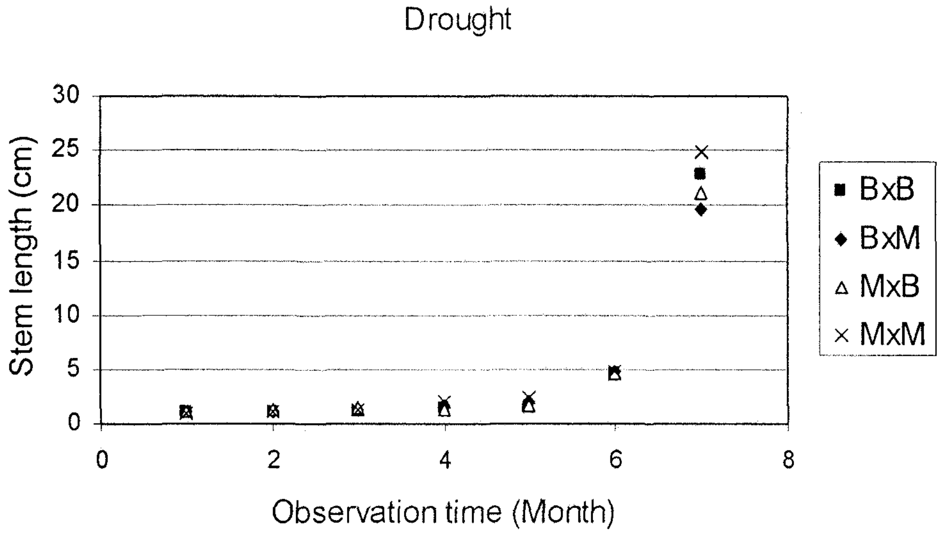


Figure 4.2. Stem length (cm) of seedlings of *Sida fallax* hybrids under well-watered and drought treatments. The graph shows observations three weeks after repotting in February 2002. BxB = cross between beach forms, BxM = cross between beach and mountain, MxB = cross between mountain and beach forms, and MxM = cross between mountain forms *S. fallax*. The first letter indicates maternal parent of the cross.

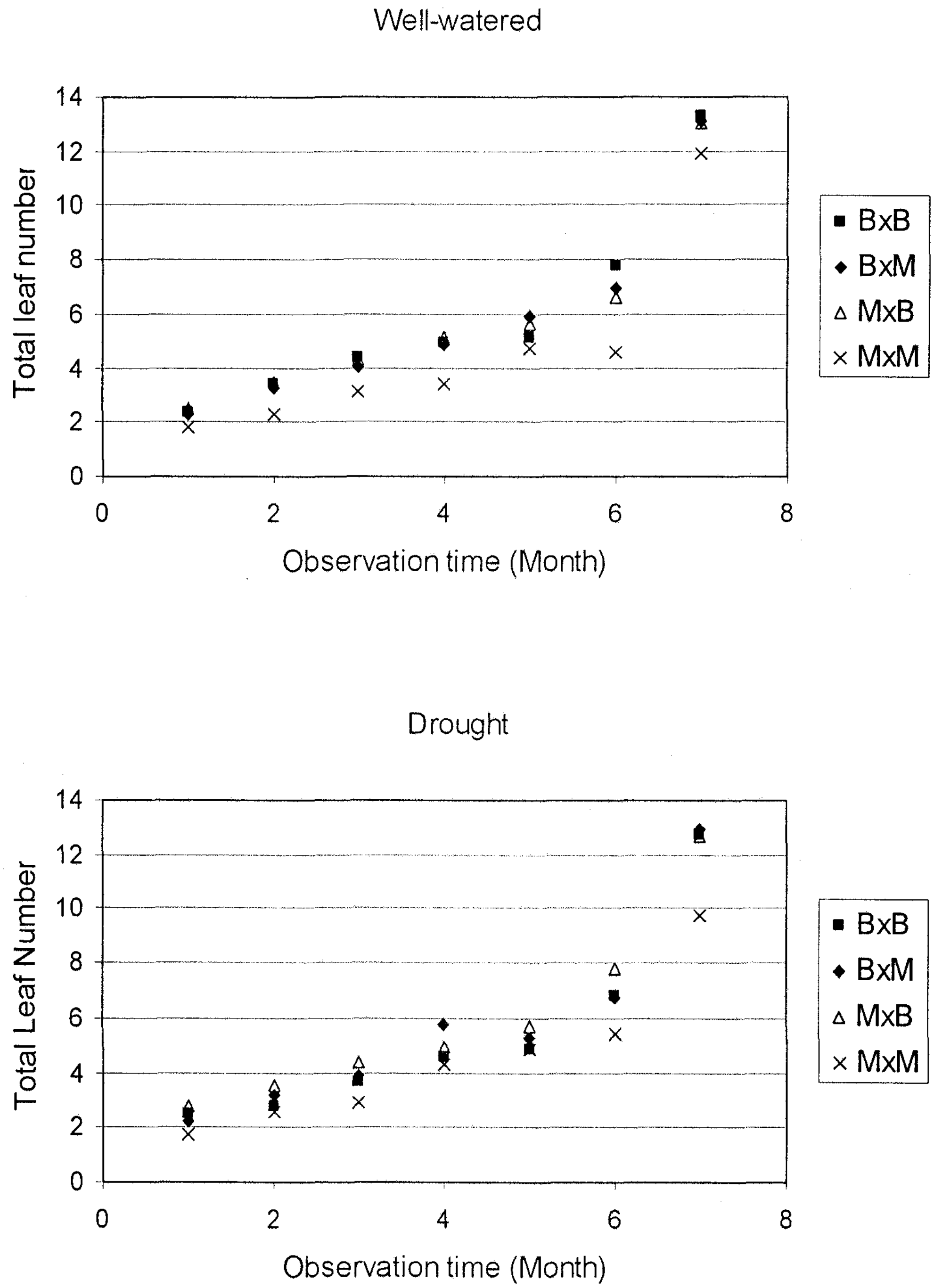


Figure 4.3. Total leaf number of seedlings of *Sida fallax* hybrids under well-watered and drought treatments. The graph shows observations three weeks after repotting in Februarys 2002. BxB = cross between beach forms, BxM = cross between beach and mountain, MxB = cross between mountain and beach forms, and MxM = cross between the mountain forms of *S. fallax*. The first letter indicates maternal parent of the cross.

Table 4.4. Mean flowering age (days) and flowering frequency (%) in *Sida fallax* hybrids under well-watered and drought treatments. Values with different letters are significantly different ($P < 0.05$). BxB = cross between beach forms, BxM = cross between beach and mountain, MxB = cross between mountain and beach forms, and MxM = cross between mountain forms of *S. fallax*. The first letter indicates maternal parent of the cross.

Cross	N of mother plants	Total N of plants observed	% of plants that flowered	Ave. age of plants that flowered (days)
Well-watered				
BxB	9	13	23.0a	236c
MxM	7	19	26.3a	243c
BxM	9	24	27.7a	240c
MxB	7	28	24.3a	245c
Drought				
BxB	9	16	18.8a	253c
MxM	7	16	6.2b	240c
BxM	9	26	11.1b	245c
MxB	7	20	9.1b	245c

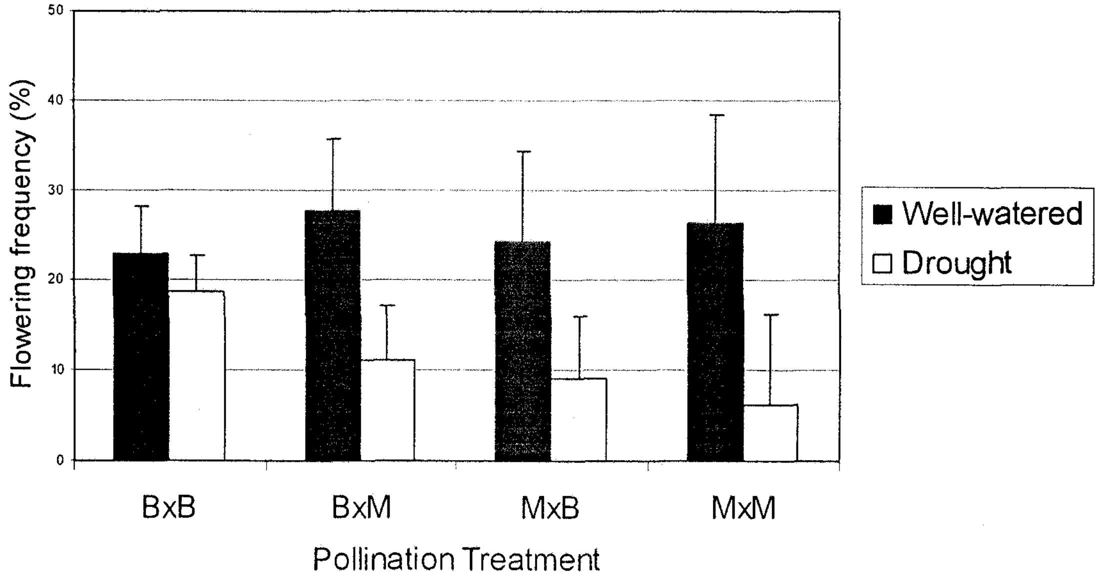


Figure 4.4. Mean flowering frequency (%) among hybrids of *Sida fallax* under well-watered and drought conditions. Error bars indicate 1 SE. BxB = cross between beach forms, BxM = cross between beach and mountain, MxB = cross between mountain and beach forms, and MxM = cross between mountain forms of *S. fallax*. The first letter indicates maternal parent of the cross.

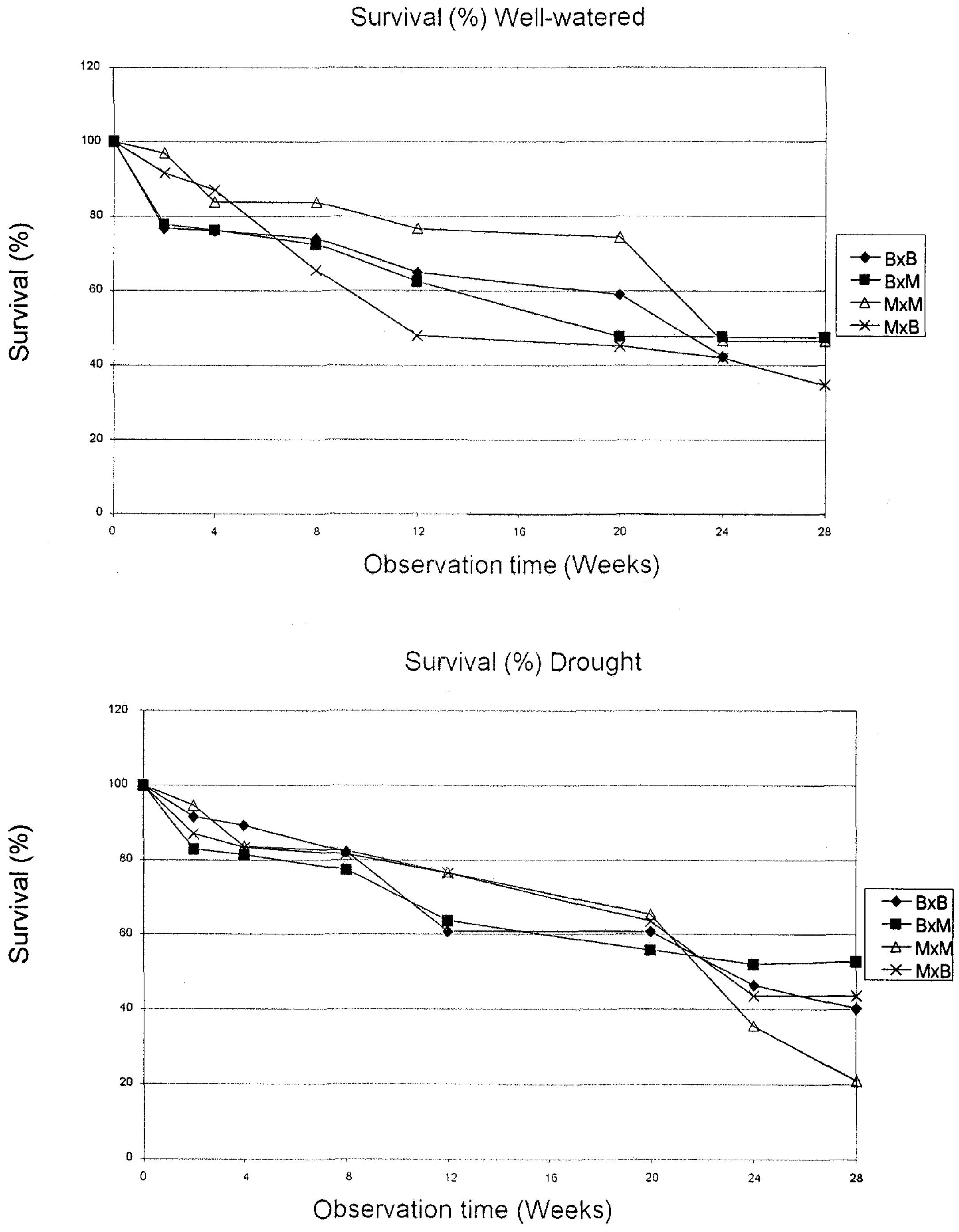


Figure 4.5. Survival rate (%) of *Sida fallax* hybrids under well-watered and drought treatments. BxB = cross between beach and mountain, BxM = cross between beach and mountain, MxB = cross between mountain and beach forms, and MxM = cross between mountain and mountain form *S. fallax*. The first letter indicates maternal parent of the cross.

Discussion

Leaf and stem growth

Leaf and stem growth rates of progeny of all crossing categories (BxB, MxM, BxM and MxB) were similar under both well-watered and drought treatments (Figure 4.2 and 4.3). Even though beach and mountain forms of *Sida fallax* differ morphologically and are found in ecologically different habitats, their growth rates were the same in experimental well-watered and drought environments. Stephens (2000) studied photosynthesis, leaf spectral characters, and water relations of beach and mountain forms of *S. fallax* and found that the beach form of *S. fallax* had higher reflectance and a higher photosynthetic rate than the mountain forms under the same light conditions (Stephens, 2000). This response was attributed to greater numbers of trichomes on the upper and lower leaf surfaces in the beach plants compared to the mountain plants (Stephenes, 2000). However, Stephens (2000) found that both ecotypes of *S. fallax* utilize tissue elasticity and osmotic adjustment to respond to seasonal water stresses (Stephens, 2000).

Overall, the growth rate based on the leaf number and stem elongation among *Sida fallax* hybrids did not differ regardless of the watering regime during the 7 month observation period.

Flowering rate

Drought stress generally resulted in low levels of flower production in *Sida fallax*. Only the beach form (BxB) maintained a similar mean flowering frequency (%) under the control (well-watered) and drought treatments (23 % and 18.75 %, respectively). This may be because the beach form (BxB) of *S. fallax* is better adapted to drought conditions compared to the mountain and hybrid forms. The coastal dry communities in which the beach form of *S. fallax* occurs naturally are generally subjected to summer drought (Wagner *et al.* 1999). Stephens (2000) reported a seasonal change in leaf traits of the beach plants under natural conditions. The increased leaf surface area in beach plants leaves coupled with the decreased reflectance of the upper leaf surface observed during the wet season would allow the beach plants to exploit the more favorable conditions (Stephens, 2000). Mountain plants (MxM) exhibited the largest difference in mean frequency of flowering in well-watered (26.3 %) versus drought treatments (6.2 %) (Table 4.4; Figure 4.4). This response is consistent with observation by Stephens (2000) that the mountain plants maintain a fairly constant leaf area under natural conditions. The mountain habitats receive more rainfall than the beach. Therefore, the drought avoidance mechanisms observed in the beach plants would not likely confer a selective advantage to the mountain plants. As might be expected, the hybrids between beach and mountain forms (BxM and MxB) appeared to be intermediate to BxB and MxM in their response to drought conditions (Table 4.4; Figure 4.4).

Among plants that flowered, the mean age (days) at flowering did not differ significantly among the hybrid categories, regarding of watering regime (Table 4.4: 234-293 days). All planting was done in August 2001 and most plants first flowered at an age of 7 to 9 months, coinciding with spring to early summer months. *Sida fallax* plants are known to bloom year round in the field but have been noted to flower more abundantly during the spring and early summer months (Pope 1968).

Survival rate

The survival rates reported in this study suggest that hybrids of *Sida fallax* are not less fit than their parents under the two conditions observed. On average, less than half of the plants in all hybrid categories survived in both well-watered and drought conditions (Figure 4.5). The range of survival rates observed at seven months was 34-47 % under the well-watered conditions and 20-52 % under the drought treatment (Figure 4.5). The differences in survival rates between the treatments and among the crossing categories were not statistically significant. Using a more extreme drought treatment might have led to higher mortality. In that case, the results could be predicted to mirror the observed flowering responses to drought: lowest mortality in the drought treatment is expected for BxB plants. The primary reasons for plant mortality in both drought and well-watered treatments over the 28 week observation period may have been the high temperature in the greenhouse (>30°C in daytime) and/or unsuccessful transplanting when the soil mix was replaced to enhance growth. The mountain form (MxM) had the lowest survival rate (20.9%) under the drought treatment (Figure 4.5). This result is consistent with Stephens' (2000) report that the mountain plants do not exhibit seasonal adaptations.

Conclusions

Initially, hybrids between beach and mountain form were expected to show lower fitness. However, they responded the same as hybrids within each form even under the drought treatment. Only the the beach form (BxB), exceeded other groups in the frequency of plants flowering under the drought treatment. Thus, these results generally agree with the conclusions of Arnold (1997); one cannot automatically assume hybrids have low fitness.

To fully test the theory that hybrids are more fit in certain habitats than either of their parents (Moor, 1977), additional studies are needed to compare the fitness of *Sida fallax* hybrids in ecotonal regions as well as parental habitats. Water availability is one of the most important determinants of the distribution of plant species, and spatial and temporal variation in water availability may often control the distribution of hybrids and the patterns of introgression (Vanbolkenburgh *et al.* 1998, Orians *et al.* 1999). Arnold (2001) and Campbell and Waser (2001) pointed out that field evaluation (i.e., reciprocal transplants) of hybrid fitness is costly in time and resources; yet, it is the most direct way to test predictions concerning the evolutionary implications of natural hybridization. Additional investigations on *S. fallax* fitness in the field, as well as a better understanding of genetic variation in beach, mountain and intermediate populations are needed to draw more reliable conclusions about the relative fitness of hybrids among different forms of this species.

Chapter 5

Experimental Hybridization Between *Sida fallax* Walp. and *Sida rhombifolia* L. (Malvaceae).

Introduction

Sida fallax Walp. (Malvaceae) is widespread on Pacific islands, including Midway Atoll, Nihoa, and all the main Hawaiian Islands, where it occurs in coastal shrubland communities (sea level to 300 m elevation), lowland dry communities (15-600 m elevation), and montane communities (500 to 2,700 m elevation) (Gagne and Cuddihy 1999). Morphological variation exists within and between populations, especially in stature, pubescence, leaf size and shape, and floral characters (Bates 1999). Two extreme ecotypes (beach and mountain) are recognized (Stephens 2000). The coastal type is prostrate scrambler or a subshrub up to 0.2 – 1.2 m tall, found on arid exposed headlands and other coastal sites. The mountain type is an erect tree-like shrub up to 2.5 m tall found in montane and mesic communities (Bates 1999).

Sida rhombifolia L. (Malvaceae) is a naturalized, polymorphic, pantropical weed (Gillett *et al.* 1991, Bates 1999). It is found in disturbed areas (0-1,240 m elevation) on all of the main islands on the Hawaiian archipelago (Bates 1999). The invasive nature of *S. rhombifolia* is well recognized in Australia and the Southern United States from Virginia to Florida and Texas (Holm *et al.* 1977, Gillett *et al.* 1991, Smith *et al.* 1992).

The purpose of this study was to investigate the possible outcomes of hybridization between *Sida fallax* and *S. rhombifolia*. Natural or artificial hybridization between *S. rhombifolia* and *S. fallax* has never been documented. Since *S. rhombifolia* possesses a strong invasive ability and co-occurs with *S. fallax*, there is interest in the ecological relationship and the potential for introgression between these two species.

Materials and Methods

Plant materials

Experiments were conducted at the Pope Laboratory greenhouse, at the University of Hawaii Manoa. The original plants used to generate hybrids and non-hybrid seeds were a subset of the plants used by Stevens (2000). These plants were grown from seeds collected from six different sites on the island of Oahu. The locations, elevations and annual rainfalls of the seed collection sites are summarized in Table 5.1 and Figure 5.1.

Plants from the mountain populations (elevation higher than 266m) had an upright growth form with nearly glabrous leaves, and plants from the coastal populations (near sea level) were prostrate with pubescent leaves (Stephens 2000). Seeds of *Sida rhombifolia* were collected at Kuaokala (Stephens 2000). Seeds from all six collection sites were germinated and grown in two-gallon pots in Sunshine soil medium (J. G. Smith & Company, Illinois) in the greenhouse during 1998 and1999 (Stephens 2000). A total of 11 mature beach and 10 mature mountain plants of *Sida fallax* from 6 populations, and 5 mature *S. rhombifolia* plants were used to attempt hybridization between *S. fallax* and *S. rhombifolia.*

Table 5.1. Collection sites of original maternal plants. Rainfall data of these sites is from Giambelluca et al. (1996).

Collection Site	Elevation (m)	Annual Rainfall (mm)
Mountain *S. fallax* (M)		
Wa'ahila Ridge	366	2000
Hawai'iloa Ridge trail	330	1250
Kuaokala Forest Reserve	266	900
Beach *S. fallax* (B)		
Makapu'u beach park	near sea level	800
Ka'ena Point Natural Area Reserve	near sea level	800
Sandy Beach Park	near sea level	800
S. rhombifolia (A)		
Kuaokala Forest Reserve	266	900

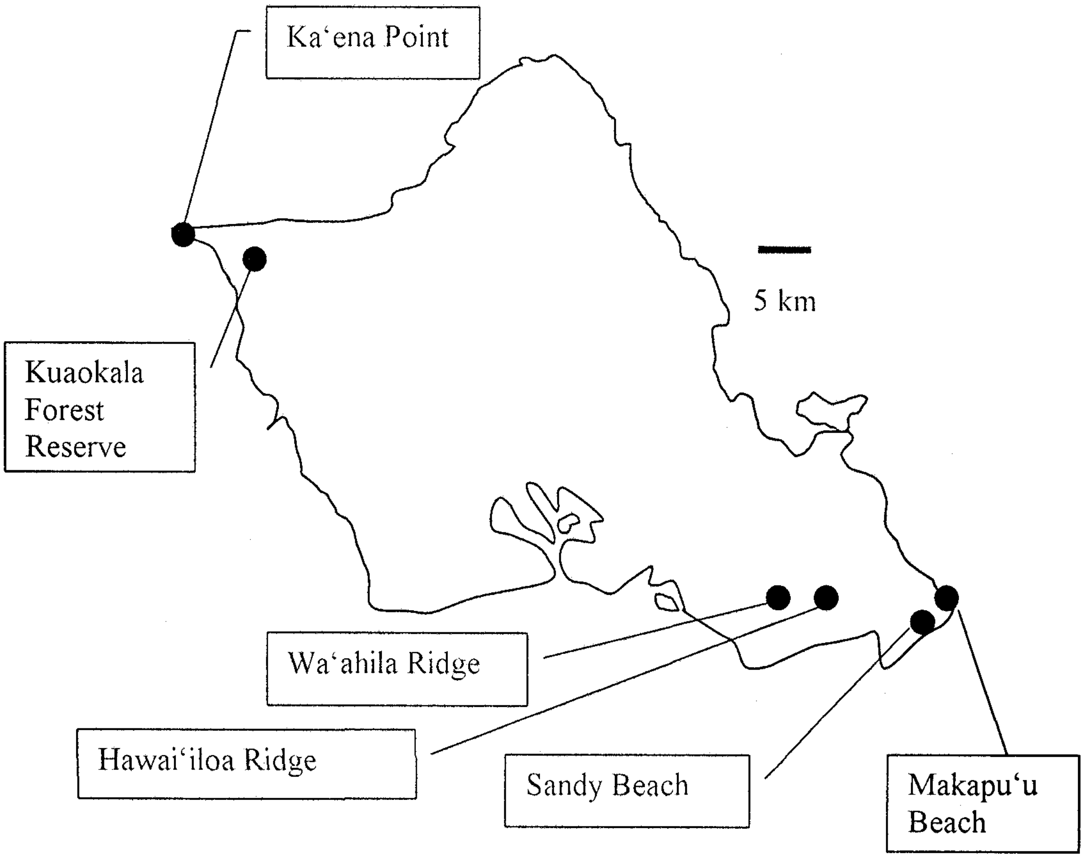


Figure 5.1. Collection sites of original plants.

Experimental pollination

A total of six combinations of hand pollinations were carried out to test crossability and to produce seeds in the greenhouse (Table 5.2). Attempts to produce interspecific hybrids were done by reciprocally crossing *Sida rhombifolia* (A, for alien) and both mountain (M) and beach (B) forms of *S. fallax* (AxM, MxA, AxB, BxA, and AxA). Self-pollination of individuals of *S. rhombifolia* was also done.

Pollination was carried out from 1999 to 2001. A sharp-tipped forceps was used to collect mature anthers from a pollen donor and transfer pollen to the stigmas of a maternal parent. Flowers were pollinated at various time of the day in order to obtain seeds from as many combinations of crosses as possible. All pollinated flowers were monitored for two to three weeks, and success or failure in seed production was recorded. Any seeds produced were collected for germination.

All data were averaged for the individual parent or the individual cross combination. Data were averaged again according to the common mother plant. Finally, these values were averaged to compare the different crossing categories using ANOVA or a student t-test.

Table 5.2. *Sida* cross pollination matrix.

	Parental plant			
Maternal Plant	M	B	A	Self
Sida rhombifolia (A)	AxM	AxB	AxA	A-self
Beach *S. fallax* (B)			BxA	
Mountain *S. fallax* (M)			MxA	

Breeding system of *Sida rhombifolia*

Based on the breeding system study (Chapter 2), *Sida fallax* is mainly outcrossing while observations of very high fruit set rates among cultivated *S. rhombifolia* plants in the absence of pollinators suggested that it is self-fertile. Thus, *S. fallax* flowers did not need to be emasculated, whereas emasculation of *S. rhombifolia* is required to test for the possibility of AxM and AxB hybrid combinations. However, free parts of the filaments and styles of *S. rhombifolia* are of equal length, so stigmas and anthers are basically touching each other at floral anthesis. This made it unfeasible to emasculate *S. rhombifolia*. Neverthless, crossing attempts were made without applying any special emasculation techniques, despite the high probability that selfing would occur when *S. rhombifolia* was used as the female parent.

Flower bagging and self-pollination experiments

To assess self-pollination and self-compatibility in *Sida rhombifolia*, two flowers per individual from five *S. rhombifolia* plants were isolated in fine nylon mesh bags. Each flower bud for testing was enclosed in a nylon mesh bag 1-2 days before opening, to ensue isolation from insects that were occasionally observed in the greenhouse. Two to three weeks after flowering, the success or failure of seed production was recorded.

In addition to the bagged selfing experiment, ten flowers from each of five unbagged *Sida rhombifolia* plants were manually self-pollinated by dusting self pollen on the stigma. An additional 10 flowers from these individual plants were also observed without any manipulation (controls).

Artificial crossablity

Pollination success was determined two or three weeks after pollination by harvesting the shizocarp (fruit) which contains the mericarps (seeds). When a shizocarp was empty (all mericarps aborted), the pollination was considered a failure. When the shizocarp contained any number of healthy looking mericarps, the pollination was considered successful. The number of pollination successes divided by the total number of pollination attempts is reported as the % pollination success for each type of cross.

Seed (mericarp) fertility and characteristics

Both fertile (good) and sterile (aborted) seeds (mericarps) were counted for each crossing category of fruit set. Determining the fertility or sterility of seeds was not difficult, as sterile ones usually appeared to be smaller in size and had an obviously wrinkled mericarp. The number of fertile mericarps (seeds) per fruit, the number of aborted mericarps (seeds) per fruit, and the mass of both fertile and sterile mericarps per fruit were obtained. The length of the awn of the mericarp was measured to the nearest 0.1 mm using digital calipers, and averaged, from theree mericarps per fruit. Mericarp color was recorded as black, brown or tan.

Germination velocity

Non-aborted seeds were tested for germination in August, 2001. Before planting, all seeds were stripped from the mericarps and nicked to enhance germination. Nicking of seeds was done with a sharp razor under a dissecting microscope. Seeds were submerged in solidified 2% water agar in covered, clear-plastic Petri dishes. The dishes were randomly positioned on lab benches in an air-conditioned room (74° F), under sun light. The dishes were monitored every day for two months, and dates of germination (=

first emergence of the radicle) were recorded. The water agar medium was replaced as needed to prevent desiccation. The germination rate was observed for 60 days, and the germinated seeds were counted at 2-day intervals (30 observations). The germination rate was determined using a modification of Timson's index of germination velocity (Timson 1965, Khan and Rizvi 1994).

$$\text{Germination velocity} = (\Sigma\, G)/t$$

(where G is the percentage of seeds germinated at any given 2-day interval, and t is the total germination period).

The maximum values possible using this index with the data was 50 (=3000/60: if 100 % germination occurred from the first observation, $\Sigma\, G$ = 100 % x 30 observations =3000, divided by t total germination period = 60 days). A higher index value means a higher germination rate.

Results

Flower bagging and self-pollination experiments

The flowers of *Sida rhombifolia* demonstrated 100 % self-pollination and self fertilization in all the selfing treatments (Table 5.3). This results support the assumption that *S. rhombifolia* is self-compatible.

Artificial crossability

All of the crossing attempts made between *Sida rhombifolia* plants (AxA), and the interspecific crossing attempts using *S. rhombifolia* as the female parent (AxB and AxM) yielded seeds (Table 5.4). Based on the flower bagging and selfing experiments, all of the seeds produced by *S. rhombifolia* were likely the products of selfing (Table 5.3). Mean pollination success was significantly lower when beach and mountain forms of *S.*

fallax were the mother plant in crosses with *S. rhombifolia* (Table 5.4, BxA = 11.6 %, MxA = 31.4 %, $p < 0.05$). These results were also significantly lower than the pollination success of crosses within *S. fallax* (Chapter 2, Table 2.3, MxM = 91.2 %, BxM = 71.0 %, BxB = 82.9 %, and MxB = 95.5 %).

Seed production and quality

All seed measurements of crosses made with *Sida rhombifolia* as maternal plants (AxA, A-self, AxB and AxM) were similar (Table 5.5). Pollinations made with beach and mountain *S. fallax* as maternal plants (BxA and MxA) yielded a significantly lower mean number of seeds (mericarps)/fruit and mean seed (mericarp) mass/fruit set than the pollinations made among *S. fallax* ecotypes. The mean number of aborted seeds (mericarps)/fruit for both BxA and MxA crosses was significantly higher than in the crosses among *S. fallax* (BxB = 0.6 BxM = 1.4, MxB = 1.8 and MxM = 0.9) and lower than selfed *S. fallax* (mean B-self = 4.66/fruit and mean M-self = 4.86/fruit) (Chapter 2, Table 2.4).

Table 5.3. Results of flower bagging and self-pollination experiments. Values of mean pollination success (%) sharing the same letter do not differ significantly (P>0.05). A = *Sida rhombifolia.*

	Number of maternal plants	Number of maternal plants with seeds	Number of flowers	Number of flowers producing seeds	Pollination success (%)
Bagged	5	5	10	10	100a
Un bagged, Self-pollinated	5	5	50	50	100a
Un bagged, unmanipulated	5	5	50	50	100a

Table 5.4. Seed production as a result of pollination between *Sida fallax* and *S. rhombifolia*. Mean pollination success rates sharing the same letter do not differ significantly ($P>0.05$). A = *Sida rhombifolia*, B = Beach and M = mountain *S. fallax* ecotypes. SD = Standard Deviation.

	Number of Maternal plants	Number of crossing combination	Total number of flowers pollinated	Mean pollination success (%)	Mean pollination success (%) SD
AxA	4	9	46	100a	0
AxB	4	7	40	100a	0
AxM	4	5	34	100a	0
BxA	9	29	145	11.6b	14.4
MxA	7	10	28	31.4b	43.5

Table 5.5. Seed parameter means. Mericarp color was coded as 1 = black, 2 = brown, 3 = light brown. Means for a given character sharing the same letter do not differ significantly ($P>0.05$). A = *Sida rhombifolia*, B = Beach and M = mountain *S. fallax* ecotypes.

Cross	Number of maternal plants	Number of flowers	Normal seeds/fruit	Aborted seeds/fruit	Seed mass/fruit (mg)	Mericarp Color	Awn (mm)
AxA	5	13	7.1b	1.1a	0.021b	2.0a	1.0b
A self	5	23	7.0b	0.8a	0.023b	2.0a	1.2b
AxM	3	6	6.7b	1.6a	0.023b	1.8b	1.1b
AxB	4	6	7.1b	1.8a	0.022b	1.8b	1.1b
BxA	10	21	4.1a	2.4b	0.012a	2.3a	0.3a
MxA	8	18	4.2a	3.1b	0.007c	1.4b	1.6b

Sida rhombifolia seeds have distinguishable characteristics when compared to *S. fallax* seeds. *Sida rhombifolia* seeds have a smooth seed coat, a pronounced wedge shape, and short, sharp awns. *Sida fallax* seeds have a relatively rough seed coat, a rounded wedge shape, and awns with various lengths. However, the seed measurements of mass, color and size of *S. rhombifolia* were within the range encompassing beach and mountain forms of *S. fallax* seeds. The seed characters of AxB and AxM did not differ from either AxA or A-self pollinations.

Germination velocity

The mean germination velocities are summarized in Table 5.6. Most seeds derived from *Sida rhombifolia* as a maternal parent (AxA, A-self, AxB and AxM) germinated within 2 days. The mean germination velocity of AxA was 50, and A-self was 49 out of the possible maximum germination velocity of 50 (Table 5.6). The mean germination velocity of progeny with *S. rhombifolia* as the maternal plant (AxB = 47.81; AxM = 46.50) was similar to AxA and A-self, and significantly greater than progeny with *S. fallax* as the maternal plant (MxA = 22.66, BxA = 19.89) (Table 5.6). It is notable that the mean germination velocity of BxA and MxA did not differ significantly from the products of infraspecific pollinations within *S. fallax* (BxB, BxM, MxB and MxM) (Chapter 2, Table 2.6).

Table 5.6. Mean germination velocity (GV) of *Sida* seeds derived from various pollination treatments. Means sharing the same letter do not differ significantly (P>0.05). B = Beach and M = mountain *S. fallax* ecotypes.

Cross	Number of maternal plants	Number of seeds set	GV Minimum	GV Maximum	Mean GV	Standard Dev
AxA	5	5	50	50	50b	0
A-self	4	9	47.828	49.762	49.077b	0.707
AxB	4	10	47.123	48.501	47.812b	0.974
AxM	4	7	43.317	48.883	46.503b	2.87
BxA	5	16	0	36.333	19.894a	14.023
MxA	2	8	18.262	27.075	22.668a	6.231

Discussion

Species may be prevented from crossing by isolating mechanisms or breeding barriers (Stace 1975). Mechanisms that limit or promote hybridization are of primary importance in determining the evolutionary consequence of a particular case of hybridization (Arnold 1997). Hybridization between two sympatrically occurring *Sida* species has been reported in Pakistan (Dawar *et al.* 1994, 1996). Therefore, I predicted that *S. fallax* and *S. rhombifolia* may be capable of hybridizing.

Based on personal observations in the greenhouse, most of the flowers of *Sida rhombifolia* produced fruits. Therefore, *S. rhombifolia* was expected to be self-compatible. Results from pollination demonstrated that *S. rhombifolia* does indeed self-fertilize. This means that seeds produced from crosses with *S. rhombifolia* as the maternal parent, may actually be the result of self-fertilization, since the maternal plant had not been emasculated. Pollination success rates, seed parameters, and germination velocity of AxA, A-self, AxB and AxM did not differ among these experimental treatments. Therefore, it is uncertain whether any of these progeny were actually the result of cross fertilization (Table 5.5, 5.6).

The results of pollinations between *Sida fallax* and *S. rhombifolia* with *S. fallax* as the maternal plant (BxA and MxA) were also indistinguishable from the results of putative stray pollinations resulting from occasional Hymenoptera entering the greenhouse that may have effected either self or cross-pollination (see Chapter 2). The mean number of aborted seeds/fruit of both BxA (2.38) and MxA (3.07) were significantly higher than *Sida fallax* crosses (mean BxB =0.60/fruit, BxM = 1.39/fruit, MxB = 1.18/fruit and MxM = 0.9/fruit) and lower than selfed *S. fallax* (mean B-self =

4.66/fruit and mean M-self = 4.85/fruit) (Table 5.5 and Chapter 2, Table 2.4). Moreover, results from germination velocity of BxA and MxA were not significantly different from crosses among *S. fallax* (BxB, BxM, MxB and MxM) and selfs (B-self and M-self) (Table 5.6 and Chapter 2, Table 2.5). These results suggest that the good seeds from BxA and MxA might be the result of stray pollinations from *S. fallax*, as suggested in the situation involving good seeds from selfed *S. fallax* (B-self and M-self) (Chapter 2).

Conclusions

This study could not convincingly demonstrate successful hybridization between *Sida* species. *Sida rhombifolia* appeared to be self-compatible and *S. fallax* was mainly self-incompatible. The experimental results suggested that hybridization between *S. fallax* and *S. rhombifolia* is unlikely, even though *Sida fallax* and *S. rhombifolia* occur in the same or similar habitats, have similar flower morphologies, and likely share the same Hymenopteran pollinators.

Sida rhombifolia germinated faster than *S. fallax* under the same conditions. By testing germination ability over a wide range of temperature and pH levels, Smith et al. (1992) demonstrated that *S. rhombifolia* is adapted to a wide range of environmental conditions. Hillebrand (1888) and Pope (1968) reported *S. rhombifolia* as possibly indigenous. Althought *S. rhombifolia* is recognized as a scattered weed in Hawaii today, the germination velocity and consistently high (selfed) seed production suggest the possibility that it could become a serious weed problem in native and disturbed ecosystems. Further investigations of interactions between natural populations of *Sida rhombifolia* and *S. fallax* on the Hawaiian Islands would be worth pursuing as a means to gain insight into issues of conservation of native Hawaiian plants and their habitats.

Chapter 6

Hypotheses Revisited and Conclusions

The purpose of this study was to investigate the possible outcomes of hybridization between morphologically distinctive beach and mountain ecotypes of indigenous *Sida fallax* and between *S. fallax* and *S. rhombifolia*. Conclusions from this study partially supported the first hypothesis but did not support the second hypothesis.

Hypothesis 1:

Artificially produced hybrids between mountain and coastal ecotypes of *Sida fallax* and between *S. fallax* and *S. rhombifolia* will exhibit morphology intermediate to the parent forms. Comparisons of morphological characteristics such as flower color, petal shape, mericarp color, awn length, leaf shape, leaf margin, and habit in ecotype hybrids and the parental ecotypes will reveal heritable phenotypic traits.

Conclusion:

Hybrids between beach and mountain forms of *Sida fallax*

This study demonstrated that these divergent forms of *Sida fallax* are able to produce viable hybrid offspring when they are cross-fertilized. This indicates a general lack of prezygotic and internal postzygotic barriers to gene exchange within the species. This study also found that hybrids between beach and mountain *Sida fallax* ecotypes did not express uniformly intermediate morphology. The morphological variability was high within beach and mountain forms of *S. fallax* and the hybrids traits expressed were a mosaic of characteristics within the range of parent forms. No novel traits were recorded in the hybrids. Out of 17 traits assessed in this study, 9 traits were the same for beach and mountain types, while 4 traits differed statistically between beach and mountain types and were intermediate in the hybrids. Three traits (leaf blade length, leaf margin, petal width) seemed to be dominant characters based on hybrid morphology. The dark spot of the flower was the only extreme trait expressed in hybrids.

Hybrids between *Sida fallax* and *S. rhombifolia*

This study did not confirm the production of hybrid progeny produced from reciprocal crossing attempts between *S. fallax* and *S. rhombifolia*. The evidence suggest that most if not all progeny from these crossing attempts were selfs or the result of stray intraspecific pollinations.

Hypothesis 2:

Artificially produced hybrids between mountain and coastal ecotypes of *Sida fallax* and between *S. fallax* and *S. rhombifolia* will be less fit than progeny derived from either ecotype of *Sida fallax*. Hybrids will show lower pollen stainability, lower seed production, lower seed mass and number of mericarps per fruit, lower germination velosity, slower growth rate, lower flowering rate and lower survival rate than the control crosses grown under the same conditions. Hybrids will also have lower survival than the control crosses when grown under stressful (drought) conditions.

Conclusion:

Hybrids between beach and mountain forms of *Sida fallax*

Pollen stainability, seed mass, number of mericarps per fruit, and germination velocity were not significantly different in the hybrids between beach and mountain ecotypes of *Sida fallax* compared to progeny derived either ecotype. Leaf and stem growth rate, flowering rate and survival rate were also similar for all progenies of crossing categories under both well-watered and drought treatment, although the beach ecotype had a higher flowering rate than the mountain ecotype and the hybrids under drought conditions.

Hybrids between *Sida fallax* and *S. rhombifolia*

Because there is no clear evidence that any interspecific hybrid progeny were produced, it is not relevant to address the fitness of the progeny from the inter-specific pollinations.

Further investigation:

Wagner *et al.* (1999) pointed out that there are numerous patterns of variation in the Hawaiian flora that cannot be satisfactorily resolved with present information. *Sida fallax* is an excellent case in point. This indigenous plant is widely distributed from China to the Pacific Islands, including Hawaii (Bates 1999). Morphological variation and fitness analysis in this study suggested traits that, with further study, may prove useful in resolving the taxonomy of this complex species. Focusing on intraspecific phylogeographic analysis may clarify interpretations of biogeographic patterns among groups of closely related populations (Maskas and Cruzan 2000). Because of the great diversity of habitats, climatic variation, and isolation of the Hawaiian Islands, additional studies that examine the native Hawaiian flora could provide valuable information concerning general biogeographic patterns and evolution.

Literature Cited

Abedin S. 1979. Malvaceae. In: Masor E, Ali SI, editors. Flora of West Pakistan 130.

Aguilar JF, Fryxell PA, Jansen RK. 2003. Phylogenetic relationship and classification of the *Sida* generic alliance (Malvaceae) based on nrDNA ITS evidence. Systematic Botany 28(2):352-364.

Albert ME, D'Antonio CM, Schierenbeck KA. 1997. Hybridization and introgression in *Carpobrotus* spp. (Aizoaceae) in California. I. Morphological evidence. American Journal of Botany 84(7):896-904.

Alice LA, Eriksson T, Eriksen B, Campbell CS. 2001. Hybridization and gene flow between distantly related species of *Rubus* (Rosaceae): Evidence form nuclear ribosomal DNA internal transcribed spacer region sequences. Systematic Botany 26(4):769-778.

Allen HH. 1949. Wild species-hybrids in the phanerogams. II. Botanical Review 15:77.

Anderson E. 1949. Introgressive hybridization. New York: John Wiley & Sons, Inc.

Anderson E, Stebbins GL, Jr. 1954. Hybridization as an evolutionary stimulus. Evolution 8:378-388.

Arnold ML. 1997. Natural hybridization and evolution. New York: Oxford University Press.

Arnold ML, Bulger MR, Burke JM, Hempel AL, Williams JH. 1999. Natural hybridization: How low can you go and still be important? Ecology 80(2):371-381.

Arnold ML, Hodges SA. 1995. Are natural hybrids fit or unfit relative to their parents? Trends in Ecology & Evolution 10(2):67-71.

Arnold ML, Kentner EK, Johnston JA, Cornman S, Bouch AC. 2001. Natural hybridization and fitness. Taxon 50:93-104.

Athens JS. 1997. Hawaiian native lowland vegetation in prehistory. In: Kirch PK, Hunt TL, editors. Historical Ecology in the Pacific Islands: Prehistoric Environmental and Landscape Change. New Heaven: Yale University Press.

Baker HG. 1951. Hybridization and natural gene-flow between higher plants. Biological Review 26:302-337.

Baldwin BG, Preston RE, Wessa BL, Wetherwax M. 2001. A biosystematic and phylogenetic assessment of sympatric taxa in *Blepharizonia* (Compositae-Madiinae). Systematic Botany 26(1):184-194.

Bates DM. 1968. Generic relationship in the Malaceae, tribe Malvae. Gentes Herbarum 10:117-135.

Bates DM. 1999. Malvaceae Mallow family. In: Wagner WL, Herbst DR, Sohmer SH, editors. Manual of the flowering plants of Hawaii. Honolulu: University of Hawaii Press. p 868-903.

Benson L, Phillips EA, Wilder A. 1967. Evolutionary sorting of characters in a hybrid swarm. American Journal of Botany 54(8):1026-1967.

Bentham G. 1861. Flora Hongkongensis: a description of the flowering plants and ferns of the Island of Hongkong. London: Lovell Reeve.

Bhandari MM. 1978. Flora of the Indian desert. Jodhpur: Scientific Publishers.

Bhandari MM. 1990. Flora of the Indian desert. Jodhpur: New Pali Road.

Boesewinkel DF, Bouman F. 1995. Seed morphology and development. In: Kigel J, Galili G, editors. Seed development and germination. New York: Marcel Dekker, Inc. p 1-24.

Borba EL, Semir J, Shepherd GJ. 2001. Self-incompatibility, inbreeding depression and crossing potential in five Brazilian *Pleurrothallis* (Orchidaceae) species. Annals of Botany 88(89-99).

Boyer JS. 1982. Plant productivity and environment. Science 218:443-338.

Briggs D, Walters SM. 1997. Plant Variation and Evolution. Cambridge: Cambridge University Press.

Brown AHD, Barrett SCH, Moran GF. 1985. Mating system estimation in forest trees: models, methods and meanings. In: Soltis DE, Soltis PS, editors. Isozymes in plant biology. Portland, Oregon: Dioscorides Press. p 73-86.

Burdon JJ, Brown AHD. 1986. Population genetics of *Echium plantagineum* L.: Target weed for biological control. Australian Journal of Biological Science 39:369-378.

Caius JF. 1986. The medicinal and poisonous plants in India. Jodhpur: Scientific Publisher.

Campbell CS, Waser NM, Melendez-Ackerman EJ. 1997. Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. American Naturalist 149:295-315.

Campbell DR, Waser NM. 2001. Genotype-by-environment interaction and the fitness of plant hybrids in the wild. Evolution 55(4):669-676.

Caraway V. 1997. Hybridization, introgression and speciation among *Dubautia* species (Asteracae: Madinae) [Masters of Science]. Honolulu: University of Hawaii.

Caraway V, Carr GD, Morden CW. 2001. Assessment of hybridization and introgression in lava-colonizing Hawaiian *Dubautia* (Asteraceae: Mandiinae) using RAPD markers. American Journal of Botany 88:1988-1694.

Carlquist S. 1974. Island biology. New York: Columbia University Press.

Carlquist S. 1980. Hawaii: a natural history. Lawai: Pacific Tropical Botanical Garden.

Carney SE, Cruzan MB, Arnold ML. 1994. Reproductive interactions between hybridizing irises: analyses of pollen-tube growth and fertilization success. American Journal of Botany 81(9):1169-1175.

Carney SE, Gardner KA, Rieseberg LH. 2000. Evolutionary changes over the fifty-year history of a hybrid population of sunflowers (*Helianthus*). Evolution 54(2):462-474.

Carr GD. 1978. Chromosome numbers of Hawaiian flowering plants and the significance of cytology in selected taxa. American Journal of. Botany 65:236-242.

Carr GD. 1985. Monograph of the Hawaiian Madiinae (Asteraceae): *Argyroxiphium*, *Dubautia*, and *Wilkesia*. Allertonia 4(1-123).

Carr GD. 1986. Adaptive radiation in the Hawaiian silversword alliance (Compositae-Madiinae). II. Cytogenetics of artificial and natural hybrids. Evolution 40:959-976.

Carr GD. 1987. Beggar's ticks and tarweeds: Masters of Adaptive Radiation. Trends in Ecology & Evolution 2:192-195.

Carr GD. 1995. A fully fertile intergeneric hybrids derivative from *Argyroxiphium sandwicense* ssp. *macrocephalum* x *Dubautia menziesii* (Asteraceae) and its relevance to plant evolution in the Hawaiian Islands. American Journal of Botany 82(12):1574-181.

Carr GD. 1998. Chromosome evolution and speciation in Hawaiian flowering plants. In: Stuessy TF, Ono M, editors. Evolution and speciation of island plants. Campridge: Campridge University Press.

Carr GD, Baldwin BG, Kyhos D. 1996. Cytogenetic implications of artificial hybrids between the Hawaiian silversword alliance and North American tarweeds (Asteraceae: Heliantheae-Madiinae). American Journal of Botany 83(5):653-660.

Carr GD, King RM, Powell MA, Robinson H. 1999. Chromosome numbers in Compositae. XVIII. American Journal of Botany 86(1003-1013).

Carr GD, Kyhos DW. 1981. Adaptive radiation in the Hawaiian silversword alliance (Compositae-Madiinae) I. Cytogenetics of spontaneous hybrids. Evolution 35:543-556.

Clausen J, Keck DD, Heisey WM. 1948. Experimental studies on the nature of species. III: Environmental responses of climatic races of *Achillea*. Carnegie Institution of Washington 58:1-129.

Cohen D. 1968. A general mode of optimal reproduction in a randomly varying environment. Journal of Ecology 56:219-228.

Craddock EM. 2000. Speciation process in the adaptive radiation of Hawaiian plants and animals. In: Hecht MK, Macintyre RJ, Clegg MT, editors. Evolutionary biology. New York: Kluwer Academic/Plenum Publishers.

Cruzan MB, Arnold ML, Carney SE, Wollengerg KR. 1993. CPDNA inheritance in interspecific crosses and evolutionary inference in Louisiana irises. American Journal of Botany 80(3):344-350.

Culliney JL, Koebele BP. 1999. A native Hawaiian Garden: How to grow and care for Island plants. Honolulu: University of Hawaii Press.

Daehler CC. 1999. Inbreeding depression in smooth cordgrass (*Spartina alterniflora*, Poaceae) invading San Francisco Bay. American Journal of Botany 86(1):131-139.

Daehler CC, Carino DA. 1998. Recent replacement of native pili grass (*Heteropogon contortus*) by invasive African grasses in the Hawaiian Islands. Pacific Science 52(3):220-227.

Daehler CC, Carino DA. 2001. Hybridization between native and alien plants and its consequences. In: Lockwood JL, Mckinney ML, editors. Biotic Homogenization: Kluwer Academic Publishers. p 85-106.

Daehler CC, Strong DR. 1997. Hybridization between introduced smooth cordgrass (*Spartina alterniflora*; Poaceae) and native California cordgrass (*S. foliosa*) in San Francisco Bay, California, UAA. American Journal of Botany 84(5):607-611.

Daehler CC, Yorkston M, Sun W, Dudley N. 1999. Genetic variation in morphology and growth characters of *Acacia koa* in the Hawaiian Islands. International Journal of Plant Sciences 160(4):767-773.

Darwin CR. 1859. On the Origin of Species by Means of Natural Selection; or, The Preservation of Favored Races in the Struggle for Life. London: John Murray.

Dawar R, Ali T, Qaiser M. 1994. Hybridization in the *Sida ovata* complex II. Evidence from breeding studies. Pakistan Journal of Botany 26:83-97.

Dawar R, Ali T, Qaiser M. 1996. Hybridization in the *Sida ovata* complex (Malvaceae) I. Evidence form morphology, chemistry and cytology. Willdenowia 25:637-646.

De Nettancourt D. 1977. Imcompatibility in angiosperms. Berlin: Heidelberg & New York: Springer Verlag.

Ellstrand NC, Elam DR. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24:217-242.

Ellstrand NC, Schiernbeck KA. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? In: Ayala FJ, Fitch WM, Clegg MT, editors. Variation and evolution in plants and microorganisms: Toward a new synthesis 50 years after Stebbins. Washington, D. C: National Academy Press.

Ellstrand NC, Whitkus R, Rieseberg LH. 1996. Distribution of spontaneous plant hybrids. Proceedings of the National Academy of Science, U.S.A 93:5090-5093.

Elton C. 1958. The ecology of invasions by animals and plants. London: Methuen.

Emms SK, Arnold ML. 1997. The effect of habitat on parental and hybrid fitness: transprant experiments with Lousiana irises. Evolution 51:1112-1119.

Endler JA. 1977. Geographic variation, speciation, and clines. Princeton, NJ: Princeton University Press.

Faure N, Serieys H, Cazaux E, Kaan F, Berville A. 2002. Partial hybridization in wide crosses between cultivated sunflower and the perennial *Helianthus* species *H. mollis* and *H. orgyalis*. Annals of Botany 89(1):31-39.

Fenner M, Kitajima K. 1999. Seed and seedling ecology. In: Pugnaire FI, Valladares F, editors. Handbook of functional plant ecology. New York: Marcel Dekker, Inc. p 589-622.

Fischer M, Matthies D. 1997. Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). American Journal of Botany 84(12):1685-1692.

Fishman L, Willis JH. 2001. Evidence for Dobzhansky-Muller incompatibilities contributing to the sterility of hybrids between *Mimulus guttatus* and *M. nasutus*. Evolution; International Journal of Organic Evolution 55(10):1932-1942.

Forno IW, Kassulke RC, Harley LS. 1992. Host specificity and aspects of the biology of *Calligrapha pantherina* (Col.: Chrysomelidae), a biological control agent of *Sida acuta* [Malvaceae] and *S. rhombifolia* in Australia. Entomophaga 37(3):409-417.

Forsyth C, Brown AHD. 1982. Germination of the dimorphic fruits of *Bidens pilosa* L. New Phytologist 90:151-164.

Fosberg FR. 1948. Derivation of the flora of the Hawaiian Islands. In: Zimmerman EC, editor. Insects of Hawaii: University of California Press. p 55-61.

Fosberg FR. 1963. Disturbance in island ecosystems. In: Gillett GW, editor. Pacific Basin Biogeography. Honolulu: Bishop Museum Press. p 557-561.

Franzotti EM, Santos CVF, Rodrigues HMSL, Mourao RHV, Andrade MR, Antoniolli AR. 2000. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). Journal of Ethnopharmacology 72:273-278.

Fritz RS, Nichols-Orians CM, Brunsfeld SJ. 1994. Interspecific hybridization of plants and resistance to herbivores: hypothesis, genetics, and variable responses in a diverse herbivore community. Oecologia 97:106-117.

Gagne WC, Cuddihy LW. 1999. Vegetation. In: Wagner WL, Herbst DR, Sohmer SH, editors. Manual of the flowering plants of Hawaii. Honolulu: University of Hawaii Press.

Gallagher KG, Schierenbeck KA, D'Antonio CM. 1997. Hybridization and introgression in *Carpobrotus* spp. (Aizoaceae) in California II. Allozyme evidence. American Journal of Botany 84(7):905-911.

Ganders FR. 1989. Adaptive radiation in Hawaiian *Bidens*. In: Giddings LV, Kaneshiro KY, Anderson WW, editors. Genetics, Speciation, and the Founder Principle. New York: Oxford University Press. p 99-112.

Giambelluca TW, Nullet M, Schroeder TA. 1996. Rainfall atlas of Hawaii. Honolulu: Water Reseources Research Center, University of Hawaii. Report nr Report No. R76.

Gillett GW. 1966. Hybridization and its taxonomic implications in the *Scaevola gaudichaudiana* complex of the Hawaiian Island. Evolution 20:506-516.

Gillett GW. 1972a. Additional experimental crosses in Hawaiian *Bidens* (Asteraceae). Pacific Science 2:415-418.

Gillett GW. 1972b. The role of hybridization in the evolution of the Hawaiian Flora. In: H. VD, editor. Taxonomy phytogeography and evolution. London: Academic Press.

Gillett JD, Harley KLS, Kassulke RC, Miranda HJ. 1991. Natural enemies of *Sida acuta* and *S. rhombifolia* (Malvaceae) in Mexico and their potential for biological control of these weeds in Australia. Entomological Society of America 20(3).

Gillispie RG. 1997. Range contraction and extinction vulnerability: What is natural? Memoir of the Museum of Victoria 56:401-409.

Grant V. 1964. Genetic and taxonomic studies of *Gilia*. XII. Fertility relationship of the polyploid cobwebby gilias. El Aliso 4:435-481.

Grant V. 1981. Plant Speciation. New York: Columbia University Press.

Hamrick JL, Linhart YB, Mitton JB. 1979. Relationship between life history characteristics and electrophoretically detectable genetic variation in plants. Annual Review of Ecology and Systematics 10:173-200.

Harrison RG. 1990. Hybrid zones: windows on evolutionary process. In: Futuyama D, Antonovics J, editors. Oxford surveys in Evolutionary Biology. Oxford: Oxford University Press. p 69-128.

Harrison RG, editor. 1993a. Hybrid zones and the evolutionary process. New York: Oxford University Press.

Harrison RG. 1993b. Hybrids and hybrid zones: historical perspective. In: Harrison RG, editor. Hybrid zones and the evolutionary process. Oxford: Oxford University Press.

Harvill AM, Stevens CE, Ware DME. 1977. Atlas of the Virginia Flora, Part 1. Petridophytes through Monocotyledons. Farmville: Virginia Botanical Associates.

Hauser EJP, Morrison JH. 1964. The cytochemical reduction of nitro blue tetrazolium as an index of pollen viability. American Journal of Botany 51(7):748-752.

Heiser CB. 1949. Study in the evolution of the sunflower species *Helianthus annuus* and *H. bolanderi*. University of California Publications in Botany 23:157-196.

Heiser CB. 1973. Introgression re-examined. The Botanical Review 39(4):347-366.

Hillebrand W. 1888. Flora of the Hawaiian Islands. Heidelberg: Privately published.

Hollingsworth ML, Bailey JP, Hollingsworth PM, Ferris C. 1999. Chloroplast DNA variation and hybridization between invasive populations of Japanese knotweed and giant knotweed (*Fallopia*, Polygonaceae). Botanical Journal of the Linnean Society 129:139-154.

Holm LG, Plucknett DL, Pancho JV. 1977. The world's worst weeds: Distribution and biology. Honolulu: The University Press of Hawaii. 621 p.

Hufford KM, Mazer S. 2003. Plant ecotypes: genetic differentiation in the age of ecological restoration. Trends in Ecology & Evolution 18(3):147-155.

Hughes JM, Baker AM, De Zylva G, Mather PB. 2001. A phylogeographic analysis of southern and eastern populations of the Australian magpie: evidence for selection in maintenance of the distribution of two plumage morphs. Biological Journal of the Linnean Society 74:25-34.

Jafri SMH. 1966. The flora of Karachi. Karachi.

Johnston JA, Grise DJ, Donovan LA, Arnold ML. 2001. Environment-dependent performance and fitness of *Iris brevicaulis*, *I. fulva* (Iridaceae), and hybrids. American Journal of Botany 88(5):933-938.

Jones JS, Leith BH, Rawlings P. 1977. Polymorphism in *Cepaea*: a problem with too many solutions. Annual Review of Ecology & Systematics 8:109-143.

Kaneshiro KY. 1990. Natural hybridization in *Drosophila*, with special reference to species form Hawaii. Canadian Journal of Zoology 68:1800-1805.

Kearns CA, Inouye DW. 1993. Techniques for pollination biology. Niwot: University Press of Colorado.

Keller LF, Grant PR, Rosemary Grant B, Petren K. 2001. Heritability of morphological traits in Darwin's Finches: misidentified paternity and maternal effects. Heredity 87:325-336.

Kepler AK. 1983. Hawaiian heritage plants. Honolulu: The Oriental Publishing Co.

Khan MA, Rizvi Y. 1994. Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii var. stocksii*. Canadian Journal of Botany 72(475-479).

Kitayama K, Pattison R, Cordell S, Webb D, Mueller-Dombois D. 1997. Ecological and genetic implications of foliar polymorphism in *Meterosideros polymorpha* Gaud. (Mytaceae) in a habitat matrix on Mauna Loa, Hawaii. Annals of Botany 80(491-497).

Kron KA, Gawen LM, Chase MW. 1993. Evidence for introgression in Azaleas (*Rhododendron*; Ericaceae): Chloroplast DNA and morphological variation in a hybrid swarm on Stone Mountain, Georgia. American Journal of Botany 80(9):1095-1099.

Lack AJ. 1995. Relationship and hybridization between British species of *Polygala*-evidence from isozymes. New Phytologist 130:217-223.

Lammers TG. 1988a. Chromosome numbers and their systematic implications in Hawaiian USA Lobelioideae Campanulacae. American Journal of Botany 75:1130-1134.

Lammers TG. 1988b. Systematics and evolution of the endemic Hawaiian genus *Clermontia* Gaudichaud (Campanulaceae: Lobelioideae) [dissertation]. Columbus: Ohio State University.

Lamoureux CH. 1973. Plants. In: Armstrong RW, editor. Atlas of Hawaii. Honolulu: Universiy of Hawaii Press. p 69-72.

Leebens-Mack J, Milligan BG. 1998. Pollination biology in hybridizing *Baptisia* (Fabaceae) populations. American Journal of Botany 85(4):100-107.

Levin DA. 1993. Local speciation in plants: The rule not the exception. Systematic Botany 18(2):197-208.

Levin DA, Francisco-Ortega J, Jansen RK. 1996. Hybridization and the extinction of rare plant species. Conservation Biology 10(10-16).

Levin DA, Schmidt KP. 1985. Dynamics of a hybrid zone in Phlox: and experimental demographic investigation. American Journal of Botany 72:1404-1409.

Loop LL, Mueller-Dombois D. 1989. Characteristics of invaded islands, with special reference to Hawaii. In: Drake JA, editor. Biological Invasions: A Global Perspective. New York: John Wiley. p 257-81.

MacDonald GA, Abbot AT. 1970. Volcanoes in the Sea. Geology of Hawaii. Honolulu: University of Hawaii Press.

Masaki S. 1967. Geographic variation and climatic adaptation in a field cricket. Evoltuion 21:725-741.

Maskas SD, Cruzan MB. 2000. Patterns of intraspecific diversification in the *Piriqueta caroliniana* complex in Southeastern North America and the Bahamas. Evoltuion 54(3):815-827.

Mayer SS. 1991a. Artificial hybridization in Hawaiian *Wikstroemia* (Thymelaeaceae). American Journal of Botany 78(1):122-130.

Mayer SS. 1991b. Morphological variation in Hawaiian *Wikstroemia* (Thymelaeaceae). Systematic Botany 16(4):693-704.

Mayr E. 1942. Systematics and the Origin of Species from the View Point of a Zoologist. New York: Columbia University Press.

McDade LA. 1997. Hybrids and phylogenetic systematics III. Comparison with distance methods. Systematic Botany 22:669-683.

McNaughton IH, Harper JL. 1960. The comparative biology of closely related species living in the same area. III. The nature of barriers isolating sympatric populations of *Papaver dubium* and *P. lecoquii*. New Phytologist 59:129-137.

Merlin MD. 1977. Hawaiian Costal plants and Scenic Shorelines. Honolulu: Oriental Publishing Co.

Molano-Flores B. 2001. Herbivory and calcium concentrations affect calcium oxalate crystal formation in leaves of *Sida* (Malvaceae). Annals of Botany 88:387-391.

Moor WS. 1977. An evaluation of narrow hybrid zones in vertebrates. Quarterly Review of Biology 52:263-277.

Motley TJ, Carr GD. 1998. Artificial hybridization in the Hawaiian endemic genus *Labordia* (Loganiaceae). American Journal of Botany 85(5):654-660.

Mueller-Dombois D. 1975. Some aspects of island ecosystem analysis. In: Golley GB, Medina E, editors. Tropical Ecological System: Trends in Aquatic and Terrestrial Research. New York: Springer Verlag New York Inc. p 353-366.

Mueller-Dombois D, Bridges KW, Carson HL, editors. 1981. Island ecosystems. Biological organization in selected Hawaiian communities. Wood Hole, Mass.: Hutchinson Ross Pulbisher. 583 p.

Neal MC. 1965. In gardens of Hawaii. 50 SP, editor: Bishop Museum Press.

Novak SJ, Mack RN. 1993. Genetic variation in *Bromus tectorum* (Poaceae): Comparison between native and introduced populations. Heredity 71:167-176.

Orians CM, Bolnick DI, Roche BM, Fritz RS, Floyd T. 1999. Water availability alters the relative performance of *Salix sericea*, *Salix eriocephala*, and their F_1 hybrids. Canadian Journal of Botany 77:514-522.

Osmond CB, Bjorkman O, Anderson DJ. 1980. Physiological processes in plant ecology. Toward a synthesis with *Atriplex*. Berlin, Heidelberg, and New York: Springer-Verlag.

Parker RN. 1918. Forest flora of Punjab with Hazara and Delhi. Lahore.

Peres CA, Patton JL, da Silva MNF. 1996. Riverine barriers and gene flow in Amazonian saddle-back tamarins. Folia Primatol 67:113-124.

Pope WT. 1968. Manual of wayside plants of Hawaii; including illustrations, descriptions, habits, uses and methods of control of such plants as have a wild nature of growth, exclusive of ferns. Rutland: C. E. Tuttle Co.

Rabakonandrianina E. 1980. Infrageneric relationships and the origin of the Hawaiian endemic genus *Lipochaeta* (Compositae). Pacific Science 34(1):29-39.

Rabakonandrianina E, Carr GD. 1981. Intergeneric hybridization, induced polyploidy, and the origin of the Hawaiian endemic *Lipochaeta* from *Wedelia* (Compositae). American Journal of Botany 68(2):206-215.

Randell R. 2000. Hybridization between naturalized and Endemic *Rubus* (Rosaceae) species in Hawaii [thesis]. Honolulu, Hawaii: University of Hawaii, Manoa.

Randell RA, Morden CW. 1999. Hawaiian plant DNA library II: Endemic, indigenous, and introduced species. Pacific Science 53(4):401-417.

Rattenbury JA. 1962. Cyclic hybridization as a survival mechanism in the New Zealand forest flora. Evolution 16:348-363.

Raven PH. 1973. Evolution of subalpine and alpine plant groups in New Zealand. New Zealand Journal of Botany 11:177-200.

Ricklefs RE. 2001. The economy of nature. New York: W. H. Freeman and Company.

Rieseberg LH. 1995. The role of hybridization in evolution: old wine in new skins. American Journal of Botany 82(7):944-953.

Rieseberg LH. 2000. Crossing relationships among ancient and experimental sunflower hybrid lineages. Evolution 54(3):859-865.

Rieseberg LH, Carney SE. 1998. Plant hybridization. New Phytologist 140(4):599-624.

Rieseberg LH, Ellstrand NC. 1993. What can molecular and morphological markers tell us about plant hybridization? Critical Reviews in Plant Sciences 12(3):213-241.

Rosenthal DM, Schwarzbach AE, Donavan LA, Raymond O, Riesberg LH. 2002. Phenotypic differentiation between three ancient hybrid taxa and their parental species. International Journal of Plant Sciences 163(3):387-389.

Runyeon-Lager H, Prentice HC. 2000. Morphometric variation in a hybrid zone between the weed, *Silene vulgaris*, and the endemic, *Silene uniflora* ssp. *petraea* (Caryophyllaceae), on the Baltic island of Oland. Canadian Journal of Botany 78:1384-1397.

Schierup MH, Christiansen FB. 1996. Inbreeding depression and outbreeding depression in plants. Heredity 77:461-468.

Schlessman CD, Lowry PPI, Lloyd DG. 1990. Functional dioecism in the New Caledonia endemic *Polyscias pancheri* (Araliaceae). Biotropica 22:133-139.

Slatkin M, Maddison WP. 1989. Cladstic measure of gene flow inferred from the phylogenies of alleles. Genetics 123:603-613.

Slatkin M, Maddison WP. 1990. Detecting isolation by distance using phylogenies of genes. Genetics 126:249-260.

Smith CA, Shaw DR, Newsom LJ. 1992. Arrowleaf sida (*Sida rhombifolia*) and prickly sida (*Sida spinosa*): germination and emergence. Weed Research 32(2):103-109.

Smith JF, Burke CC, Wagner WL. 1996. Interspecific hybridization in natural populations of *Cyrtandra* (Gesneriaceae) in the Hawaiian Islands; evidence from RAPD markers. Plant systematics and Evolution 200:61-77.

Snustad PD, Simmons MJ, Jenkins JB. 1997. Principles of genetics. New York: John Wiley & Sons, Inc.

Stace CA, editor. 1975. Hybridization and the Flora of the British Isles. London: Academic Press.

Standley LA. 1990. Allozyme evidence for the hybrid origin of maritime species *Carex salina* and *Carex recta* (Cyperaceae) in eastern North America. Systematic Botany 15:182-191.

Stanley RG, Linskens HF. 1974. Pollen, biology, biochemistry, and management. New York: Springer Verlag.

Stebbins GL, Jr. 1974. Flowering plants: Evolution above the species level. Cambridge, MA: Belknap Press of Harvard University Press.

Stebbins GLJ. 1950. Variation and evolution in plants. New York: Columbia University Press.

Stephens ML. 2000. The comparative ecophysiology of mountain and coastal populations of *Sida fallax* Walp. (Malvacea) in Hawaii [thesis]. Honolulu: University of Hawaii.

Sun W, Brewbaker JL, Austin MT. 1996. Genetic variations of *Acacia koa* seed, seedling, and early growth traits. In: Ferentinos L, Evans DO, Association HFI, editors. Koa: a decade of growth: proceedings of the symposium. Honolulu: Hawaii Forest Industry Association. p 33-38.

Taylor L. 1998 August 28. More variety with less land. Star-Bulletin.

Templeton AR, Routman E, Phillips CA. 1995. Separating population structure from population history; a cladistic analysis of geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140:767-782.

Timson J. 1965. New method of recording gemination data. Nature 207:2016-217.

Tswei S. 2001 October 5. Landscaper harmonizes with native Hawaiian flora. Star-Bulletin.

Valverde PL, Vite F, Zavala-Hurtado JA. 1996. A morphometric analysis of a putative hybrid between *Agave narnorata* Roezl and *Agave kerchovei* Lem: *Agave peacockii* Croucher. Botanical Journal of the Linnean Society 122:155-161.

Vanbolkenburgh E, Stahlberg R, Bultynck L. 1998. Physiological mechanisms controling the rate of leaf growth. In: Lambers H, Poorter H, Van Vuuren MMI, editors. Inherent variation in plant growth. Leiden, The Netherlands: Backhuys Publishers. p 41-56.

Wagner WL. 1991. Evolution of waif floras: A comparison of the Hawaiian and Marquesan Archipelagoes. In: Dudley EC, editor. The unity of evolutionary biology: the proceedings of the fourth International Congress of Systematics and Evolutionary Biology. Portland: Dioscorides Press. p 267-284.

Wagner WL, Herbst DR, Sohmer SH, editors. 1999. Manual of flowering plants of Hawaii. 2nd ed. Honolulu: University of Hawaii Press.

Wallace AR. 1889. Darwinism, and exposition of the theory of natural selection with some applications. London: Macmillan.

Wang H, Byrd DW, Joward JL, McArthur ED, Graham JH, Freeman DC. 1998. Narrow hybrid zone between two subspecies of big sagerbush (*Artemisia tridentata*:

Asteraceae). V. Soil properties. International Journal of Plant Sciences 159(1):139-147.

Wang H, McArthur ED, Sanderson SC, Graham JH, Freeman DC. 1997. Narrow hybrid zone between two subspecies of big sagebush (*Artemisia tridentata*: Astraceae). IV. Reciprocal transplant experiments. Evolution 51:95-102.

Weber E, D'Antonio CM. 1999. Phenotypic plasticity in hybridizing *Carpobrotus* spp. (Aizoaceae) from costal California and its role in plant invasion. Canadian Journal of Botany 77(1411-1418).

Weller SG, Sakai A, K., Wagner WL. 2001. Artificial and natural hybridization in *Schiedea* and *Alisinidendron* (Caryophyllacae: Alsinoideae): the importance of phylogeny, genetic, divergence, breeding system, and population size. Systematic Botany 26(3):571-584.

Wendt T, Canela MBF, Gelli de Raria AP, Rios RI. 2001. Reproductive biology and natural hybridization between two endemic species of *Pitcairnia* (Bromeliaceae). American Journal of Botany 88(10):1760-1767.

Wills RT, Keighery GJ. 1994. Ecological impact of plant disease on plant communities. Journal of the Royal Society of Western Australia 77(4):127-131.

Wills RT, Robinson CJ. 1994. Threats to flora-based industries in Western Australia form plant disease. Journal of Royal Society of Western Australia 77(4):159-162.

Wilson P. 1992. On inferring hybridity from morphological intermediacy. Taxon 41:11-23.

Wunderlin RP, Hansen BF, Briges EL. 1996. Atlas of Florida vascular plants.

Yang TW, Yang YA, Xiong Z. 2000. Paternal inheritance of chloroplast DNA in interspecific hybrids in the genus *Larrea* (Zygohylaceae). American Journal of Botany 87(10):1452-1458.