INBREEDING DEPRESSION, OUTBREEDING DEPRESSION AND HETEROSIS IN RARE SPECIES IN THE GENUS *SCHIEDEA* (CARYOPHYLLACEAE) ON O‘AHU

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ABSTRACT

The Hawaiian flora is rare, with approximately 10% extinct, 30% federally listed as endangered or threatened, and 89% endemic. Reintroductions are a tool for recovery efforts for species with few *in situ* individuals to establish healthy populations. Determining whether reintroductions should represent a single or multiple population(s) is based on factors including population demography and trends, gene flow, breeding and mating systems, inter-population and intra-population genetic variation, and variation in habitats between populations. Concerns about inbreeding depression and outbreeding depression were investigated in three critically endangered species of *Schiedea* (Caryophyllaceae). *Ex situ* living collections were hand-pollinated with pollen from the same plant, a plant from the same population, or a plant from another population. Progeny were propagated and outplanted into suitable habitat within the historic range of each species. One outplanting site was used for each species except *S. kaalae*, where two plantings were conducted because populations occurred in two mountain ranges with different forest types (wet vs. mesic). Early and later life stages were measured, including estimates for the number of flowers and seeds a plant produced, and cumulative and relative performance of cross treatments were calculated. Neither inbreeding depression nor outbreeding depression was detected in the relative performance of any stage for all three species. Heterosis was present in *S. kaalae* and *S. obovata* and progeny from crosses between populations were more relatively fit than progeny from crosses within populations (which had the same relative fitness as selfed progeny). Local adaptation to habitat differences may have been present in these two species due to the different forest types between the two mountain ranges (*S. kaalae*), and a rainfall gradient across the distribution of *S. obovata.* Plants of *S. kaalae* with maternal sources from the Waiʻanae

Mountains had a lower chance of survival than expected at the Koʻolau Mountain outplanting site. Plants of *S. obovata* with maternal sources from the wetter end of the rainfall gradient produced less seeds than the plants with maternal sources from the drier end of the gradient, where the outplanting site was located. Management recommendations include outplanting heterotic individuals from all available populations.

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CHAPTER 1. INTRODUCTION

The angiosperm flora of the Hawaiian Islands has one of the highest rates of endemism (89%) in the world, and over half of the taxa are at risk (Wagner et al. 1999, Sakai et al. 2002). Approximately 10% of the flora is extinct (Wagner et al. 1999), and over thirty percent of the extant flora is federally listed as threatened or endangered (out of 1094 species; USFWS 2010). The conservation of Hawai‘i’s rare flora is necessary and challenging. Reasons for the decline of native species are both numerous and uncertain, centered around the introduction of many invasive species (Cuddihy and Stone 1993). More recent studies have investigated direct impacts of specific introduced species on native Hawaiian flora, including impacts of slugs (Joe and Daehler 2008) and rats (Athens 2009, Shiels 2011) on the reproductive and recruitment success of native plants, in addition to effects of feral ungulates (Diong 1982, Nogueira-Filho et al. 2009, Weller et al. 2011) and invasive plants (Loope 1992, Thaxton et al. 2010, Weller et al. 2011) on native habitats and plant communities. These negative impacts on the native flora, which eventually can contribute to increased rarity, can also trigger additional genetic and demographic concerns with decreasing population sizes (Lande 1988, Ellstrand and Elam 1993, Groom 1998). For example, smaller populations that rely on biotic pollination may not be able to find a mate, or there may not be enough flowering plants in the population to attract a pollinator. This can lead to lower reproductive success (Allee effect; Groom 1998). This has been documented in Hawai‘i in the threatened Haleakalā silversword (*Argyroxiphium sandwicense* subsp. *macrocephalum*; Forsyth 2003). Without controlling the introduction or impact of exotic species, genetic factors could escalate the negative consequences of population decline.

Loss of habitat, loss of pollinators, and a decrease in population size have led to concern that much of Hawai‘i’s flora is experiencing increased rates of selfing, genetic drift, and consequently the increased expression of inbreeding depression; the decrease in fitness that usually occurs among progeny when normally outcrossed species are forced to inbreed (Charlesworth and Charlesworth 1987, Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005). A decrease in the number of individuals and genetic variability in a population can lead to increased homozygosity and the fixation of

deleterious alleles in a population. These factors may consequently limit a population's ability to withstand changes in the environment and stochastic events, and the population may become more prone to extirpation. Populations of the same species may also become more differentiated from each other with increased levels of selfing (Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005). Several studies have quantified the amount of inbreeding and genetic variation within and among populations of rare Hawaiian plant species through genetic analyses with microsatellites, isozymes, and random amplified polymorphic DNA markers (Gemmill et al. 1998, Morden and Loeffler 1999, Friar et al. 2000, Friar et al. 2001). In order to determine if inbreeding depression is occurring, common garden experiments can be conducted to quantify fitness differences in the progeny of selfed and outcrossed individuals within a population (Kramer and Havens 2009). These studies have been conducted in several species of the endemic genus, *Schiedea* (Sakai et al. 1989, Norman et al. 1995, Sakai et al. 1997, Culley et al. 1999, Weller et al. 2005). There are no known studies that have investigated inbreeding depression in a species in the Hawaiian flora with a common garden experiment in the field as opposed to in the greenhouse. If and when inbreeding depression is detected in small, declining populations, restoration of the species through reintroduction (or augmentation) may be necessary to prevent extinction.

If individuals in small populations are suffering from inbreeding depression, mixing populations at a reintroduction site or augmenting an existing population may increase the amount of genetic variation and heterozygosity within the new population, reduce genetic drift, increase heterosis, and increase the ability of the population to tolerate environmental stochasticity (Vergeer et al. 2005, Guerrant and Kaye 2007, Menges 2008, Frankham 2010). Outplantings with large numbers of plants may circumvent density-dependent factors that may affect small populations, such as susceptibility to plant invasions or a decrease in attractiveness to pollinators, as mentioned above (Groom 1998, Forsyth 2003, Hobbs and Yates 2003). When natural populations are degraded, reintroductions (referring to any outplanting, including augmentation, within the historic range of a species) may need to be located in protected areas. In Hawai‘i, this would most likely include habitat that is ungulate-free via large fences (Menges 2008, Weller et al. 2011). Factors such as breeding system, pollinator

relationships, morphology, genetic diversity and relatedness, habitat differences, and recent and historic levels of gene flow between populations need to be considered in determining the appropriate individual founders to use in reintroduction attempts (Guerrant 1996, Guerrant and Kaye 2007, Menges 2008, Godefroid et al. 2011). Depending on the number and relatedness of remaining populations and individuals, reintroductions could be established with all individuals from a single population or a combination of multiple populations. It is recommended to use a single population for reintroduction (other than when it is the only population remaining) when there is *ex situ* material from a relatively large number of individuals that are representative of the natural population. When there are few individuals available and/or they are not representative of the genetic diversity present in the natural population, it may be more successful to include multiple source populations at the reintroduction. Additionally, if the augmentation is into altered habitat, or the reintroduction is either outside of historic distribution or not very similar to the habitat of a single population, it may be more successful to reintroduce from multiple populations. Whether or not to mix populations at a reintroduction is an important consideration to be made carefully, as hybrid breakdown due to outcrossing is possible if individuals are either too genetically distinct or adapted to different habitats (Guerrant 1996, Menges 2008).

The potential negative consequences to mixing different individuals or small populations of the same species that have been isolated for an uncertain number of generations also need to be considered in reintroduction efforts. Outbreeding depression is the decrease in fitness of offspring due to crossing of individuals either within or between populations, and may occur in hybrid offspring whose parents were located at varying distances within populations, from different, geographically distant populations, or from different habitats (regardless of geographic distance) (Waser and Price 1989, Lynch 1991, Ellstrand and Elam 1993). Outbreeding depression may occur due to the breakup of co-adapted gene complexes or as a result of local adaptation to environmental conditions specific to the individual source populations (Lynch 1991, Ellstrand and Elam 1993, Waser and Price 1993, Frankham et al. 2002). Outbreeding depression can occur both within a population and/or between populations, and the mechanism can be either environmental and/or physiological. There is more support, however, for within-

population outbreeding depression to be a result of adaptation to specific environmental factors than the disruption of allelic coadaptation, in which the latter would indicate the capability of genetic drift to isolate individuals in close proximity to one another (Schemske 1984, Slatkin 1985, Waser and Price 1989). Waser and Price (1994) and Waser, Price and Shaw (2000) demonstrated an optimal crossing distance between individuals in two separate species by comparing offspring fitness of hybrids whose parents were separated by various geographic distances. Their results suggest that a balance between inbreeding depression (following selfing or crossing with close relatives) and outbreeding depression (following crossing with more distant relatives) could contribute to a distance between parents that would produce maximum fitness in their progeny. Producing offspring with maximum relative fitness should be considered in designing a reintroduction for rare species.

Heterosis, in addition to considering levels of inbreeding depression and outbreeding depression when planning a reintroduction, is another factor to be considered upon outcrossing. Heterosis is the increase in progeny fitness upon outcrossing, and is measured as an increase in the fitness of the first filial generation in relation to parental fitness or the increase in fitness of progeny from crosses between populations as compared to crosses within populations (Glemin et al. 2003, Charlesworth and Willis 2009). Heterosis is not simply a reversal in the reduction of fitness upon inbreeding, but rather an increase upon outcrossing between populations, due to an increase in heterozygosity and the reduction of fixed, deleterious alleles; most likely due to increased drift in small populations. Inbreeding depression occurs when a normally outcrossed population is forced to self and there is genetic variation within a population for traits related to fitness, while heterosis may occur if here is genetic variation for fitness-related traits between populations (Keller and Waller 2002, Charlesworth and Willis 2009). Outbreeding depression is a reduction in fitness upon outcrossing, either within or between populations. Outbreeding depression may also take more than one generation of outcrossing to express the extent of the reduction in fitness if the mechanism causing the reduction is the breakup of coadapted gene complexes, as these may continue with continued outcrossing (Lynch 1991). Erickson and Fenster (2006), however, followed crosses for six generations and discovered that initial indications of outbreeding

depression in early generations diminished as consequent generations of recombination allowed plants to recover from the initial breakup of coadapted gene complexes. It will be difficult to plan a successful reintroduction without a general understanding of if and how these factors are expressed in a particular species.

Predicting the levels of heterosis, inbreeding depression and outbreeding depression when considering genetic sources for a reintroduction of a rare plant species is difficult, primarily due to the lack of information regarding the basic biology of most species (Guerrant 1996). The breeding system and mating system of the species can provide insight into the amount of inbreeding depression expected and the likelihood of outbreeding depression during restoration efforts (Dudash and Murren 2008). If a species is primarily selfing, a long history of inbreeding will have resulted in expression of recessive deleterious alleles, and purging of strongly deleterious alleles is likely to have already occurred (Charlesworth and Charlesworth 1987, Husband and Schemske 1996). Mildly deleterious alleles, however, may still be present within the population, allowing for the expression of inbreeding depression in later life stages (Lande and Schemske 1985, Husband and Schemske 1996). As a result, inbreeding depression may be cryptic and difficult to assess in species with a long history of selfing (Lande and Schemske 1985, Husband and Schemske 1996, Weller et al. 2005). Additionally, predominantly selfing species may have more between-population variation due to reduced gene flow (Schemske 1984), and heterosis is still possible upon outcrossing between these populations (Lande and Schemske 1985). For predominantly outcrossing species, the chance of expressing inbreeding depression may increase as selfing increases (Charlesworth and Charlesworth 1987, Husband and Schemske 1996). Outcrossing species are more likely to have less genetic variation differentiating populations than selfing species (Dudash and Murren 2008). Populations may show less outbreeding depression upon mixing of populations if there has been recent gene flow between the parent populations (Ellstrand and Elam 1993). Populations are predicted to have stable mating systems when high selfing rates are accompanied by low inbreeding depression, and low selfing rates are accompanied by high inbreeding depression (Lande and Schemske 1985). If individuals of small populations of a particular taxon (selfers or outcrossers) are suffering from inbreeding depression, the chance of establishing a new

self-sustaining population (via reintroduction) may increase with the combination of source material from multiple small populations. The goal of this study was to determine how the balance of these genetic concepts could contribute to the design and success of a reintroduction of a rare plant species.

Heterosis, inbreeding depression and outbreeding depression have been investigated in plant populations, including rare species (Quilichini et al. 2001, Kephart 2004, Erickson and Fenster 2006, Raabová et al. 2009) by comparing relative fitness of selfed and outcrossed progeny, identifying levels of inbreeding and/or outbreeding depression. Quilichini et al. (2001) detected outbreeding depression in some stages within a single population of a rare plant species on a Mediterranean island (Corsica). Raabova (2009) observed heterotic progeny in a rare herb in some fitness stages and stressed the importance of distance between populations contributing to the success of the cross. Kephart (2004) grew progeny in a field setting of another rare species in the family Caryophyllaceae and showed greater fitness in outcrossed versus selfed progeny. All studies stressed the importance of hesitation upon outcrossing until species-specific studies have determined the benefit of (or lack there of) outcrossing and the difficulty in predicting the outcome without understanding the biology of the species of concern. Information from these studies can be used to guide the management of reintroductions, particularly with regard to mixing of gene pools. If wild plants from different populations are represented in an outplanting and outbreeding depression is expressed, the offspring may be less vigorous and the reintroduction could fail. Godefroid et al. (2011) completed a large synthesis of the success of plant reintroductions, and recommended an increased focus on the basic biology of the species being outplanted. Frankham et al. (2011) identified issues that may raise the likelihood that outbreeding depression is a concern, including if populations: 1) are actually distinct species; 2) have fixed chromosomal differences; 3) have been genetically isolated for at least 500 years; 4) are in different environments. The problem facing reintroduction plans today is that these factors are mostly unknown for these rare taxa. The maintenance of outcrossing is also largely unknown for many of these taxa. While abiotic pollination can be inferred by floral morphology and pollen size and dispersal, the dependence on a biotic pollinator is

another factor that should be assessed as part of the reintroduction planning for a species that has any level of outcrossing (anything other than a species with obligate autogamy).

If a mixed source reintroduction is the best chance to maximize offspring fitness, one additional factor needs to be considered: the sexual reproduction among individuals. Outcrossing may be necessary for reproduction to occur. If the plants are animal pollinated, an appropriate pollinator must be present to ensure cross-pollination. Presumed modes of pollination (bird, insect, or wind) are often described for the Hawaiian flora, though detailed knowledge of relationships between effective pollinators and plant taxa is limited (Wagner et al 1999). Determining what is effectively pollinating a species is difficult, but observing which birds and insects visit flowers can help determine if the habitat is also host to appropriate floral visitors.

Study Taxa: The endemic Hawaiian genus, *Schiedea* (Caryophyllaceae), was chosen for this study due to its rarity as well as its diversity in habitat, breeding systems, and mating patterns. *Schiedea* is one of the largest adaptive radiations of vascular plants in the Hawaiian Islands; comprised of 34 species that evolved from a single hermaphroditic ancestor (Wagner et al. 1999, Willyard et al. 2011). Research on *Schiedea* has focused on the phylogeny, genetic diversity, and evolution of dimorphic breeding systems (Weller and Sakai 1990, Weller et al. 1995, Weller et al. 1996, Weller et al. 1998, Weller et al. 2001, Sakai et al. 2006). Six species have shown high levels of inbreeding depression, with low levels of selfing in hermaphroditic species, or high levels of selfing in hermaphrodites in dimorphic species (Sakai et al. 1989, Norman et al. 1995, Sakai et al. 1997, Culley et al. 1999, Rankin et al. 2002, Wagner et al. 2005, Weller and Sakai 2005, Sakai et al. 2008). A seventh species showed low levels of inbreeding depression and high levels of selfing (Weller et al. 2005). Three species have been chosen for this study, all in sections of *Schiedea* that have been relatively understudied in comparison to species in other sections and found on the island of O‘ahu. All three species are federally listed endangered, have *ex situ* collections that can be used for reintroductions, and have historic ranges where protected (fenced) gulches can serve as outplanting sites (Keir and Weisenberger 2011). Two species, *S. nuttallii* Hooker and *S. kaalae* Wawra, are sister species, and both are predicted to be predominantly outcrossing species, though the latter

is predicted to have higher rates of outcrossing than the former (Weller et al. 1998). *S. nuttallii* is relatively shorter-lived and known to have sustained recently large population crashes, while *S. kaalae* has only been known from small populations dispersed throughout many locations on the island (Keir and Weisenberger 2011). The last species, *S. obovata* (Sherff) W.L. Wagner & Weller, is in another section of *Schiedea*, and is predicted to be a predominantly selfing species (Weller et al. 1998).

All species in the *S. nuttallii* clade in section *Mononeura* (Wagner et al. 2005; but see also Willyard et al. 2011) are hermaphroditic, and have been understudied in comparison to other sections. There have been shifts to obligate autogamy in this section, suggesting high rates of selfing (Wagner et al. 2005). Levels of inbreeding depression have not been assessed for any of these species. *S. nuttallii* is a perennial subshrub in this section. It has slightly protandrous flowers that are likely insect pollinated and capable of self-fertilization; wind tunnel studies showed that *S. nuttallii* is incapable of wind pollination (Wagner et al. 2005, Weller et al. 1998). Pollen: ovule ratios of *S. nuttallii* indicate that this species is in the range characteristic of facultative selfing and outcrossing (Cruden 1977, Weller et al. 1998). Hermaphroditism could be stable in this species, either through a combination of high levels of inbreeding depression and low levels of selfing, or low levels of inbreeding depression and high levels of selfing.

Schiedea nuttallii has recently decreased in numbers and is restricted to the northern Waiʻanae Mountains of Oʻahu. One extant population (Pahole) and one isolated individual (Kahanahāiki) in mesic habitat currently represent this species in this study. There is a third location, one isolated individual also in Pahole Gulch, which is not represented in this study due to unique morphology not seen in other known conspecifics. It is unclear if the plant is *S. nuttallii* or possibly a hybrid with another species of *Schiedea*. The species was federally listed as endangered in 1996 (Russell & Bruegmann 1996). The Pahole population has consisted of approximately one dozen mature individuals for the past fifteen years (Keir and Weisenberger 2011). The only sign of recruitment during this time period was in 2009 when three seedlings were observed, however all three died prior to maturation. The Kahanahāiki population has declined within the past few years from dozens to only one individual, with a maximum known

population size of 30 mature and 18 immature plants in 2001 (Keir and Weisenberger 2011). Both populations (Pahole and Kahanahāiki) have been fenced and have been ungulate-free for the past 13 years. Cuttings and fruit have been collected from some individuals no longer in the natural populations. *S. nuttallii* has been known from a couple other locations within the last 20 years. *Ex situ* representation from these areas, however, was unknown at the time of this study. Since then, representation of an additional location has been located and will be incorporated into management suggestions. The following specific questions were addressed in this study:

1. Is inbreeding depression and/or heterosis detectable in *S. nuttallii*? If this species has moderate levels of outcrossing, it would suggest that its mating system is not evolutionarily stable. Though it is likely that selfing rates have increased as population size has decreased, obligate autogamy in closely related species suggests that selfing rates are likely higher in this section. Consequently, inbreeding depression, if present, may only be at later life stages. Heterosis will likely be present as well due to the very small population sizes.
2. Is outbreeding depression a concern in planning reintroductions of this species? Due to similar habitats and close proximity of remaining these remaining habitats, it is unlikely that outbreeding depression is present.
3. What combination of founders (wild plants either extant or extirpated but with *ex situ* representation) will yield the most fit progeny with the goal of creating a new viable population of this species? It is likely that a combination of the remaining wild plants will provide optimal fitness of progeny at a reintroduction site.

No studies have investigated inbreeding depression in the two species in section *Alsinidendron*, though it is sister to section *Nothoschiedea*, where it has been studied (Weller et al. 2005). *Schiedea obovata* is a short-lived, perennial, woody herb with a hermaphroditic breeding system (Weller et al. 1995, Wagner et al. 2005) and the second species of *Schiedea* in this study. Flowers have apparent adaptations for bird-pollination, including fleshy white sepals, slight protandry, and a sweet black nectar with concentrations and amounts compatible with pollination by passerine birds (Weller et al. 1998). However, native honeycreepers and honeyeaters on O‘ahu have undergone

massive population crashes and are extinct, rare, or uncommon (Pratt 1994, Shehata et al. 2001, Fleischer et al. 2008). It is unlikely that birds are currently maintaining any level of outcrossing in this species (Chapter 5). In the absence of pollination, plants easily self-fertilize and produce viable seed and vigorous progeny capable of reproduction (Wagner et al. 2005, Keir and Weisenberger 2011). *S. obovata* has a facultatively autogamous breeding system based on floral morphology and observations in cultivation. Pollen:ovule ratios indicate that the breeding systems of this species is somewhere between facultative autogamy and xenogamy. Among the four species of the sections *Alsinidendron* and *Nothoschiedea*, *S. obovata* stands apart by having a pollen:ovule ratio that is over eleven times higher than the other three species, suggesting that it might be better adapted for outcrossing (Cruden 1977, Weller et al. 1998). The breeding and mating systems of *S. obovata* are difficult to assess, especially without any known observations of bird pollination. Current information about the species is somewhat contradictory, and in light of recent population crashes of native honeycreepers and honeyeaters and *S. obovata*, interactions between *S. obovata* and its potential pollinators remain unknown.

The range of *S. obovata* once extended throughout ridges along the entire Waiʻanae Mountain Range of Oʻahu. Within the last couple of decades, *S. obovata* has been found only along ridges of the northern Waiʻanae Mountains. In the late 1970s, Keawapilau gulch had approximately 100 individuals, but no plants were monitored in this area until recently. During this same time, Pahole gulch had approximately 70 plants, with the last known plant dying in 2003 (Keir and Weisenberger 2011, Lau 2011). Currently, one population (NW Makaleha) of approximately 100 individuals (30 mature) and a few isolated individuals in surrounding areas (Keawapilau) represent the extant, *in situ* distribution for this species. The populations are on an elevational incline over 200m long from the lowest, most northern population, Kahanahāiki to the most southern population, W Makaleha. This species was federally listed as endangered in 1991 (Ellshoff et al. 1991). Cuttings and fruit have been collected from some individuals no longer found in the natural populations. All *ex situ* plants for this species (including plants used in this study) are derived from populations in the northern Waiʻanae Mountains.

The goal of this study is to determine what source(s) should be used in reintroduction efforts of *S. obovata*, based on potential population differentiation and the expression of inbreeding depression. In 2003, the Makua Implementation Plan for Makua Military Reservation stated the following: *"Outplanting considerations: Since A. obovatum* [synonym *S. obovata*] *is a naturally selfing plant (Weller pers. comm. 2000), plants from different stocks should not be mixed together in outplantings."* (MIT 2003). Arguments toward establishing populations with mixed sources stem from concerns of outbreeding depression if gene flow has not occurred among small selfing populations, and plants have become adapted to local conditions. This goal and these considerations have invoked the following questions:

4. Is inbreeding depression and/or heterosis detectable in *S. obovata*? If this species is highly selfing, it is doubtful that inbreeding depression will be detect unless only at later life stages. Due to population decline, heterosis will be present.
5. Is outbreeding depression a concern in planning reintroductions of this species? There may be more genetic isolation between populations of this species due to its predicted high levels of selfing, suggesting a potential for outbeeeding depression.
6. Are there differences in fitness of progeny from populations distributed along an elevation gradient grown in a common outplanting site (garden)? Progeny may be less fit the further their source population is from the common garden site along the gradient.
7. What combination of founders (wild plants either extant or extirpated, but with *ex situ* representation) will yield the most fit progeny with the goal of creating a new viable population of this species? Crosses between nearby populations, especially when there is only one individual per population, will likely increase fitness of progeny. Crosses between further populations may be more likely to express outbreeding depression in progeny.

The third species of interest in this study is *Schiedea kaalae,* a perennial course shrub in the section *Mononeura* (sister to *S. nuttallii*). It has slightly protandrous flowers that are likely insect pollinated and capable of self-fertilization (Wagner et al. 2005,

Weller et al. 1998). Pollen:ovule ratios of *S. kaalae* indicate that this species has a facultative xenogamous mating system (Cruden 1977, Weller et al. 1998). Hermaphroditism could be stable in this species, either through a combination of high levels of inbreeding depression and low levels of selfing, or low levels of inbreeding depression and high levels of selfing (Lande and Schemske 1985). Though inflorescence morphology and pollen:ovule ratios suggest facultative outcrossing as the predominant mating system (and consequently high inbreeding depression and low selfing rates), historically small population sizes may maintain higher than predicted levels of selfing in this species. If this is true, low inbreeding depression and high selfing rates may be the most recent mating system in this species.

Schiedea kaalae is located in both the Koʻolau and Waiʻanae Mountain Ranges of Oʻahu, where it was known from mesic gulches (Waiʻanae Mts.) to wet forests (Koʻolau Mts.) on steep, shaded slopes (Wagner et al. 1999). This species is extremely rare and was federally listed as endangered in 1991 (Ellshoff et al. 1991). Relatively little is known about previous population sizes, and no recent observations (within the last 30 years) have documented populations greater than 20 individuals. Notes from Wayne N. Takeuchi, a botanist who made extensive collections on Oʻahu, suggested he found more plants than he had expected when he saw the ʻĒkahanui population of approximately 10 plants in 1987, though he concluded that the species was not common: "this sp. is not as rare as previously supposed, but still uncommon" (Takeuchi and Paquin 1987). Lifespan is uncertain in *S. kaalae*, and no plant has been documented from seedling through maturity and to death (Keir and Weisenberger 2011). It is assumed from observing mature plants over the last decade that this species is long-lived (> 10 years). Isolated plants are able to self-fertilize and produce viable seeds that yield reproductive progeny. There were four known locations in the Koʻolau Mts.: three are extant (one with only a single individual) and three were available for this study (Kaipapaʻu, Maʻakua and Kahana). There were ten known locations in the Waiʻanae Mts.: three are extant (two with only one individual) and seven are represented in this study (Pālāwai, ʻĒkahanui-A, ʻĒkahanui-B, Huliwai, Kaluaʻa, Pahole-A and Pahole-B). Fruit collections and cuttings have been taken from many individuals, some of which are now extirpated. The following questions were addressed in this study:

8. Is inbreeding depression and/or heterosis detectable in *S. kaalae*? With predicted high selfing levels but historically small population sizes, inbreeding depression will again be at later life stages if at all. Heterosis will be present.
9. Is outbreeding depression a concern in planning reintroductions of this species? Outbreeding depression will be present in crosses between populations in different mountain ranges, as plants exist in different habitats and have likely not had recent gene flow.
10. Is there local adaptation of populations to their home mountain range? Individuals will be more fit in their home mountain range relative to the other mountain range.
11. What combination of wild plants will yield the most fit progeny with the goal of creating a new viable population of this species? Again, due to reduced population size, a mix of wild plants from each mountain range (but populations from different ranges kept separate) will provide the best chance for reintroduction success.

To address these questions, fitness of progeny from crosses within and between populations and the fitness of selfed progeny were assessed in field settings comparable to locations where reintroductions would take place. A field setting (one in each mountain range for *S. kaalae*) was used to test fitness of progeny from crosses to mimic conditions likely to occur during reintroduction efforts, and to estimate levels inbreeding depression under field conditions rather than greenhouse conditions likely to provide unrealistic estimates of the genetic effects of selfing and outcrossing on fitness (Dudash 1990, Husband and Schemske 1996). *Ex situ* hand-pollinations were conducted to produce progeny from selfed treatments, crosses within populations, and crosses between populations. The number of fruit and seeds per flower pollinated, seed mass, and seed viability were quantified. Progeny were propagated and outplanted, and their survival and maturation, growth, and male and female fitness estimates were measured and compared. Pollinator observations were conducted at existing locations to estimate levels of outcrossing. Reintroduction plans were designed for each species.

Potential Pollinator Observations: Floral visitor observations were conducted throughout the flowering period at already established reintroduction sites and selected wild populations of all three taxa (Table 1.1). Reintroduction sites had been initiated and maintained by the O‘ahu Army Natural Resources Program (OANRP: University of Hawai‘i – Pacific Cooperative Studies Unit). Observations consisted of recording the faunal taxa that visited plants within a ten or thirty minute block of time. The amount of plants flowering at a particular observation site varied in attempt to determine if the pollinator attractiveness of the site was size and density dependent. Observations were made during daylight hours only (dawn to dusk) when the human eye could see without artificial light. A camera (Moultrie Feeders Infrared Game Spy Model #MFH-I65, EBSCO Industries, Inc.) took pictures once an hour for one night at the ‘Ēkahanui outplanting (study) site for *S. kaalae*. Studies have indicated that native Pyralid moths could be potential pollinators for *S. lydgatei* (Norman, Weller & Sakai 1997), supporting the necessity of dusk and potentially evening observations. Taxa, length of visit per flower, behavior of visitor, number of flowers visited, number of flowers on the observed plant and the number of flowering plants were recorded at each observation. Floral morphology suggests that *S. nuttallii* and *S. kaalae* are potentially insect-pollinated, and observations were made at close distance (ca. 1m). Floral morphology and nectar quantities and composition of *S. obovata* suggested possible bird pollination. Observations on this taxon were made at a distance of 10m with binoculars in silence and without moving. A five-minute buffer, in position to observe, prefaced every 30-minute observation.

Table 1.1. Locations for Potential Pollinator Observations by Plant Species

Species	Population	*In situ* or Reintroduction Site	# Plants*
S. obovata	W Makaleha	Reintroduction	40
S. obovata	Kahanahāiki	Reintroduction	~100
S. kaalae	‘Ēkahanui	*In situ*	13
S. kaalae	‘Ēkahanui	Study Outplanting Site	700+
S. kaalae	Kalua’a	Reintroduction	88
S. kaalae	Makaua (Koolau Mts.)	Reintroduction	12
S. nuttallii	Pahole	Reintroduction	36

* Only mature founders are documented*

CHAPTER 2. *Schiedea nuttallii*

Introduction

The angiosperm flora of the Hawaiian Islands has one of the highest rates of endemism (89%) in the world, and over half of the taxa are at risk (Wagner et al. 1999, Sakai et al. 2002). Approximately 10% of the flora is extinct (Wagner et al. 1999), and over thirty percent of the flora is federally listed as threatened or endangered (out of 1094 species; USFWS 2010). The conservation of Hawai‘i’s rare flora is necessary and challenging. Reasons for the decline of native species are both numerous and uncertain, often centered around the introduction of many invasive species, including other plants, invertebrates, and large ungulates, which can also cause vast habitat degradation (Diong 1982, Loope et al. 1988, Loope 1992, Cuddihy and Stone 1993, Joe and Daehler 2008, Athens 2009, Weller et al. 2011). Loss of native habitats, loss of pollinators, and decreases in population size have led to concern that much of Hawai‘i’s flora is experiencing increased rates of selfing, genetic drift, and consequently the increased expression of inbreeding depression (the decrease in fitness that usually occurs among progeny when normally outcrossed species are forced to inbreed; Charlesworth and Charlesworth 1987, Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005). A decrease in the number of individuals and genetic variability in a population can lead to increased homozygosity, which may consequently limit the population’s ability to withstand changes in the environment and stochastic events, and the population may become more prone to extirpation (Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005).

If individuals in small populations are suffering from expression of inbreeding depression, mixing populations at a reintroduction site or augmentation site may increase the amount of genetic variation and heterozygosity within the new population, reduce genetic drift, increase heterosis, and increase the ability of the population to tolerate environmental stochasticity (Vergeer et al. 2005, Guerrant and Kaye 2007, Menges 2008, Frankham 2010). Heterosis can be measured as the increase in fitness of progeny from crosses between populations compared to crosses within populations (Glemin et al. 2003, Charlesworth and Willis 2009). Heterosis is due to an increase in heterozygosity and the

reduction of fixed, deleterious alleles, which is most likely due to increased drift in small populations. Inbreeding depression occurs when a normally outcrossed population is forced to self and there is genetic variation within a population for traits related to fitness, while heterosis may occur if there is genetic variation for fitness-related traits between populations (Keller and Waller 2002, Charlesworth and Willis 2009). These genetic interactions are not the only factors that need to be considered in recovery efforts of rare species.

Ecological and biological dynamics other than inbreeding depression and heterosis may be important as well in the recovery of rare plants. Factors such as breeding system, pollinator relationships, morphology, habitat differences, genetic diversity and relatedness, and recent and historic levels of gene flow between populations need to be considered in determining the appropriate individual founders to use in reintroduction attempts (Guerrant 1996, Guerrant and Kaye 2007, Menges 2008, Godefroid et al. 2011). Reintroductions, located within historic ranges of the species and protected from detrimental herbivory (*e.g.* ungulate-free and fenced areas in Hawai‘i), may be necessary to re-establish species severely threatened with extinction due to decreased population sizes and expression of inbreeding depression (Menges 2008, Weller et al. 2011). Reintroductions with large numbers of plants may circumvent density-dependent factors that may affect small populations, such as susceptibility to plant invasions or a decrease in attractiveness to pollinators (Groom 1998, Forsyth 2003, Hobbs and Yates 2003). The number of plants to reintroduce will ultimately depend on the effective population size of the rare species at the location chosen for outplanting (Guerrant 1996). Depending on the number and relatedness of remaining wild populations, reintroductions could be established with all individuals from a single population or from using individuals from multiple populations. Whether genotypes from different populations are mixed together in recovery efforts may depend on many of the factors listed here. Whether they are known or not, the balance among the history of isolation, potential adaptation to different habitats, and the potential benefits and risks of outcrossing should be explored prior to reintroduction attempts.

The potential negative consequences to mixing different individuals or small populations of the same species that have been isolated for an uncertain number of generations also need to be considered in reintroduction efforts. Outbreeding depression is the decrease in fitness of offspring due to crossing of individuals either within or between populations, and may occur in hybrid offspring whose parents were located at varying distances within populations, from different, geographically distant populations, or from different habitats regardless of geographic distance (Waser and Price 1989, Lynch 1991, Ellstrand and Elam 1993). Outbreeding depression may occur due to the breakup of co-adapted gene complexes or as a result of local adaptation to environmental conditions specific to the individual source populations (Lynch 1991, Ellstrand and Elam 1993, Waser and Price 1993, Frankham et al. 2002). Heterosis, inbreeding depression and outbreeding depression have been investigated in plant populations, including rare species (Quilichini et al. 2001, Kephart 2004, Erickson and Fenster 2006, Raabová et al. 2009) by comparing the relative fitness of selfed and outcrossed progeny, and identifying levels of inbreeding depression and/or outbreeding depression. Information from these studies can be used to guide the management of reintroductions, particularly with regard to mixing of gene pools. If wild plants from different populations (founders) are represented in an outplanting and outbreeding depression is expressed, the offspring may be less vigorous and the reintroduction could fail. Frankham et al. (2011) identified issues that may raise the likelihood that outbreeding depression is a concern, including if populations: 1) are actually distinct species; 2) have fixed chromosomal differences; 3) have been genetically isolated for at least 500 years; and/or 4) are in different environments. The problem facing reintroduction plans in Hawai‘i today is that these factors are mostly unknown among rare taxa.

Predicting the levels of heterosis, inbreeding depression and outbreeding depression when considering genetic sources for a reintroduction of a rare plant species is difficult, primarily due to the lack of information regarding the basic biology of most species (Guerrant 1996). The breeding system and mating system of the species can provide insight into the amount of inbreeding depression expected and the likelihood of outbreeding depression during restoration efforts (Dudash and Murren 2008). If a species is primarily selfing, a long history of inbreeding will have resulted in expression of

recessive deleterious alleles, and purging of strongly deleterious alleles is likely to have already occurred (Lande & Schemske 1985, Charlesworth & Charlesworth 1987, Husband & Schemske 1996). Mildly deleterious alleles, however, may still be present within the population, allowing for the expression of inbreeding depression in later life stages (Lande and Schemske 1985, Husband and Schemske 1996). As a result, inbreeding depression may be cryptic and difficult to assess in species with a long history of selfing (Lande and Schemske 1985, Husband and Schemske 1996, Weller et al. 2005). For predominantly outcrossing species, the chance of expressing inbreeding depression may increase as selfing increases (Lande & Schemske 1985, Charlesworth & Charlesworth 1987, Husband & Schemske 1996). Populations may show less outbreeding depression upon mixing of populations if there has been recent gene flow between the parent populations, and the persistence of outbreeding depression throughout successive generations may decrease (Edmands and Timmerman 2003). If individuals of small populations of a particular taxon (selfers or outcrossers) are suffering from inbreeding depression, the chance of establishing a new self-sustaining population (via reintroduction) may increase with the combination of source material from multiple small populations. The goal of this study was to determine how the balance of these genetic issues could influence the design and success of the reintroduction of a rare plant species.

Study Taxon: The endemic Hawaiian genus *Schiedea* (Caryophyllaceae) was chosen for this study due to its rarity as well as its diversity in habitat, breeding systems, and mating patterns. *Schiedea* is one of the largest adaptive radiations of vascular plants in the Hawaiian Islands, comprised of 34 species that evolved from a single hermaphroditic ancestor (Wagner et al. 1999, Willyard et al. 2011). Research on *Schiedea* has focused on the phylogeny, genetic diversity, and evolution of dimorphic breeding systems (Weller and Sakai 1990, Weller et al. 1995, Weller et al. 1996, Weller et al. 1998, Weller et al. 2001, Sakai et al. 2006, Sakai et al. 2008). Six species have shown high levels of inbreeding depression, with either low levels of selfing in hermaphroditic species, or high levels of selfing in hermaphrodites in dimorphic species (Sakai et al. 1989, Norman et al. 1995, Sakai et al. 1997, Culley et al. 1999, Rankin et al. 2002, Weller and Sakai 2005, Weller et al. 2005). A seventh species showed low levels of inbreeding depression and high levels of selfing (Weller et al. 2005). All species in the *S. nuttallii* clade, section

Mononeura (Wagner et al. 2005; but see also Willyard et al. 2011) are hermaphroditic, and have been understudied in comparison to other sections. Levels of inbreeding depression have not been assessed for any of these species, though shifts in breeding systems from outcrossing to obligate selfing have been documented (Wagner et al. 2005). *Schiedea nuttallii* Hooker is a perennial subshrub in this clade and was the subject of this investigation. It has slightly protandrous flowers that are likely insect pollinated and capable of self-fertilization; wind tunnel studies showed that *S. nuttallii* is incapable of wind pollination (Weller et al. 1998, Wagner et al. 2005). Pollen:ovule ratios of *S. nuttallii* indicate that this species is in the range of facultatively selfing and outcrossing (Cruden 1977, Weller et al. 1998). Hermaphroditism could be stable in this species, either through a combination of high levels of inbreeding depression and low levels of selfing, or low levels of inbreeding depression and high levels of selfing.

Schiedea nuttallii has recently decreased in numbers and is currently restricted to the northern Waiʻanae Mountains of Oʻahu (Table 2.1; Fig. 2.1). One extant population (Pahole) and one isolated individual (Kahanahāiki) in mesic habitat represent this species in this study. There is a third location with one isolated individual, also in Pahole Gulch, which is not represented in this study due to unique morphology not seen in other known conspecifics. It is unclear if the plant is *S. nuttallii* or possibly a hybrid with another species of *Schiedea*. The species was federally listed as endangered in 1996 (Russell & Bruegmann 1996). Pahole has consisted of approximately one dozen mature individuals for the past fifteen years (Keir and Weisenberger 2011). The only sign of recruitment during this time was in 2009 when three seedlings were observed, however all three died prior to maturation. The Kahanahāiki population has declined within the past few years from dozens to only one individual, with a maximum known population size of 30 mature and 18 immature plants in 2001 (Keir and Weisenberger 2011). Both populations (Pahole and Kahanahāiki) have been fenced and ungulate-free for the past 13 years. Cuttings and fruit have been collected from some individuals no longer in the natural populations. *S. nuttallii* has been known from a couple other locations within the last 20 years. The following questions were addressed in this study:

1. Is inbreeding depression and/or heterosis detectable in *S. nuttallii*?

2. Is outbreeding depression a concern in planning reintroductions?
3. What combination of founders (wild plants either extant or extirpated but with *ex situ* representation) will yield the most relatively fit progeny?

To address these questions, fitness of progeny from crosses within and between populations and the fitness of selfed progeny were assessed in field settings comparable to locations were reintroductions would take place. A field setting was used to test fitness of progeny from crosses to mimic conditions likely to occur during reintroduction efforts, and to estimate levels inbreeding depression under field conditions rather than greenhouse conditions likely to provide unrealistic estimates of the genetic effects of selfing and outcrossing on fitness (Dudash 1990, Husband and Schemske 1996).

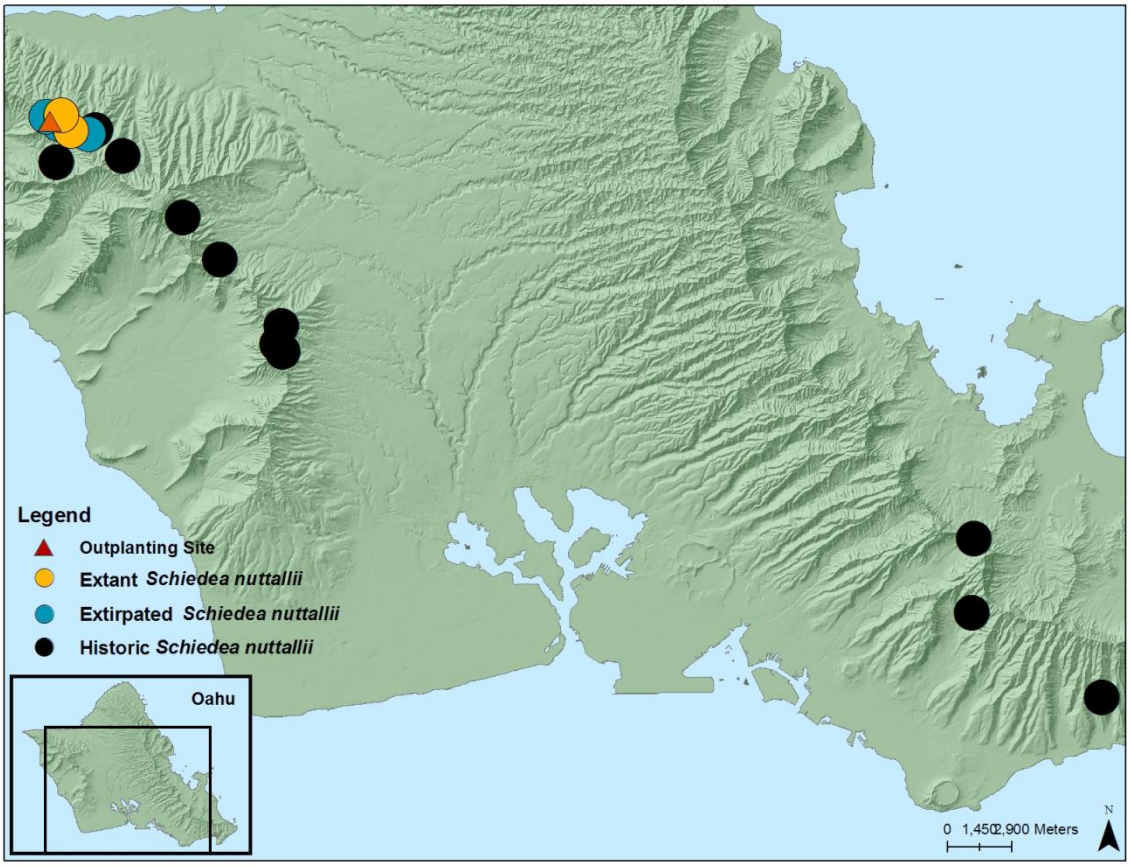

Fig. 2.1. Historic (last seen prior to 1984) and Recent Distribution of *S. nuttallii* on O‘ahu.

Table 2.1. Historic and Current Distribution of *S. nuttallii* and Representation in Study. Temperature data from PRISM Climate Group (PRISM 2004). Rainfall data from Giambelluca et al. (2011). Slope, canopy cover, topography, and aspect from Keir and Weisenberger (2011) and Weisenberger (personal observations).

Population	Maximum Known Individuals*	N Extant Plants*	N Plants Represented in Crosses - Female: Male Parent	Average Annual Max. Temp. (°C)	Average Annual Rainfall (mm)	Elev. (m)	Slope	Canopy Cover	Topography	Aspect
Pahole**	17	9	5: 9	24	1766	719	Steep & Moderate	Intermediate	Upper Slope	NE
Kahanahāiki **	67	0	5: 17	24	1561	689	Moderate	Intermediate	Upper Slope	N
Keawapilau	4	0	0	24	1781	658	Moderate	Closed	Upper Slope	N
Pahole Gulch	1	1	0	25	1588	512	Moderate	Closed	Lower Slope	NE
Outplanting Site	NA	NA	NA	24	1619	668	Moderate	Intermediate	Upper Slope	N

* Only mature founders are documented; ** Available for this study

Methods

Hand Pollinations: Plants from the Pahole and Kahanahāiki populations of *S. nuttallii* were used in a crossing program. Nursery plants used as a seed source for this study were propagated from clonal cuttings from wild plants; except in one case, in which seeds were collected. Two replicate plants (clones of each other and the wild plant) representing five founders each from the two populations were included in the study (Table 2.1). Plants were grown under pollinator-free conditions at Pahole Mid-elevation Nursery in Mokulēʻia Forest Reserve (northern Waiʻanae Mts., 656m elev.) on Oʻahu.

Flowers were emasculated just prior to dehiscence of the anthers. Stigmas were receptive for pollinating two days after emasculation, when stigmas had increased in length and width and the stigmatic papillae were elongated. Only fresh pollen available the day of pollination was used in pollinations. Pollen was applied by gently touching receptive stigmas with dehisced anthers containing pollen, and the yellow pollen easily stood out on white stigmas. Stigmas were examined to ensure that pollen had been deposited along the length of the receptive stigmatic surface. Pollination treatments included flowers that were unmanipulated (control), emasculated and not pollinated (emasculated), hand-pollinated with pollen from the same plant (selfed), or pollinated with pollen from a different plant. Crosses with pollen from a different plant were either from within a population (within) or between the two populations (between). A minimum of 25 pollinations were attempted for every cross treatment for both populations. Fruit were collected at maturity when capsules were tan and the calyx, which had turned purple and fleshy, had begun to desiccate. The seeds of each fruit collection were counted and held in storage (20% relative humidity at -18C) for approximately two months prior to germination.

The number of fruit and seeds per pollination (including the control and emasculated treatments) were determined for each treatment and nursery plant as a total for each plant. Emasculated flowers did not produce any seeds, and were consequently removed from analyses. Mean mass (mg) per seed was also calculated as the mass of the number of seeds produced for each nursery plant by cross treatment over the number of seeds produced. The dry weight of seeds was obtained upon removal from storage and

just prior to sowing. Seed viability was calculated as the number of seeds that germinated (root and shoot visible) out of the total sown for a sample of fruits collected on different days per nursery plant. All seeds that did not germinate within four months had rotted prior to dissection. The number of seeds that germinated, therefore, was equal to the number of seeds that were viable. The control treatments were excluded from the analyses following measurement of seed mass.

Propagation: Seeds were sown in January 2009. Enough seeds were germinated to obtain 25 plants per treatment to outplant in a field setting. Seeds were germinated on 1% water agar (Sigma-Aldrich® A1296) in Petri dishes in a Controlled-Environment Chamber (Pervical Scientific, Inc. E36V). After germination, seedlings were propagated in a 3:1 mix of perlite to vermiculite and remained in the chamber until they were approximately 5 cm tall. Seedlings were then moved onto a mist bench for one month prior to transplanting into individuals pots (RS400: 4" round, standard pot, Exacta Sales, Inc.). After transplantation, 25 plants each from the three pollination treatments for both populations were randomly selected to be in the field experiment and were grown at O'ahu Army Natural Resources Program's (OANRP) East Range nursery (Wahiawa, 247m elevation, 1283mm annual rainfall, 26.1C annual maximum temperature [WorldClimate.com copyright 1996-2011]) until they were outplanted seven months later.

Outplant Site: Prior to flowering, plants were outplanted into Kahanahāiki Gulch in Mākua Military Reservation, northern Wai'anae Mountains in October 2009. The location was selected by the Rare Plant Program Manager of OANRP (Matthew J. Keir) and was located between the Pahole and Kahanahāiki population (Fig. 2.2). There are no quantifiable habitat differences among these three locations. Plants were monitored every month for one year. The total number of inflorescences per plant (0 inflorescences = immature; ≥1 inflorescences = mature) and date of mortality were recorded for each plant. In addition, five plants each from each treatment were randomly selected to count either the number of flowers or number of fruit on one inflorescence. Typically, the first inflorescence on the plant was chosen for counting. If the chosen plant died before counts were made, another unmeasured plant was randomly selected for measurement. In these

cases, the inflorescence chosen may not have been the first to flower. At least three fruit per plant were collected from as many plants as possible to estimate seed set (number of seeds per fruit). Plant height and the length of the longest leaf were measured every other month for the first six months on every plant, with a final measurement 12 months following planting. Size was measured as a product of final plant height (cm) and number of branches (all plants at the time of initial measurements had only one branch). Total growth from the first month after planting to the 12^{th} month after planting was calculated as (final size – initial size)/initial size. Height was measured from the base of the stem to the most distal apical meristem. Several variables were measured to calculate an overall fitness estimate for each plant, including the number of inflorescences per plant, number of flowers and fruit per inflorescence, and number of seeds per fruit. Female fitness was estimated as the total number of seeds each plant produced, calculated as the product of the total number of inflorescences per plant, the mean seeds/fruit, and the mean number of fruit per inflorescence. Male fitness was estimated as the total number of flowers each plant produced, calculated as the product of the total number of inflorescences per plant and the mean number of flowers per inflorescence. Differences among the cross treatments (selfed, crosses within a population, crosses between populations) and between the two populations were investigated.

Analyses: Data were analyzed using SPSS 19 (IBM®). All means, standard errors, and sample sizes are listed in Table 2.2. Data were transformed (log10 x+1 [fruit per inflorescence] or sqrt x+1 [seed/pollination, final size and total growth]) for homogeneity of variance and normality when necessary. General Linear Model 3 (GLM3, two-way (factorial) ANOVA) was used for all parametric tests. Tukey's *post hoc* test was used whenever samples sizes were close to equality. Helmert Contrast (Field 2009) was used to determine if means varied between individual cross treatments as a gradual increase in fitness from selfed to between-population crosses was hypothesized. Kruskal-Wallis and Mann-Whitney U tests were used to analyze all non-parametric data. A three-way loglinear analysis was used on the survival data. The odds ratio was calculated as the difference in the chance of survival of plants between maternal source populations ((N Pahole-source plants alive/ N Pahole-source plants dead) / (N Kahanahāiki-source plant alive / N Kahanahāiki-source plants dead)). Cumulative fitness was calculated for both

maternal source populations as the product of number of seeds per pollination, survival, number of inflorescences per plant, number of fruit per inflorescence, and number of seeds per fruit. Means for each founder (maternal family) were averaged to produce a population mean for each stage. Due to the low number of founders and low production of seeds per founder, cumulative fitness could only be calculated per population and not by founder. Statistical analyses, therefore, could not be conducted as there were only two populations in the study.

To quantify levels of heterosis, inbreeding depression and outbreeding depression, Ågren and Schemske's (1993) "relative performance of crosstypes" (*RP*) was calculated for each variable in both populations as well as for cumulative fitness in both populations as follows:

1. Inbreeding / Outbreeding Depression:

   $RP_i = 1 - w_s / w_w$ if $w_s \leq w_w$,

   and

   $RP_i = w_w / w_s - 1$ if $w_s > w_w$,

   where w_s is the fitness of the selfed progeny and w_w is the fitness of the progeny from crosses within populations.
2. Heterosis / Outbreeding Depression:

   $RP_h = 1 - w_w / w_b$ if $w_w \leq w_b$,

   and

   $RP_h = w_b / w_w - 1$ if $w_w > w_b$,

   where w_b is the fitness of the progeny from crosses between populations.
3. Selfed Progeny / Progeny of Crosses Between Populations:

   $RP_b = 1 - w_s / w_b$ if $w_s \leq w_b$,

   and

   $RP_b = w_b / w_s - 1$ if $w_s > w_b$,

   where w_s is the fitness of the selfed progeny and w_b is the fitness of the progeny from crosses between populations.

RP values range between -1 and +1, where positive values for RP_i indicate that progeny from crosses within populations outperform selfed progeny; inbreeding depression is

consequently present. Negative values indicate outbreeding depression within populations. For RP_h, positive values indicate heterosis and progeny from crosses between populations outperform progeny from crosses within populations. A negative RP_h value therefore indicates outbreeding depression between populations.

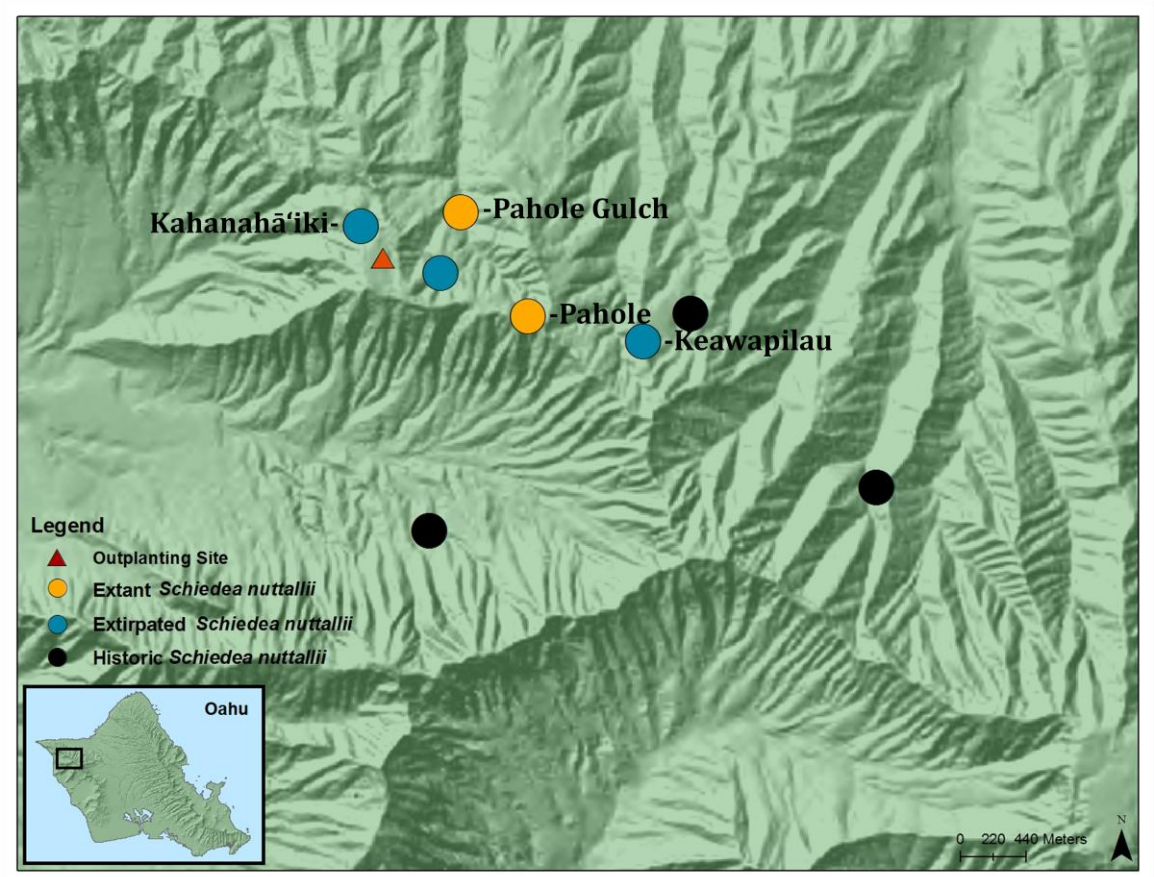

Fig. 2.2. Outplanting Site and Adjacent Source Populations

Table 2.2. Statistics of *S. nuttallii* by Cross Treatment and Population. Cross Abbreviations: X Within = Crosses within populations; X Between = crosses between populations. Total growth was calculated as (final – initial size)/initial size. Size of plant = height (cm) of plant x braches (n). Seeds per plant (N) are shown excluding all immature plants (plants that did not produce an inflorescence).

Variable	**Maternal**	**Cross**	**Mean**	**SE**	**N**
Fruit per Pollination (N)	Kahanahāiki	Control	0.133	0.138	5
Fruit per Pollination (N)	Kahanahāiki	Emasculated	0.000	0.138	5
Fruit per Pollination (N)	Kahanahāiki	Selfed	0.730	0.138	5
Fruit per Pollination (N)	Kahanahāiki	X Within	0.673	0.138	5
Fruit per Pollination (N)	Kahanahāiki	X Between	1.000	0.138	5
Fruit per Pollination (N)	Pahole	Control	0.441	0.116	7
Fruit per Pollination (N)	Pahole	Emasculated	0.167	0.126	6
Fruit per Pollination (N)	Pahole	Selfed	0.910	0.138	5
Fruit per Pollination (N)	Pahole	X Within	0.792	0.126	6
Fruit per Pollination (N)	Pahole	X Between	0.847	0.138	5
Seeds per Pollination (N)	Kahanahāiki	Control	0.367	1.057	5
Seeds per Pollination (N)	Kahanahāiki	Selfed	4.155	1.057	5
Seeds per Pollination (N)	Kahanahāiki	X Within	4.253	1.057	5
Seeds per Pollination (N)	Kahanahāiki	X Between	7.089	1.057	5
Seeds per Pollination (N)	Pahole	Control	1.700	1.057	5
Seeds per Pollination (N)	Pahole	Selfed	3.570	0.965	6
Seeds per Pollination (N)	Pahole	X Within	4.410	1.057	5
Seeds per Pollination (N)	Pahole	X Between	6.085	1.057	5
Seed Mass (mg)	Kahanahāiki	Control	0.236	0.042	1
Seed Mass (mg)	Kahanahāiki	Selfed	0.156	0.021	4
Seed Mass (mg)	Kahanahāiki	X Within	0.191	0.019	5
Seed Mass (mg)	Kahanahāiki	X Between	0.188	0.019	5
Seed Mass (mg)	Pahole	Control	0.238	0.019	5
Seed Mass (mg)	Pahole	Selfed	0.207	0.019	5
Seed Mass (mg)	Pahole	X Within	0.207	0.019	5
Seed Mass (mg)	Pahole	X Between	0.209	0.019	5
Seed Viability (%)	Kahanahāiki	Selfed	0.669	0.06	7
Seed Viability (%)	Kahanahāiki	X Within	0.938	0.065	6
Seed Viability (%)	Kahanahāiki	X Between	0.991	0.065	6
Seed Viability (%)	Pahole	Selfed	0.920	0.065	6
Seed Viability (%)	Pahole	X Within	1.000	0.06	7
Seed Viability (%)	Pahole	X Between	0.95	0.065	6
Total Growth	Kahanahāiki	Selfed	76.722	27.912	9
Total Growth	Kahanahāiki	X Within	123.308	23.225	13
Total Growth	Kahanahāiki	X Between	169.444	27.912	9
Total Growth	Pahole	Selfed	175.368	19.211	19
Total Growth	Pahole	X Within	145.333	19.737	18
Total Growth	Pahole	X Between	162.067	21.621	15

N Inflorescences	Kahanahāiki	Selfed	1.92	0.507	25
N Inflorescences	Kahanahāiki	X Within	2.32	0.507	25
N Inflorescences	Kahanahāiki	X Between	2.24	0.507	25
N Inflorescences	Pahole	Selfed	2.4	0.507	25
N Inflorescences	Pahole	X Within	1.6	0.507	25
N Inflorescences	Pahole	X Between	1.76	0.507	25
Flws per Inflorescence (N)	Kahanahāiki	Selfed	28.667	13.78	6
Flws per Inflorescence (N)	Kahanahāiki	X Within	30.571	12.757	7
Flws per Inflorescence (N)	Kahanahāiki	X Between	39.25	11.934	8
Flws per Inflorescence (N)	Pahole	Selfed	56.75	16.877	4
Flws per Inflorescence (N)	Pahole	X Within	59.2	15.095	5
Flws per Inflorescence (N)	Pahole	X Between	63.286	12.757	7
Fruit per Inflorescence (N)	Kahanahāiki	Selfed	12.75	7.880	4
Fruit per Inflorescence (N)	Kahanahāiki	X Within	5.714	5.957	7
Fruit per Inflorescence (N)	Kahanahāiki	X Between	14	6.434	6
Fruit per Inflorescence (N)	Pahole	Selfed	30.222	5.253	9
Fruit per Inflorescence (N)	Pahole	X Within	16.333	6.434	6
Fruit per Inflorescence (N)	Pahole	X Between	12.571	5.957	7
Flowers per Plant (N)	Kahanahāiki	Selfed	55.04	23.357	25
Flowers per Plant (N)	Kahanahāiki	X Within	70.926	23.357	25
Flowers per Plant (N)	Kahanahāiki	X Between	87.92	23.357	25
Flowers per Plant (N)	Pahole	Selfed	136.2	23.357	25
Flowers per Plant (N)	Pahole	X Within	94.72	23.357	25
Flowers per Plant (N)	Pahole	X Between	111.383	23.357	25
Seeds per Fruit (N)	Kahanahāiki	Selfed	4.712	1.277	6
Seeds per Fruit (N)	Kahanahāiki	X Within	4.871	1.042	9
Seeds per Fruit (N)	Kahanahāiki	X Between	4.342	1.106	8
Seeds per Fruit (N)	Pahole	Selfed	4.067	0.989	10
Seeds per Fruit (N)	Pahole	X Within	4.607	1.042	9
Seeds per Fruit (N)	Pahole	X Between	6.078	1.182	7
Seeds per Plant (N)	Kahanahāiki	Selfed	205.978	47.826	14
Seeds per Plant (N)	Kahanahāiki	X Within	117.72	44.737	16
Seeds per Plant (N)	Kahanahāiki	X Between	226.924	46.204	15
Seeds per Plant (N)	Pahole	Selfed	526.823	47.826	14
Seeds per Plant (N)	Pahole	X Within	250.836	51.658	12
Seeds per Plant (N)	Pahole	X Between	313.775	46.204	15
Cumulative Fitness	Kahanahāiki	Selfed	191.456		
Cumulative Fitness	Kahanahāiki	X Within	120.180		
Cumulative Fitness	Kahanahāiki	X Between	337.875		
Cumulative Fitness	Pahole	Selfed	258.008		
Cumulative Fitness	Pahole	X Within	334.514		
Cumulative Fitness	Pahole	X Between	384.461		
Cumulative Fitness	Species Total	Selfed	432.441		
Cumulative Fitness	Species Total	X Within	258.898		

Cumulative Fitness	Species Total	X Between	428.050		
			%	**N Alive**	**N Total**
Survival	Kahanahāiki	Selfed	0.40	10	25
Survival	Kahanahāiki	X Within	0.52	13	25
Survival	Kahanahāiki	X Between	0.36	9	25
Survival	Pahole	Selfed	0.76	19	25
Survival	Pahole	X Within	0.72	18	25
Survival	Pahole	X Between	0.60	15	25

Results

Pre-planting: Maternal source population did not affect the percent of flowers that formed fruit; there were no differences between the two populations at this stage (Table 2, Fig. 3; Mann-Whitney U = 352.0, z = 0.193, $p = 0.847$). Cross treatment did affect the percent of pollinations that formed fruit (Kruskal-Wallis: $\chi^2 = 25.94$, df = 4, $p < 0.001$). The control and emasculated treatments had fewer fruit per flower than all the hand-pollination treatments (Fig. 2.3; Control: Mann-Whitney U = (Self/Crossed Within/Crossed Between) 21.5/30.0/13.0, z = 2.63/2.285/3.279, $p = 0.009/0.027/0.001$; Emasculated: Mann-Whitney U = 12.0/13.0/6.0, z = 3.344/3.395/3.822, $p = 0.002/0.001/0.0001$).

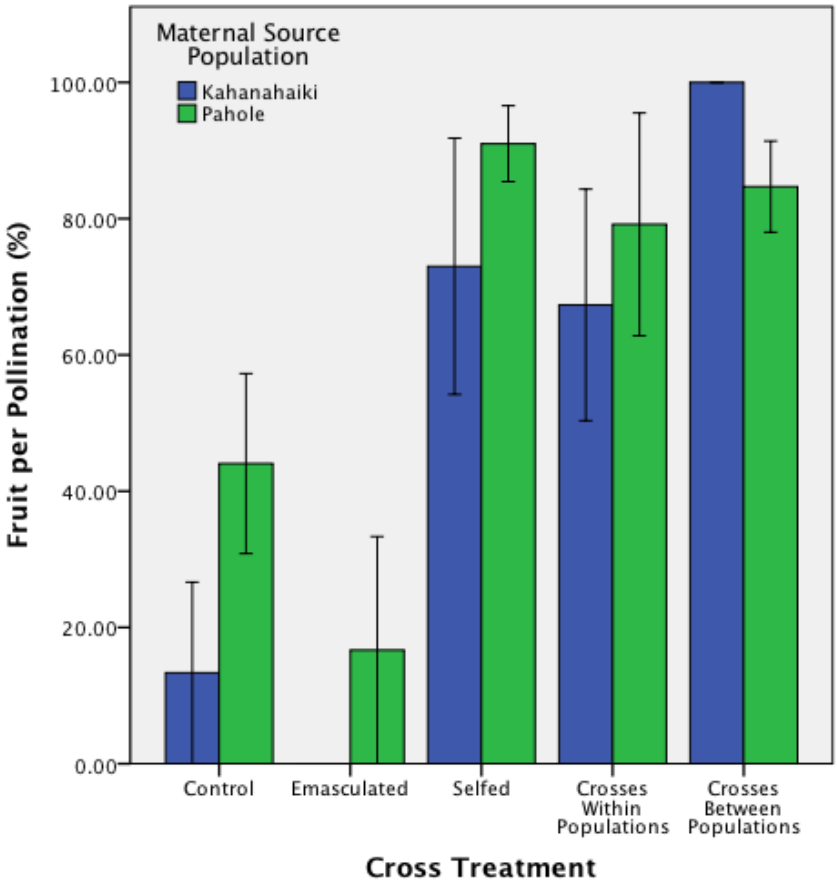

Fig. 2.3. Percent fruit per flower in nursery hand-pollinations. Error bars ± 1 SE.

Neither maternal source population nor the interaction of maternal source population and cross treatment affected the number of seeds per pollination (F = 0.196, df = 1, p = 0.661; F = 0.871, df = 3, p = 0.466, respectively). Seed production per pollination was affected by cross treatment. The control had fewer seeds per pollination than the other treatments (Fig. 2.4; F = 10.853, df = 3, p < 0.001, Helmert Contrast estimate = -0.977 ± 0.192, p < 0.001). When the control was removed from the analysis, the cross treatment did not affect the number of seeds produced per pollination (F = 2.871, df = 2, p = 0.075). No seeds were produced in the emasculation treatment.

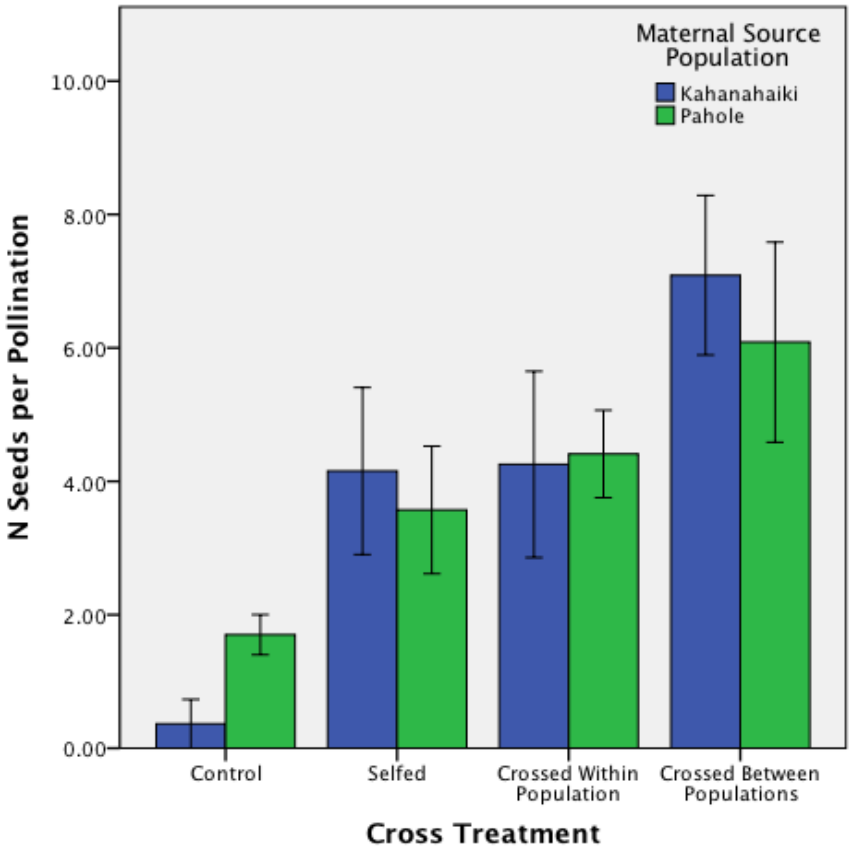

Fig. 2.4. Mean number of seeds per pollination in nursery hand-pollinations. Error bars ± 1 SE.

Neither maternal source population, cross treatment nor their interaction affected seed mass (Fig. 2.5; $F = 1.916$, df = 1, $p = 0.178$; $F = 1.455$, df = 3, $p = 0.249$; $F = 0.411$, df = 3, $p = 0.747$, respectively). The control treatment for Kahanahāiki was eliminated from analyses due to low sample size (n = 1), and the mean of this one sample was comparable to other means (Table 2.2). Maternal source population and cross treatment did not affect seed viability (Fig. 2.6; Mann-Whitney U = 144.0, z = 1.260, $p = 0.208$; Kruskal-Wallis, $\chi^2 = 0.195$, df = 2, $p = 0.912$, respectively). Seeds from all populations and cross treatments had similar mass and viability.

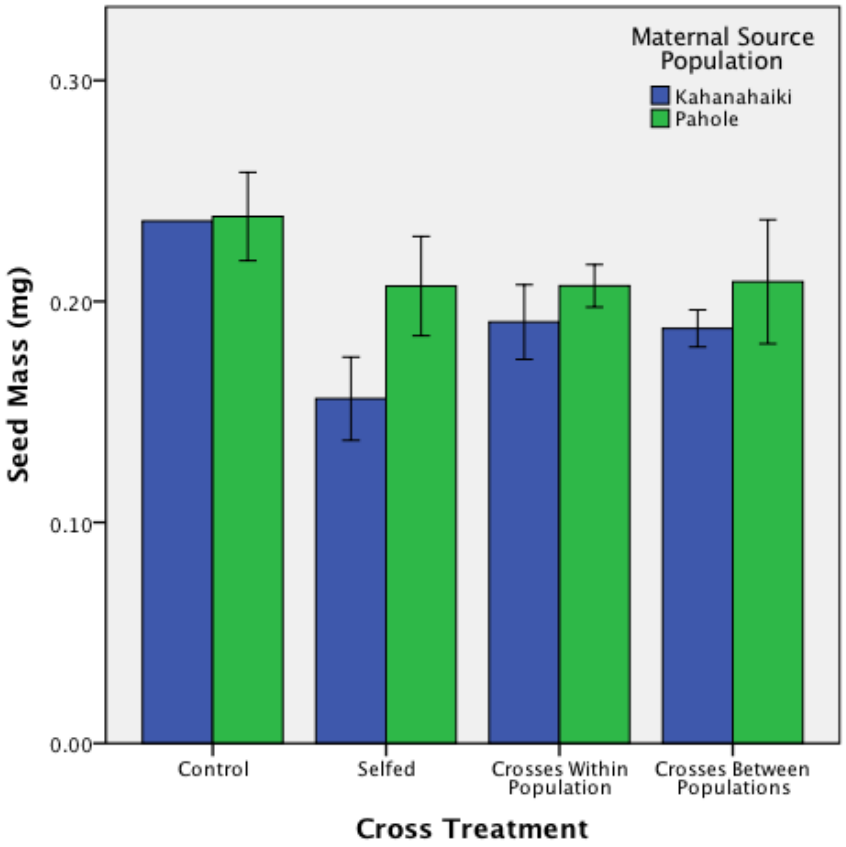

Fig. 2.5. Mean mass per seed in nursery hand-pollinations. Error bars ± 1 SE.

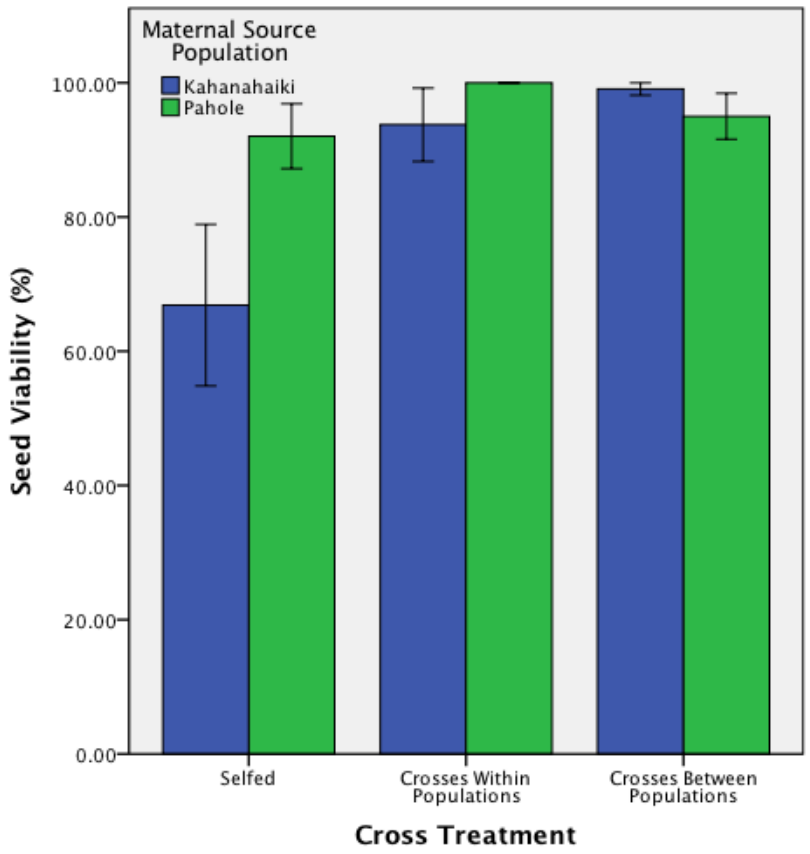

Fig. 2.6. Percent seed viability of nursery hand-pollinations. Error bars ± 1 SE.

Post-planting: Maternal source population affected the total growth of plants, as plants from the Pahole population grew larger than plants from the Kahanahāiki population (Fig. 2.7; $F = 8.673$, df = 1, $p = 0.004$). Neither cross treatment nor its interaction with maternal source population affected plant growth ($F = 1.668$, df = 2, $p = 0.195$; $F = 2.887$, df = 2, $p = 0.062$, respectively). When means were compared as population x cross treatments interactions, the selfed progeny from Pahole grew over twice as large as the selfed progeny from Kahanahāiki (GLM1, $F = 2.738$, df = 5, $p = 0.025$; Tukey HSD mean diff. = 4.591 ± 1.456, $p = 0.027$).

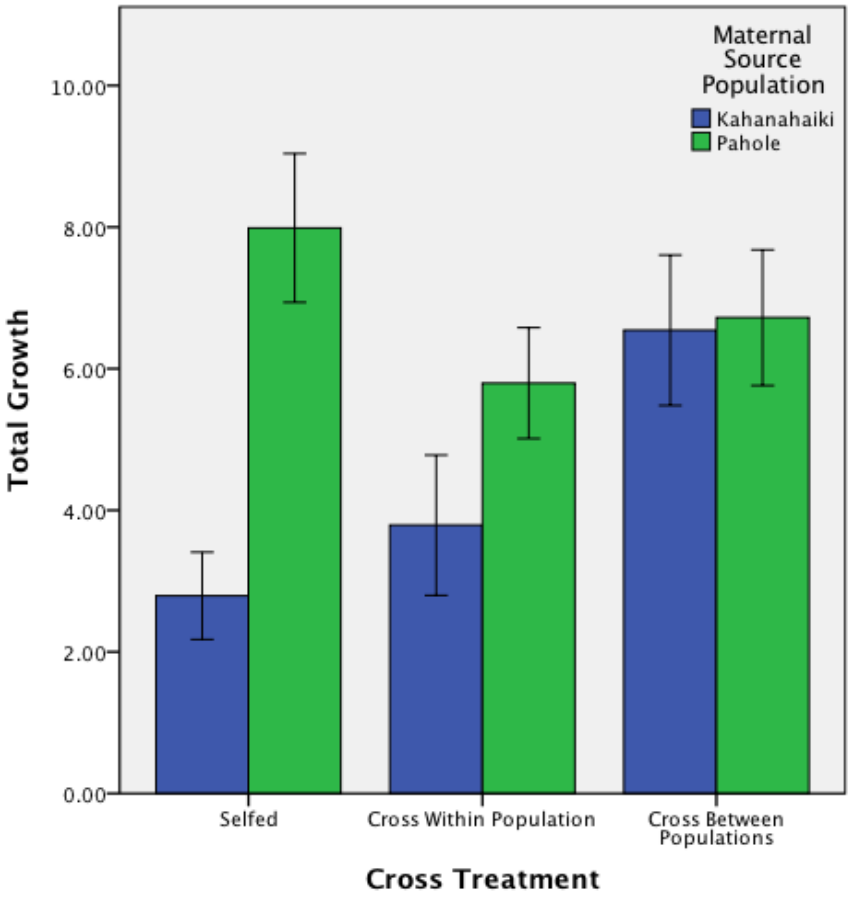

Fig. 2.7. Mean total growth of plants. Total growth was calculated as (final – initial size)/initial size. Size of plant = height (cm) of plant x braches (n). Error bars = ± 1 SE.

Fifty-six percent of the plants survived the first year (Table 2.2). The final model produced by the loglinear analysis of source population, cross treatment, and survival was a good fit to the data ($\chi 2 = 3.023$, df = 8, $p = 0.933$ for the likelihood ratio statistic of the final model; the expected values generated by the model were not significantly different from the observed data). The test of the value of the two-way interactions was significant, and removing the two-way interactions would have been detrimental to the model (K-way Effects, likelihood ratio, $\chi 2 = 13.240$, df = 5, $p = 0.021$). The two-way interaction between population and survival was significant (Fig. 2.8; partial $\chi 2 = 11.132$, df = 1, $p = 0.001$). The odds ratio indicated that plants from the Pahole population source had three times the probability of surviving than plants from the Kahanahāiki source population. The two-way interaction between cross treatment and survival was not significant (partial

χ^2 = 2.275, df = 2, p = 0.321). A three-way interaction between source population, cross treatment, and survival was also not significant (K-way Effects, likelihood ratio, χ^2 = 0.747, df = s, p = 0.688). Height at 1 month did not predict mortality (linear regression, Exp (B) = 0.962 ± 0.028, Wald Statistic = 1.951, df = 1, p = 0.163).

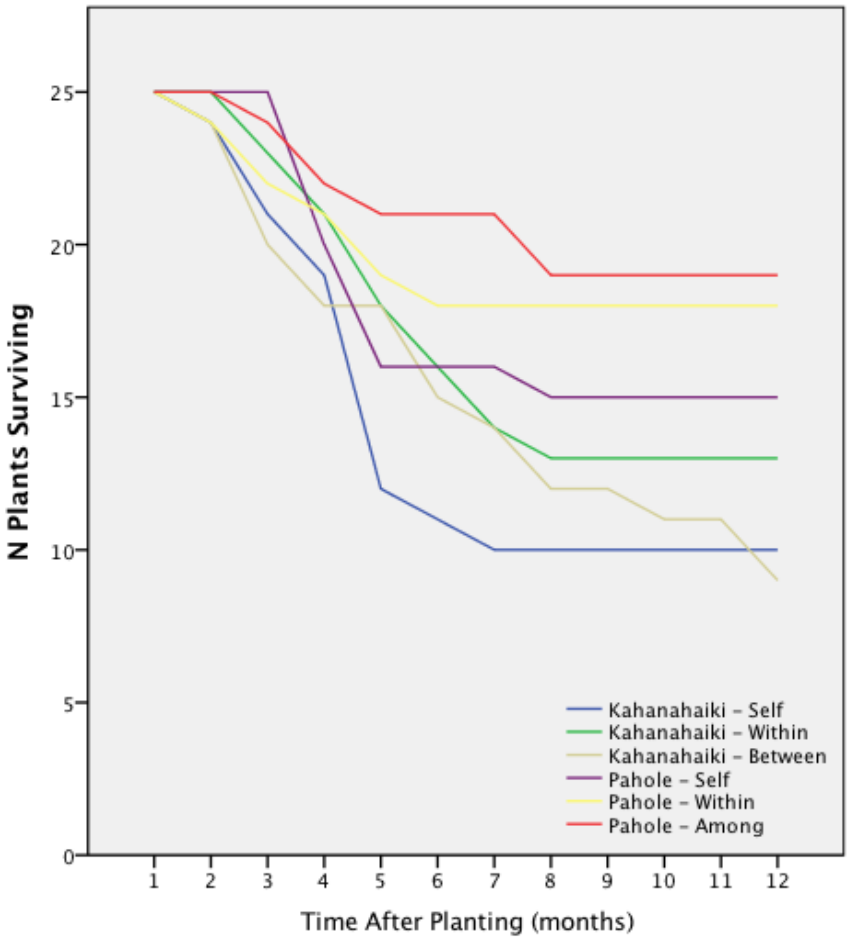

Fig. 2.8. Population decline over time at outplanting.

Fifty-four percent of the plants matured (60% Kahanahāiki-source; 55% Pahole-source; 56% of both selfed and crossed-within-a-population treatments; 60% of crossed-between-populations treatment). Neither maternal source population nor cross treatment affected the chance that a plant would become mature (χ^2pop = 0.436, df = 1, p = 0.509; χ^2cross = 0.218, df = 0, p = 0.897). There was also no effect of maternal source

population or cross treatment on the number of inflorescences a plant produced over the first year after planting (Fig. 2.9; Mann-Whitney U = 2690, z = 0.481, $p = 0.630$; Kruskal-Wallis; $\chi^2 = 0.110$, df = 2, $p = 0.947$). Plants from both populations and in all treatments produced approximately two inflorescences during this time.

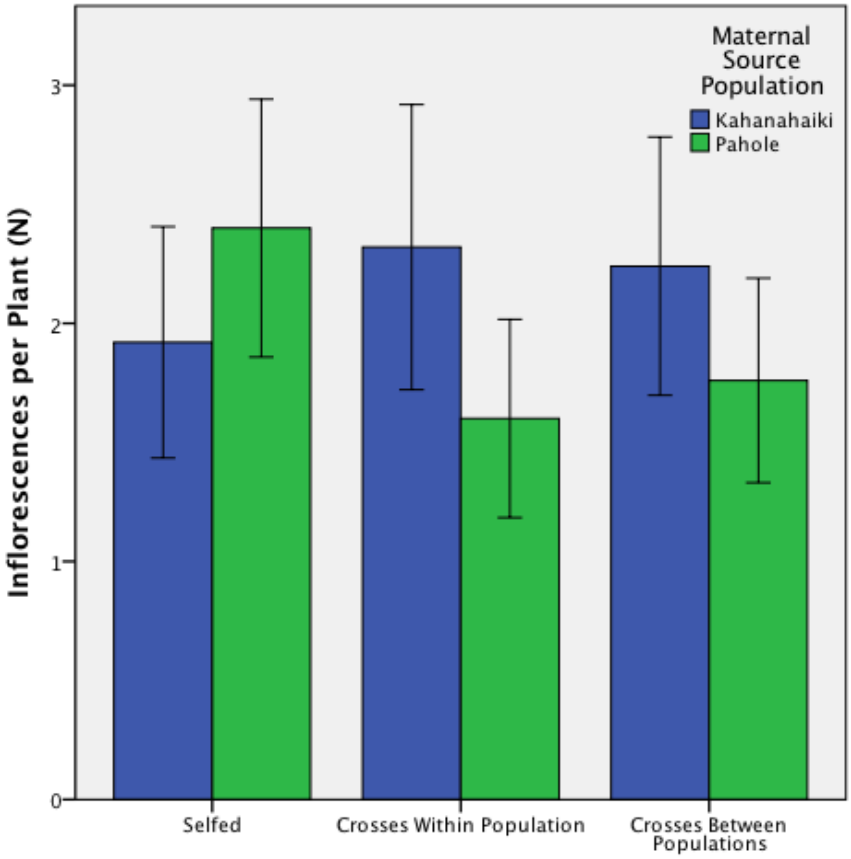

Fig. 2.9. Mean number of inflorescences per plant by cross treatment and maternal source population. Error bars ± 1 SE.

Neither cross treatment nor the interaction between maternal source population and cross treatment affected the number of flowers per inflorescence (Fig. 2.10; $F = 0.220$, df = 2, $p = 0.804$; $F = 0.018$, df = 2, $p = 0.982$, respectively). Plants from the Pahole population produced more flowers (approximately twice as many) per inflorescence than plants from the Kahanahāiki population ($F = 5.571$, df = 1, $p = 0.025$).

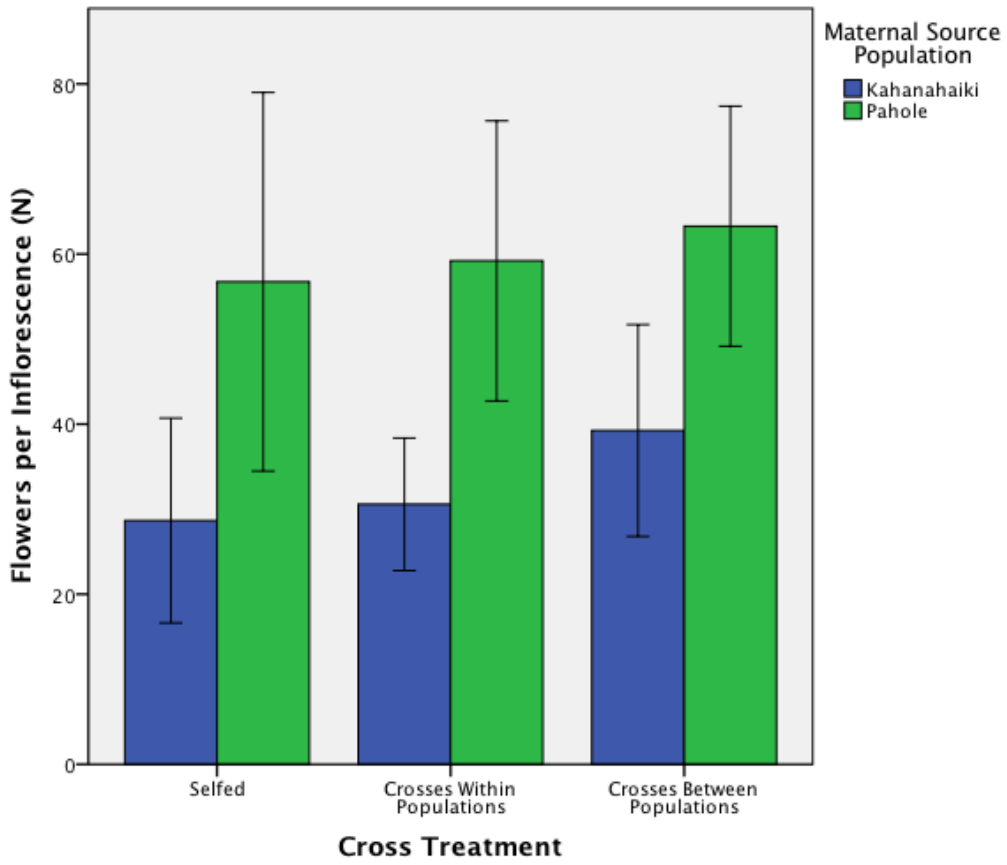

Fig. 2.10. Mean number of flowers per inflorescence by cross treatment and maternal source population. Error bars ± 1SE.

Neither maternal source population nor cross treatment affected the number of flowers per plant (Mann-Whitney U = 2520, z = 1.145, $p = 0.252$; Kruskal-Wallis, $\chi^2 = 0.587$, df = 2, $p = 0.746$, respectively). All population x cross treatment combinations produced a similar amount of flowers (Kruskal-Wallis, $\chi^2 = 3.055$, df = 5, $p = 0.692$), despite the fact that the number of flowers produced by progeny of the Pahole selfed treatment was over twice as much as the number of flowers produced by Kahanahāiki plants from the selfed treatment (Table 2.2; Fig. 2.11). When immature plants are excluded, plants from Pahole produced more flowers than plants from Kahanahāiki (GLM3, $F = 15.576$, df =1, $p < 0.001$), but cross treatment did not affect this stage ($F = 0.184$, df = 2, $p = 0.832$). When each population x cross combination was compared to

every other combination (with the immature plants removed from analyses), the selfed progeny from Kahanahāiki did produce fewer flowers per plant than the selfed progeny from Pahole (GLM1, $F = 3.916$, df = 5, $p = 0.003$; Tukey HSD mean diff. = 144.92 ± 39.88, $p = 0.006$).

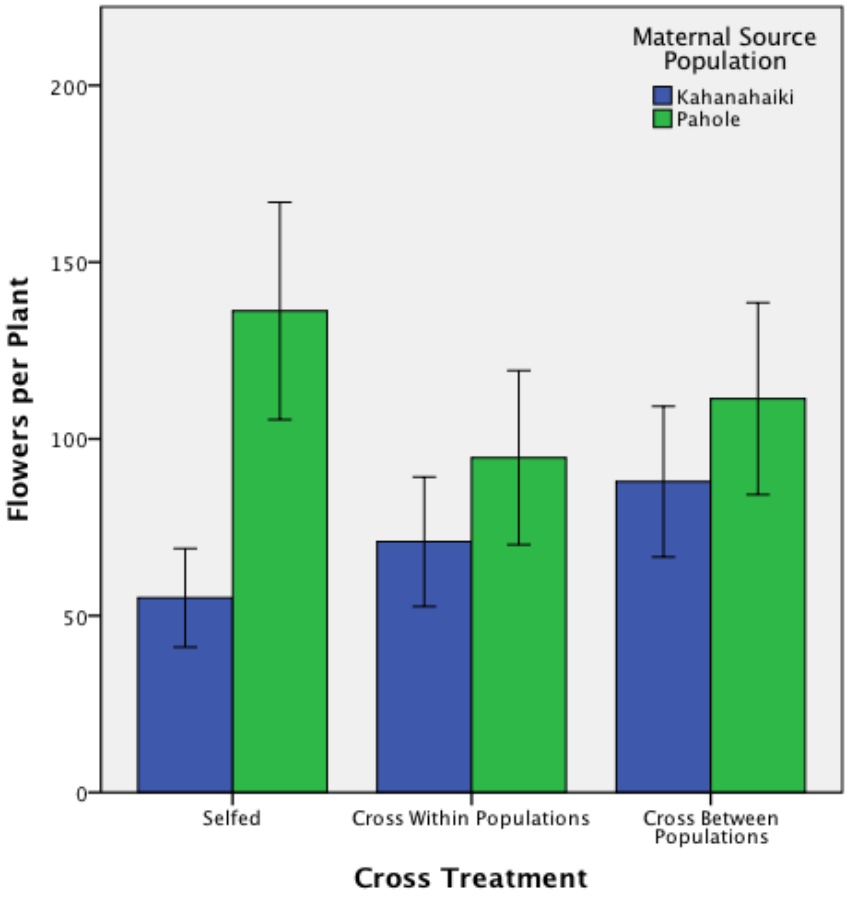

Fig. 2.11. Mean estimated number of flowers produced per plant by cross treatment and maternal source populations. Error bars ± 1SE.

Neither maternal source population, cross treatment, nor their interaction affected the number of fruit per inflorescence (Fig. 2.12; $F = 2.009$, df = 1, $p = 0.166$; $F = 2.033$, df = 2, $p = 0.147$; $F = 1.335$, df = 2, $p = 0.277$, respectively), despite the fact that there was over twice as many fruit per inflorescence produced by selfed progeny from Pahole when compared to selfed progeny from Kahanahāiki. Neither maternal source population,

cross treatment, nor their interaction affected the number of seeds per fruit (Fig. 2.13; F = 0.093, df = 1, p = 0.762; F = 0.259, df = 1, p = 0.773; F = 0.636, df = 2, p = 0.534, respectively).

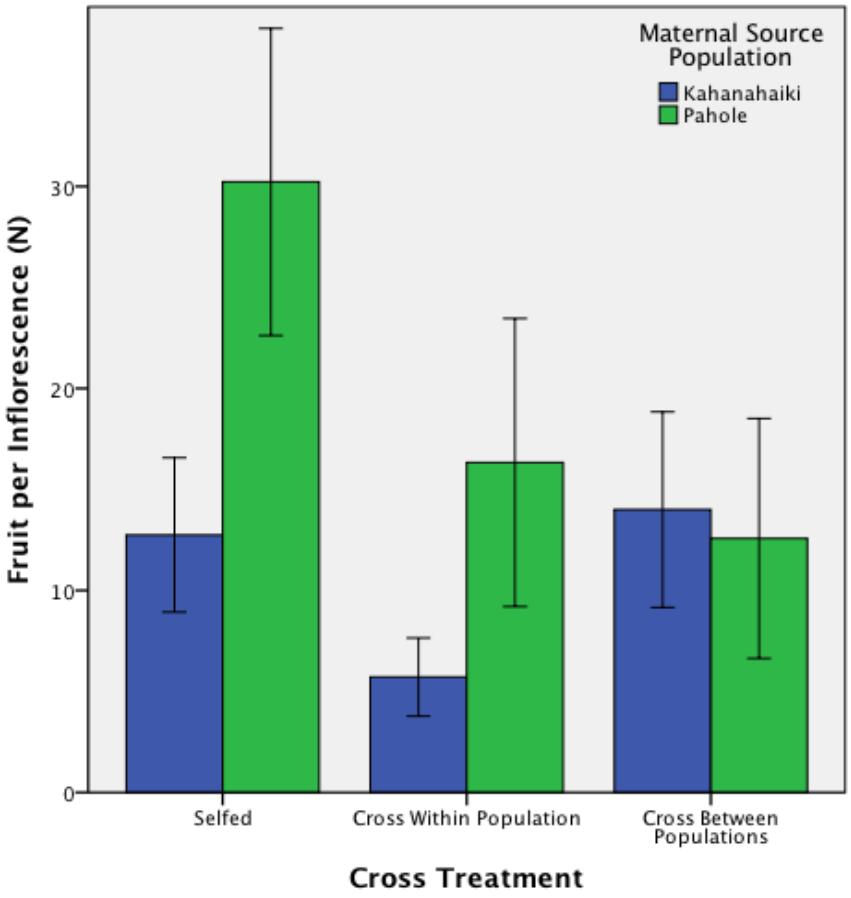

Fig. 2.12. Mean number of fruit produced per inflorescence by cross treatment and maternal source population. Error bars ± 1SE.

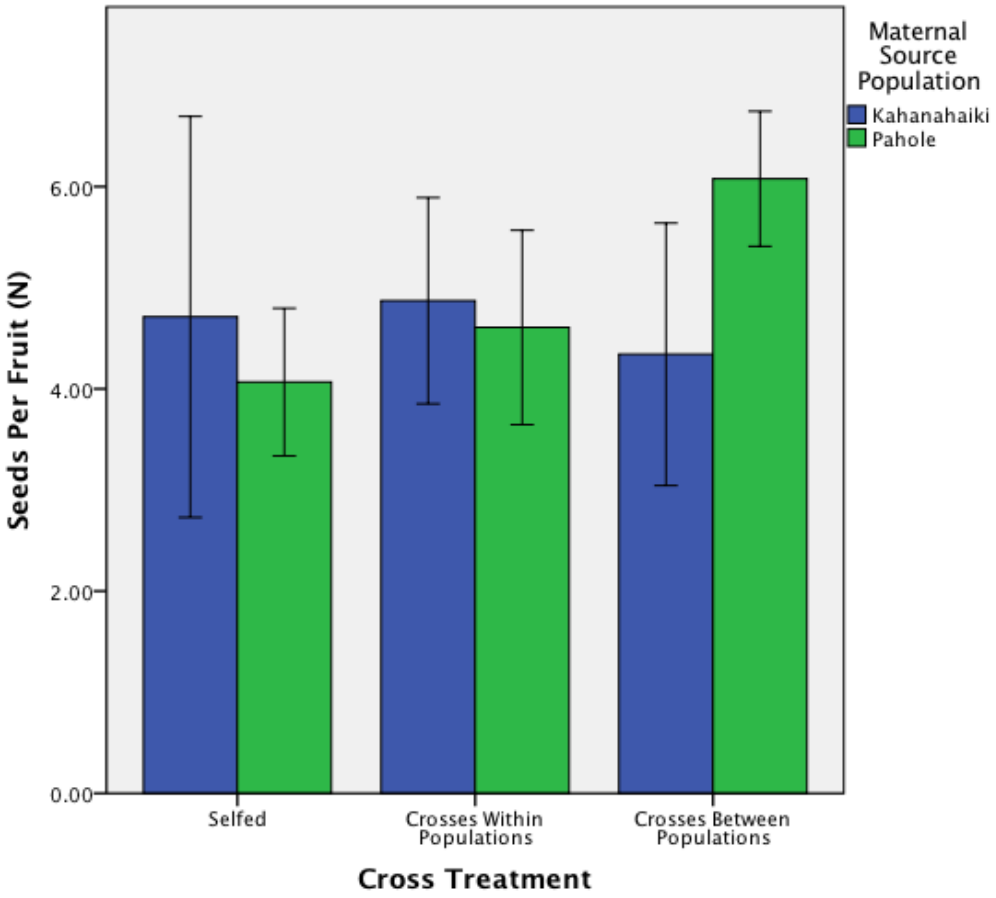

Fig. 2.13. Mean number of seeds per fruit of outplants by cross treatment and maternal source population. Error bars ± 1 SE.

Neither maternal source population nor cross treatment affected the estimated number of seeds produced per plant (Fig. 2.14; Mann-Whitney U = 2512, z = 1.176, p = 0.239; Kruskal-Wallis, χ^2 = 2.650, df = 2, p = 0.266). The estimated number of seeds produced per plant was re-analyzed after excluding the immature plants that never produced inflorescences (Fig. 2.15). When immature plants were excluded, maternal source population and cross treatment affected the estimated number of seeds produced (F = 22.873, df = 1, p < 0.001; F = 5.810, df = 2, p = 0.004, respectively). The interaction of these factors did not affect the estimated number of seeds per plant when immature plants were excluded (F = 1.777, df = 2, p = 0.176). Plants from the Pahole maternal source produced more seeds per plant than the plants from the Kahanahāiki maternal source. Plants from the selfed treatment produced more seeds per plant than plants from

the crosses within and between populations (Helmert Contrast Estimate = 0.209 ± 0.077, $p = 0.008$). Additionally, the selfed plants from the Pahole population produced more seeds than the selfed plants from the Kahanahāiki population ($t = 3.900$, df = 26, $p = 0.001$), as well as from the Kahanahāiki plants from the crosses within population and crosses between populations ($t = 6.189$, df = 26, $p < 0.001$; $t = 3.571$, df = 27, $p = 0.001$, respectively). Lastly, the selfed plants from the Pahole population produced more seeds per plant than the Pahole plants from the crosses within the population ($t = 3.088$, df = 24, $p = 0.005$). Pahole plants from crosses between populations were the only plants that did not produce significantly fewer seeds per plant than selfed plants from Pahole ($t = 2.406$, df = 27, $p = 0.115$).

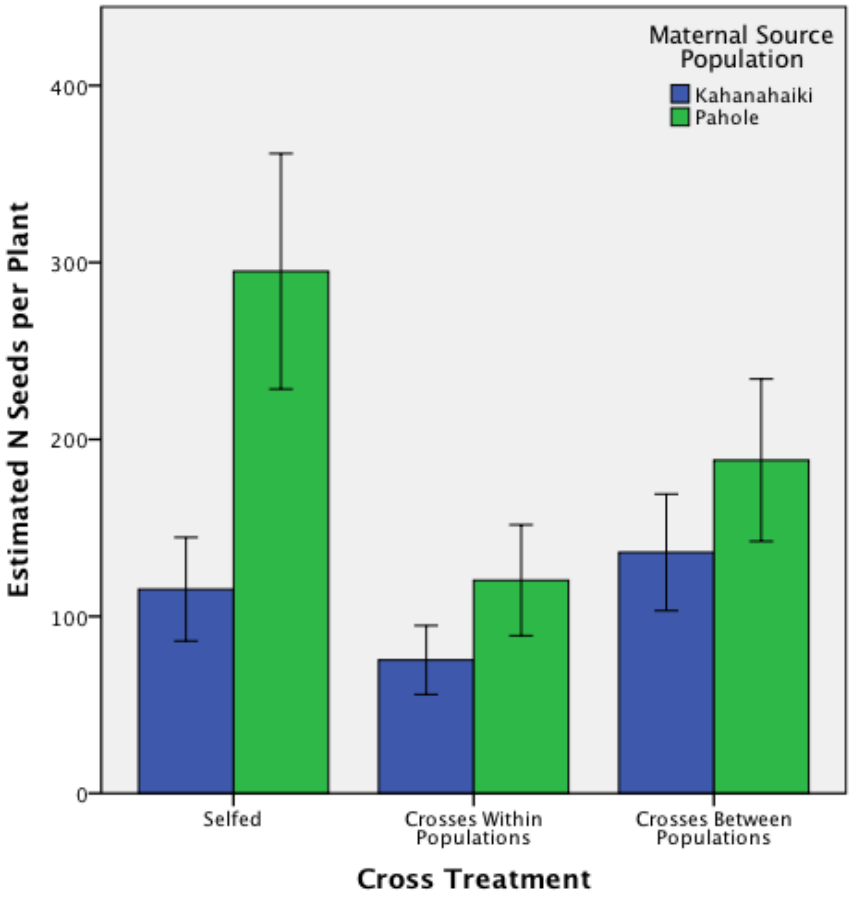

Fig. 2.14. Mean estimated number of seeds per plant by cross treatment and maternal source population. Error bars ± 1 SE. This value was calculated as the product of inflorescences/plant and mean fruit per inflorescence and mean seeds per fruit.

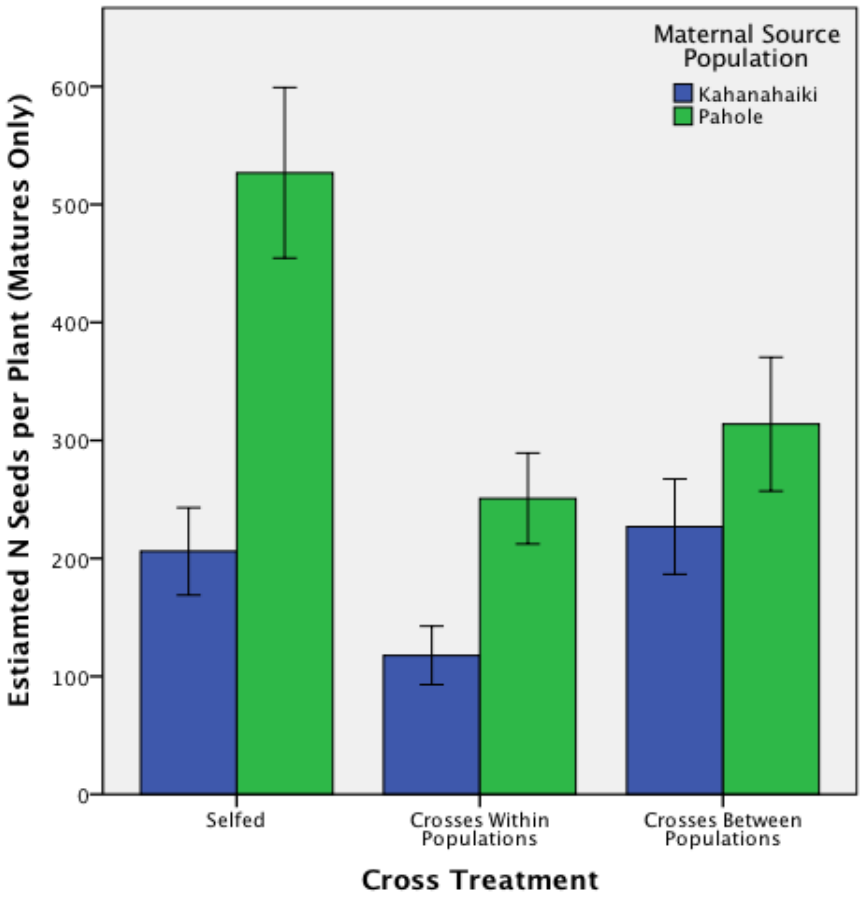

Fig. 2.15. Mean estimated number of seeds per mature plant by cross treatment and maternal source population. Error bars ± 1 SE. This value was calculated from mature plants only. This value was calculated as the product of inflorescences/plant and mean fruit per inflorescence and mean seeds per fruit.

Cumulative Fitness: Cumulative fitness for the selfed progeny and progeny from interpopulation crosses were almost identical, and progeny from intrapopulation crosses had lower fitness (Table 2.3). There were no significant differences between the cumulative fitness of progeny from Pahole (325.66 ± 36.77) compared to those from Kahanahāiki (216.50 ± 64.08; $t = 1.477$, df = 4, $p = 0.214$). Despite the inability to analyze the *RP* statistics, some values could be interpreted. Most values were less than +0.5 or greater than -0.5, and consequently were close to zero and therefore not indicators

of inbreeding depression, outbreeding depression, or heterosis. This was true for number of seeds per pollination (nursery), number of inflorescences per plant, number of seeds per fruit, and survival. The RP_h (intrapopulation crosses/ interpopulation crosses) value for number of fruit per inflorescence in the Kahanahāiki progeny was high (0.7353), suggesting heterosis; but heterosis was not significant in the analysis of means (GLM3). *RP* values for cumulative fitness for the species were consistent with the majority of *RP* values; there was no strong indication of inbreeding depression, outbreeding depression, or heterosis (Table 2.3).

Table 2.3. Relative performance of crosstypes for progeny: RP_i (selfed/crosses within populations); RP_b (selfed/crosses between populations); RP_h (crosses within populations/crosses between populations). Positive RP_i indicates inbreeding depression, negative RP_i indicates outbreeding depression, positive RP_h indicates heterosis, and negative RP_h indicates outbreeding depression.

Variable	**Maternal Population**	RP_i **(s/w)**	RP_b **(s/b)**	RP_h **(w/b)**
Seeds per Pollination (GH)	Kahanahāiki	-0.225	0.419	0.550
Seeds per Pollination (GH)	Pahole	0.355	0.509	0.239
N Inflorescences	Kahanahāiki	0.433	0.240	0.341
N Inflorescences	Pahole	0.067	0.103	0.039
Fruit per Inflorescence	Kahanahāiki	-0.667	0.206	0.735
Fruit per Inflorescence	Pahole	-0.351	-0.515	-0.253
Seeds per Fruit	Kahanahāiki	0.075	-0.237	-0.237
Seeds per Fruit	Pahole	-0.021	0.261	0.277
Survival	Kahanahāiki	0.216	-0.249	-0.411
Survival	Pahole	0.186	0.000	-0.186
Cumulative Fitness	**Kahanahāiki**	-0.593	0.644	0.433
Cumulative Fitness	**Pahole**	0.229	0.329	0.130

Discussion

As expected, control flowers of plants in pollinator-free enclosures produced fewer seeds than hand-pollinated flowers, indicating the ineffectiveness of flowers to self-fertilize in the absence of pollinators. Additonally, emasculated flowers did not produce seeds, demonstrating an inability to self-fertilize in the absence of pollen. Plants with their maternal source from Pahole were relatively more fit than plants from Kahanahāiki. Inbreeding depression was not detected at any life stage. Heterosis and outbreeding depression were not detected at early stages (fruit and seeds per pollination, seed mass, and seed viability) or most later life stages (growth, survival, maturity, number of fruit per inflorescence, number of flowers per inflorescence and plant, and seeds per fruit). Heterosis may have been present in the number of fruit per inflorescence in the Kahanahāiki population, but could not be determined due to the lack of sample size in the relative performance (*RP*) calculations and its nonsignificance in the analysis of variance. Outbreeding depression was present in the estimated number of seeds produced per plant (when immatures were excluded from analyses) within the Pahole population. Cumulative fitness estimates do not support inbreeding depression, outbreeding depression, or heterosis for this species. Management recommendations were based on these results.

Plants from the Pahole population were more vigorous and more fecund than those from Kahanahāiki. Plants from the Pahole population grew larger, had a three times greater chance of suriviving the first year following outplanting, and produced more flowers and seeds per plant. The differences in vigor and fecundity are likely due to genetic differences between the two populations, rather than better adaptation of the Pahole plants to the habitat conditions of the outplanting site. There were no obvious habitat differences (aspect, soil substrate, slope, temperature and elevation) among the three locations (outplantig site, Kahanahāiki population, Pahole population) which could indicate why the plants from Pahole were more vigorous and fecund in the outplanting site. Though the common garden site was geographically between the natural sites, it was in Kahanahāiki Gulch, suggesting that the site could be more advantageous for Kahanahāiki plants; this, however, was not detected.

Female and male fitness estimates with respect to cross treatment provided insight into the occurrences of inbreeding depression, outbreeding depression and heterosis in *S. nuttallii*. When immature plants were excluded from the analyses, the selfed plants produced more seeds per plant than plants from the crosses within and between populations. This trend is almost entirely due to selfed Pahole plants outperforming most other population-cross treatment groupings, including the selfed Kahanahāiki plants. Potential outbreeding depression (including within the population) was detected in the female fitness of the Pahole population. Male fitness showed no inbreeding depression, outbreeding depression or heterosis, but instead reflected the vast difference in selfed progeny between the populations; Pahole selfed progeny had higher male fitness than Kahanahāiki selfed progeny. What was more interesting was that the crosses within and between populations had similar fitness values, highlighting the superiority of the Pahole selfed stock to the Kahanahāiki selfed stock. Outbreeding depression was not detected in Kahanahāiki plants for either male or female fitness estimates. The superiority of the selfed Pahole plants, coupled with slight and/or possible outbreeding depression upon any outcrossing and the lack of inbreeding depression, may be an indication of high selfing rates within the Pahole population for many generations. The Pahole population has consisted of anywhere from 8-17 plants within the last twenty years (Keir and Weisenberger 2011). Plants are currently distributed within an area of approximately 900m^2 (OANRP personal obs. 2011). The Kahanahāiki population, in contrast, has been known to have recently declined from the approximately 50+ plants (21 mature) to one plant just within the last ten years. The population area over the last ten years was smaller than Pahole, approximately 200m^2 (OANRP personal obs. 2011). It is likely that plants from Pahole historically have had higher levels of inbreeding for a longer period of time in comparison to the Kahanahāiki population. A longer time period of higher levels of inbreeding in the Pahole population could have led to the purging of deleterious alleles (Lande and Schemske 1985), as well as the creation of coadapted gene complexes (Shields 1982). Conversely, the size and area of the Kahanahāiki population is more consistent with higher levels of outcrossing between individuals within the population relative to Pahole, though not high enough to detect inbreeding depression or substanital

heterosis upon outcrossing. The recent extreme drop in population size may have been too fast to allow for consequent generations with higher levels of inbreeding.

All species in section *Mononeura* are hermaphroditic, and two species have evolved obligate selfing (Wagner et al. 2005), suggesting that high selfing rates and low inbreeding depression may be present in this section (Wagner et al. 2005). Data for *S. nuttalli*, the first obtained for any species in section *Mononeura*, demonstrate that inbreeding depression could not be detected, and support previous hypotheses for species in this section. It is likely that the absence of detectable inbreeding depression results from selfing rates that may have been high enough to have purged recessive deleterious alleles (Lande and Schemske 1985). *Schiedea nuttallii* is morphologically adapted for biotic pollination (Weller et al. 1998), and is recently known from only small populations. Few floral visitors were observed for this species (Chapter 5), and it is unknown whether this species historically had more floral visitors when native insect species may have been more abundant and may have visited *S. nuttallii* more frequently. If historic rates of visitation by pollinators were consistent with current low rates of visitation, these limited outcrossing conditions may have favored purging of deleterious alleles. This would explain the lack of inbreeding depression despite high levels of inbreeding (Husband and Schemske 1996). In view of the absence of evidence for inbreeding depression, the historical and more recent accelerated decline of populations of *S. nuttallii* is possibly more due to continued threats to recruitment from invasive species (Diong 1982, Loope et al. 1988, Loope 1992, Cuddihy and Stone 1993, USFWS 1999, Joe and Daehler 2008, Athens 2009) than inbreeding depression. In addition to the mitigation of some of these threats, the reintroduction of plants with purged genetic load may actually increase the success of the outplanting (Dudash and Murren 2008).

Outbreeding depression (both within and between populations) could be due to local adaptation or the breakup of coadapted gene complexes (due to genetic drift) (Waser and Price 1983, 1989). Since the selfed progeny from Pahole outperformed the intrapopulation-crossed progeny at the outplanting site, local adaptation to the parental habitat is not likely a reason for outbreeding depression, unless the habitat at the outplanting site was identical to one area within the Pahole population, but not the other

area. Consequently, these results may have been more consistent with the breakup of coadapted gene complexes of the parents at the Pahole population as the cause of this outbreeding depression. High selfing and limited gene flow throughout the large area of the Pahole population could have led to a break up of coadapted gene complexes upon crossing. It may be possible that heterosis from between-population crosses could dilute or eliminate any outbreeding depression that may have occurred from crossing the populations, which may explain why it is only detected in intrapopulation crosses for Pahole (Lynch 1991, Fenster and Galloway 2000a, Charlesworth and Willis 2009). If there is genetic divergence between the populations, crossing the populations could provide an influx of novel alleles to aid in masking any fixed deleterious alleles more than what would result from crosses of plants within the same population. On the other hand, this slight indication of outbreeding depression may simply not amount to anything relevant, as overall there were no significant trends of heterosis or outbreeding depression. These conclusions can be applied to the species biology and distribution to guide management recommendations.

Management Recommendations and Further Research: Management suggestions have been developed for reintroductions efforts for this species. Edmands (2002, 2007) suggested that populations should not be mixed unless inbreeding depression had been detected. Frankham et al. (2011) took a much less conservative approach and developed a key for determining the likelihood of outbreeding depression. If the chance of outbreeding depression was low, gene flow should be re-established between populations. Following the key (and inferring that gene flow between the populations occurred within the last 500 years because several historic populations of *S. nuttallii* were known throughout the geographic distance between them), gene flow should be re-established between these two populations. The benefit from heterosis should also influence management suggestions. Heterotic individuals may be the most successful in new populations (Lande and Schemske 1985), and consequently contribute to the success of the reintroduction, especially when there is genetic representation from a very low number of wild plants and several unmititgated threats to the survival of the outplants. In

this study, the effects of heterosis and outbreeding depression were likely absent, but could have possibly been opposing. The presence of outbreeding depression and heterosis could be determined by continuing the cross treatments in the next filial generations. If outbreeding depression was present, the reduction of fitness could increase upon further generations of outcrossing (Lynch 1991, Fenster and Galloway 2000a, b, Quilichini et al. 2001). These additional crosses should be carried out at the common garden (outplanting) site. Current recommendations are as follows:

1. Neither inbreeding depression nor outbreeding depression could be detected in Kahanahāiki plants, though the cumulative relative fitness suggests that heterosis may be present upon outcrossing. Pahole plants, however, seemed overall relatively more fit at the outplanting site than Kahanahāiki plants. OANRP has not been able to successfully reintroduce Kahanahāiki stock (Keir and Weisenberger 2011). Pahole plants should be incorporated into an existing reintroduction of Kahanahāiki stock, or a new reintroduction mixing the populations could be designed. Additionally, another reintroduction of Kahanahāiki plants only could be initiated in Kahanahāiki to preserve any genetic variation that may be unique to Kahanahāiki. There are 25 plants represented *ex situ* from Kahanahāiki that can be used at this reintroduction. Both reintroductions should be monitored for recruitment and replacement and significant differences between the two plantings could suggest abandonment of the less successful outplanting.
2. Because of their overall lower relative fitness and lack of heterosis upon mixing, plants from Kahanahāiki should not be introduced into a Pahole Gulch reintroduction. Rather, the current population of *S. nuttallii* should be augmented with plants from cultivation that originated from Pahole. An alternative to augmentation would be the creation of a new population in Pahole Gulch with Pahole plants only.

CHAPTER 3: *Schiedea obovata*

Introduction

The angiosperm flora of the Hawaiian Islands has one of the highest rates of endemism (89%) in the world, and over half of the taxa are at risk (Wagner et al. 1999, Sakai et al. 2002). Approximately 10% of the flora is extinct (Wagner et al. 1999), and over thirty percent of the flora is federally listed as threatened or endangered (out of 1094 species; USFWS 2010). The conservation of Hawai‘i’s rare flora is necessary and challenging. Reasons for the decline of native species are both numerous and uncertain, often centered around the introduction of many invasive species, including other plants, invertebrates, and large ungulates, which can also cause vast habitat degradation (Diong 1982, Loope et al. 1988, Loope 1992, Cuddihy and Stone 1993, Joe and Daehler 2008, Athens 2009, Weller et al. 2011). Loss of native habitats, loss of pollinators, and decreases in population size have led to concern that much of Hawai‘i’s flora is experiencing increased rates of selfing, genetic drift, and consequently the increased expression of inbreeding depression (the decrease in fitness that usually occurs among progeny when normally outcrossed species are forced to inbreed; Charlesworth and Charlesworth 1987, Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005). A decrease in the number of individuals and genetic variability in a population can lead to increased homozygosity, which may consequently limit the population’s ability to withstand changes in the environment and stochastic events, and the population may become more prone to extirpation (Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005).

If individuals in small populations are suffering from expression of inbreeding depression, mixing populations at a reintroduction site or augmentation site may increase the amount of genetic variation and heterozygosity within the new population, reduce genetic drift, increase heterosis, and increase the ability of the population to tolerate environmental stochasticity (Vergeer et al. 2005, Guerrant and Kaye 2007, Menges 2008, Frankham 2010). Heterosis can be measured as the increase in fitness of progeny from crosses between populations compared to crosses within populations (Glemin et al. 2003, Charlesworth and Willis 2009). Heterosis is due to an increase in heterozygosity and the

reduction of fixed, deleterious alleles, which is most likely due to increased drift in small populations. Inbreeding depression occurs when a normally outcrossed population is forced to self and there is genetic variation within a population for traits related to fitness, while heterosis may occur if there is genetic variation for fitness-related traits between populations (Keller and Waller 2002, Charlesworth and Willis 2009). These genetic interactions are not the only factors that need to be considered in recovery efforts of rare species.

Ecological and biological dynamics other than inbreeding depression and heterosis may be important as well in the recovery of rare plants. Factors such as breeding system, pollinator relationships, morphology, habitat differences, genetic diversity and relatedness, and recent and historic levels of gene flow between populations need to be considered in determining the appropriate individual founders to use in reintroduction attempts (Guerrant 1996, Guerrant and Kaye 2007, Menges 2008, Godefroid et al. 2011). Reintroductions, located within historic ranges of the species and protected from detrimental herbivory (*e.g.* ungulate-free and fenced areas in Hawai‘i), may be necessary to re-establish species severely threatened with extinction due to decreased population sizes and expression of inbreeding depression (Menges 2008, Weller et al. 2011). Reintroductions with large numbers of plants may circumvent density-dependent factors that may affect small populations, such as susceptibility to plant invasions or a decrease in attractiveness to pollinators (Groom 1998, Forsyth 2003, Hobbs and Yates 2003). The number of plants to reintroduce will ultimately depend on the effective population size of the rare species at the location chosen for outplanting (Guerrant 1996). Depending on the number and relatedness of remaining wild populations, reintroductions could be established with all individuals from a single population or from using individuals from multiple populations. Whether genotypes from different populations are mixed together in recovery efforts may depend on many of the factors listed here. Whether they are known or not, the balance among the history of isolation, potential adaptation to different habitats, and the potential benefits and risks of outcrossing should be explored prior to reintroduction attempts.

The potential negative consequences to mixing different individuals or small populations of the same species that have been isolated for an uncertain number of generations also need to be considered in reintroduction efforts. Outbreeding depression is the decrease in fitness of offspring due to crossing of individuals either within or between populations, and may occur in hybrid offspring whose parents were located at varying distances within populations, from different, geographically distant populations, or from different habitats regardless of geographic distance (Waser and Price 1989, Lynch 1991, Ellstrand and Elam 1993). Outbreeding depression may occur due to the breakup of co-adapted gene complexes or as a result of local adaptation to environmental conditions specific to the individual source populations (Lynch 1991, Ellstrand and Elam 1993, Waser and Price 1993, Frankham et al. 2002). Heterosis, inbreeding depression and outbreeding depression have been investigated in plant populations, including rare species (Quilichini et al. 2001, Kephart 2004, Erickson and Fenster 2006, Raabová et al. 2009) by comparing the relative fitness of selfed and outcrossed progeny, and identifying levels of inbreeding depression and/or outbreeding depression. Information from these studies can be used to guide the management of reintroductions, particularly with regard to mixing of gene pools. If wild plants from different populations (founders) are represented in an outplanting and outbreeding depression is expressed, the offspring may be less vigorous and the reintroduction could fail. Frankham et al. (2011) identified issues that may raise the likelihood that outbreeding depression is a concern, including if populations: 1) are actually distinct species; 2) have fixed chromosomal differences; 3) have been genetically isolated for at least 500 years; and/or 4) are in different environments. The problem facing reintroduction plans in Hawai‘i today is that these factors are mostly unknown among rare taxa.

Predicting the levels of heterosis, inbreeding depression and outbreeding depression when considering genetic sources for a reintroduction of a rare plant species is difficult, primarily due to the lack of information regarding the basic biology of most species (Guerrant 1996). The breeding system and mating system of the species can provide insight into the amount of inbreeding depression expected and the likelihood of outbreeding depression during restoration efforts (Dudash and Murren 2008). If a species is primarily selfing, a long history of inbreeding will have resulted in expression of

recessive deleterious alleles, and purging of strongly deleterious alleles is likely to have already occurred (Lande & Schemske 1985, Charlesworth & Charlesworth 1987, Husband & Schemske 1996). Mildly deleterious alleles, however, may still be present within the population, allowing for the expression of inbreeding depression in later life stages (Lande and Schemske 1985, Husband and Schemske 1996). As a result, inbreeding depression may be cryptic and difficult to assess in species with a long history of selfing (Lande and Schemske 1985, Husband and Schemske 1996, Weller et al. 2005). For predominantly outcrossing species, the chance of expressing inbreeding depression may increase as selfing increases (Lande & Schemske 1985, Charlesworth & Charlesworth 1987, Husband & Schemske 1996). Populations may show less outbreeding depression upon mixing of populations if there has been recent gene flow between the parent populations, and the persistence of outbreeding depression throughout successive generations may decrease (Edmands and Timmerman 2003). If individuals of small populations of a particular taxon (selfers or outcrossers) are suffering from inbreeding depression, the chance of establishing a new self-sustaining population (via reintroduction) may increase with the combination of source material from multiple small populations. The goal of this study was to determine how the balance of these genetic issues could influence the design and success of the reintroduction of a rare plant species.

Study Taxon: The endemic Hawaiian genus *Schiedea* (Caryophyllaceae) was chosen for this study due to its rarity as well as its diversity in habitat, breeding systems, and mating patterns. *Schiedea* is one of the largest adaptive radiations of vascular plants in the Hawaiian Islands, comprised of 34 species that evolved from a single hermaphroditic ancestor (Wagner et al. 1999, Willyard et al. 2011). Research on *Schiedea* has focused on the phylogeny, genetic diversity, and evolution of dimorphic breeding systems (Weller and Sakai 1990, Weller et al. 1995, Weller et al. 1996, Weller et al. 1998, Weller et al. 2001, Sakai et al. 2006, Sakai et al. 2008). Six species have shown high levels of inbreeding depression, with either low levels of selfing in hermaphroditic species, or high levels of selfing in hermaphrodites of dimorphic species (Sakai et al. 1989, Norman et al. 1995, Sakai et al. 1997, Culley et al. 1999, Rankin et al. 2002, Weller and Sakai 2005). A seventh species, *S. viscosum* (section *Nothoschiedea*), showed low levels of inbreeding depression and high levels of selfing (Weller et al. 2005).

No studies have investigated inbreeding depression in the two species of section *Alsinidendron*, though it is sister to section *Nothoschiedea*. *Schiedea obovata* (Sherff) W.L. Wagner & Weller is a short-lived, perennial, woody herb with a hermaphroditic breeding system (Weller et al. 1995, Wagner et al. 2005). Flowers have apparent adaptations for bird-pollination, including fleshy white sepals, slight protandry, and a sweet black nectar with quantities compatible with pollination by passerine birds (Weller et al. 1998). However, native honeycreepers and honeyeaters on O‘ahu have undergone massive population crashes and are extinct, rare, or uncommon (Pratt 1994, Shehata et al. 2001, Fleischer et al. 2008). It is unlikely that native birds are currently maintaining any level of outcrossing in this species (Chapter 5). Solitary plants easily self-fertilize and produce viable seed and vigorous progeny capable of reproduction (Wagner et al. 2005, Keir and Weisenberger 2011). *Schiedea obovata* has a facultatively autogamous breeding system based on floral morphology and observations in cultivation. Pollen:ovule ratios indicate that the breeding systems of this species is somewhere between facultative autogamy and xenogamy. Among the four species of the sections *Alsinidendron* and *Nothoschiedea*, *S. obovata* stands apart by having a pollen:ovule ratio that is over eleven times higher than the other three species, suggesting that it might be better adapted for outcrossing (Cruden 1977, Weller et al. 1998). The breeding and mating systems of *S. obovata* are difficult to assess, especially without any known observations of bird pollination. Current information about the species is somewhat contradictory, and in light of recent population crashes of native honeycreepers and honeyeaters and *S. obovata*, interactions between *S. obovata* and its potential pollinators remain unknown.

Schiedea obovata once occurred along ridges throughout the entire Wai‘anae Mountain Range of O‘ahu (Fig. 3.1). Within the last couple of decades, *S. obovata* has been found only in the northern Wai‘anae Mountains and was federally listed as endangered in 1991 (Ellshoff et al. 1991). In the late 1970s, Keawapilau gulch had approximately 100 individuals, but no plants were observed in this area until recently. During this same time, Pahole gulch had approximately 70 plants, with the last known plant dying in 2003 (Keir and Weisenberger 2011, Lau 2011). Currently, one population (NW Makaleha) of approximately 100 individuals (30 mature) and a few isolated individuals in surrounding areas (Keawapilau) represent the extant, *in situ* distribution for

this species. The populations are on an elevational gradient of over 200m from the lowest, most northern population, Kahanahāiki to the most southern population, W Makaleha. Cuttings and fruit have been collected from some individuals no longer found in the natural populations. All *ex situ* plants for this species (including plants used in this study) are derived from populations in the northern Waiʻanae Mountains.

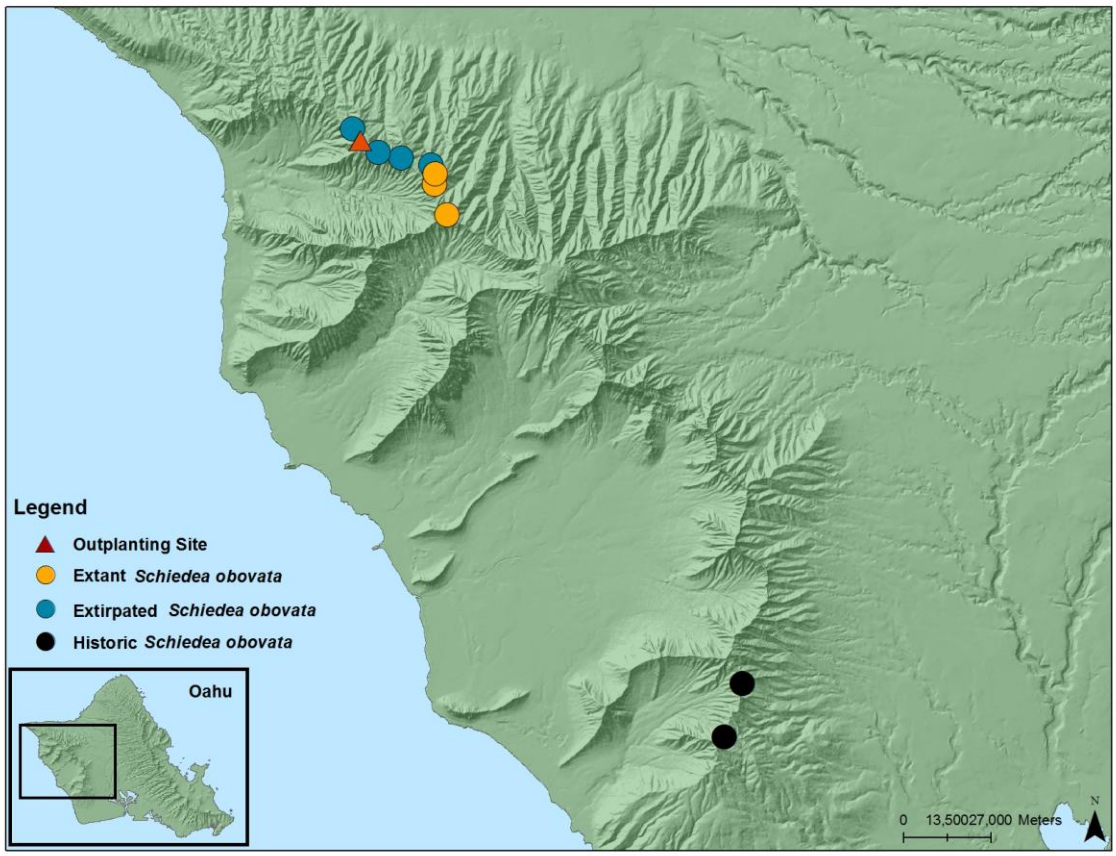

Fig. 3.1. Historic (last seen prior to 1984) and Recent Distribution of *S. obovata*. The outplanting site (red triangle) was chosen as suitable, fenced habitat that was adjacent to similar habitat appropriate for future reintroduction sites planned by land managers (OANRP). Suitable, accessible, and fenced habitat was not available near the southern extant populations.

The goal of this study is to determine what source(s) should be used in reintroduction efforts of *S. obovata*, based on potential population differentiation and the expression of inbreeding depression. In 2003, the Makua Implementation Plan for Makua Military Reservation stated the following: *"Outplanting considerations: Since A.*

obovatum [synonym *S. obovata*] *is a naturally selfing plant (Weller pers. comm. 2000), plants from different stocks should not be mixed together in outplantings."* (MIT 2003). Arguments toward establishing populations with mixed sources stem from concerns that outbreeding depression may occur if gene flow has not occurred among small selfing populations, and plants have become adapted to local conditions. This goal and these considerations have invoked the following questions:

1. Is inbreeding depression and/or heterosis detectable in *S. obovata*?
2. Is outbreeding depression a concern in planning reintroductions?
3. What combination of founders (wild plants either extant or extirpated but with *ex situ* representation) will yield the most relatively fit progeny?
4. Are there differences in fitness of progeny from populations distributed along an elevation gradient grown in a common outplanting site (garden)?

To address these questions, fitness of progeny from crosses within and between populations and the fitness of selfed progeny were assessed in field settings comparable to locations were reintroductions would take place. A field setting was used to test fitness of progeny from crosses to mimic conditions likely to occur during reintroduction efforts, and to estimate levels inbreeding depression under field conditions rather than greenhouse conditions likely to provide unrealistic estimates of the genetic effects of selfing and outcrossing on fitness (Dudash 1990, Husband and Schemske 1996).

Methods

Hand Pollinations: Plants were grown under pollinator-free conditions at Pahole Mid-elevation Nursery (northern Waiʻanae Mts., 656m elev.) in Mokulēʻia Forest Reserve on Oʻahu. Nursery plants used as a seed source for this study were propagated from fruit collections from wild plants (founders). One founder was represented from seed collected from plants maintained at a greenhouse at the University of California at Irvine for several generations (Keawapilau-Kapuna Ridge). Thirteen founders from a total of six populations are represented in this study (Table 3.1). Twenty-five plants (progeny of the

13 founders, including some siblings from the same fruit collection) were available for pollinations in the nursery. Only two of the six populations are extant. Three populations had more than one founder available to implement the crosses within populations. One additional population of three plants (Keawapilau) was recently re-discovered but not available to incorporate in this study.

Table 3.1. Historical and Current Distribution of *S. obovata* and Representation in Study. Average maximum annual temperature (F) from 1971-2000 from PRISM Climate Group (PRISM 2004). Average annual rainfall (mm) data from PRISM Climate Group[1] (PRISM 2004) and Giambelluca et al. (2011)[2]. Elevation data via ArcGIS from hand-plotted or GPS points of population locations. Populations ordered from southeast to northwest.

Population	Elevation (m)	Temp. (˚C)	Rainfall (mm) [1]	Rainfall (mm)[2]	Maximum Known Individuals*	N Extant Plants*	N Founders Represented in Crosses
West Makaleha	792	23	2022	1764.8	6	1	2
NW West Makaleha	762	24	1962	1651.3	40	40	5
Keawapilau **	683	24	1880	1612.2	100+	3	0**
Keawapilau-Kapuna Ridge	655	24	1781	1565.5	1	0	1
Pahole Rim	700	24	1766	1505.5	10	0	1
Pahole	640	24	1667	1425.9	~60	0	3
Outplanting Site	646	24	1561	1350.9	NA	NA	NA
Kahanahāiki	573	25	1561	1334.7	3	0	1

* Only mature founders are listed.
** Propagules were not available to incorporate this population into this study.

Hand-pollinations were conducted from November 2007 through summer 2008. Flowers were emasculated 1-2 days prior to anthesis by removing the calyx and anthers to ensure that self-fertilization did not occur. The calyx was peeled back and removed and anthers were removed with forceps. Stigmas were receptive for pollination five days after emasculation, when stigmas had increased in length and width and the stigmatic papillae were elongated. Only fresh pollen available the day of pollination was used in pollinations. Pollen was applied by gently touching receptive stigmas with dehisced

anthers containing pollen, and the purple-gray pollen easily stood out on white stigmas. Stigmas were examined to ensure that pollen had been deposited along the length of the receptive stigmatic surface. Pollination treatments included flowers that were unmanipulated (control-calyx), calyx removed but not pollinated (control-no calyx), hand-pollinated with pollen from the same plant (selfed), or pollinated with pollen from a different plant. Crosses with pollen from a different plant were either from within a population (within) or between two populations (between). A minimum of 25 pollinations were attempted for every cross treatment, including every combination of populations for between-population crosses (distances (m) between populations are located in Table 3.2). Self-pollinations were attempted on the progeny of at least one founder in each of the populations. Crosses within populations were performed for the West Makaleha, NW Makaleha, and Pahole population sources. All inflorescences that contained pollinated flowers or control flowers were contained in organza drawstring bags (handmade) to eliminate cross-pollination via biotic pollinators. Fruit were collected at maturity when capsules were tan and the calyx, which had turned purple and fleshy, had begun to desiccate. The seeds of each fruit collection were counted and held in storage (20% relative humidity at -18C) for approximately eight months prior to germination.

The number of fruit per pollination (including controls) and seeds per pollination and fruit were determined for each treatment and nursery plant as a total for each plant. Emasculated flowers did not produce any seeds, and were consequently removed from analyses. Mean mass (mg) per seed was also calculated as the mass of the number of seeds produced for each nursery plant by cross treatment over the number of seeds produced. The dry weight of seeds was obtained upon removal from storage and just prior to sowing. Seed viability was calculated as the number of seeds that germinated (root and shoot visible) out of the total sown for a sample of fruits collected on different days per nursery plant. All seeds that did not germinate within four months had rotted prior to dissection. The amount of seeds that germinated, therefore, was equal to the number of seeds that were viable. The control treatments were excluded from the analyses following measurement of seed mass.

Propagation: Seeds were sown in January 2009. Enough seeds germinated in each population to obtain 15 plants per treatment to outplant in a field setting. Seeds were germinated on 1% water agar (Sigma-Aldrich® A1296) in Petri dishes in a Controlled-Environment Chamber (Pervical Scientific, Inc. E36V). After germination, seedlings were planted in a 3:1 mix of perlite: vermiculite and placed on a mist bench for two months prior to transplanting into individual pots (RS400: 4" round, standard pot, Exacta Sales, Inc.). The plants were grown at O'ahu Army Natural Resources Program's (OANRP) East Range nursery (Wahiawa, 247m elevation, 1283mm annual rainfall, 26.1C annual maximum temperature [WorldClimate.com copyright 1996-2011]) until they were outplanted seven months later. Fifteen plants of every cross treatment from every population were randomly selected without respect to founder for outplanting.

Outplanting Site: Plants were outplanted prior to flowering into Kahanahāiki Gulch in Mākua Military Reservation, northern Wai'anae Mts. in October 2009. Plants were randomly packed into tubs of 20 plants each and each tub was placed within the designated planting area. The location was selected by the Rare Plant Program Manager of OANRP (Matthew J. Keir) and was located between the two most northwestern populations (Fig. 3.2). The outplanting site was chosen as suitable, fenced habitat that was adjacent to similar habitat appropriate for future reintroduction sites planned by OANRP. The location was also adjacent to existing single-source reintroductions that have maintained high survival rates and moderate maturation rates in the first and second filial generations (Keir and Weisenberger 2011). Plants were monitored every month for one year. The total number of inflorescences per plant and the date of mortality were recorded for each plant. In addition, three plants each from each treatment and population were randomly selected to count the number of flowers on one inflorescence. Another three plants per population and treatment were randomly chosen the count the total number of fruit produced on one inflorescence. Typically, the first inflorescence on the plant was chosen for counting. If the chosen plant died before counts were made, another unmeasured plant was randomly selected for measurement. In these cases, the inflorescence chosen may not have been the first to flower. At least three fruits per plant were collected from three randomly chosen plants from every treatment per population, including every population x population cross, to estimate the number of seeds per fruit.

Plant height and the total number of leaves were measured every other month for the first six months on every plant, with a final measurement 12 months following planting. Size was measured as a product of final plant height (cm), number of leaves, and number of branches (all plants at the time of initial measurements had only one branch). Total growth from the first month after planting to the 12^{th} month after planting was calculated as (final size – initial size)/ initial size. Height was measured from the base of the stem to the most distal apical meristem.

Several variables were measured to calculate male and female fitness estimates for each plant including the number of inflorescences per plant, number of flowers and fruit per inflorescence, and number of seeds per fruit. Female fitness was estimated as the total number of seeds each plant produced, calculated as the product of the total number of inflorescences per plant, the mean number of fruit per inflorescence and the mean number seeds per fruit. Male fitness was estimated as the total number of flowers each plant produced, calculated as the product of the total number of inflorescences per plant and the mean number of flowers per inflorescence. If a treatment only had two replicates for either mean fruit per inflorescence or seeds per fruit, the mean for that maternal source was used as the third replicate. If less than two replicates were available for either or both variables, the cross was eliminated from the analyses. The only treatment eliminated was the selfed treatment of the W Makaleha maternal source population. Differences among the cross treatments (selfed, crosses within a population, crosses between populations) and populations were investigated.

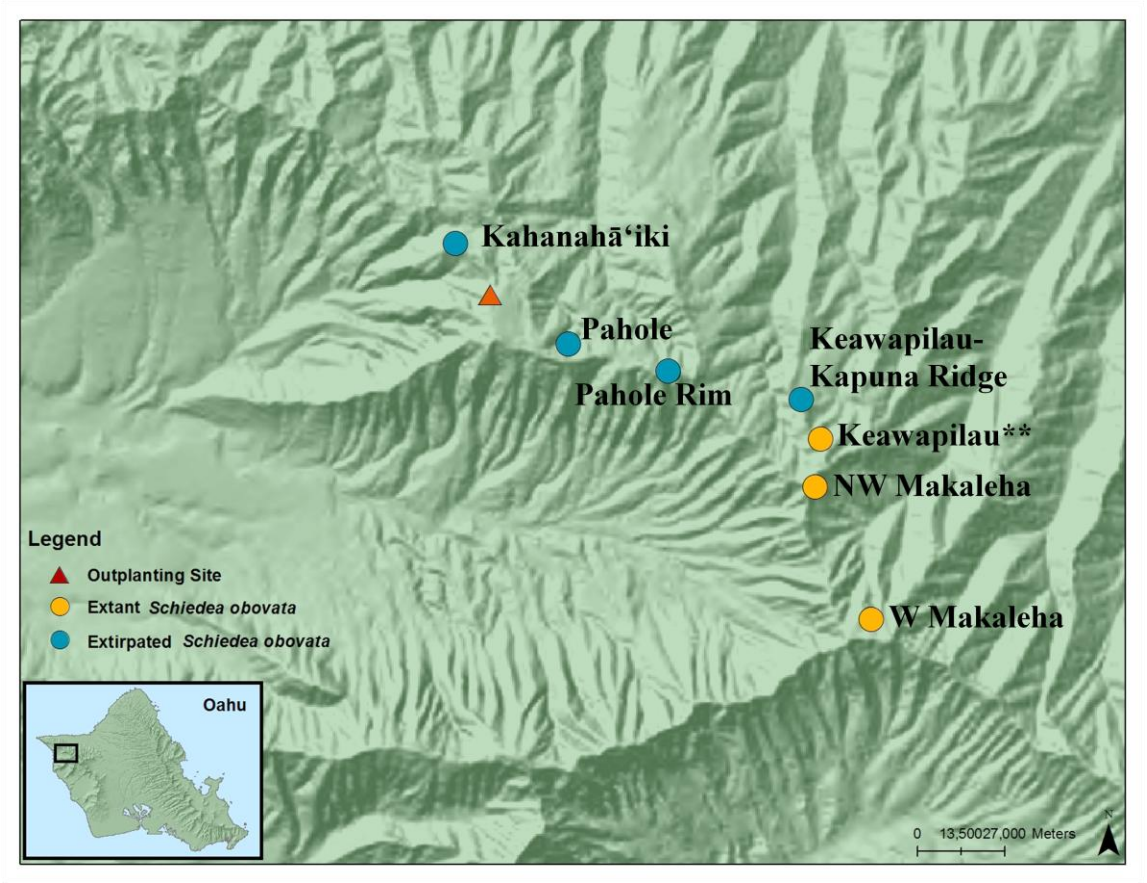

Fig. 3.2. Outplanting site for *S. obovata* and *in situ* populations. **Not represented in this study.

Table 3.2. Linear Distance between Populations from North to South (meters)

Population	Codes	Distance (m)
Kahanahāiki – Outplanting Site	MMR-A – outplanting site	382
Outplanting Site - Pahole	outplanting site – PAH-C	559
Kahanahāiki – Pahole	MMR-A – PAH-C	910
Pahole – Pahole Rim	PAH-C – PAH-A	600
Pahole Rim - Keawapilau	PAH-A – PIL-A	839
Keawapilau – NW Makaleha	PIL-A – LEH-B	530
NW Makaleha – W Makaleha	LEH-B – LEH-A	880

Analyses: Data were analyzed using SPSS 19 (IBM®). Data were transformed (log10 x+1 OR sqrt x+1) for homogeneity of variance and normality when necessary. General Linear Model 3 (GLM3, two-way (factorial) ANOVA) was used for all parametric tests.

Kruskal-Wallis and Mann-Whitey U tests were used to analyze all non-parametric data. A three-way loglinear analysis was used to analyze survival and the percentage of plants that produced at least one inflorescence (matured). The maturity data were also further analyzed with z-scores from cross-tabulation tables to describe specific interactions. The Bonferroni correction was applied to the significance value (alpha) to prevent inflation of the Type I error (.05/number of comparisons). Hochberg's GT2 (Field 2009) was the *post hoc* test most commonly used as sample sizes varied slightly and this test was more conservative than other *post hoc* tests. The Helmert Contrast (Field 2009) was used to determine if means varied between individual cross treatments as a gradual increase in values for traits from selfed to between-population crosses was hypothesized. Cumulative fitness was calculated for each maternal source population as the product of the mean number of seeds per pollination, survival, number of inflorescences per plant, number of fruit per inflorescence, and number of seeds per fruit. Means for each founder (maternal family) were averaged to produce a population mean for the three populations that had more than one founder. Founder means for survival were calculated if more than two progeny per founder were present. All other stages were calculated if a founder was represented by at least one progeny (plant). To quantify levels of heterosis, inbreeding depression and outbreeding depression, Ågren and Schemske's (1993) "relative performance of crosstypes" (*RP*) was calculated for each variable in both populations as well as for cumulative fitness in both populations as follows:

1. Inbreeding / Outbreeding Depression:

   $RP_i = 1 - w_s / w_w$ if $w_s \leq w_w$,

   and

   $RP_i = w_w / w_s - 1$ if $w_s > w_w$,

   where w_s is the fitness of the selfed progeny and w_w is the fitness of the progeny from crosses within populations.

2. Heterosis / Outbreeding Depression:

   $RP_h = 1 - w_w / w_b$ if $w_w \leq w_b$,

   and

   $RP_h = w_b / w_w - 1$ if $w_w > w_b$,

   where w_b is the fitness of the progeny from crosses between populations.

3. Selfed Progeny / Progeny of Crosses Between Populations:

$RP_b = 1 - w_s / w_b$ if $w_s \leq w_{b,}$

and

$RP_b = w_b / w_s - 1$ if $w_s > w_{b,}$

where w_s is the fitness of the selfed progeny and w_b is the fitness of the progeny from crosses between populations. This latter statistic was necessary due to the absence of within-population crosses when there was only one plant at a source population. Inbreeding depression and heterosis cannot be differentiated in this one ratio, but outbreeding depression can be detected, as well as a benefit to outcrossing, as the only options for these populations are to self or outcross with other populations.

RP values range between -1 and +1, where positive values for RP_i indicate that progeny from crosses within populations outperform selfed progeny; inbreeding depression is consequently present. Negative values indicate outbreeding depression within populations. For RP_h, positive values indicate heterosis and progeny from crosses between populations outperform progeny from crosses within populations. A negative RP_h value therefore indicates outbreeding depression between populations. A GLM1 (one-way ANOVA) was used to compare cumulative fitness means among cross treatments. A one-sample *t* test was used to compare RP_i, RP_h, and RP_b values for each selected variable, including cumulative fitness, to determine if they were significantly different from zero. A GLM1 was performed to determine if there were differences among the populations for each *RP* value (combining all variables).

Results

Pre-planting: Cross treatment, including both controls (with and without an intact calyx) did not affect the number of flowers that formed a fruit (Fig. 3.3; Appendix A; Kruskal-Wallis: $\chi^2 = 7.718$, df = 4, $p = 0.102$). The maternal source population affected the percentage of flowers that formed a fruit (Kruskal-Wallis: $\chi^2 = 20.488$, df = 5, $p = 0.001$). Plants with maternal sources from Kahanahāiki produced fewer fruit per flower than plants with maternal sources from NW Makaleha (Mann-Whitney U = 323, z = 3.620, *p*

< 0.001) and Pahole Rim (Mann-Whitney U = 343, z = 3.208, p = 0.001). Paternal source population did not affect the percentage of flowers that formed a fruit (Kruskal-Wallis: χ^2 = 10.636, df = 5, p = 0.059).

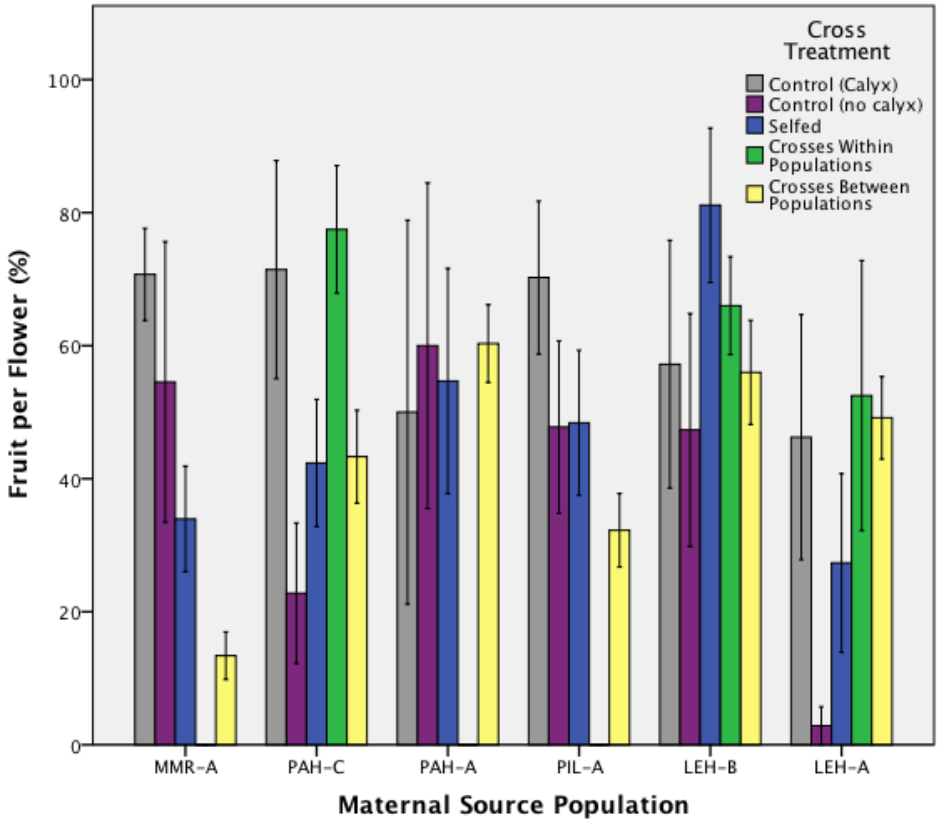

Fig. 3.3. Mean percentage of flowers that formed fruit by maternal source population and cross treatment. Error bars ± 1 SE. Populations displayed left to right from northwest to southeast geographic distribution. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Cross treatment affected the number of seeds produced per pollination (Fig. 3.4; Kruskal-Wallis: χ^2 = 15.065, df = 4, p = 0.005). The control with the calyx intact produced more seeds per pollination than the selfed treatment (Mann-Whitney U = 293, z = 2.770, p = 0.005) and the interpopulation cross treatment (Mann-Whitney U = 1358, z = 3.788, p < 0.001). Both the control without the calyx and the intrapopulation cross

treatment were not significantly different to the control with the calyx with the Bonferroni correction. Maternal source population also affected the number of seeds produced per pollination (Kruskal-Wallis: χ^2 = 19.431, df = 5, p = 0.002). Plants with maternal sources from the Pahole population produced more seeds per pollination than the plants with maternal sources from the Kahanahāiki population (Mann-Whitney U = 364, z = 3.249, p = 0.001). Paternal source population did affect the amount of seeds produced per pollination (Kruskal-Wallis: χ^2 = 11.324, df = 5, p = 0.045). Plants with paternal sources from the Keawapilau populations produced more seeds per pollination than the plants with paternal sources from the Pahole population (Mann-Whitney U = 161, z = 3.389, p = 0.001).

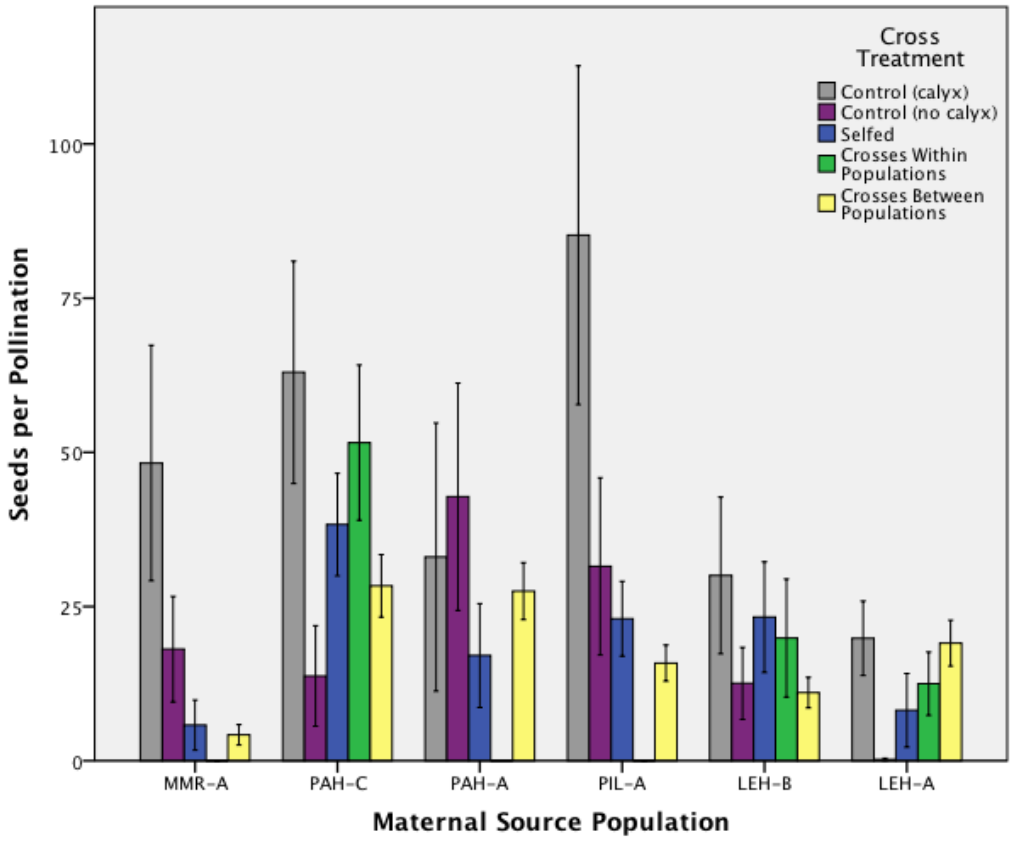

Fig. 3.4. Mean number of seeds per pollination by maternal source populations and cross treatment. Error bars ± 1 SE. Populations displayed left to right from northwest to southeast geographic distribution. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Both maternal source population and cross treatment affected the number of seeds a fruit produced (Fig. 3.5; GLM3, $F = 6.259$, df = 5, $p < 0.001$; $F = 4.053$, df = 4, $p = 0.004$, respectively), but not their interaction (GLM3, $F = 0.675$, df = 4, $p = 0.825$). The control (calyx intact) treatment yielded more seeds per fruit than any treatment where the calyx was removed; hence, removing the calyx, as anticipated, decreased the number of seeds per fruit (Helmert Contrast $F = 4.071$, df = 4, $p = 0.003$; contrast estimate = 0.338 ± 0.09, $p < 0.001$). Plants from the Pahole maternal source population produced more seeds per fruit than plants from W Makaleha, NW Makaleha, and Kahanahāiki maternal source populations (Hochberg's GT2, mean diff. = 0.353 ± 0.09, $p = 0.001$; 0.500 ± 0.08, $p < 0.001$; 0.469 ± 0.10, $p < 0.001$, respectively). Additionally, plants with maternal sources from NW Makaleha also produced fewer seed per fruit than plants with maternal sources from Keawapilau (Hochberg's GT2, mean diff. = 0.379 ± 0.08, $p < 0.001$). The paternal source population did not affect the number of seeds produced per fruit (GLM3, $F = 1.157$, df = 5, $p = 0.336$).

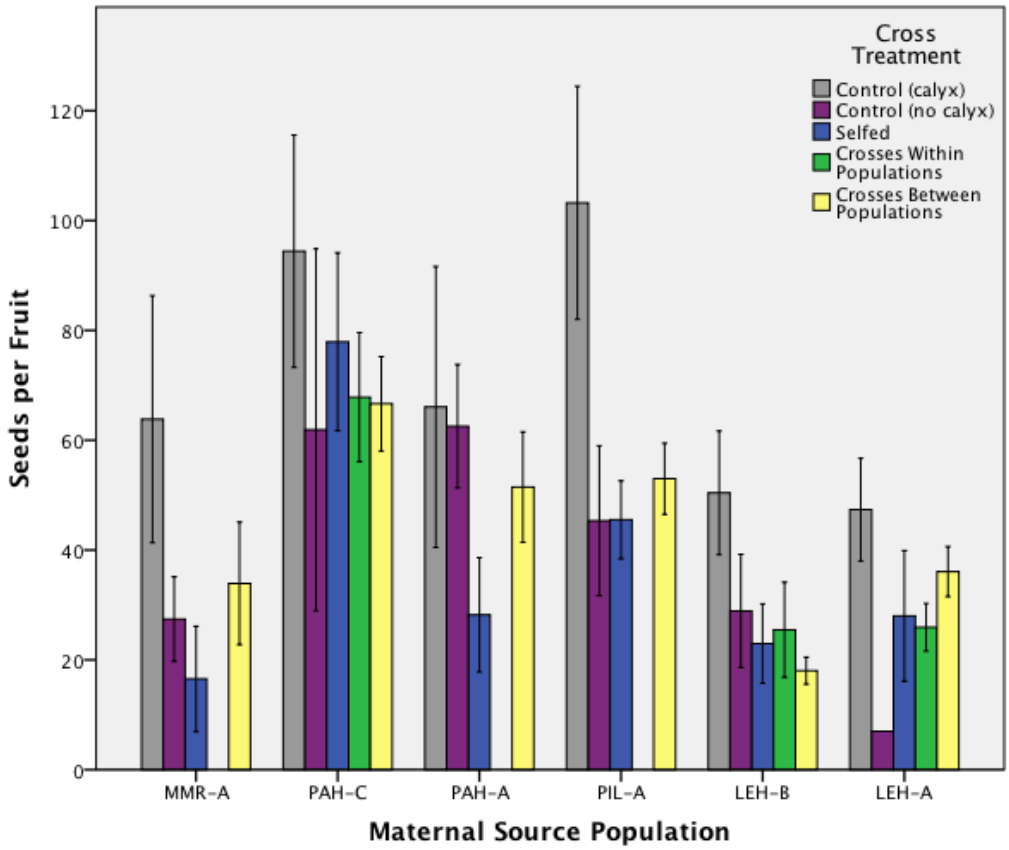

Fig. 3.5. Mean number of seeds per fruit by maternal source populations and cross treatment. Error bars ± 1 SE. Populations displayed left to right from northwest to southeast geographic distribution. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Cross treatment affected seed mass from the nursery hand-pollinations (Fig. 3.6; GLM3, $F = 13.765$, df = 4, $p < 0.001$). Seeds in the control (with calyx intact) had more mass than seeds in all other treatments (Helmert Contrast $F = 14.454$, df = 4, $p < 0.001$; contrast estimate = 0.047 ± 0.007, $p < 0.001$). Crosses within populations produced seeds with less mass than crosses between populations (contrast estimate = 0.022 ± 0.007, $p = 0.004$), although seed mass following selfing did not differ from seed mass following outcrossing (contrast estimate = 0.003 ± 0.008, $p = 0.724$). Maternal source population also affected seed mass, though the interaction between maternal source and cross treatment did not (GLM3, $F = 20.071$, df = 5, $p < 0.001$; $F = 0.886$, df = 17, $p = 0.591$, respectively). Seeds from the NW Makaleha maternal source had more mass than the

seeds from all other maternal source populations (Hochberg's GT2: mean diff. = 0.0462 ± 0.0639, $p < 0.001$). Paternal source population did not affect seed mass (GLM3, $F = 1.091$, df = 5, $p = 0.373$).

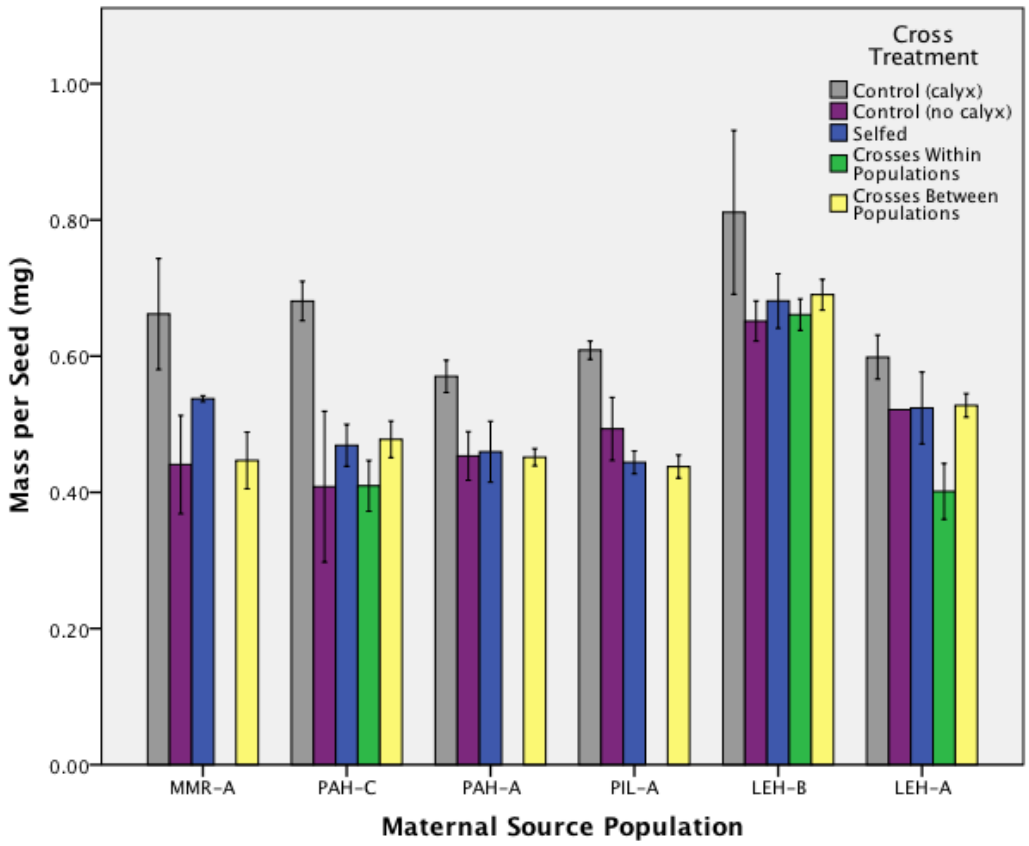

Fig. 3.6. Mean mass per seed (mg) by maternal source populations and cross treatment. Error bars ± 1 SE. Populations displayed left to right from northwest to southeast geographic distribution. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Cross treatment did not affect seed viability (Fig. 3.7; Kruskal-Wallis, $\chi^2 = 13.171$, df = 9, $p = 0.155$). Maternal source population did affect seed viability (Kruskal-Wallis, $\chi^2 = 19.7$, df = 5, $p = 0.001$). Seeds from plants with maternal sources from Kahanahāiki were less viable than seeds from plants with maternal sources from W Makaleha, NW Makaleha, and Keawapilau (Mann-Whitney U = 489.5, z = 3.843, $p <$

0.001; Mann-Whitney U = 772, z = 3.941, $p < 0.001$; Mann-Whitney U = 767.5, z = 3.064, $p = 0.002$, respectively). The paternal source populations did not affect viability (Kruskal-Wallis, $\chi^2 = 4.925$, df = 5, $p = 0.425$).

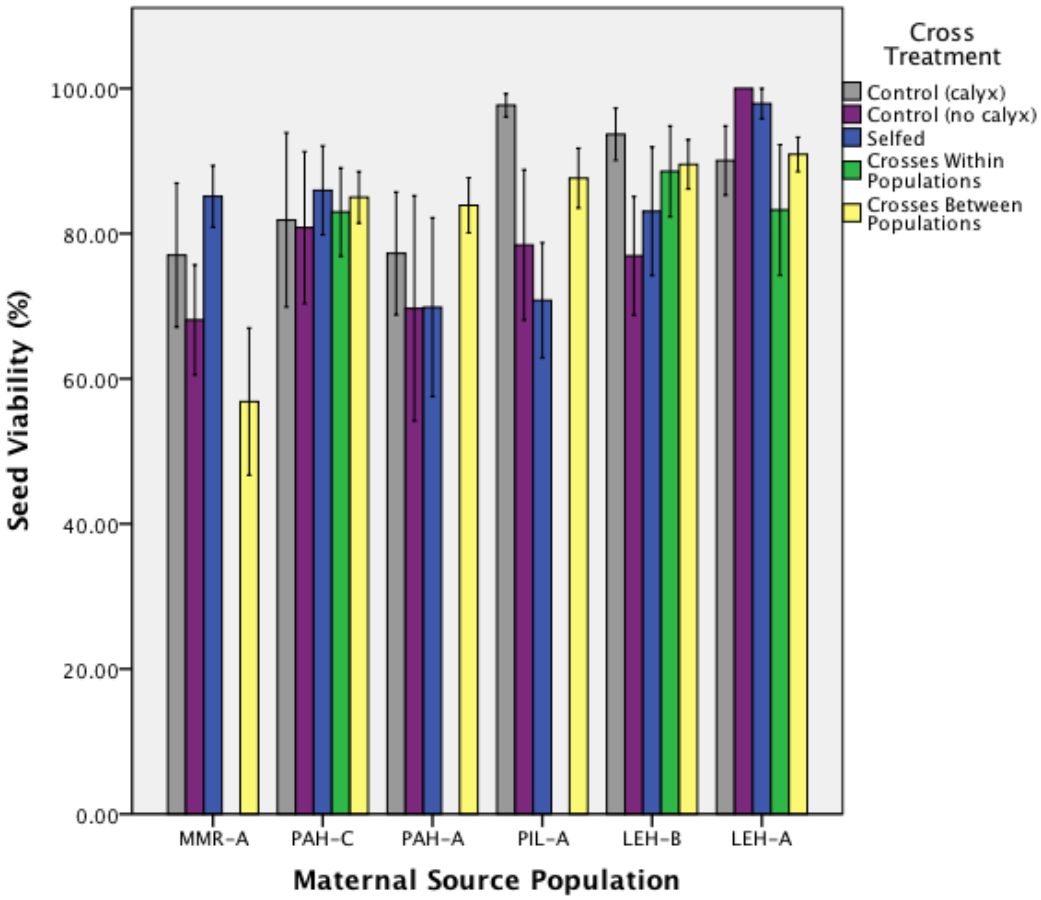

Fig. 3.7. Mean percentage of viable seed per collection by maternal source populations and cross treatment. Error bars ± 1 SE. Populations displayed left to right from northwest to southeast geographic distribution. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Post-planting: Nearly all plants (mean value of 93.30%, range 88.88-98.85% by maternal source) survived the first year after planting. The final model produced by the loglinear analysis of maternal source population, cross treatment, and survival is a good fit of the data ($\chi^2 = 5.081$, df = 12, $p = 0.955$ for the likelihood ratio statistic of the final model; the

expected values generated by the model are not significantly different from the observed data). There is no three-way interaction among these factors (K-way Effects, likelihood ratio, $\chi^2 = 1.984$, df = 10, $p = 0.996$). The test of the value of the two-way interactions is significant, and removing the two-way interactions would be detrimental to the model (K-way Effects, likelihood ratio, $\chi^2 = 73.593$, df = 17, $p < 0.001$). The two-way interaction between maternal source population and survival is significant (Fig. 3.8; partial $\chi^2 = 11.655$, df = 5, $p = 0.04$). Fewer plants died than expected from the Kahanahāiki maternal source population ($z = 2.000$, $p < 0.05$). Cross treatment did not affect survival of outplants (Fig. 3.9; loglinear analysis, partial $\chi^2 = 3.097$, df = 2, $p = 0.213$). Another final model was produced by a three-way loglinear analysis of maturation, cross treatment and paternal source population. The final model is a good fit of the data ($\chi^2 = 4.680$, df = 32, $p = 1.000$ for the likelihood ratio statistic of the final model; the expected values generated by the model are not significantly different from the observed data). The test of the value of the three-way interaction is not significant (K-way Effects, likelihood ratio, $\chi^2 = 0.000$, df = 10, $p = 1.000$). There are also no significant two-way interactions ($\chi^2 = 4.633$, df = 17, $p = 0.999$).

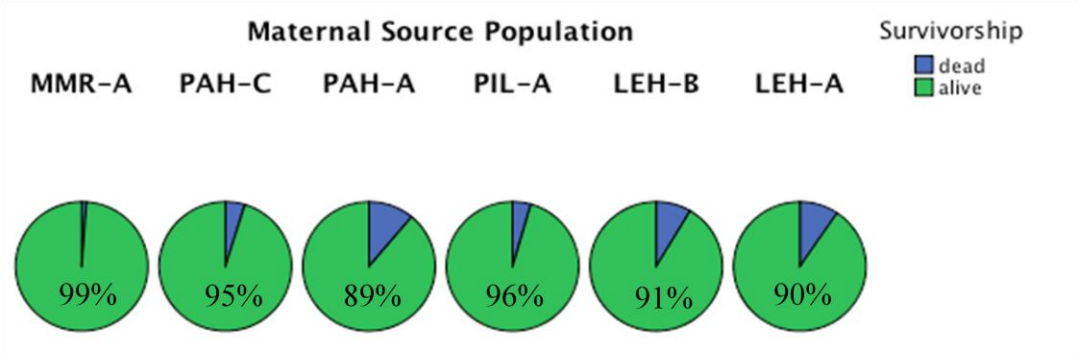

Fig. 3.8. Percentage of plants in each maternal source population that survived the first year after outplanting. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

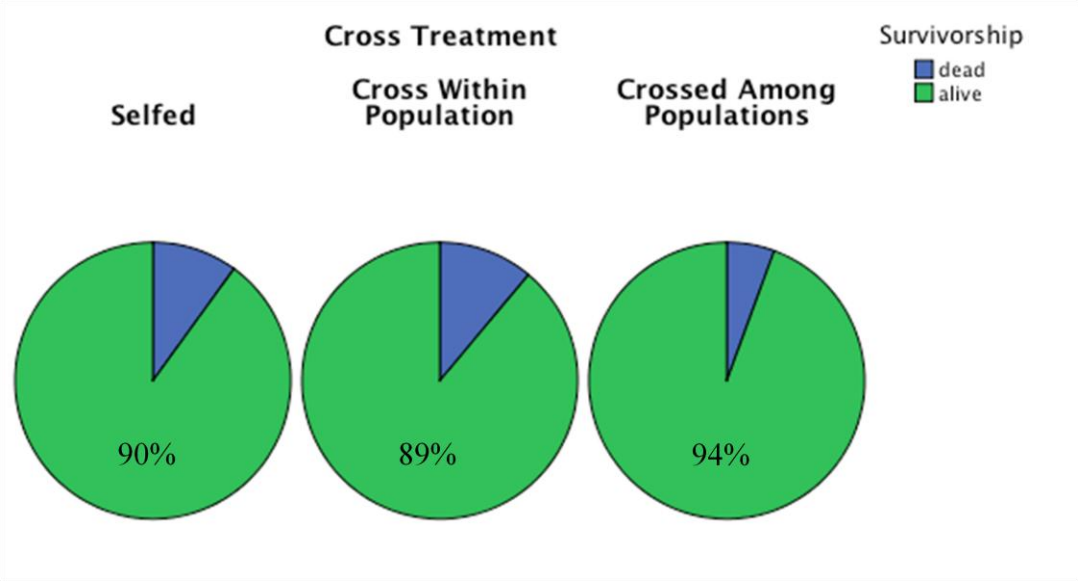

Fig. 3.9. Percentage of plants in each cross treatment that survived the first year following outplanting.

There was no interaction effect between the maternal source population and the cross treatment or paternal source population in total plant growth. Maternal source population and cross treatment affected plant growth (Fig. 3.10; GLM3, $F = 4.900$, df = 5, $p < 0.001$; $F = 17.072$, df = 2, $p < 0.001$, respectively). Plants from interpopulation crosses grew more than the plants from crosses within populations (Helmert Contrast $F = 18.054$, df = 2, $p < 0.001$, contrast estimate = 0.421 ± 0.073, $p < 0.001$). Plants from the W Makaleha maternal source population grew less than the plants from the NW Makaleha (Hochberg's GT2, mean diff. = 0.278 ± 0.061, $p < 0.001$), Kahanahāiki (mean diff. = 0.393 ± 0.063, $p < 0.001$), and Pahole Rim populations (mean diff. = 0.306 ± 0.064, $p < 0.001$). Plants from the Keawapilau maternal source population grew less than the plants from the Kahanahāiki maternal source population (Hochberg's GT2, mean diff. = 0.2541 ± 0.064, $p = 0.001$). Paternal source population had no effect on plant growth (GLM1, $F = 1.848$, df = 5, $p = 0.102$). Plants from W Makaleha were analyzed within their population to determine if the outcrossing yielded faster-growing plants as this population produced the slowest growing plants. Both the selfed plants and plants from crosses within the W Makaleha population were smaller than the plants from the crosses between populations (GLM1, $F = 9.188$, df = 2, $p < 0.001$; Tukey HSD; mean diff. (self /

crosses within) = 0.563 ± 0.169 / 0.525 ± 0.169, p = 0.003 / 0.007). When means from crosses from individual paternal populations were compared, paternal source did not affect growth (GLM1, F = 2.355, df = 6, p = 0.05).

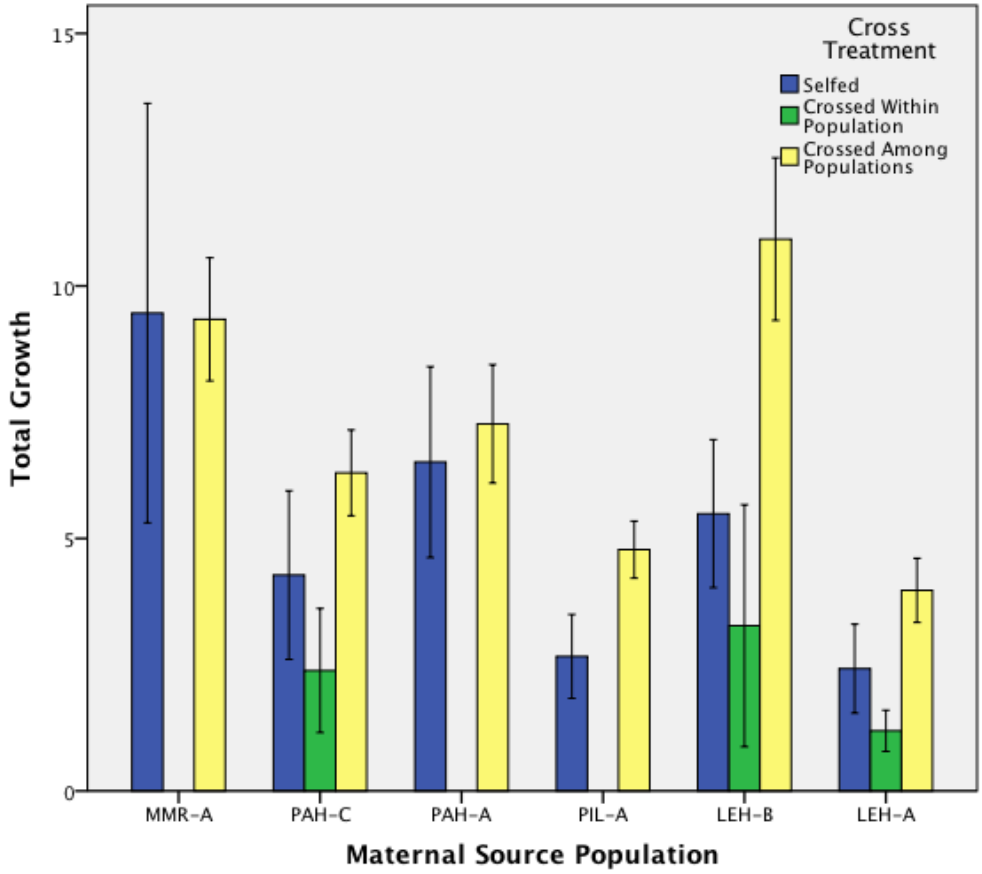

Fig. 3.10. Mean growth ((final size-initial size)/initial size) of plants by cross treatment and maternal source population. Size calculated as plant height (cm) x N leaves x N branches. Error bars ± 1SE. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Most plants (mean = 73.5; range = 65.6-79.0%) produced inflorescences during the first year following planting. Neither maternal source population, paternal source population, nor cross treatment affected the likelihood that a plant matured (the

production of at least one inflorescence; Pearson χ^2= 5.592, df = 5, p = 0.348; χ^2 = 9.724, df = 7, p = 0.205; χ^2 = 0.657, df = 2, p = 0.720, respectively).

There was no effect of maternal source population on the number of inflorescences per plant (Fig. 3.11; Kruskal-Wallis, χ^2 = 9, df = 4, p = 0.109). The cross treatment also did not have an effect (Kruskal-Wallis, χ^2 = 4.143, df = 2, p = 0.126). Lastly, the paternal source population did not have an effect, despite a significant difference among all populations (Kruskal-Wallis, χ^2 = 15.058, df = 2, p = 0.035). No two-way comparison of any paternal source populations yielded a significant effect when the family-wise error rate was adjusted to account for all comparisons (p = 0.002). Neither the maternal source population, cross treatment, nor their interaction had an effect on the number of flowers per inflorescence (Fig. 3.12; GLM3, F (5,2,7) = 0.841, 1.638, 0.858, p > 0.05). The paternal source population also did not affect the number of flowers per inflorescence (GLM3, F = 1.149, df = 7, p = 0.338), nor was the interaction of paternal and maternal source population significant (F = 0.474, df = 26, p = 0.985).

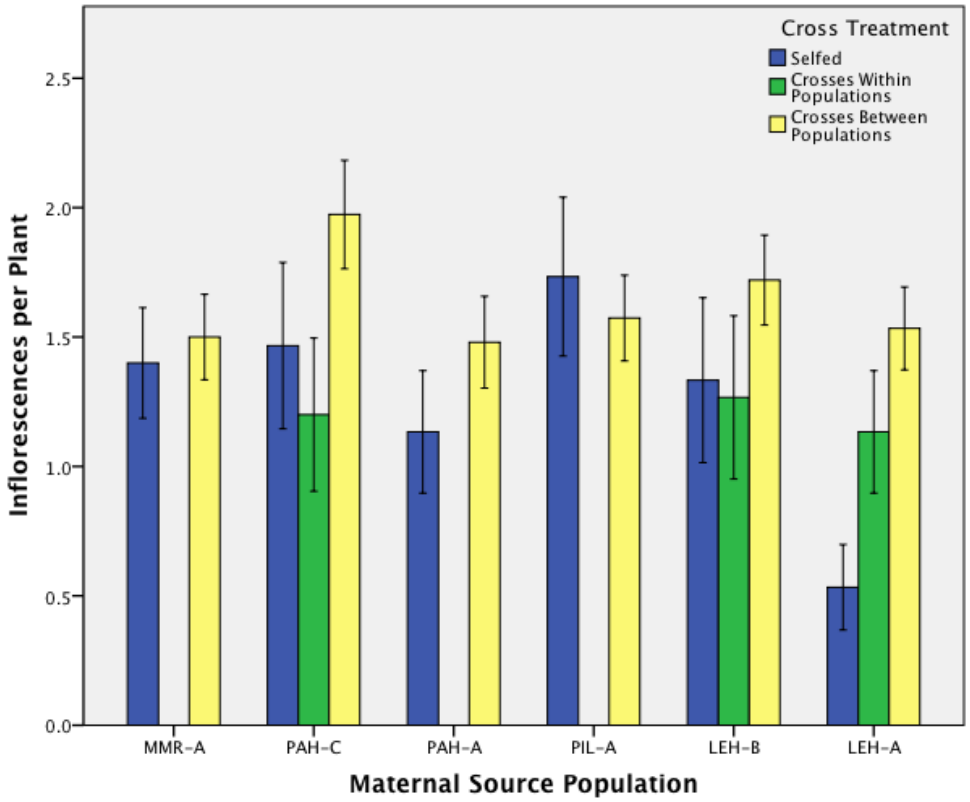

Fig. 3.11. Mean number of inflorescences per plant by maternal source and cross treatment. Error bars ± 1SE. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

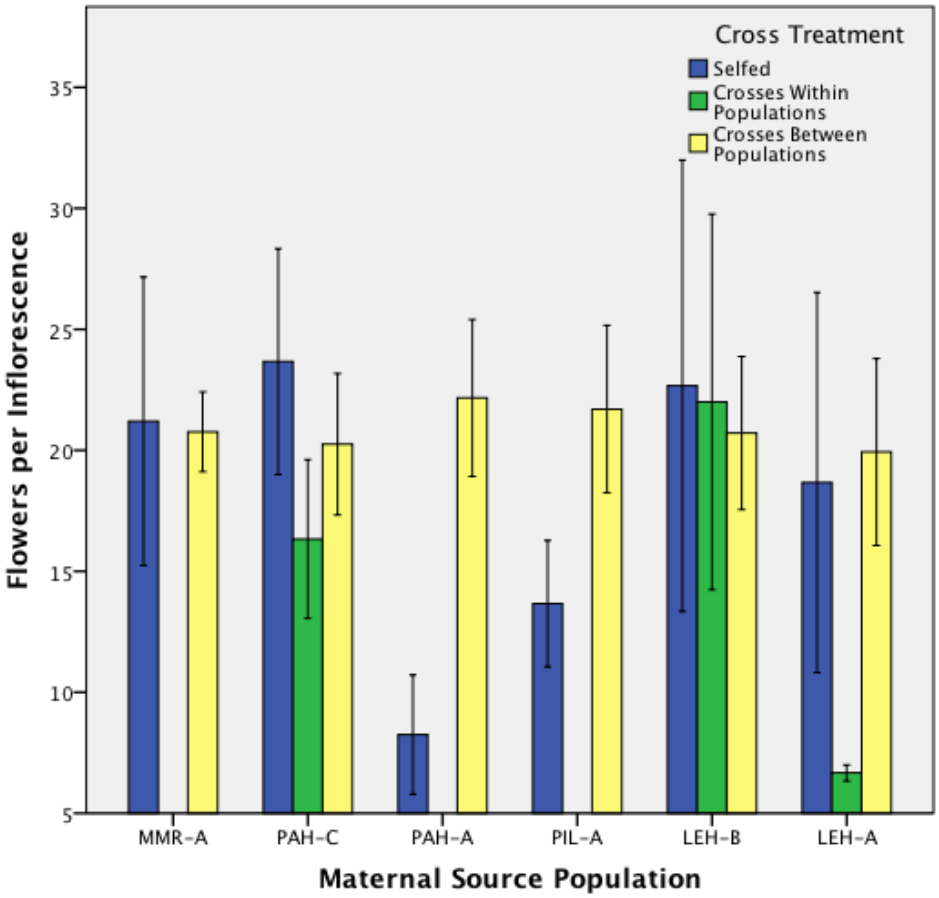

Fig. 3.12. Mean number of flowers per inflorescence by maternal source and cross treatment. Error bars ± 1SE. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Maternal source population affected the estimated number of flowers per plant (Fig. 3.13; Kruskal-Wallis, $\chi^2 = 13.876$, df = 5, $p = 0.016$). Progeny from the Pahole maternal population had more flowers than progeny from W Makaleha (Mann-Whitney U = 4145, z = 3.130, $p = 0.002$). Cross treatment also affected this variable (Kruskal-Wallis, $\chi^2 = 14.978$, df = 2, $p = 0.001$). Crosses between populations produced plants with more flowers than crosses within populations or selfed treatments (Mann-Whitney U = 7323, z = 3.037, $p = 0.002$; Mann-Whitney U = 19511, z = 2.714, $p = 0.007$, respectively). Paternal source population did not affect the estimated number of flowers

per plant (Kruskal-Wallis, χ^2 = 6.777, df = 5, p = 0.238). There was no effect of maternal population (GLM3, F = 2.356, df = 5, p = 0.05), cross treatment (F = 2.603, df = 2, p = 0.077), or their interaction on the number of fruit produced per inflorescence (Fig. 3.14; F = 0.175, df = 7, p > 0.990). There was also no effect of paternal population or its interaction with maternal population (GLM3, F = 1.444, df = 7, p = 0.193; F = 0.567, df = 26, p = 0.954, respectively).

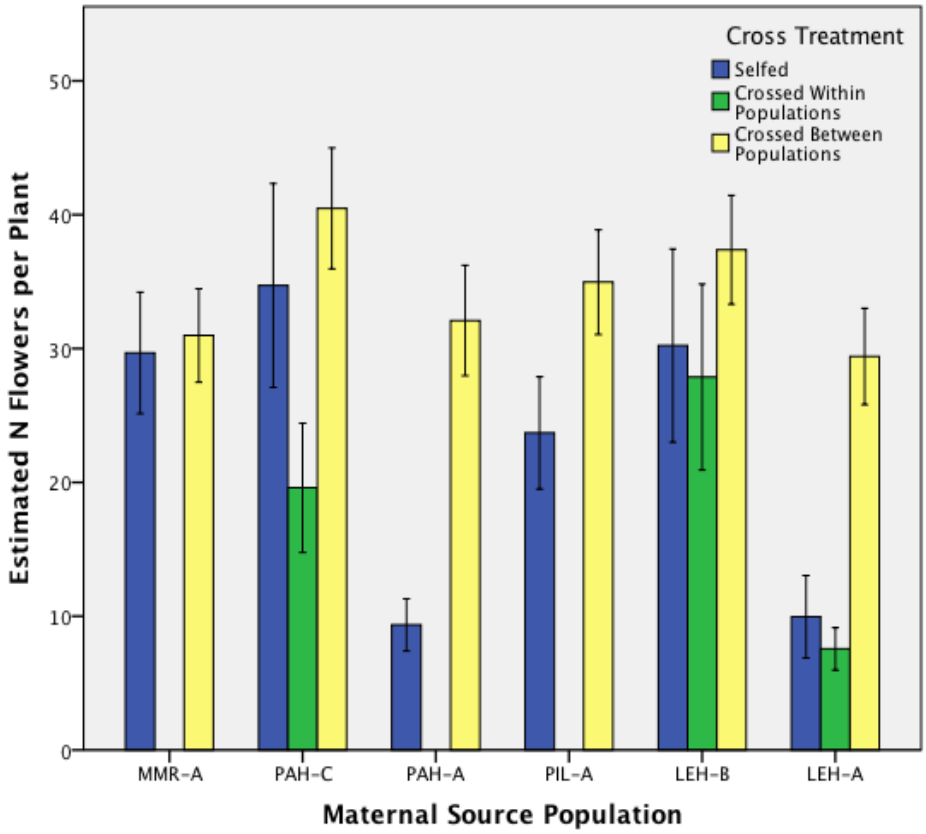

Fig. 3.13. Mean estimated number of flowers produced per plant by maternal source and cross treatment. Error bars ± 1SE. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

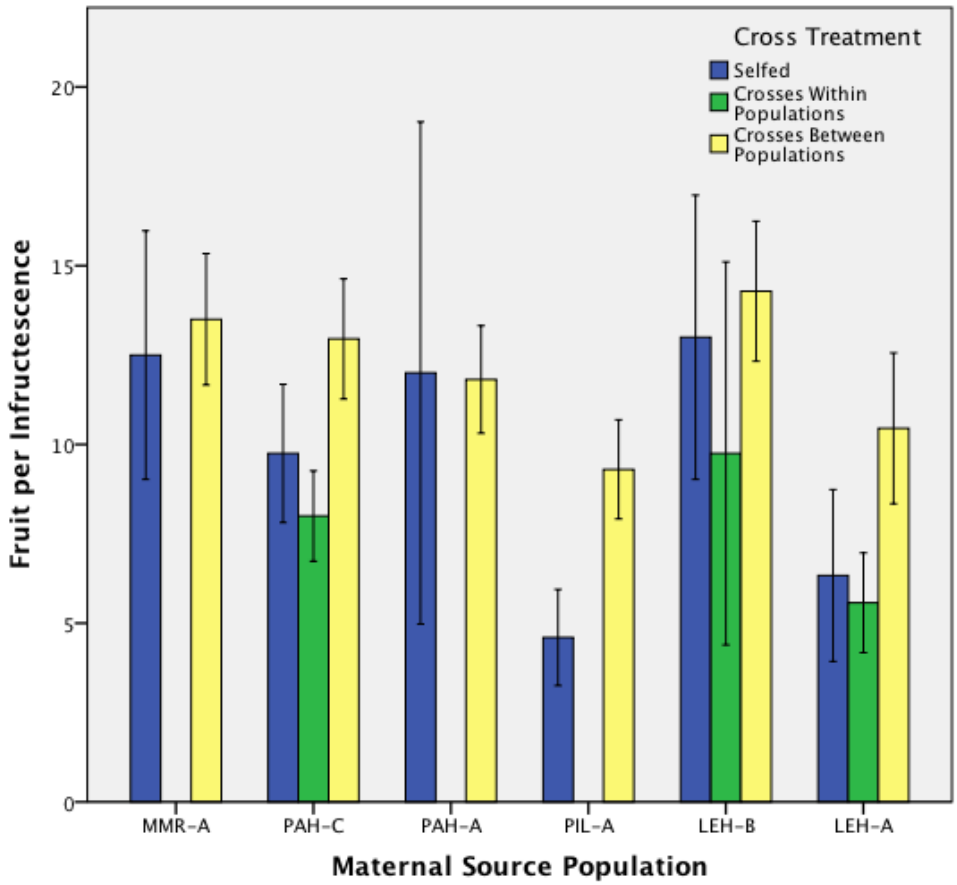

Fig. 3.14. Mean number of fruit produced per inflorescence by maternal source and cross treatment. Error bars ± 1SE. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Maternal source population affected the number of seeds per fruit (Fig. 3.15; GLM3, $F = 8.013$, df = 5, $p < 0.001$). Plants from the NW Makaleha maternal source population had fewer seeds per fruit than the plants from the Kahanahāiki and Pahole maternal source populations (Hochberg's GT2, mean diff. 47.34 ± 10.21, $p < 0.001$; 39.42 ± 9.29, $p = 0.001$, respectively). Neither cross treatment nor the interaction between cross treatment and maternal source population affected the number of seeds per fruit ($F = 2.053$, df = 2, $p = 0.132$; $F = 0.715$, df = 7, $p = 0.660$, respectively). The paternal source population affected the number of seeds per fruit (GLM3, $F = 4.797$, df = 7, $p < 0.001$). Plants with paternal sources from NW Makaleha produced fewer seeds per

fruit than plant with paternal sources from Pahole (Tukey HSD, mean diff. = 33.539 ± 10.802, $p = 0.028$).

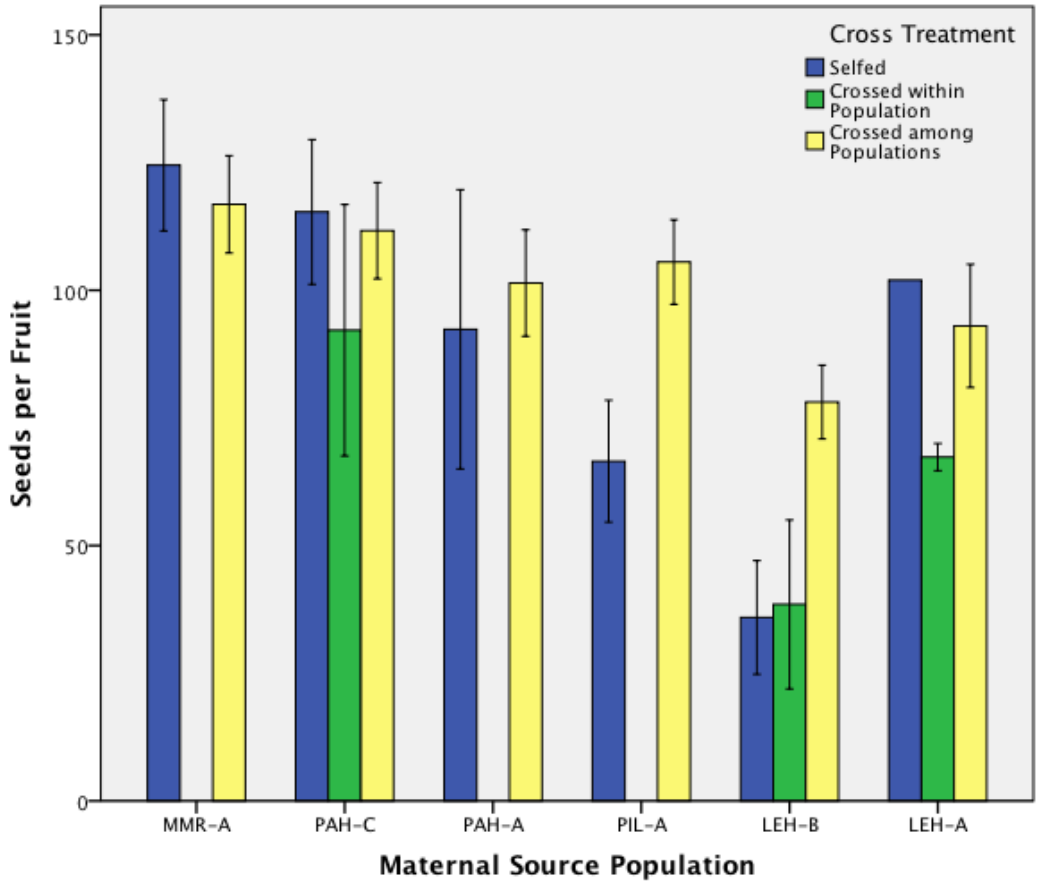

Fig. 3.15. Mean number of seeds produced per fruit by maternal source and cross treatment. Error bars ± 1SE. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Maternal source population affected the estimated total number of seeds a plant produced (Fig. 3.16; Kruskal-Wallis, $\chi^2 = 35.373$, df = 5, $p < 0.001$). Plants with maternal sources from the W Makaleha, NW Makaleha, and Keawapilau populations produce fewer seeds than the plants with maternal sources from the Kahanahāiki (Mann-Whitney U; z = 3.49, $p < 0.001$; z = 3.15, $p = 0.002$; z = 3.13, $p = 0.002$, respectively) and Pahole

populations (Mann-Whitney U, z = 3.90, $p < 0.001$; z = 3.14, $p = 0.002$; z = 3.25, $p = 0.001$, respectively). The selfed treatment of the W Makaleha maternal source was excluded from analyses because only one fruit was obtained. Cross treatment had an effect on the number of seeds produced by plant (Fig. 3.16; Kruskal-Wallis, $\chi^2 = 25.316$, df = 2, $p < 0.001$). Plants from crosses within a population produced fewer seeds than plants from selfs and crosses between populations (Mann-Whitney U = 1431, z = 2.793, $p = 0.005$; Mann-Whitney U = 6059, z = 4.442, $p < 0.001$, respectively). Also, plants from the selfed treatment produced fewer seeds than plants from the crosses between populations treatment (Mann-Whitney U = 16463, z = 2.742, $p = 0.006$). Plants with paternal sources from Kahanahāiki produced more seeds than plants from every other paternal source population except Pahole Rim (Fig. 3.17; W Makaleha: Mann-Whitney U = 1892, z = 3.38, $p = 0.001$; NW Makaleha: Mann-Whitney U = 1697, z = 4.23, $p < 0.001$; Pahole: Mann-Whitney U = 1915, z = 3.39, $p = 0.001$; Keawapilau: Mann-Whitney U = 1798, z = 3.85, $p < 0.001$. Additionally, plants with paternal sources from NW Makaleha also produced fewer seeds than plants with paternal sources from the Pahole Rim population (Mann-Whitney U = 1911, z = 3.196, $p = 0.001$).

When immature plants were removed from the analyses, the interaction between maternal source population and cross treatment could be examined, and it was significant (Fig. 3.18; GLM3, $F = 3.018$, df = 6, $p = 0.007$). The number of seeds produced decreased from the selfed progeny from the northwestern maternal source populations to the southeastern maternal source populations (the selfed treatment for W Makaleha was eliminated from analyses due to low sample size). The interaction between maternal and paternal source population was also significant (Fig. 3.19; GLM3, $F = 5.173$, df = 25, $p < 0.001$). Progeny with paternal sources from both the Makaleha populations produced more seeds per plant when they were crossed with maternal sources from the Kahanahāiki and Pahole populations (and the Pahole Rim population for paternal sources from NW Makaleha only) than when crossed with any of the other populations.

The three populations for which crosses were conducted within each population were analyzed separately (Fig. 3.20). Cross treatment again affected the estimated number of seeds each outplant produced (Kruskal-Wallis, $\chi^2 = 24.609$, df = 2, $p < 0.001$).

The progeny from crosses between populations produced more seeds per plant than the progeny from crosses within populations and the selfed progeny (Mann-Whitney U = 2862, z = 4.636, $p < 0.001$; Mann-Whitney U = 2521, z = 2.265, $p = 0.024$, respectively). However, there was only a marginally significant difference between the estimated number of seeds produced by selfed progeny and progeny from intrapopulation crosses (Mann-Whitney U = 496, z = 1.961, $p = 0.050$), in which plants from the selfed progeny may have produced slightly more seeds per plant than plants from the intrapopulation crosses. This trend of selfed progeny out-performing progeny from crosses within populations appears to be isolated to the Pahole population.

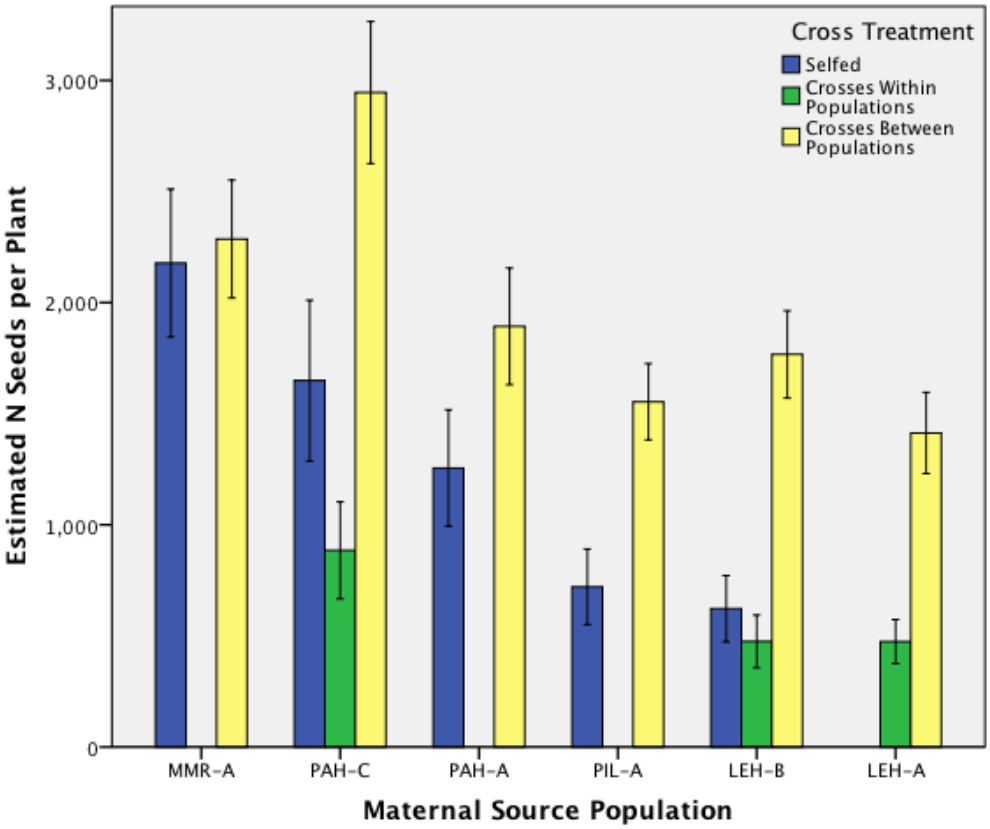

Fig. 3.16. Mean estimated number of seeds produced per plant by maternal source and cross treatment. Error bars ± 1SE. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

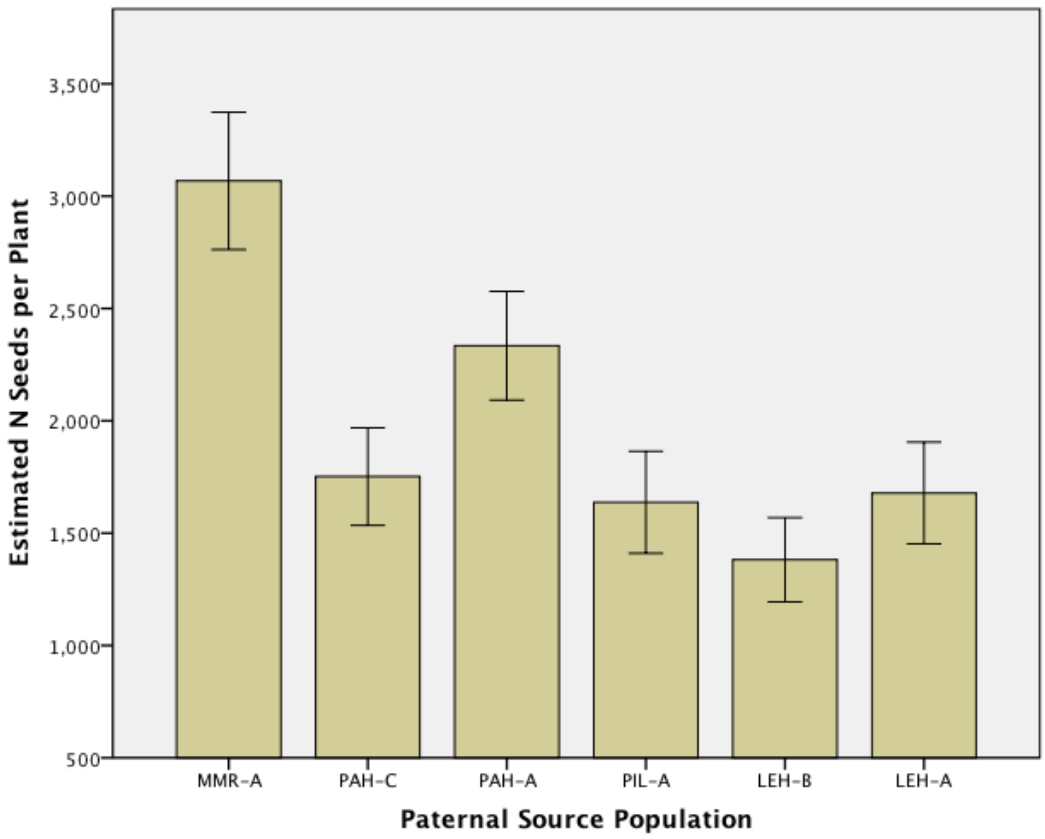

Fig. 3.17. Mean estimated number of seeds produced per plant by paternal source population. Error bars ± 1SE. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

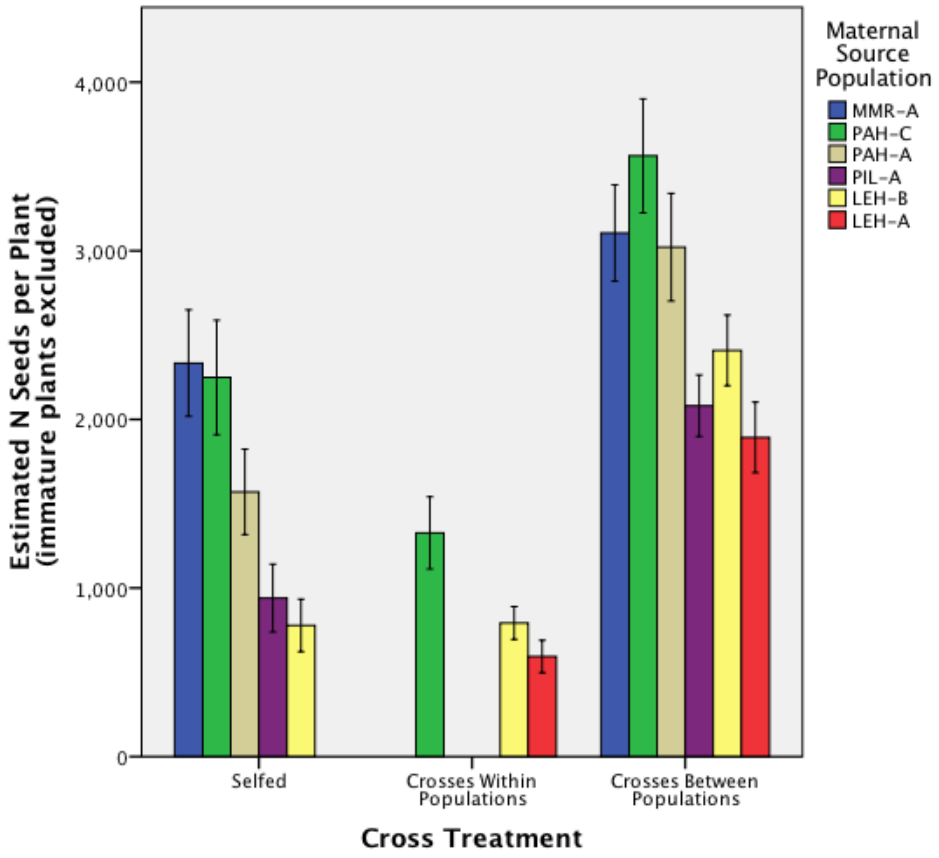

Fig. 3.18. Mean estimated number of seeds produced per plant by maternal source and cross treatment for mature plants only (immatures excluded). Error bars ± 1SE. Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha. Maternal source populations are ordered along from left to right representing geographical distribution from northwest to southeast.

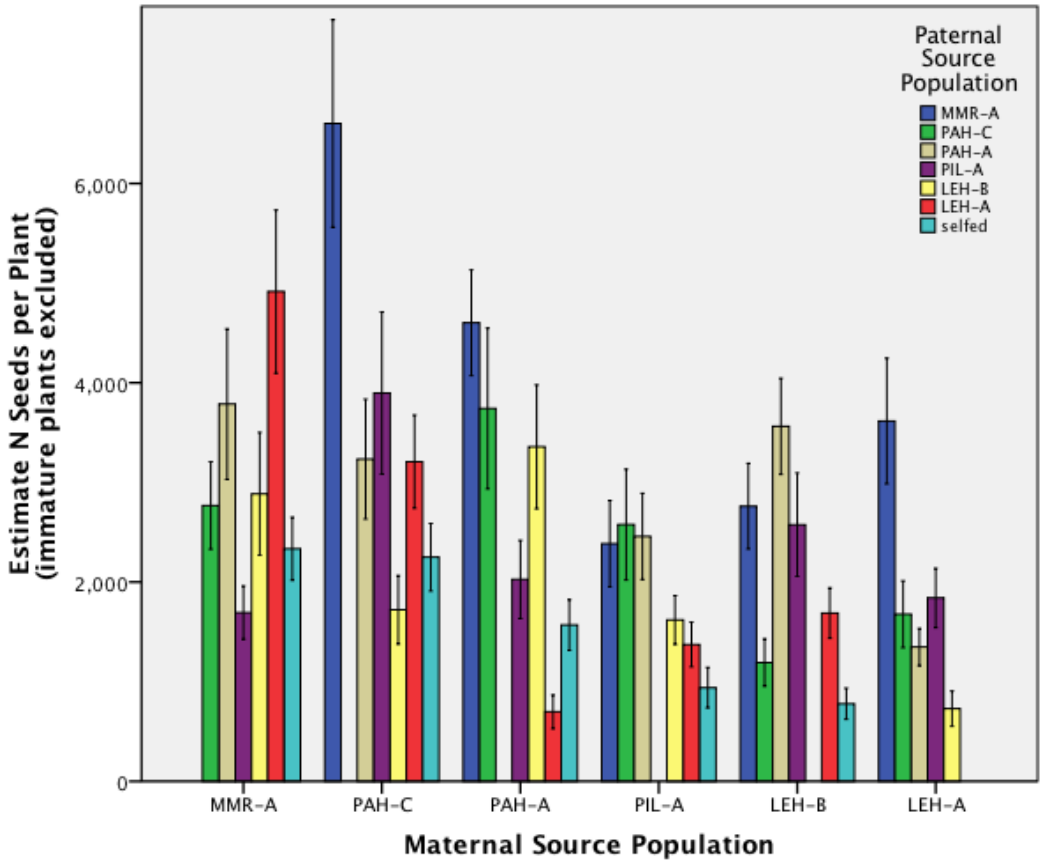

Fig. 3.19. Mean estimated number of seeds produced per plant by maternal source and paternal source for mature plants only (immatures excluded). Error bars ± 1SE. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha. Maternal source populations are ordered along the x-axis from left to right representing geographical distribution from northwest to southeast.

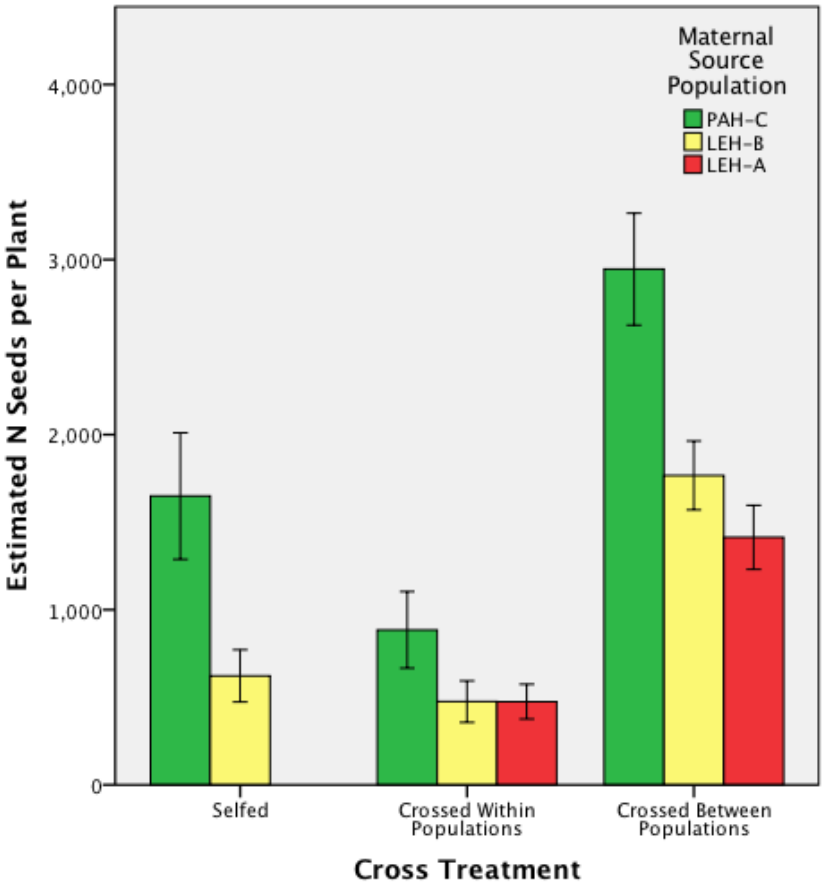

Fig. 3.20. Mean estimated number of seeds produced per plant by maternal source population and cross treatment for the populations represented in crosses within populations only. Error bars ± 1SE. PAH-C = Pahole, LEH-B = NW Makaleha, LEH-A = W Makaleha.

Relative Performance (*RP*) and Cumulative Fitness: Cumulative fitness was calculated for each population. Cross treatment did not affect cumulative fitness (Fig. 3.21; GLM1, $F = 1.279$, df = 2, $p = 0.314$). A comparison of mean cumuative fitness by maternal source population could not be analyzed because there was only one value per cross treatment for each population (Table 3.3).

Table 3.3. Cumulative fitness (seeds per pollination (GH) * survival * N inflorescences* N fruit per inflorescence * N seeds per fruit. Values are means per population. Cross treatment for Selfed, progeny of crosses within populations (Within Populations) and progeny of crosses between populations (Between Populations).

Maternal Population	Cross Treatment	Cumulative Fitness
Kahanahāiki	Selfed	12594.21
Kahanahāiki	Between Populations	9861.20
Pahole	Selfed	58741.58
Pahole	Within Populations	42432.95
Pahole	Between Populations	77701.83
Pahole Rim	Selfed	18644.99
Pahole Rim	Between Populations	43411.33
Keawapilau	Selfed	10624.92
Keawapilau	Between Populations	23699.88
NW Makaleha	Selfed	12616.81
NW Makaleha	Within Populations	8231.91
NW Makaleha	Between Populations	19698.46
W Makaleha	Selfed	2452.91
W Makaleha	Within Populations	4623.64
W Makaleha	Between Populations	26146.59
Total	Selfed	19407.85
Total	Within Populations	12191.73
Total	Between Populations	35630.93

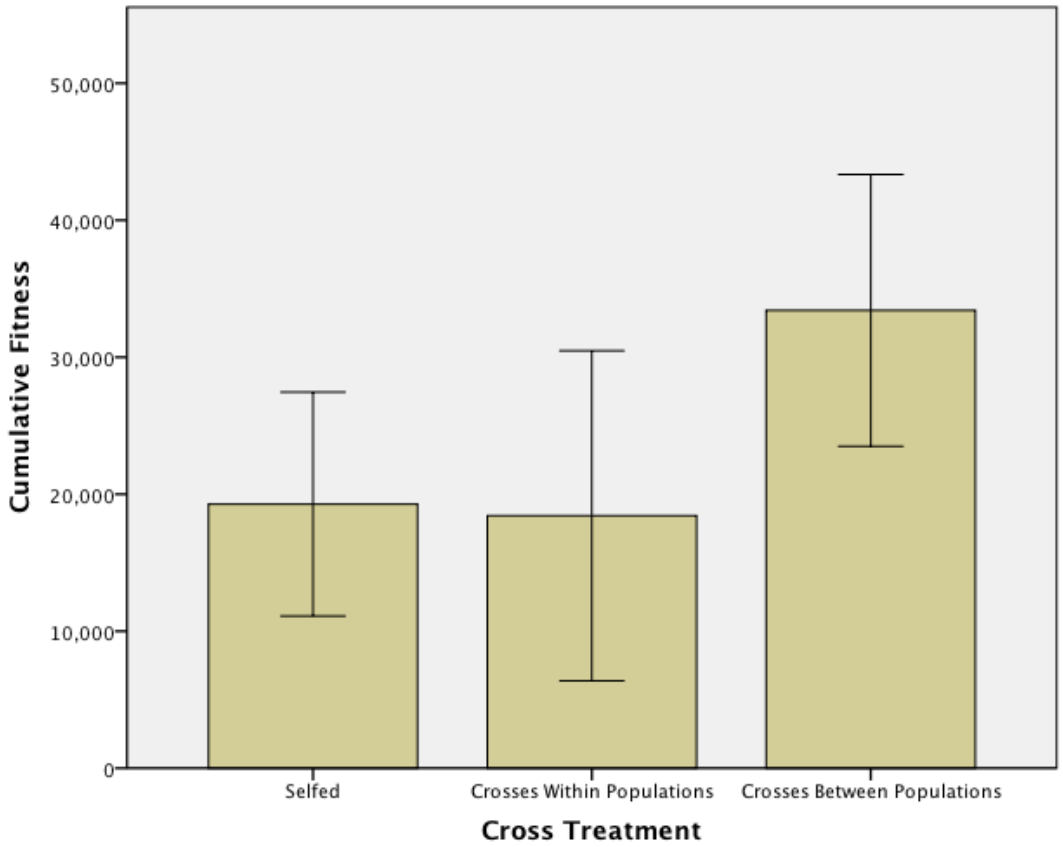

Fig. 3.21. Cumulative fitness by cross treatment. Cumulative fitness was the product of the mean of population means of number of seeds per pollination, survival, N inflorescences, N fruit per inflorescence, and N seeds per fruit. Error bars ± 1 SE.

Neither inbreeding depression nor outbreeding depression was detected at any life stages (Table 3.4; Fig. 3.22). Heterosis was detected in many of the life stages, including the number of inflorescences per plant ($RP_h > 0$; $t = 6.967$, df = 2, $p = 0.020$) and the number of seeds per plant (RP_h; $t = 36.150$, df = 2, $p = 0.001$). Heterosis was also inferred for the number of flowers per plant (RP_b; $t = 3.025$, df = 5, $p = 0.029$). Since the RP_i ratio was negative at this stage, the selfed progeny outperformed the progeny from crosses within populations. The statistically positive RP_b value could not be due to inbreeding depression but rather heterosis, which likely was not significantly greater than zero due to a lower sample size (n = 3 (RP_h) and n = 6 (RP_b). Maternal population did not affect any of the *RP* values; there were no differences in the values among populations (Fig. 3.23; RP_i; $F = 2.185$, df = 2, $p = 0.141$; RP_h; $F = 0.505$, df = 2, $p = 0.612$; RP_b; $F = 2.041$, df =

5, $p = 0.096$). There was variation among the different stages for the heterosis estimate, RP_h (GLM1, $F = 6.638$, df = 6, $p = 0.002$). Heterosis was stronger in the number of seeds produced per plant than in the earlier survival stage (Games-Howell; mean diff. = 0.6712 ± 0.0263, $p < 0.001$). Neither heterosis, inbreeding depression nor outbreeding depression was detected in the cumulative realtive fitness estimates (Fig. 3.24).

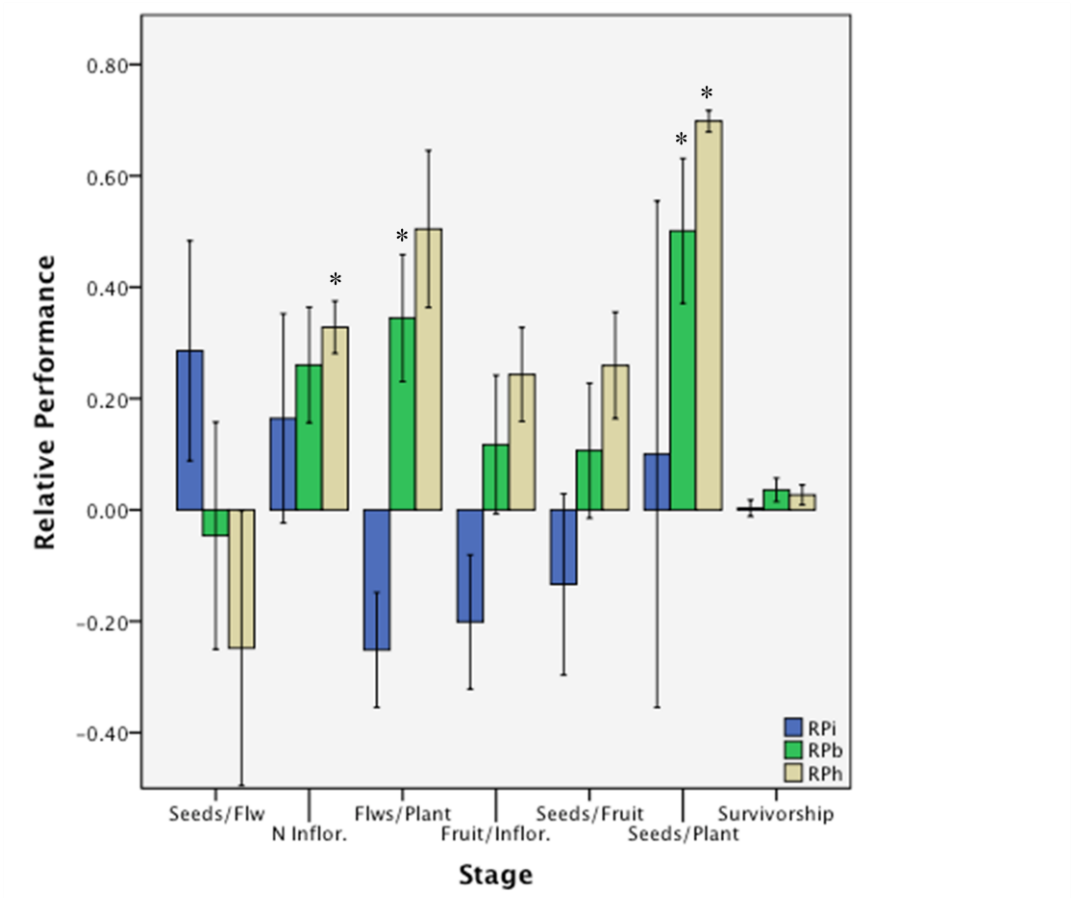

Fig. 3.22. Mean relative performance (*RP*; of all population mean *RP*) by cross type and stage. RP_i = selfed progeny / progeny from crosses within populations; RP_b = selfed progeny / progeny of crosses between populations; RP_h = progeny from crosses within populations / crosses between populations. Error bars ± 1 SE. * *RP* is not zero ($p < 0.05$).

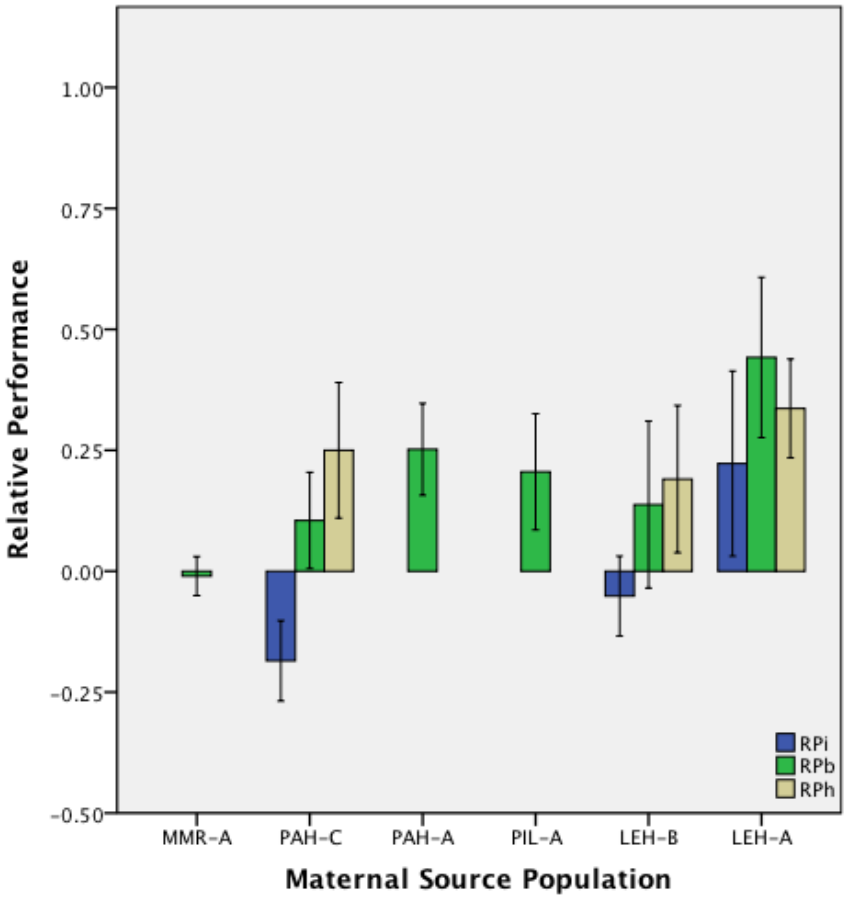

Fig. 3.23. Mean relative performance (*RP*; of all stage means) by cross type and maternal source population. RP_i = selfed progeny / progeny from crosses within populations; RP_b = selfed progeny / progeny of crosses between populations; RP_h = progeny from crosses within populations / crosses between populations. Error bars ± 1 SE. Only PAH-C, LEH-B, and LEH-A had RP_i and RP_h values. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha.

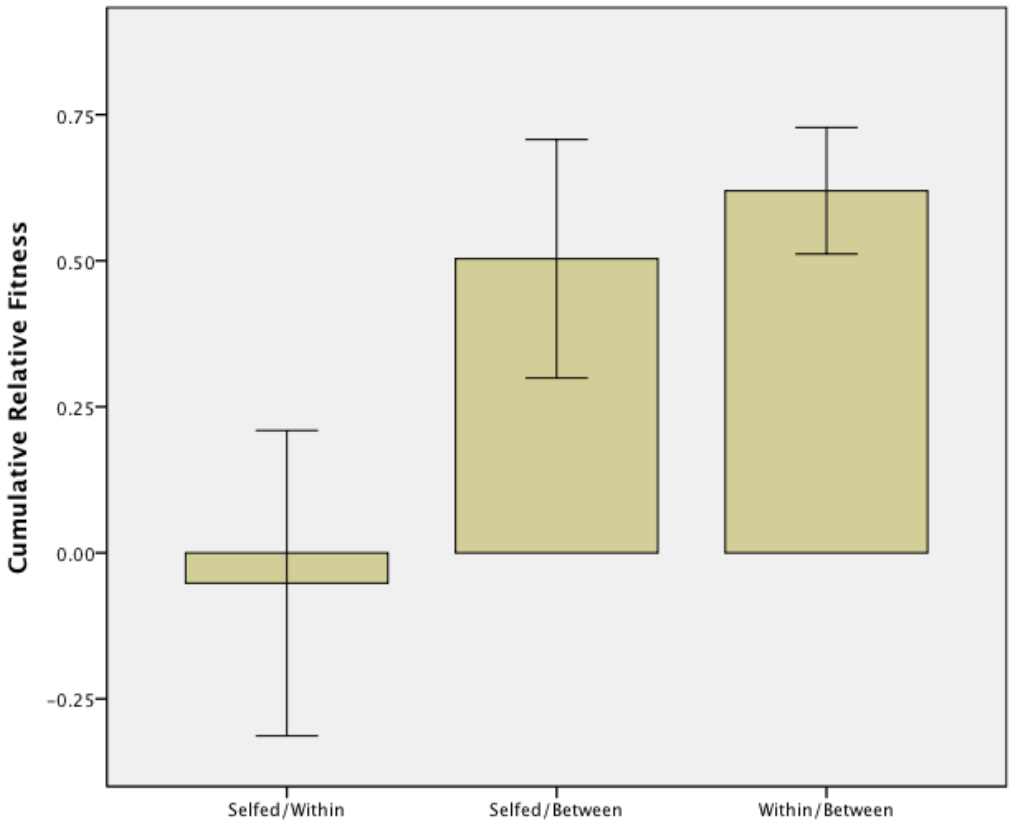

Fig. 3.24. Cumulative relative fitness. Error bars ± 1 SE.

Table 3.4. Relative performance of crosstypes for progeny: RP_i (selfed/crosses within populations); RP_b (selfed/crosses between populations); RP_h (crosses within populations/crosses between populations). Positive RP_i indicates inbreeding depression, negative RP_i indicates outbreeding depression, positive RP_h indicates heterosis, and negative RP_h indicates outbreeding depression.

Stage	Maternal Population	RP_i	RP_b	RP_h
Seed / Pollination (GH)	Kahanahāiki		-0.226	
Seed / Pollination (GH)	Pahole	0.1489	-0.329	-0.429
Seed / Pollination (GH)	Pahole Rim		0.379	
Seed / Pollination (GH)	Keawapilau		-0.313	
Seed / Pollination (GH)	NW Makaleha	0.034	-0.541	-0.556
Seed / Pollination (GH)	W Makaleha	0.675	0.753	0.240
N Inflorescences	Kahanahāiki		0.067	
N Inflorescences	Pahole	-0.145	0.312	0.412
N Inflorescences	Pahole Rim		0.234	
N Inflorescences	Keawapilau		-0.092	

N Inflorescences	NW Makaleha	0.135	0.414	0.322
N Inflorescences	W Makaleha	0.503	0.627	0.250
Flowers / Plant	Kahanahāiki		0.042	
Flowers / Plant	Pahole	-0.436	0.142	0.516
Flowers / Plant	Pahole Rim		0.709	
Flowers / Plant	Keawapilau		0.322	
Flowers / Plant	NW Makaleha	-0.078	0.191	0.255
Flowers / Plant	W Makaleha	-0.2408	0.661	0.743
Fruit / Inflorescence	Kahanahāiki		0.074	
Fruit / Inflorescence	Pahole	-0.1410	0.249	0.355
Fruit / Inflorescence	Pahole Rim		-0.015	
Fruit / Inflorescence	Keawapilau		0.505	
Fruit / Inflorescence	NW Makaleha	-0.434	-0.386	0.078
Fruit / Inflorescence	W Makaleha	-0.029	0.276	0.297
Seeds / Fruit	Kahanahāiki		-0.062	
Seeds / Fruit	Pahole	-0.249	-0.1053	0.160
Seeds / Fruit	Pahole Rim		0.090	
Seeds / Fruit	Keawapilau		0.370	
Seeds / Fruit	NW Makaleha	0.187	0.554	0.451
Seeds / Fruit	W Makaleha	-0.340	-0.207	0.167
Seeds / Plant	Kahanahāiki		0.047	
Seeds / Plant	Pahole	-0.464	0.440	0.700
Seeds / Plant	Pahole Rim		0.337	
Seeds / Plant	Keawapilau		0.536	
Seeds / Plant	NW Makaleha	-0.237	0.648	0.731
Seeds / Plant	W Makaleha	1.000	1.000	0.664
Survival	Kahanahāiki		-0.014	
Survival	Pahole Rim		0.030	
Survival	Pahole	-0.011	0.026	0.036
Survival	Keawapilau		0.110	
Survival	NW Makaleha	0.033	0.083	0.052
Survival	W Makaleha	-0.012	-0.020	-0.007
Cumulative Fitness	Kahanahāiki		-0.217	
Cumulative Fitness	Pahole	-0.359	0.161	0.463
Cumulative Fitness	Pahole Rim		0.574	
Cumulative Fitness	Keawapilau		0.555	
Cumulative Fitness	NW Makaleha	-0.138	0.150	0.267
Cumulative Fitness	W Makaleha	0.745	0.914	0.664

Discussion

No early life stages showed heterosis, inbreeding depression or outbreeding depression. As expected, removing the calyx in the bud stage reduced the number of seeds per fruit and seed mass. Heterosis was present in the total growth of plants and the number of inflorescences, flowers, and seeds produced per plant. Neither inbreeding depression nor outbreeding depression were detected at any stage. A trend of selfed progeny out-performing progeny from crosses within populations appears to be isolated to the Pahole population for several stages including female and male fitness (number of seeds per plant and number of flowers per plant, respectively), fruits per inflorescence, and seeds per fruit, though these differences were not significant. Plants from maternal source populations located furthest from the outplanting site had the lowest relative fitness. This suggests a home site advantage that may be due to the elevational and rainfall gradient throughout the recent distribution of *S. obovata*.

Plants with the lowest relative fitness were those from W Makaleha, the maternal source population located at the greatest distance from Kahanahāiki (the outplanting site). One reason for the low relative fitness of these plants could be local adaptation. Alternatively, variation in fitness could be due to random genetic drift resulting from small population size (Ellstrand and Elam 1993, Frankham et al. 2002). Plants from W Makaleha, however, also produced fewer flowers per plant than plants from Pahole and the smallest number of flowers for all maternal source populations. Plants with paternal parents from W Makaleha also produced the fewest seeds per plant. Reductions in fitness across many life history stages was unlikely to result from genetic drift, and suggests the possibility of habitat differences between the outplanting site and the W Makaleha site as the cause of reduced fitness at the outplanting site. Additionally, the only extant populations of *S. obovata* are located in the southeastern portion of the distribution (W and NW Makaleha plus a rediscovered Keawapilau population one gulch west of Makaleha), indicating that they are likely not less vigorous or fecund than the plants of the extirapted populations to the northwest. Futher supporting the first hypothesis that reduced relative fitness of the progeny of the W Makaleha population could be attibuted to local adaptation, the populations with the highest relative fitness were at the

northwestern end of the distribution, and surrounding the outplanting site. There is no natural geographic barrier separating any populations in the recent distribution of *S. obovata*, although they occur along an elevational gradient with an increase in over 244m elevation from the most northwestern site to the most southeastern site.

With the increase in elevation, the rainfall gradually increases from Kahanahāiki to W Makaleha (Table 3.1, PRISM 2004, Giambelluca et al. 2011). When populations are crossed, heterosis remained the most dominant influence on fitness, and the crosses with northwestern sites were the most fit for the three southeastern maternal source populations. This suggests that there was not a break-up in coadapted gene complexes by crossing the more distant populations, and oubreeding depression was not detected. It is most likely that the southeastern maternal source populations are better adapted to higher elevations (and the increased rainfall and lower temperatures associated with this gradient), and the northwestern sources outperformed the southeastern sources at the outplanting site. The lower rainfall at the site of the outplanting site relative to the W. Makaleha population might explain the reduced fitness of these plants. Furthermore, the progeny from the northwestern maternal source populations had a "home site" advantage at the outplanting site, and were possibly better suited for environmental conditions that are correlated with lower elevations, such as higher mean daily temperatures and lower mean rainfall (Timm and Diaz 2009, Diaz et al. 2011). The plants from the Kahanahāiki maternal source populations (most northwestern) were also the only plants where selfed progeny had higher cumulative fitness than outcrossed progeny (one cumulative fitness value per cross treatment, results were not analyzed so significance is unknown); suggesting a preference for crossing with plants from that gulch.

Maternal effects occurred throughout all life stages of *S. obovata*, but their significance is unclear because these effects were highly variable among populations. Interpretation of maternal effects was also difficult because populations were not grown in their home sites. In the NW Makaleha maternal source population, for example, low seed number was associated with high mean seed mass. This trend was apparent in the next generation, as outplants from the NW Makaleha maternal source population also had fewer seeds per fruit than any other maternal source population (though seed mass was

not calculated for progeny). Higher seed mass may contribute to higher levels of recruitment (Gonzalez-Rodriguez et al. , Westoby et al. 1996), and the NW Makaleha source population is the only extant *in situ* population of this species with more than 10 individuals (~30 mature + >100 immature). It is possible that seed mass plays a role in the success of this population. Conversely, early life stages suggested decreased fitness in Kahanahāiki plants because they produced fewer seeds per pollination and seeds were less viable than many other maternal source populations. The plants from the Kahanahāiki population that grew and survived, however, had high seed production per fruit and per plant, suggesting that differences at early and late life history traits could be compensatory within populations.

Inbreeding depression was not predicted at early life stages for selfing species (Husband and Schemske 1996), and *S. obovata* was predicted to have high selfing rates based on floral morphology (Weller et al. 1998) and observations in cultivation. Inbreeding depression, however, was not present at later life stages as well, suggesting that substantial purging may have occurred, excluding any deleterious alleles that had become fixed in the populations. The lack of inbreeding depression likely coincides with high levels of selfing (despite any indications to the contrary, including high nectar production). A reintroduction of plants with purged genetic load may actually increase the success of the outplanting (Dudash and Murren 2008). Because potential inbreeding depression was measured from seed production of maternal sources to seed production of the progeny, the likelihood of detecting inbreeding depression at later life history stages was high relative to many studies that focus on only part of the life cycle. Similarly, opportunities for detecting outbreeding depression were high, but in accord with the substantial evidence for heterosis, there was no evidence for outbreeding depression. The lack of outbreeding depression was surprising considering it was predicted for species that occupy dissimilar habitats (Edmands 2007, Frankham et al. 2011), but the variation in habitat described here may be small in comparison to the variation necessary for hybrid breakdown. Furthermore, concerns of land managers for mixing populations of this “naturally-selfing” species (MIT 2003) now seem unwarranted as the benefit of outcrossing (heterosis) is the dominant factor in outcrossed progeny fitness.

Heterosis also dominates despite the potential local adaptation of maternal source populations furthest from the outplanting site (experimental garden) and in the wetter habitat, as they displayed reduced progeny fitness at the outplanting site. This may be due to the fact that populations have become so reduced in size within the last several decades that substantial drift has occurred (Frankham et al. 2002). The benefit of alleviating the fixation of slightly deleterious alleles in the few remaining founders is larger than the disruption of local adaptation, at least in the first generation. With high levels of selfing, there is concern that the benefit of heterosis may decline after the first generation, as continued selfing expresses the mildly deleterious alleles that were masked by outcrossing. Heterotic individuals, however, may be the most successful in new populations (Lande and Schemske 1985), and consequently contribute to the success of the reintroduction. Due to the their increased relative fitness, outplanting heterotic individuals may be necessary in creating sucessful reintroductions to overcome unmanaged threats, such as introduced slugs (Joe and Daehler 2008). If the reintroductions are located in habitat closer to the average rainfall and temperature for the species, it is possible that local adaptation may go undetected, and have less of an influence on successive generations.

Management Recommendations: Management suggestions have been developed for reintroductions efforts for this species. Edmands (2002, 2007) suggested that populations should not be mixed unless inbreeding depression has been detected. Frankham et al. (2011) takes a less conservative approach and had developed a key for determining the likelihood of outbreeding depression. If the chance of outbreeding depression is low, gene flow should be re-established between populations. Following the key (and inferring that gene flow between the populations occurred within the last 500 years because several historic populations of *S. obovata* were known throughout this region that might have linked populations), gene flow should be re-established between all populations. The benefit from heterosis should also influence management suggestions. In this study, heterosis was detected upon interpopulation outcrossing, but populations furthest from the outplanting site and in moderately wetter habitat had reduced relative fitness in comparison the population sources closer to the outplanting site. Recommendations are as follows:

1. Propagules that originate from higher elevations should not be moved to lower elevations. Kahanahāiki progeny are the only plants that should be planted into Kahanahāiki gulch.
2. Stock from Kahanahāiki and Pahole should be planted into Pahole up to Keawapilau, and possibly as far as Makaleha. However, caution should be exercised in planting this stock above their average elevation. While the stock from all populations exhibited heterosis and there were no indications of outbreeding depression, propagules from lower elevations may have reduced fitness at higher elevations. The balance between heterosis and costs of local adaptation need to be quantified. Climate change theory, however, predicts plants will move up along elevational gradients (Vitt et al. 2010, Loss et al. 2011), therefore plants from lower elevations may eventually have higher fitness at sites that are now relatively cooler and wetter.
3. Stock from Keawapilau to West Makaleha should be mixed throughout their range, because plants from this region are all adapted to relatively mesic environments.
4. Reintroductions should be initiated with seeds from wild plants or from *ex situ* interpopulation hand-pollinations. Since pollinator visitation is low (Chapter 5), and mixed-population progeny are more fit than selfed progeny, seeds produced from interpopulation crosses are likely to be most successful in reintroduction programs. Enough pollinations must be carried out, however, to overcome reductions in seed production due to emasculation.
5. Makaha, a gulch on the west facing side of the Waianae Mts. and south of the W Makaleha population, has been a proposed outpanting site for *S. obovata* by OANRP. It is 792m in elevation (the same elevation as W Makaleha), with a mean maximum annual temperature of 75.2F and an average annual rainfall of 1921mm (PRISM 2004). These environmental conditions are consistent with the majority of wild population sites and intermediate between the Kahanahāiki and Makaleha population sites. Ideally, an additional site in Makaha that has a higher annual rainfall should also be established and both sites should be established with heterotic individuals from all sources as outlines in recommendation #4.

CHAPTER 4: *Schiedea kaalae*

Introduction

The angiosperm flora of the Hawaiian Islands has one of the highest rates of endemism (89%) in the world, and over half of the taxa are at risk (Wagner et al. 1999, Sakai et al. 2002). Approximately 10% of the flora is extinct (Wagner et al. 1999), and over thirty percent of the flora is federally listed as threatened or endangered (out of 1094 species; USFWS 2010). The conservation of Hawai‘i’s rare flora is necessary and challenging. Reasons for the decline of native species are both numerous and uncertain, often centered around the introduction of many invasive species, including other plants, invertebrates, and large ungulates, which can also cause vast habitat degradation (Diong 1982, Loope et al. 1988, Loope 1992, Cuddihy and Stone 1993, Joe and Daehler 2008, Athens 2009, Weller et al. 2011). Loss of native habitats, loss of pollinators, and decreases in population size have led to concern that much of Hawai‘i’s flora is experiencing increased rates of selfing, genetic drift, and consequently the increased expression of inbreeding depression (the decrease in fitness that usually occurs among progeny when normally outcrossed species are forced to inbreed; Charlesworth and Charlesworth 1987, Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005). A decrease in the number of individuals and genetic variability in a population can lead to increased homozygosity, which may consequently limit the population’s ability to withstand changes in the environment and stochastic events, and the population may become more prone to extirpation (Ellstrand and Elam 1993, Frankham et al. 2002, Frankham 2005).

If individuals in small populations are suffering from expression of inbreeding depression, mixing populations at a reintroduction site or augmentation site may increase the amount of genetic variation and heterozygosity within the new population, reduce genetic drift, increase heterosis, and increase the ability of the population to tolerate environmental stochasticity (Vergeer et al. 2005, Guerrant and Kaye 2007, Menges 2008, Frankham 2010). Heterosis can be measured as the increase in fitness of progeny from crosses between populations compared to crosses within populations (Glemin et al. 2003, Charlesworth and Willis 2009). Heterosis is due to an increase in heterozygosity and the

reduction of fixed, deleterious alleles, which is most likely due to increased drift in small populations. Inbreeding depression occurs when a normally outcrossed population is forced to self and there is genetic variation within a population for traits related to fitness, while heterosis may occur if there is genetic variation for fitness-related traits between populations (Keller and Waller 2002, Charlesworth and Willis 2009). These genetic interactions are not the only factors that need to be considered in recovery efforts of rare species.

Ecological and biological dynamics other than inbreeding depression and heterosis may be important as well in the recovery of rare plants. Factors such as breeding system, pollinator relationships, morphology, habitat differences, genetic diversity and relatedness, and recent and historic levels of gene flow between populations need to be considered in determining the appropriate individual founders to use in reintroduction attempts (Guerrant 1996, Guerrant and Kaye 2007, Menges 2008, Godefroid et al. 2011). Reintroductions, located within historic ranges of the species and protected from detrimental herbivory (*e.g.* ungulate-free and fenced areas in Hawai‘i), may be necessary to re-establish species severely threatened with extinction due to decreased population sizes and expression of inbreeding depression (Menges 2008, Weller et al. 2011). Reintroductions with large numbers of plants may circumvent density-dependent factors that may affect small populations, such as susceptibility to plant invasions or a decrease in attractiveness to pollinators (Groom 1998, Forsyth 2003, Hobbs and Yates 2003). The number of plants to reintroduce will ultimately depend on the effective population size of the rare species at the location chosen for outplanting (Guerrant 1996). Depending on the number and relatedness of remaining wild populations, reintroductions could be established with all individuals from a single population or from using individuals from multiple populations. Whether genotypes from different populations are mixed together in recovery efforts may depend on many of the factors listed here. Whether they are known or not, the balance among the history of isolation, potential adaptation to different habitats, and the potential benefits and risks of outcrossing should be explored prior to reintroduction attempts.

The potential negative consequences to mixing different individuals or small populations of the same species that have been isolated for an uncertain number of generations also need to be considered in reintroduction efforts. Outbreeding depression is the decrease in fitness of offspring due to crossing of individuals either within or between populations, and may occur in hybrid offspring whose parents were located at varying distances within populations, from different, geographically distant populations, or from different habitats regardless of geographic distance (Waser and Price 1989, Lynch 1991, Ellstrand and Elam 1993). Outbreeding depression may occur due to the breakup of co-adapted gene complexes or as a result of local adaptation to environmental conditions specific to the individual source populations (Lynch 1991, Ellstrand and Elam 1993, Waser and Price 1993, Frankham et al. 2002). Heterosis, inbreeding depression and outbreeding depression have been investigated in plant populations, including rare species (Quilichini et al. 2001, Kephart 2004, Erickson and Fenster 2006, Raabová et al. 2009) by comparing the relative fitness of selfed and outcrossed progeny, and identifying levels of inbreeding depression and/or outbreeding depression. Information from these studies can be used to guide the management of reintroductions, particularly with regard to mixing of gene pools. If wild plants from different populations (founders) are represented in an outplanting and outbreeding depression is expressed, the offspring may be less vigorous and the reintroduction could fail. Waser and Price (1994) and Waser, Price and Shaw (2000) demonstrated an optimal crossing distance between individuals in two separate species by comparing offspring fitness of hybrids whose parents were separated by various geographic distances. Their results suggest that a balance between inbreeding depression (following selfing or crossing with close relatives) and outbreeding depression (following crossing with more distant relatives) could contribute to a distance between parents that would produce maximum fitness in their progeny. Producing offspring with maximum relative fitness should be considered in designing a reintroduction for rare species. Frankham et al. (2011) identified issues that may raise the likelihood that outbreeding depression is a concern, including if populations: 1) are actually distinct species; 2) have fixed chromosomal differences; 3) have been genetically isolated for at least 500 years; and/or 4) are in different environments. The problem

facing reintroduction plans today is that these factors are mostly unknown among rare taxa.

Predicting the levels of heterosis, inbreeding depression and outbreeding depression when considering genetic sources for a reintroduction of a rare plant species is difficult, primarily due to the lack of information regarding the basic biology of most species (Guerrant 1996). The breeding system and mating system of the species can provide insight into the amount if inbreeding depression expected and the likelihood of outbreeding depression during restoration efforts (Dudash and Murren 2008). If a species is primarily selfing, a long history of inbreeding will have resulted in expression of recessive deleterious alleles, and purging of strongly deleterious alleles is likely to have already occurred (Lande & Schemske 1985, Charlesworth & Charlesworth 1987, Husband & Schemske 1996). Mildly deleterious alleles, however, may still be present within the population, allowing for the expression of inbreeding depression in later life stages (Lande and Schemske 1985, Husband and Schemske 1996). As a result, inbreeding depression may be cryptic and difficult to assess in species with a long history of selfing (Lande and Schemske 1985, Husband and Schemske 1996, Weller et al. 2005). For predominantly outcrossing species, the chance of expressing inbreeding depression may increase as selfing increases (Lande & Schemske 1985, Charlesworth & Charlesworth 1987, Husband & Schemske 1996). Populations may show less outbreeding depression upon mixing of populations if there has been recent gene flow between the parent populations. If individuals of small populations of a particular taxon (selfers or outcrossers) are suffering from inbreeding depression, the chance of establishing a new self-sustaining population (via reintroduction) may increase with the combination of source material from multiple small populations. The goal of this study was to determine how the balance of these genetic issues could influence to the design and success of the reintroduction of a rare plant species.

Study Taxon: The endemic Hawaiian genus *Schiedea* (Caryophyllaceae) was chosen for this study due to its rarity as well as its diversity in habitat, breeding systems, and mating patterns. *Schiedea* is one of the largest adaptive radiations of vascular plants in the Hawaiian Islands, comprised of 34 species that evolved from a single hermaphroditic

ancestor (Wagner et al. 1999, Willyard et al. 2011). Research on *Schiedea* has focused on the phylogeny, genetic diversity, and evolution of dimorphic breeding systems (Weller and Sakai 1990, Weller et al. 1995, Weller et al. 1996, Weller et al. 1998, Weller et al. 2001, Wagner et al. 2005, Sakai et al. 2006, Sakai et al. 2008). Six species have shown high levels of inbreeding depression, with either low levels of selfing in hermaphroditic species, or high levels of selfing in hermaphrodites of dimorphic species (Sakai et al. 1989, Norman et al. 1995, Sakai et al. 1997, Culley et al. 1999, Rankin et al. 2002, Weller and Sakai 2005). A seventh species showed low levels of inbreeding depression and high levels of selfing (Weller et al. 2005). All species in the *S. nuttallii* clade in section *Mononeura* (Wagner et al. 2005; but see also Willyard et al. 2011) are hermaphroditic, and have been understudied in comparison to other sections. Levels of inbreeding depression have not been assessed for any of these species. *Schiedea kaalae* Wawra is a perennial coarse shrub in section *Mononeura*. It has slightly protandrous flowers that are likely insect pollinated and capable of self-fertilization (Wagner et al. 2005, Weller et al. 1998). Pollen:ovule ratios of *S. kaalae* indicate that this species has a facultative xenogamous mating system (Cruden 1977, Weller et al. 1998). Hermaphroditism could be stable in this species, either through a combination of high levels of inbreeding depression and low levels of selfing, or low levels of inbreeding depression and high levels of selfing (Lande and Schemske 1985). Though inflorescence morphology and pollen:ovule ratios suggest facultative outcrossing as the predominant mating system (and consequently high inbreeding depression and low selfing rates), historically small population sizes may maintain higher than predicted levels of selfing in this species. If this is true, low inbreeding depression and high selfing rates may be the most recent mating system that has been occurring in this species.

Schiedea kaalae is located in both the Koʻolau and Waiʻanae Mountain Ranges of Oʻahu, where it was known from mesic gulches (Waiʻanae Mountains) to wet forests (Koʻolau Mountains) on steep, shaded slopes (Table 4.1; Fig. 4.1) (Wagner et al. 1999). This species is extremely rare and was federally listed as endangered in 1991 (Ellshoff et al. 1991). Relatively little is known about previous population sizes, and no recent observations (within the last 30 years) have documented populations greater than 20 individuals. Notes from a field collection by Wayne N. Takeuchi, a botanist who made

extensive collections on Oʻahu, suggested more plants were discovered than he had expected when he saw the ʻĒkahanui population of approximately 10 plants in 1987, though he concluded that the species was not common: "this sp. is not as rare as previously supposed, but still uncommon" (Takeuchi and Paquin 1987; collection no. 3442). Lifespan is uncertain in *S. kaalae,* and no plant has been documented from seedling through maturity and to death (Keir and Weisenberger 2011). It is assumed from observing mature plants over the last decade that this species is long-lived (>10 years). Isolated plants are able to self-fertilize and produce viable seeds that yield reproductive progeny. There were four known locations in the Koʻolau Mountains; three are extant (one with only a single individual) and three were available for this study (Kaipapaʻu, Maʻakua and Kahana). There were ten known locations in the Waiʻanae Mountains; three are extant (two with only one individual) and seven are represented in this study (Pālāwai, ʻĒkahanui-A, ʻĒkahanui-B, Huliwai, Kaluaʻa, Pahole-A and Pahole-B). Fruit collections and cuttings have been taken from many individuals, some of which are now extirpated.

The following questions were addressed in this study:

1. Is inbreeding depression and/or heterosis detectable in *S. kaalae*?
2. What combination of founders (wild plants either extant or extirpated but with *ex situ* representation) will yield the most relatively fit progeny?
3. Is there outbreeding depression, and a distance between source populations that once exceeded, results in expression of outbreeding depression in the progeny?
4. Is there a home site advantage for populations in particular mountain ranges? Should founders from both mountain ranges be outplanted together?

To address these questions, fitness of progeny from crosses within and between populations and the fitness of selfed progeny were assessed in field settings comparable to locations were reintroductions would take place in both mountain ranges. A field setting was used to test fitness of progeny from crosses to mimic conditions likely to occur during reintroduction efforts, and to estimate levels inbreeding depression under field conditions rather than greenhouse conditions likely to provide unrealistic estimates

of the genetic effects of selfing and outcrossing on fitness (Dudash 1990, Husband and Schemske 1996). If there is outbreeding depression and plants perform better in their respective mountain ranges, then outbreeding depression is likely due to local adaptation. If plants from both mountain ranges perform equally well in both mountain ranges, then outbreeding depression, if detected, is more likely due to the breakup of coadapted gene complexes.

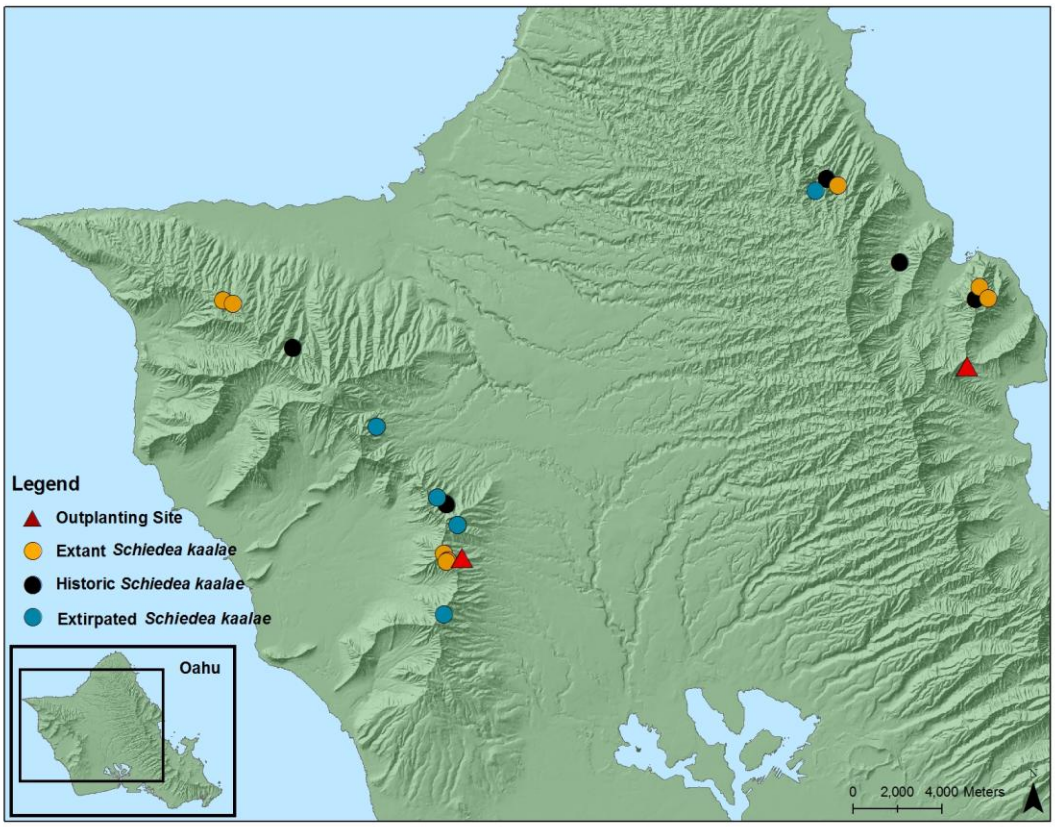

Fig. 4.1. Historic (last seen prior to 1984) and Recent Distribution of *Schiedea kaalae* on Oʻahu, Hawaiʻi

Methods

Hand Pollinations: Nursery plants used as seed source for this study were propagated from clonal cuttings and seed, representing 23 wild plants (founders; Table 4.1). Plants were grown under pollinator-free conditions at Pahole Mid-elevation Nursery in Mokulēʻia Forest Reserve (656m elev.) and Harold. L. Lyon Arboretum in Mānoa Valley

on O‘ahu (~200m elev.). All plants were initially located at Harold. L. Lyon Arboretum in the spring 2008. High summer temperatures forced the relocation of the plants to Pahole Mid-elevation Nursery in July 2008 where pollinations were completed in September 2008.

Flowers were emasculated just prior to dehiscence of the anthers. Stigmas were receptive for pollination two days after emasculation, when stigmas had increased in length and width and the stigmatic papillae were elongated. Only fresh pollen available the day of pollination was used in pollinations. Pollen was applied by gently touching receptive stigmas with dehisced anthers containing pollen, and the yellow pollen easily stood out on white stigmas. Stigmas were examined to ensure that pollen had been deposited along the length of the receptive stigmatic surface. Pollination treatments included flowers that were unmanipulated (control), emasculated and not pollinated (emasculated), hand-pollinated with pollen from the same plant (selfed), or pollinated with pollen from a different plant. Crosses with pollen from a different plant were either from within a population (within) or between the two populations (between). A minimum of 25 pollination were attempted for every cross treatment, including every combination of populations for interpopulation cross. Self-pollinations were attempted for the progeny of at least one founder in each of the populations. The crosses within a population treatment were performed for the ‘Ēkahanui and Kahana population sources only. The lack of populations with more than one founder severely limited the ability to distinguish between inbreeding depression and heterosis. Capsules were collected at maturity (tan, dry) when seeds were ripe (black). The seeds of each fruit collection were counted and held in storage (20% relative humidity at -18C) for approximately one to six months prior to germination.

The number of fruit per flower and seeds per pollination (including controls) were determined for each treatment and nursery plant as a total for each plant. Emasculated flowers did not produce any seeds, and were consequently removed from analyses. Mean mass (mg) per seed was also calculated as the mass of the number of seeds produced for each nursery plant by cross treatment over the number of seeds produced. The dry weight of seeds was obtained upon removal from storage and just prior to sowing. Seed viability

was calculated as the number of seeds that germinated (root and shoot visible) out of the total sown for a sample of fruits collected on different days per nursery plant. All seeds that did not germinate within four months had rotted prior to dissection (inviable). The control treatments were excluded from the analyses following measurement of seed mass.

Table 4.1. Historic and Current Distribution of *S. kaalae* and Representation in Study

Population	Population Code	Max. Known Plants*	N Extant Plants*	N Plants Represented in Crosses	Mountain Range** & (Elev. [m])	Annual Average Max. Temp. (F) & Rainfall (mm)
'Ēkahanui	EKA-A/B	15	13	11	W (787)	73.40F & 1178mm
Pālāwai	PAL-A	1	0	1	W (791)	75.20F & 1073mm
Huliwai	HUL-A	1	0	1	W (702)	75.20F & 1226mm
Mohiākea	SBW-A	1	0	0	W (725)	75.20F & 1395mm
Pahole	PAH-A	2	1	1	W (540)	77.00F & 1588mm
Pahole	PAH-B	1	1	1	W (576)	77.00F & 1567mm
Kaluaʻa	KAL-A	1	0	1	W (643)	75.20F & 1256mm
Maʻakua	MAA-A	30	6	2	K (220)	78.80F & 4088mm
Kaipapaʻu	PAP-A	2	0	2	K (377)	77.00F & 5122mm
Kahana	KNA-A	10	7	3	K (408)	75.20F & 2056mm
ʻĒkahanui	Study site	NA	NA	NA	W (591)	75.20 & 1142mm
Hakipuʻu	Study site	NA	NA	NA	K (194)	78.8F & 2664mm

* Only mature founders are shown here
** W = Waiʻanae Mts., K = Koʻolau Mts.

Propagation: Seeds were removed from storage, weighed, and sown in January 2009. Enough seeds were germinated to obtain 20 plants per treatment to be outplanted in a field setting. Seeds were germinated on 1% water agar (Sigma-Aldrich® A1296) in Petri dishes in a Controlled-Environment Chamber (Pervical Scientific, Inc. E36V). Seedlings were propagated in a 3:1 mix of perlite to vermiculite and placed onto a mist bench for two months prior to being transplanted each into their own pot (RS400: 4" round, standard pot, Exacta Sales, Inc.) where they were grown at O'ahu Army Natural Resources Program's (OANRP) East Range nursery (Wahiawa, 247m elevation, 1283mm annual rainfall, 26.1C annual maximum temperature [WorldClimate.com copyright 1996-2011]) until the field outplanting seven months later. After transplanting, 20 plants each from the selfed and within-population crosses ('Ēkahanui and Kahana only), as well as every population x population cross were randomly selected to be in the field experiment. Some crosses were not available because one or more variables (flower production to seed viability) were too low to produce enough plants for some of the population sources. Crosses where the Ma'akua population was used as the seed and/or pollen parent were limited by flower production in the nursery ([female x male]; PAH-B & PAP-A x MAA-A AND MAA-A x PAL-A & MAA-A [within]). The crosses that were completed (25 pollination attempts), but for which there were not at least three replicates at both outplanting sites included: EKA-A x PAL-A; KAL-A x EKA-A; KNA-A x Self & KNA-A; MAA-A x Self & HUL-A; PAL x MAA-A.

Outplanting Sites: Two outplanting sites were established in the Waianae and Koolau mountain ranges. Sites were selected by the OANRP Rare Plant Program Manager (Matthew J. Keir) and chosen based on suitable habitat, appropriate distance from extant populations, and protection from ungulates through fencing. The outplanting site in the Wai'anae Mountain Range was located in 'Ēkahanui Gulch (State of Hawaii) 834m down slope from the extant population. The outplanting site in the Ko'olau Mountain Range was located in Hakipu'u Gulch (Kualoa Ranch) 3550m away from the nearest known population, and is considered to be outside of the known range of this species (Fig. 4.1). For each site planting occurred in October 2009 at the onset of the rainy winter season and prior to flowering. Plants were randomly packed into tubs of 20 plants each and each tub was placed within the designated planting area. Ten plants of each treatment and

population cross combination (except where noted above) were planted at both sites (randomly split from the 20 plants that were chosen originally). A total of 713 plants were planted at Hakipuʻu and 734 plants were planted at ʻĒkahanui.

Plants were monitored every month for one year. The total number of inflorescences per plant (0 inflorescences = immature; ≥ 1 inflorescences = mature) and date of mortality were recorded for each plant. In addition, three plants each from each cross treatment of every source population were randomly selected to count either the number of flowers or number of fruit on one inflorescence. Typically, the first inflorescence on the plant was chosen for counting. If the chosen plant died before counts were made, another unmeasured plant was randomly selected for measurement. In these cases, the inflorescence chosen may not have been the first to flower. At least three fruit per plant were collected from three randomly chosen plants from every treatment per population, including every population x population combination to estimate seed set (number of seeds per fruit). Plant height (measured from the base of the stem to the most distal apical meristem), the length of the longest leaf (measured from the base of the petiole to the tip of the leaf), and the total number of leaves were measured bimonthly for the first six months on every plant, with a final measurement 12 months following planting. Size was measured as a product of final plant height (cm), number of leaves, and length of longest leaf. Total growth from the first month after planting to the 12^{th} month after planting was calculated as (final size – initial size)/ initial size.

An overall fitness estimate for each plant was calculated using the number of inflorescences per plant, number of flowers and fruit per inflorescence, and number of seeds per fruit. Female fitness was estimated as the total number of seeds each plant produced, calculated as the product of the total number of inflorescences per plant, the mean number of fruit per inflorescence and the mean number seeds per fruit. If a treatment only had two replicates for either mean fruit per inflorescence or seeds per fruit, the mean for that maternal source was used as the third replicate (Table 4.2). If fewer than two replicates were available for either or both variables, the cross was eliminated from the analyses. Crosses eliminated at the Hakipuʻu site only were: EKA-A x EKA-A, EKA-B x PAH-A & Self, HUL-A x Self & EKA-A, MAA-A x PAP-A, PAH-

B x PAH-A, PAL x HUL-A). Male fitness was estimated as the total number of flowers each plant produced, calculated as the product of the total number of inflorescences per plant and the mean number of flowers per inflorescence. Differences among the cross treatments (selfed, crosses within a population, crosses between populations) and populations were investigated for female and male fitness. Maternal source populations were also grouped by mountain range (Waiʻanae or Koʻolau). The distance between any two given populations was used to discern how fitness relates to the proximity of parent sources (selfed, crosses within a population, crosses among close populations, crosses among far populations, and crosses among mountain ranges). To simplify these categories they were grouped by distance (Table 4.3). Crosses within populations were eliminated from the female and male fitness calculations, number of fruit per inflorescence, and number seeds per fruit due to low sample size.

Table 4.2: Crosses where fitness calculations were comprised of either mean Seed Set (S) or Fruit Set (F) and consisted of 2 values from that cross plus the mean for the maternal source. Outplant sites include ʻĒkahanui (E) and Hakipuʻu (H).

Maternal Population	Paternal Population	Adjusted Means	Outplant Site
ʻĒkahanui-A	Kaluaʻa	F	H
ʻĒkahanui-A	Pahole-A	S (F)	H, E
ʻĒkahanui-A	Maʻakua	S	H
ʻĒkahanui-A	Self	S + F (F)	H, E
ʻĒkahanui-A	Huliwai	S + F	H
ʻĒkahanui-B	ʻĒkahanui-A	S	H
ʻĒkahanui-B	Pahole-B	S	H
ʻĒkahanui-B	Maʻakua	F	H
Huliwai	Pālāwai	S + F	H
Huliwai	Maʻakua	S	H
Kaluaʻa	ʻĒkahanui-A	S + F	E
Kaluaʻa	Pahole-B	S	H
Kaluaʻa	Pahole-A	S	H
Kahana	Pālāwai	S	E
Maʻakua	Kahana	S	H
Maʻakua	ʻĒkahanui-A	S	H
Maʻakua	Pahole-B	S (S + F)	H, E

Maʻakua	Kaipapaʻu	S	E
Pahole-A	Pahole-B	S	H
Pahole-A	Self	S	H
Pahole-A	Huliwai	S	H
Pahole-B	Kahana	S	H
Pahole-B	Pālāwai	S	H
Pahole-A	ʻĒkahanui-A	S	H
Pālāwai	Pahole-B	S	H
Pālāwai	Maʻakua	S + F	E
Pālāwai	Self	S	E
Kaipapaʻu	Pahole-B	S	H
Kaipapaʻu	Self	S	H

Table 4.3. Distance Categories for Maternal x Paternal Populations

Category	Distance (ft)
Selfed	0
Crosses Within a Population	< 400
Crosses Among Close Populations	400-5000
Crosses Among Far Populations	5000-20000
Crosses Among Mountain Ranges	> 20000

Analyses: Data were analyzed using SPSS 19 (IBM®). All means, standard errors, and sample sizes for all stages are in Appendix 2A. The data for the number of inflorescences a plant produced were transformed (log10 x+1) for homogeneity of variance and normality, and the transformation for the growth of plants was (sqrt(log10(x+2)). General Linear Model 3 (GLM3, two-way (factorial) ANOVA) was used for all parametric tests. Kruskal-Wallis and Mann-Whitney U tests were used to analyze all non-parametric data. A three-way loglinear analysis was used on the survival and maturity data. The maturity data were also further analyzed with z-scores from cross-tabulation tables to describe specific interactions. The Bonferroni correction was applied to the significance value (alpha) in order to prevent inflation of the Type I error (.05/number of comparisons). Tukey's *post hoc* test was used whenever samples sizes were close to equality. Hochberg's GT2 (Field 2009) was the *post hoc* test most commonly used when sample sizes varied slightly because this test is more conservative under these conditions. The

Games-Howell *post hoc* test was used following analyses of variances with unequal variances. The Helmert Contrast (Field 2009) was used to determine if means varied between individual cross treatments. Cumulative fitness was calculated as the product of the mean number of seeds per pollination, survival, number of inflorescences per plant, number of fruit per inflorescence, and number of seeds per fruit. Means for each founder (maternal family) were averaged to produce a population mean for the three populations that had more than one founder. Founder means for survival were calculated if more than two progeny per founder were present. All other stages were calculated if a founder was represented by at least one progeny (plant). To quantify levels of heterosis, inbreeding depression and outbreeding depression, Ågren and Schemske's (1993) "relative performance of crosstypes" (*RP*) was calculated for each stage in all populations as well as for cumulative fitness in all populations as follows:

4. Inbreeding / Outbreeding Depression:

   $RP_i = 1 - w_s / w_w$ if $w_s \leq w_w$,

   and

   $RP_i = w_w / w_s - 1$ if $w_s > w_w$,

   where w_s is the fitness of the selfed progeny and w_w is the fitness of the progeny from crosses within populations.

5. Heterosis / Outbreeding Depression:

   $RP_h = 1 - w_w / w_b$ if $w_w \leq w_b$,

   and

   $RP_h = w_b / w_w - 1$ if $w_w > w_b$,

   where w_b is the fitness of the progeny from crosses between populations.

6. Selfed Progeny / Progeny of Crosses Between Populations:

   $RP_b = 1 - w_s / w_b$ if $w_s \leq w_b$,

   and

   $RP_b = w_b / w_s - 1$ if $w_s > w_b$,

where w_s is the fitness of the selfed progeny and w_b is the fitness of the progeny from crosses between populations. This latter statistic was necessary due to the absence of within-population crosses when there was only one plant at a source population.

Inbreeding depression and heterosis cannot be differentiated in this one ratio, but outbreeding depression can be detected, as well as a benefit to outcrossing, as the only options for these populations are to self or outcross with other populations.

RP values range between -1 and +1, where positive values for RP_i indicate that progeny from crosses within populations outperform selfed progeny; inbreeding depression is consequently present. Negative values indicate outbreeding depression within populations. For RP_h, positive values indicate heterosis and progeny from crosses between populations outperform progeny from crosses within populations. A negative RP_h value therefore indicates outbreeding depression between populations. A GLM1 (one-way ANOVA) was used to compare cumulative fitness means among cross treatments. A one-sample *t* test was used to compare RP_i, RP_h, and RP_b values for each selected variable, including cumulative fitness, to determine if they were significantly different from zero. A GLM1 was performed to determine if there were differences among the populations for each *RP* value (combining all variables).

Results

Pre-planting: Whether or not the maternal source was from the Koʻolau Mountains or Waiʻanae Mountains did not affect on the percent of pollinations that formed a fruit (Fig. 4.2; Mann-Whitney U = 7541, z = 1.491, $p = 0.136$). Maternal source population affected the number of fruits per pollination (Fig. 4.3; Kruskal-Wallis, $\chi^2 = 33.027$, df = 9, $p < 0.001$). Plants from the Kaluaʻa maternal population had fewer pollinations yield fruit than the plants from the Huliwai and ʻĒkahanui-B lines (Mann-Whitney U = 47, z = 3.866, $p < 0.001$; Mann-Whitney U = 46.5, z = 3.413, $p = 0.001$, respectively). Distance between parent populations (including controls) affected the number of fruit per pollination (Kruskal-Wallis, $\chi^2 = 27.519$, df = 5, $p < 0.001$). The control treatment produced fewer fruit per flower than all treatments (Mann-Whitney U [self] = 42, z = 3.205, $p = 0.001$; Mann-Whitney U [crosses within populations]= 49, z = 2.088, $p < 0.037$; Mann-Whitney U [400-5000m] = 53, z = 3.898, $p < 0.001$; Mann-Whitney U [5000-20000m] = 56, z = 4.021, $p < 0.001$; Mann-Whitney U [>20000m] = 69.5, z =

4.190, $p < 0.001$). Flowers that were selfed formed less fruit than flowers that received pollen from other populations (Mann-Whitney U = 2729, z = 3.258, $p = 0.001$). Paternal source population also affected the number of fruit per pollination (Fig. 4.4; Kruskal-Wallis, $\chi^2 = 49.416$, df = 10, $p < 0.001$). Plants with the paternal parent from Pālāwai had fewer pollinations form fruit than plants with the paternal parent from Huliwai (Mann-Whitney U = 100, z = 3.506, $p < 0.001$) and from Kaipapaʻu (Mann-Whitney U = 21.5, z = 4.635, $p < 0.001$). Plants with paternal parents from Maʻakua also had fewer fruit per pollination than plants with the paternal parent from Kaipapaʻu (Mann-Whitney U = 59.5, z = 3.761, $p < 0.001$).

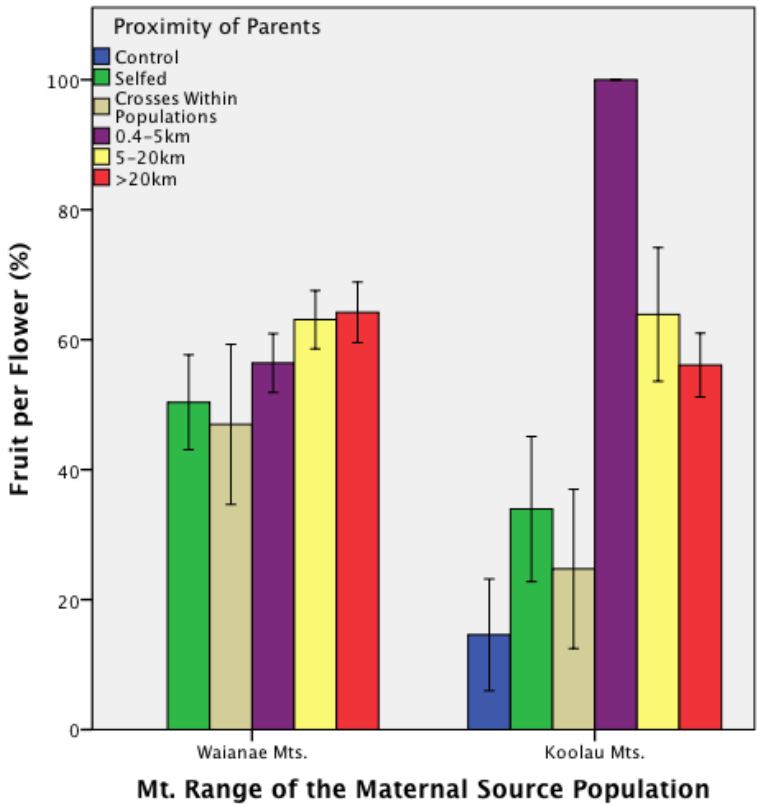

Fig. 4.2. Mean percentage of flowers that formed fruit by maternal source mountain range and geographic distance between parent source populations. Error bars ± 1SE. All values (n = 3) for the control on Waiʻanae Mt. plants were zero.

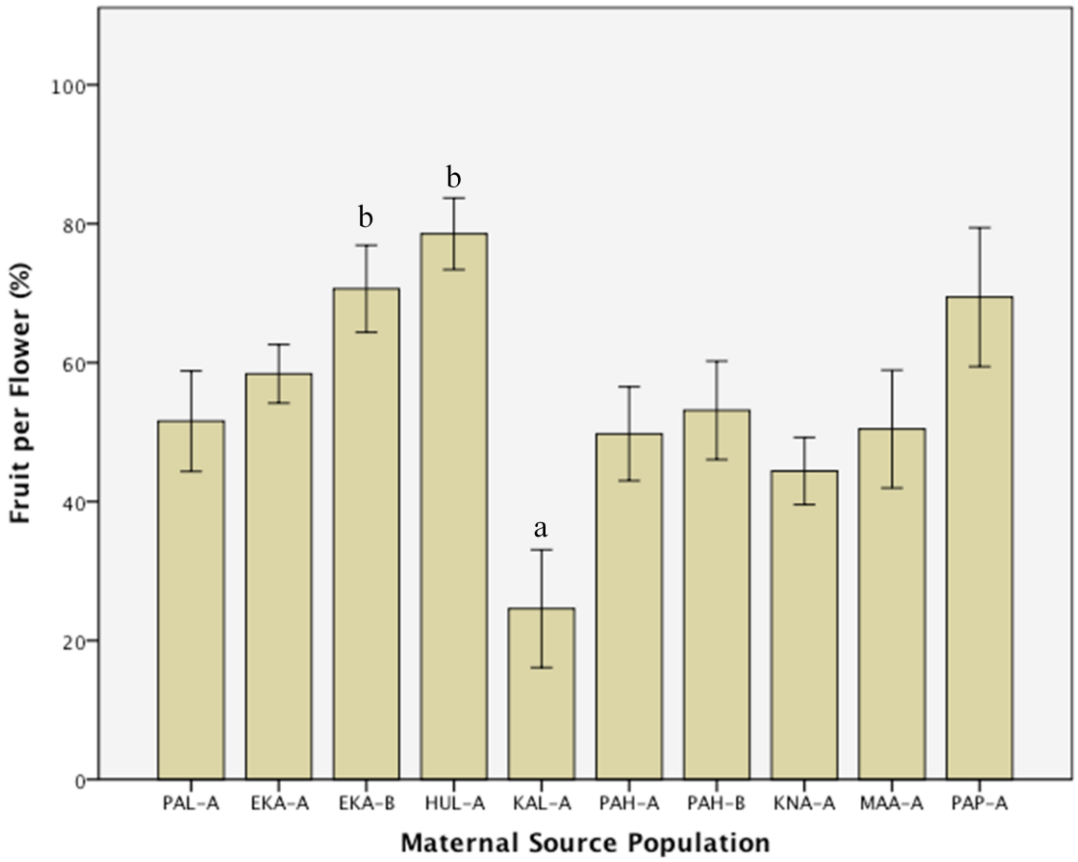

Fig. 4.3. Mean number of fruit per pollination by maternal source population. Error bars ± 1 SE. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Values labeled a and b are significantly different from each other ($p \leq 0.001$).

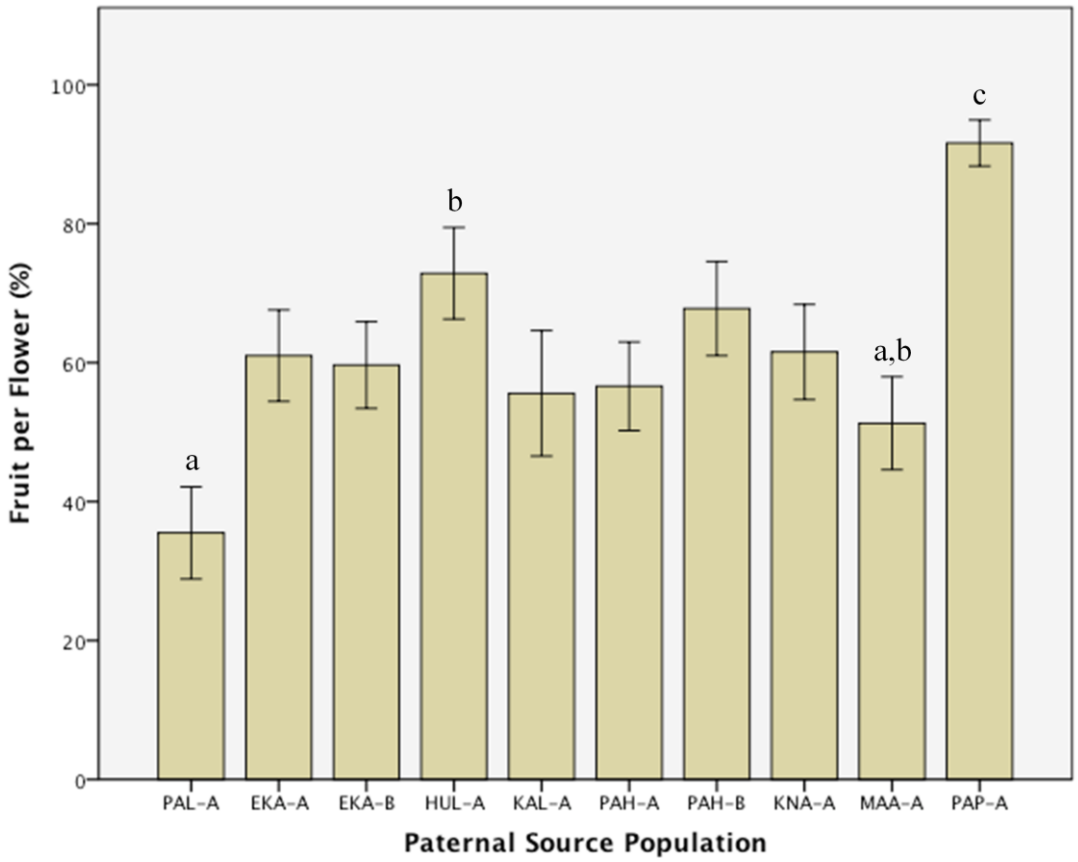

Fig. 4.4. Mean number of fruit per pollination by paternal source population. Error bars ± 1 SE. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Values labeled by different letters are significantly different from each other ($p < 0.001$).

The mountain range of the maternal source did not affect the number of seeds per pollination (Fig 4.5; Mann-Whitney U = 7232, z = 1.969, $p = 0.05$), but the maternal source population did affect the number of seeds per pollination (Fig. 4.6; Kruskal-Wallis, $\chi^2 = 52.771$, df = 9, $p < 0.001$). Plants with maternal sources from Kaluaʻa and Kahana produced fewer seeds per pollination than plants with maternal sources from Huliwai (Mann-Whitney U = 27.5; 181.5, z = 4.224; 5.079, $p < 0.001$, respectively) and ʻĒkahanui-B (Mann-Whitney U =27.5; 143, z = 3.788; 4.598, $p < 0.001$, respectively) and Kaipapaʻu (Mann-Whitney U = 39.5; 175, z = 2.859; 3.316, $p = 0.004$; 0.001,

respectively). The distance between parent populations also affected seed set (Fig. 4.5; Kruskal-Wallis, $\chi^2 = 29.611$, df = 5, $p < 0.001$). Plants from crosses within populations produced fewer seeds per pollination than plants from crosses between populations 5000-20000m apart (Mann-Whitney U = 385.5, z = 3.002, $p = 0.003$). The control treatment produced the least amount of seeds per pollination; less than the selfed treatment (Mann-Whitney U = 38, z = 3.333, $p = 0.001$), the crosses 400-5000m apart (Mann-Whitney U = 43.5, z = 4.053, $p < 0.001$), crosses 5000-20000m apart (Mann-Whitney U = 52, z = 4.058, $p < 0.001$) and crosses > 20000m apart (Mann-Whitney U = 62, z = 4.250, $p < 0.001$). Paternal source population also affected the number of seeds per pollination (Fig. 4.7; Kruskal-Wallis, $\chi^2 = 49.881$, df = 10, $p < 0.001$). Plants with a paternal parent from the Kaipapaʻu population produced more seeds per pollination than plants with a paternal parent from the ʻĒkahanui-B (Mann-Whitney U = 65.5, z = 3.596, $p < 0.001$), Maʻakua (Mann-Whitney U = 52, z = 3.866, $p < 0.001$) and Pālāwai (Mann-Whitney U = 26.5, z = 4.429, $p < 0.001$).

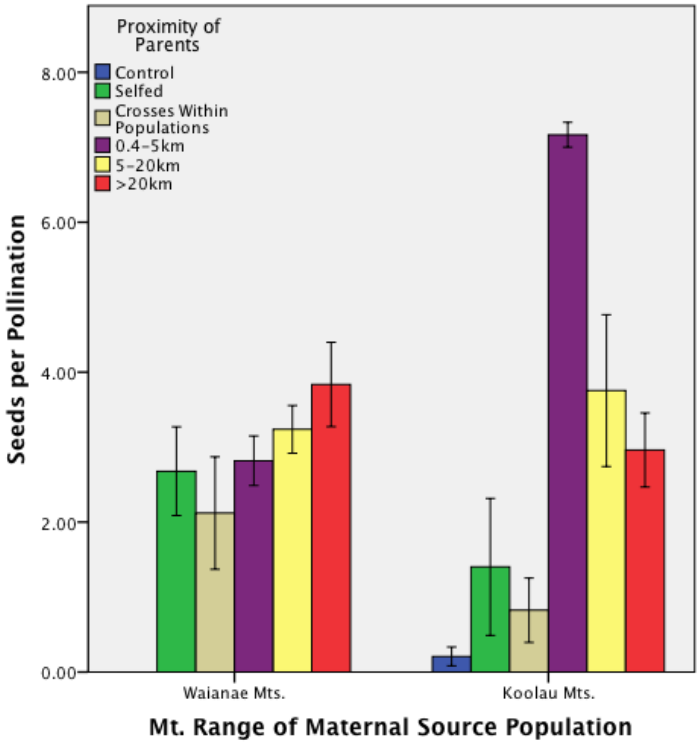

Fig. 4.5. Mean seeds per pollination (pollination) by geographic distance between parents and mountain range of maternal source. Error bars ± 1 SE.

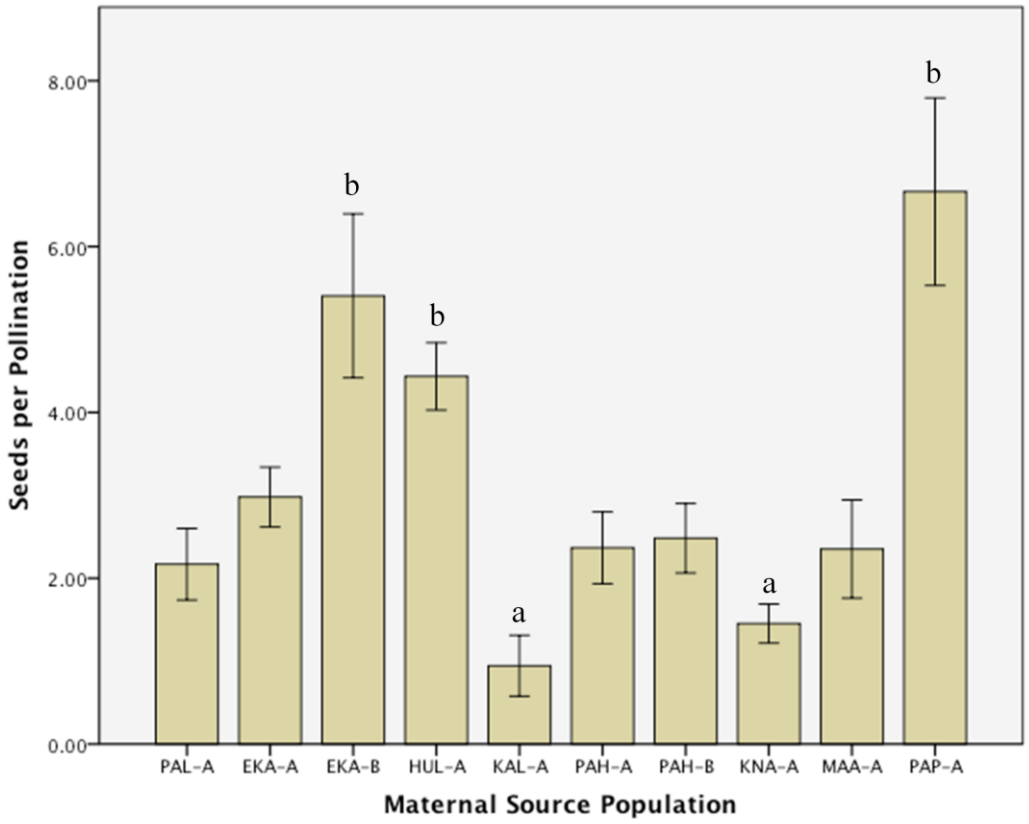

Fig. 4.6. Mean seeds per pollination by maternal source population. Error bars ± 1SE. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Values labeled different letters are significantly different from each other.

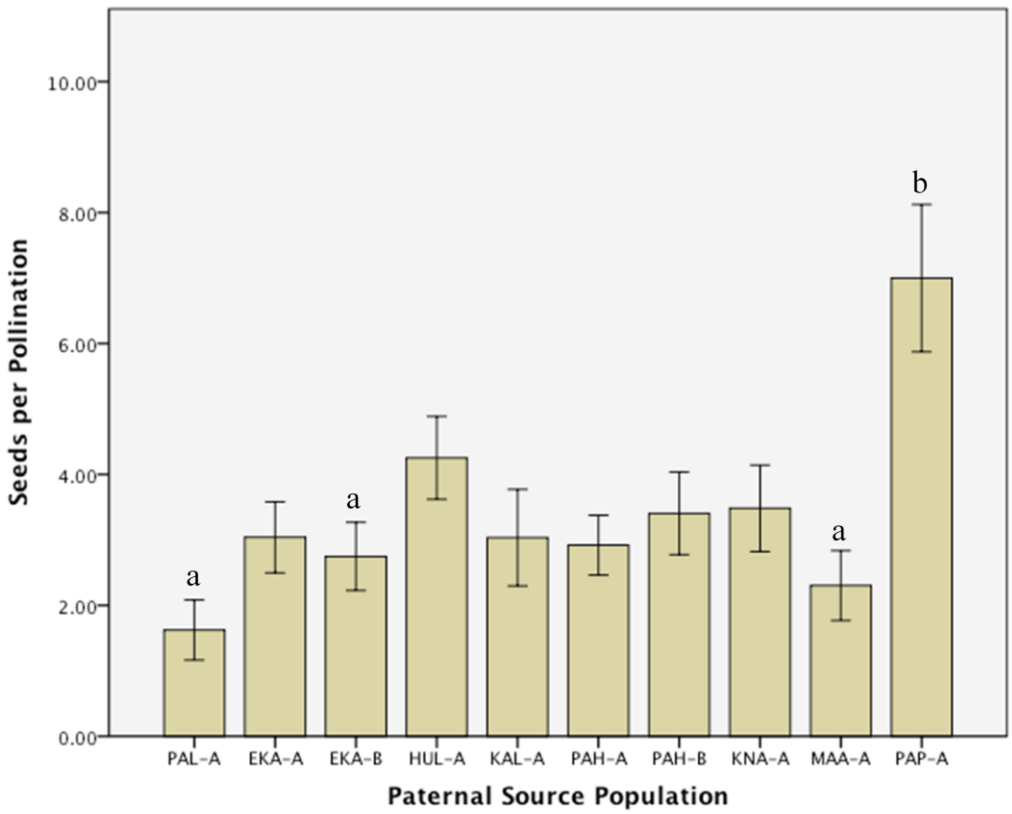

Fig. 4.7. Mean seeds per pollination by paternal source population. Error bars ± 1SE. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Values labeled different letters are significantly different from each other.

The mountain range of the maternal source population, the proximity of parents, and their interaction did not affect the mass per seed (GLM3, F = 0.032, df = 1, p = 0.859; F = 1.796, df = 4, p = 0.130; F = 1.348, df = 4, p = 0.253, respectively). Neither the paternal source population nor its interaction with maternal source population affected seed mass (GLM3, F = 1.040, df = 11, p = 0.414, F = 0.658, df = 79, p = 0.979, respectively). Maternal source population, however, did affect seed mass (Fig. 4.8; GLM3, F = 3.807, df = 9, $p < 0.001$). Seeds with the Pālāwai maternal population weighed more than seeds with the ʻĒkahanui-A (Hochberg's GT2, mean diff. = 0.119 ± 0.027, p = 0.001), and Kaluaʻa (mean diff. = 0.172 ± 0.042, p = 0.003).

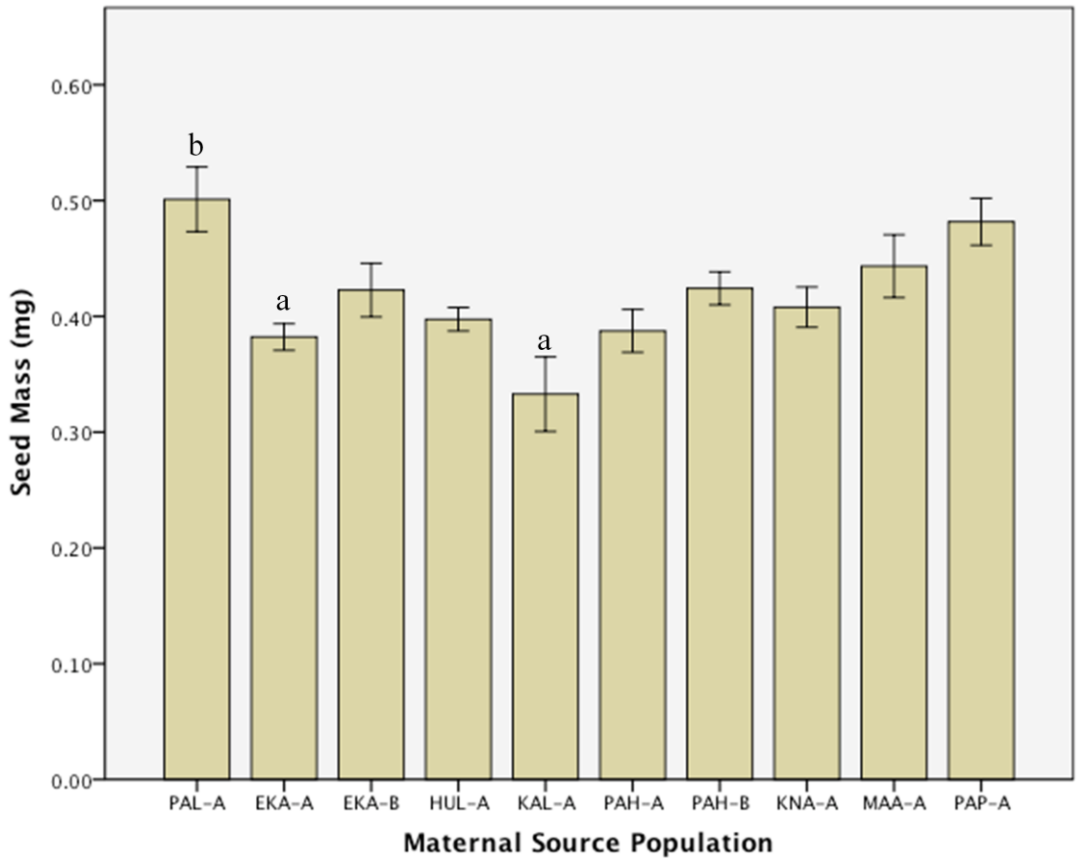

Fig. 4.8. Mean seed mass (mg) per nursery plant by maternal source population. Error bars ± 1 SE. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Values labeled different letters are significantly different from each other.

The mountain range of the maternal source significantly affected seed viability (Fig. 4.9; Mann-Whitney U = 20326, z = 4.072, $p < 0.001$). Seeds from plants with maternal sources from the Waiʻanae Mountains had higher viability than seeds of plants with maternal sources from the Koʻolau Mountains. The proximity of the parental sources did not affect the viability of the seeds collected (Kruskal-Wallis, $\chi^2 = 14.494$, df = 4, $p = 0.006$ [but no individual mean comparisons significant with the Bonferroni correction and alpha < 0.005]), and neither did paternal source population (Kruskal-Wallis, $\chi^2 = 15.032$, df = 11, $p = 0.181$). Maternal sources, however, did affect seed viability (Fig. 4.10; Kruskal-Wallis, $\chi^2 = 73.196$, df = 9, $p < 0.001$). Seeds from the

ʻĒkahanui-A and ʻĒkahanui-B maternal source population had lower viability than seeds from the Pahole-A and Pahole-B lines (Mann-Whitney U, ʻĒkahanui-A: z = 3.342, p = 0.001; z = 4.469, $p < 0.001$; ʻĒkahanui-B: z = 4.486, $p < 0.001$; z = 5.487, $p < 0.001$). Seeds from the ʻĒkahanui-B population also had lower viability than seeds from the Huliwai population (z = 4.118, $p < 0.001$). Seeds from the Kahana maternal source population had lower viability than seeds from Huliwai, Maʻakua, Pahole-A, and Pahole-B (z = 4.970, $p < 0.001$; 3.438, $p = 0.001$; z = 4.899, $p < 0.001$; z = 6.016, $p < 0.001$). Seeds with maternal parents from Kaipapaʻu also had lower viability than seeds with maternal parents from Huliwai, Pahole-A and Pahole-B (z = 3.289, $p = 0.001$; 3.821, $p < 0.001$; z = 4.825, $p < 0.001$). Lastly, seeds with maternal parents from Kaluaʻa and Pālāwai have lower viability than seeds with maternal parents from Pahole-B (z = 3.191, $p = 0.001$; z = 3.382, $p = 0.001$).

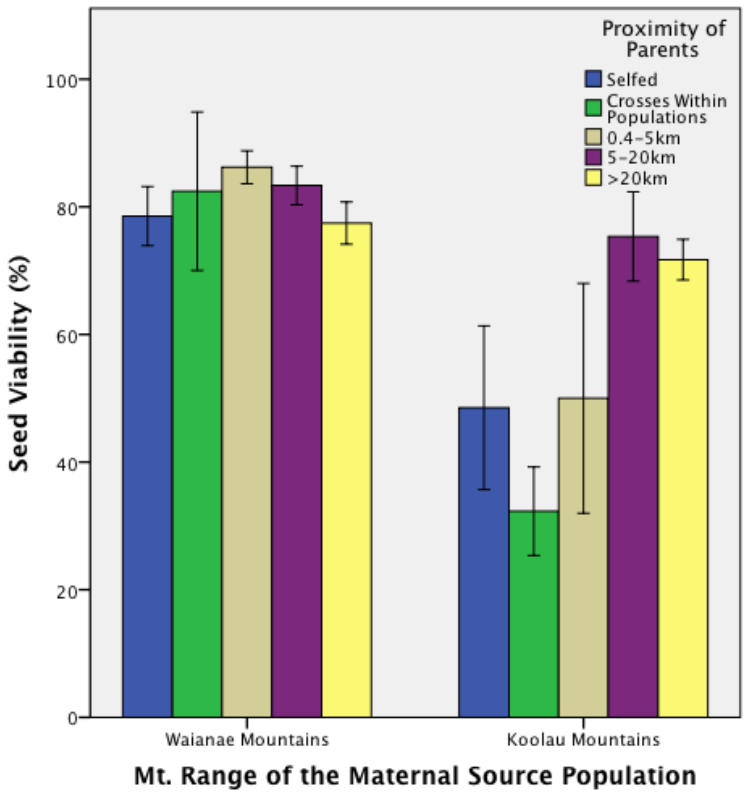

Fig. 4.9. Mean seed viability (%) by mountain range of maternal source and proximity of parent populations. Error bars ± 1SE.

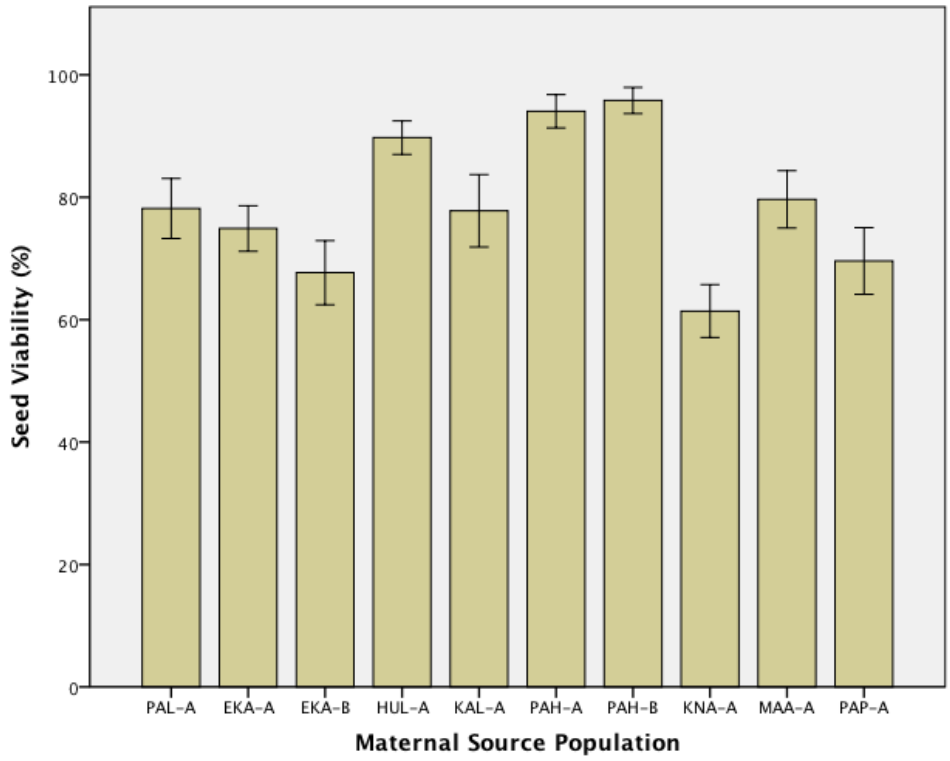

Fig. 4.10. Mean seed viability (%) by maternal source population. Error bars ± 1SE. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Refer to text for populations significantly different from each other.

Post-Planting: Nearly all plants (84.5%, range 50%-100% by maternal source) survived the first year. A final model was produced by a three-way loglinear analysis of the mountain range of the maternal source population, outplanting site, and survival. The final model is a good fit of the data ($\chi^2 = 0$, df = 0, $p = 1$ for the likelihood ratio statistic of the final model; the expected values generated by the model are not significantly different from the observed data). The test of the value of the three-way interaction was significant (K-way Effects, likelihood ratio, $\chi^2 = 5.572$, df = 1, $p = 0.018$). A larger percentage of plants with maternal sources from the Waiʻanae Mountains died at the Hakipuʻu outplanting site than at ʻĒkahanui; which is located in the Waiʻanae Mts (parameter estimate = 0.106 ± 0.044, z = 2.393, $p = 0.017$). The test of the value of the

two-way interactions was significant, and removing the two-way interactions would have been significantly detrimental to the model (K-way effects, likelihood ratio, $\chi^2 = 40.682$, df = 3, $p < 0.001$). The two-way interaction between outplanting site and survival was significant and a larger percentage of plants died at the Hakipu‘u outplant site than at the ‘Ēkahanui outplant site (Fig. 4.11; partial $\chi^2 = 38.063$, df = 1, $p < 0.001$). The mountain range of the maternal source population, however, did not affect survival ($\chi^2 = 2.629$, df = 1, $p = 0.105$).

Another final model produced by the loglinear analysis of the maternal source population, proximity of parents, and survival was a good fit of the data $\chi^2 = 51.512$, df = 45, $p = 0.234$ for the likelihood ratio statistic of the final model; the expected values generated by the model were not significantly different from the observed data). The test of the value of the three-way interaction was not significant (K-way Effects, likelihood ratio, $\chi^2 = 47.616$, df = 36, $p = 0.093$). The test of the value of the two-way interactions was significant, and removing the two-way interactions would have been significantly detrimental to the model (K-way effects, likelihood ratio, $\chi^2 = 503.412$, df = 49, $p < 0.001$). Proximity of parental populations affected survival (Fig. 4.12; partial $\chi^2 = 27.450$, df = 4, $p < 0.001$). When plants were crossed from parental populations within 400-5000m of each other, significantly more died than expected (partial estimate = 0.489 ± 0.205, z = 2.119, $p = 0.034$). When plants had parents whose sources were >20,000m away, fewer plants died than expected (partial estimate 0.249 ± 0.095, z = 2.616, $p < 0.009$). The maternal source population, however, did not affect survival ($\chi^2 = 3.896$, df = 9, $p = 0.918$).

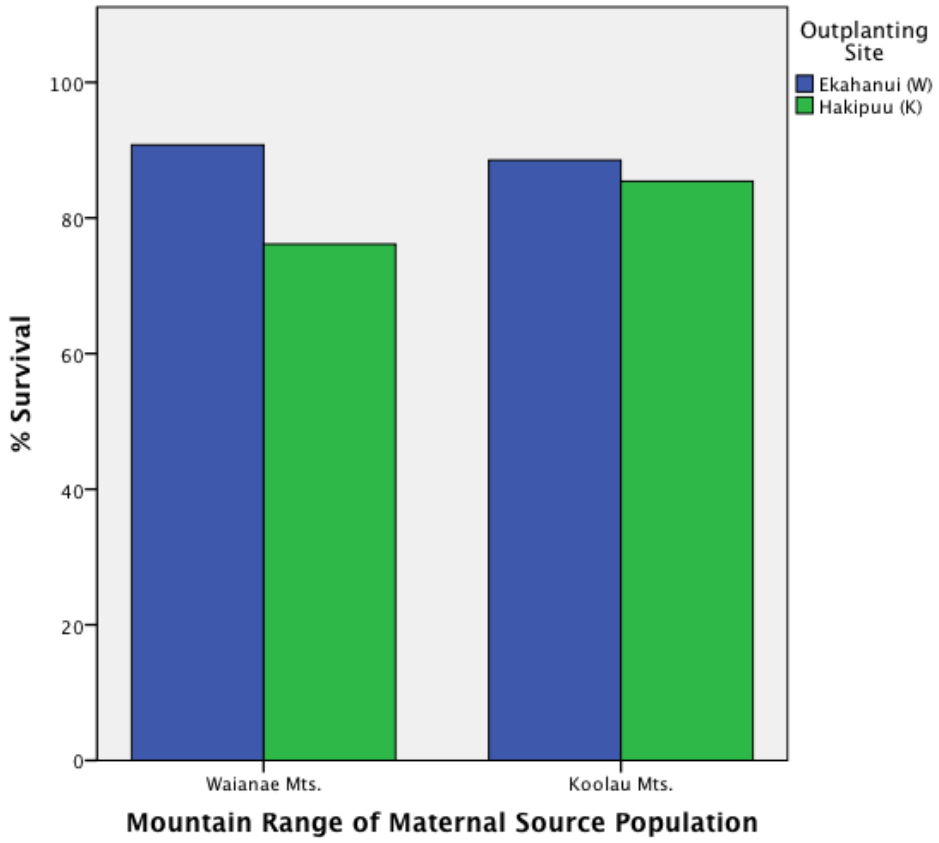

Fig. 4.11. Percent of plants that survived the first year by outplanting site and mountain range of maternal source.

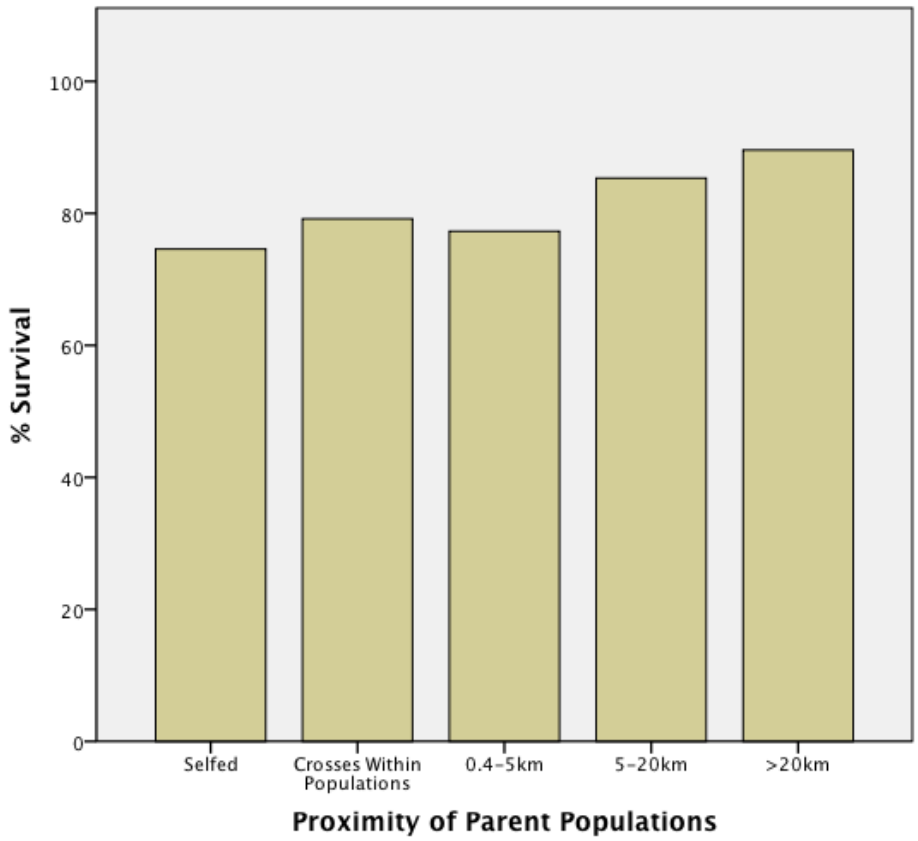

Fig. 4.12. Percent of plants that survived the first year by geographic distance between parent populations.

Outplanting site and the interaction between outplanting site and the mountain range of the maternal source populations did not affect the total growth of the plants ((final - initial size)/initial size) (GLM3, $F = 1.334$, df = 1, $p = 0.248$, $F = 1.844$, df = 1, $p = 0.175$, respectively). Mountain range of the maternal source populations did affect change in growth, and plants from maternal source populations in the Koʻolau Mountains grew more than plants from the Waiʻanae Mountains (Fig. 4.13; GLM3, $F = 14.853$, df = 1, $p < 0.001$). Proximity of parents did not affect growth (GLM3, $F = 1.455$, df = 4, $p = 0.214$), but the interaction between proximity and mountain range of maternal source populations was significant for growth (Fig. 4.13; GLM3, $F = 14.502$, df = 4, $p < 0.001$). The largest distance category (>20000m; crosses among mountain ranges), had opposite effects for growth depending on the source mountain range. Plants with maternal populations from the Koʻolau Mountains grew more than plants with maternal sources

from the Wai‘anae Mountains throughout all distances between source populations except the greatest distance of >20000m (between mountain ranges). When plants were from crosses between populations in different mountain ranges, the plants with maternal parents from the Wai‘anae Mountains were larger than plants from crosses between populations within the Wai‘anae Mountains. On the contrary, when the maternal source population was from the Ko‘olau Mountains and crossed with a Wai‘anae Mountain paternal source, the plants were smaller than plants whose parent source populations were both from within the Ko‘olau Mountains. Lastly, the maternal source population affected how much plants grew (Fig. 4.14; GLM3, $F = 5.091$, df = 9, $p < 0.001$). Plants with maternal sources from Pahole-A and Pahole-B grew less than plants with maternal sources from Pālāwai (all Wai‘anae Mt. populations; Hochberg’s GT2 mean diff. = 0.1619 ± 0.0319 & 0.1381 ± 0.0312, $p < 0.001$ & $p = 0.001$, respectively). Additionally, plants with the Pahole-A source also grew less than plants from the Kaipapa‘u source (Ko‘olau Mountains; Hochberg’s GT2 mean diff. = 0.1406 ± 0.0306, $p < 0.001$).

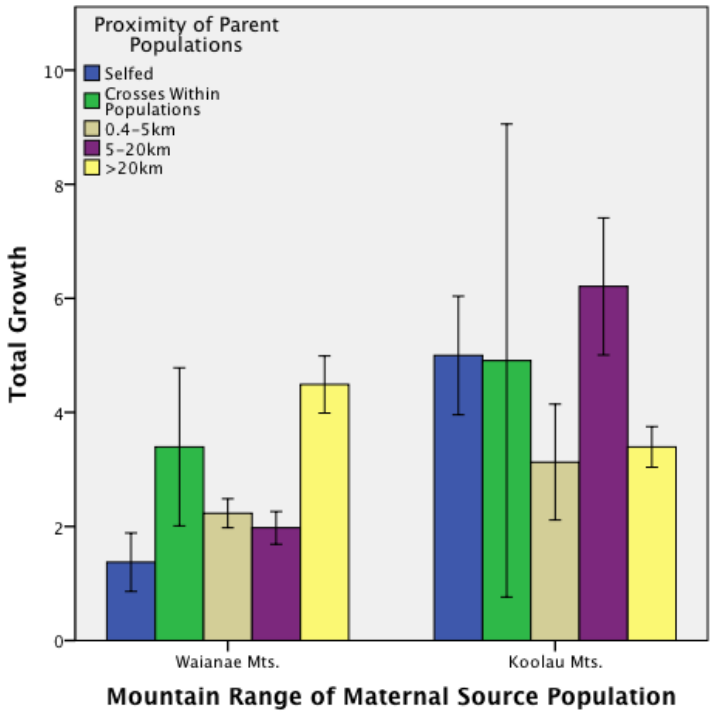

Fig. 4.13. Mean growth of plants by maternal source mountain range and geographic distance between parent populations. Size calculated as height (cm) of plant * N leaves * length (cm) of longest leaf. Growth calculated as (final size – initial size)/ initial size. Error bars ± 1SE.

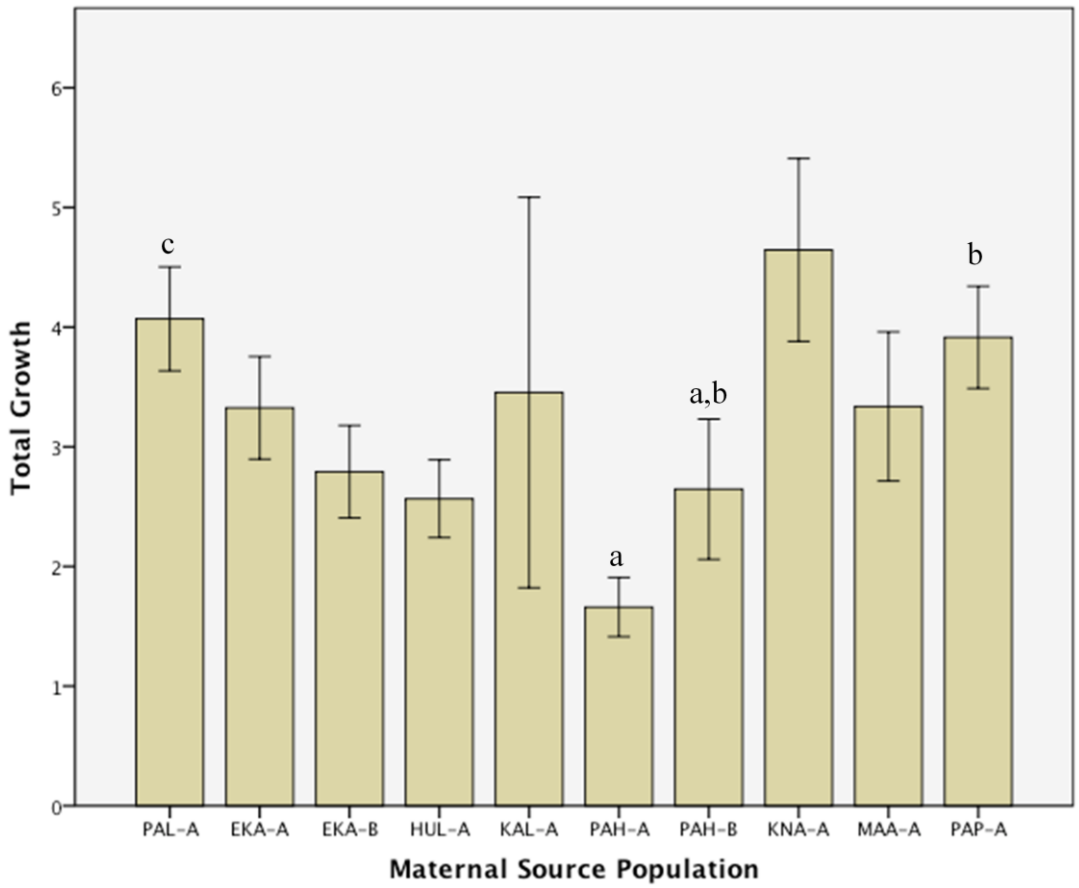

Fig. 4.14. Mean growth of plants by maternal source population. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Size calculated as height (cm) of plant * N leaves * length (cm) of longest leaf. Growth calculated as (final size – initial size)/ initial size. Error bars ± 1SE. Values labeled different letters are significantly different from each other.

A large majority (92.6%) of the plants reached maturity during the first year. The final model produced by a three-way loglinear analysis of maturation, outplanting site and mountain range of maternal source was a good fit of the data ($\chi^2 = 3.564$, df = 2, $p = 0.168$ for the likelihood ratio statistic of the final model; the expected values generated by the model were not significantly different from the observed data). The test of the value

of the three-way interaction was not significant (K-way Effects, likelihood ratio, χ^2 = 3.519, df = 1, p = 0.061). The test of the value of the two-way interactions was significant, and removing the two-way interactions would have been detrimental on the model (Fig. 4.15; K-way Effects, likelihood ratio statistic, χ^2 = 34.319, df = 3, $p < 0.001$). The two-way interaction between outplanting site and whether or not a plant matured in the first year after planting was significant (partial χ^2 = 27.729, df = 1, $p < 0.001$). More plants were immature at the Hakipuʻu outplanting site than expected (z =3.5, $p < 0.001$) and fewer plants were immature at the ʻĒkahanui outplanting site than expected (z = -3.5, $p < 0.001$). The two-way interaction between maturation and the mountain range of the maternal population was significant (partial χ^2 = 6.604, p = 0.010). Fewer plants than expected were immature when their maternal line was from the Koʻolau Mts (z = -2.0, p = $p < 0.05$).

The final model produced by a three-way loglinear analysis of maturation, maternal source population, and proximity of parental populations was a good fit of the data (χ^2 = 57.950, df = 45, p = 0.093 for the likelihood ratio statistic of the final model; the expected values generated by the model were not significantly different from the observed data). The test of the value of the three-way interaction was not significant (K-way Effects, likelihood ratio, χ^2 = 45.468, df = 36, p = 0.134). The test of the value of the two-way interactions was significant, and removing the two-way interactions would have been detrimental to the model (K-way Effects, likelihood ratio statistic, χ^2 = 567.195, df = 49, $p < 0.001$). The two-way interaction between maturation and maternal source population was not significant (partial χ^2 = 12.482, df = 9, p = 0.187). The two-way interaction between maturation and proximity of parent populations, however, was significant (Fig. 4.16; partial χ^2 = 62.811, df = 4, $p < 0.001$). More plants than expected failed to mature from the selfed treatment, crosses within populations, and crosses between parent populations 400-5000m away (z = 6.3, $p < 0.001$; z = 3.5, $p < 0.001$; z = 2.7, $p < 0.01$). Plants from crosses between the mountain ranges (>20000m) had fewer plants remain immature than expected (z = 4.6, $p < 0.001$). The interaction between paternal source population and maturation was not significant (partial χ^2 = 21.994, df = 9, p = 0.051).

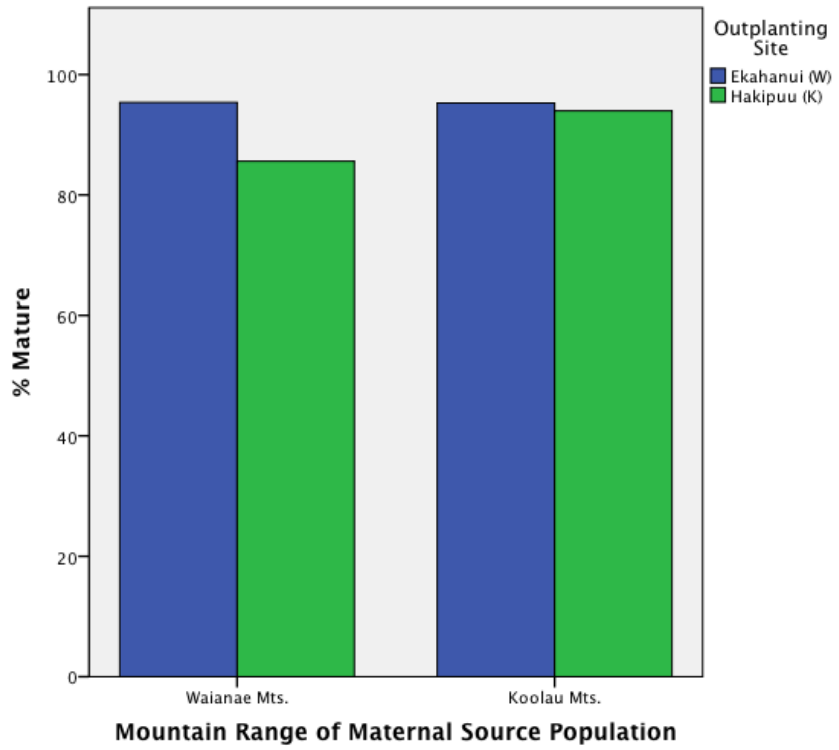

Fig. 4.15. Maturation of plants by outplanting site and mountain range of the maternal source population. Error bars ± 1 SE.

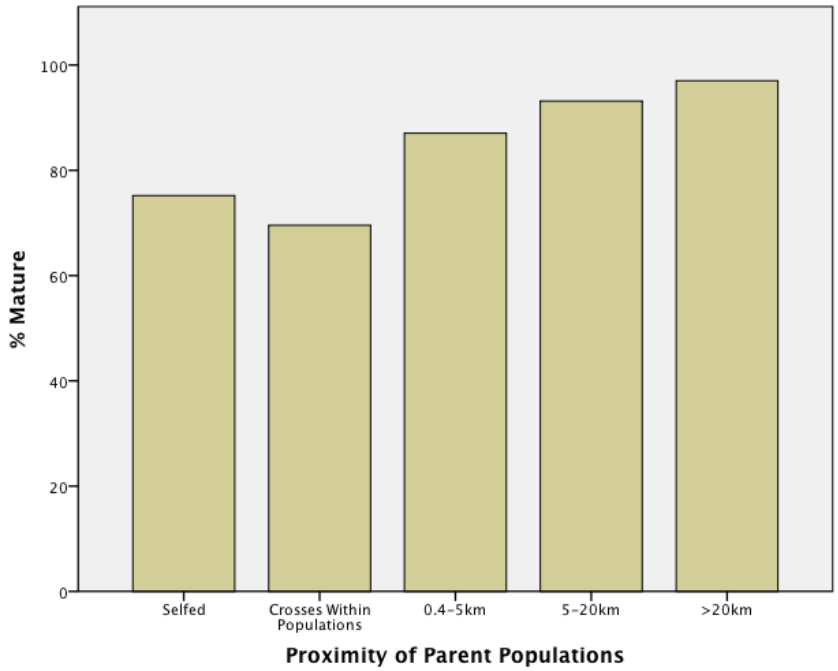

Fig. 4.16. Maturation of plants by proximity of parent populations. Error bars ± 1 SE.

Outplanting site did not affect the number of inflorescences a plant produced (GLM3, $F = 3.536$, df = 1, $p = 0.060$). Plants with a maternal source from the Ko‘olau Mountains had more inflorescences in the first year after outplanting than plants with a maternal source from the Wai‘anae Mountains (Fig. 4.17; GLM3, $F = 12.920$, df = 1, $p < 0.001$). The interaction between outplanting site and mountain range of maternal population did not affect the number of inflorescences a plant produced (GLM3, $F = 2.514$, df = 1, $p = 0.113$).

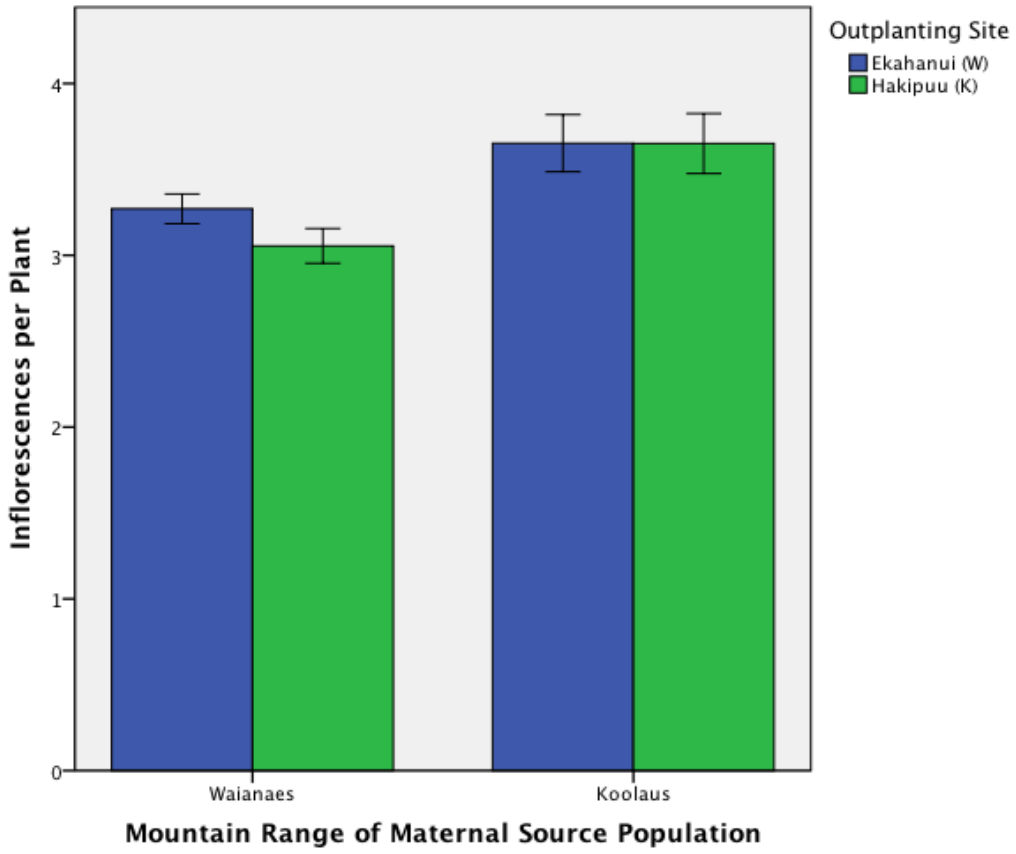

Fig. 4.17. Mean number of inflorescences per plant within the first year after planting by mountain range of the maternal source population and outplanting site. Error bars ± 1SE.

Proximity of parents affected the number of inflorescences a plant produced in the first year (Fig. 4.18; Welch’s $F = 33.440$, df = 4, $p < 0.001$). Plants from selfed treatments produced fewer inflorescences than any other treatment (Planned contrast = .6157, $t =$

5.016, df = 117, $p < 0.001$). Plants from crosses among close populations (400-5000m) produced fewer inflorescences than plants from crosses of parent populations > 5000m apart (Planned contrast = .1981, $t = 5.913$, df = 443, $p < 0.001$). Lastly, plants from crosses of parent populations 5000-20000m apart produced fewer inflorescences than plants from crosses of parent populations >20000m apart (between mountain ranges) (Planned contrast = 0.0644, $t = 4.477$, df = 797, $p < 0.001$). The maternal and paternal source populations affected the number of inflorescences produced per plant (GLM3, $F = 5.974$, df = 9, $p < 0.001$; $F = 7.765$, df = 9, $p < 0.001$, respectively), but their interaction was not significant ($F = 1.614$, df = 57, $p = 0.051$). Plants with their maternal source from Huliwai produced fewer inflorescences than plants with maternal sources from Kahana (Bonferroni, mean diff. = 0.1196 ± 0.0279, $p = 0.001$). Plants with their paternal source from Huliwai also produced the smallest number of inflorescences, and significantly fewer than plants with paternal sources from Kaluaʻa (mean diff. = 0.1116 = 0.0260, $p = 0.001$), Kahana (mean diff. = 0.1484 ± 0.0251), and Kaipapaʻu (mean diff. = 0.1175 ± 0.0254, $p < 0.001$).

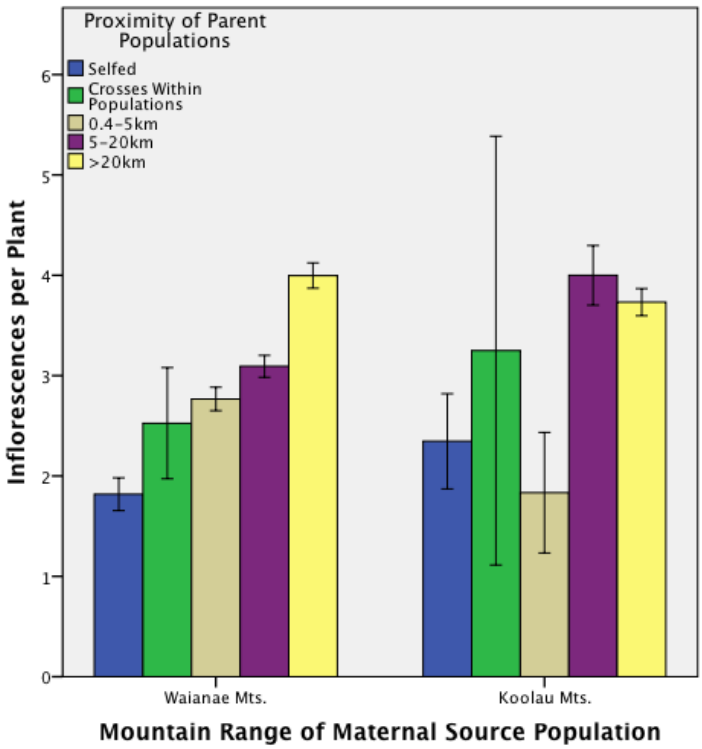

Fig. 4.18. Mean number of inflorescences per plant by mountain range of maternal source population and geographic distance between parent populations. Error bars ± 1 SE.

Plants growing in the ʻĒkahanui outplanting site had more flowers per inflorescence than the plants from the Hakipuʻu outplanting (Fig. 4.19; Mann-Whitney U = 23571, z = 6.137, $p < 0.001$). Mountain range of maternal source had no effect on the number of flowers per inflorescence (Mann-Whitney U, z = 1.061, $p = 0.289$). Maternal source population had no affect the number of flowers produced per inflorescence (Kruskal-Wallis, $\chi^2 = 10.926$, df = 9, $p = 0.281$). Proximity of parents did not affect the number of flowers per inflorescence after the Bonferroni correction for multiple comparisons (Kruskal-Wallis, $\chi^2 = 12.261$, df = 4, $p = 0.016$).

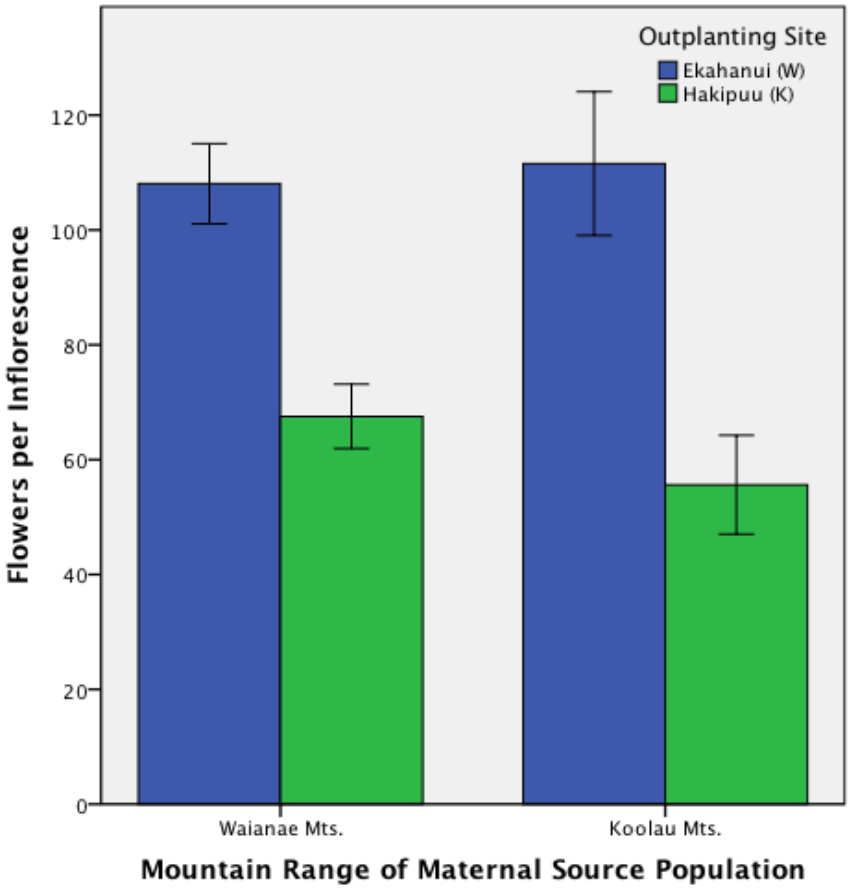

Fig. 4.19. Mean number of flowers per inflorescence by outplanting site and mountain range of maternal source population. Error bars ± 1 SE.

Plants at ʻĒkahanui produced more flowers per plant than plants at Hakipuʻu (Mann-Whitney U = 161984, z = 11.406, $p < 0.001$), regardless of the mountain range of the maternal population (Mann-Whitney U = 183504, z = 1.055, $p = 0.291$). Proximity of parent populations affected the number of flowers a plant produced (Fig. 4.20; Kruskal-Wallis, $\chi^2 = 136.156$, df = 4, $p < 0.001$). Plants from selfed treatments produced fewer flowers per plant than plants from crosses between parent sources that are ≥ 400m apart (Mann-Whitney U [400-5000m] = 11924.5, z = 4.388, $p < 0.001$; Mann-Whitney U [5000-20000m] = 13369.5, z = 6.828, $p < 0.001$; Mann-Whitney U [>20000m] = 15611.5, z = 9.425, $p < 0.001$). Maternal source population affected the number of flowers a plant produced (Fig. 4.21; Kruskal-Wallis, $\chi^2 = 60.401$, df = 9, $p < 0.001$). Plants with their maternal parent from Pahole-A produced more flowers per plant than plants with their maternal parent from ʻĒkahanui-B (Mann-Whitney U = 8923, z = 5.965, $p < 0.001$) and Huliwai (Mann-Whitney U = 6156, z = 6.702, $p < 0.001$). Plants with maternal parents from the Kaipapaʻu populations also produced more flowers per plant than plants with their maternal parent from Huliwai (Mann-Whitney U = 9336.5, z = 3.793, $p < 0.001$). Paternal source population affected the number of flowers a plant produced (Fig. 4.22; Kruskal-Wallis, $\chi^2 = 63.648$, df = 8, $p < 0.001$). Plants with paternal parents from Huliwai produced fewer flowers per plant than plants with paternal parents from Kahana (Mann-Whitney U = 8719, z = 5.841, $p < 0.001$), Maʻakua (Mann-Whitney U = 4277, z = 4.123, $p < 0.001$), and Pahole-A (Mann-Whitney U = 10036, z = 3.661, $p < 0.001$). Plants with paternal parents from Pālāwai also produced fewer flowers per plant than plants with paternal parents from Kahana (Mann-Whitney U = 5752, z = 4.763, $p < 0.001$) and Maʻakua (2780, z = 3.574, $p < 0.001$). Finally, plants with paternal parents from ʻĒkahanui-A, Pahole-B, and Kaipapaʻu also produced fewer flowers per plants than plants with paternal parents from Kahana (Mann-Whitney U = 8132, z = 4.848, $p < 0.001$; Mann-Whitney U = 10206.5, z = 3.824, $p < 0.001$; 10162, z = 4.352, $p < 0.001$, respectively).

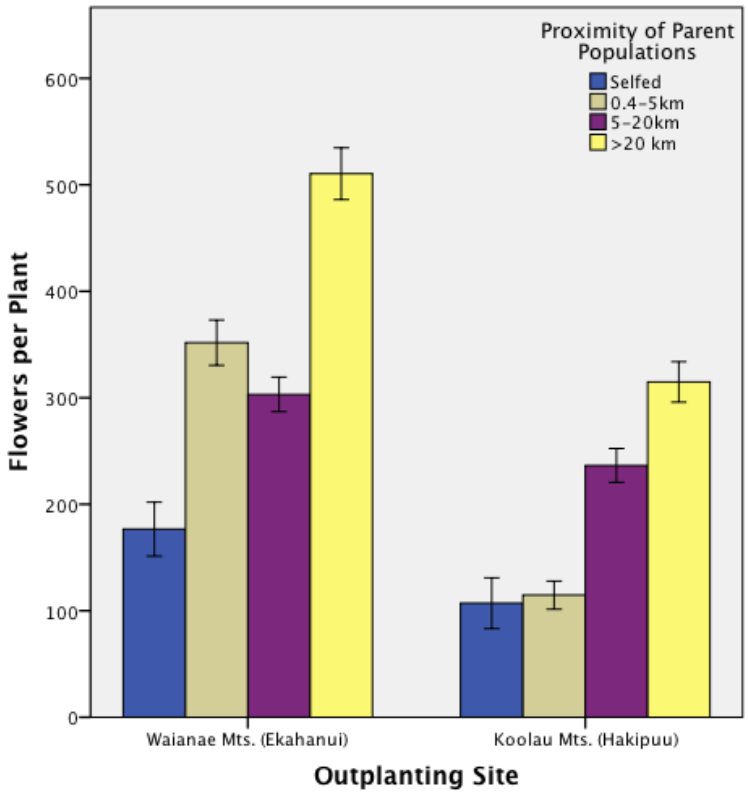

Fig. 4.20. Mean estimated number of flowers per plant by geographic distance between parent populations and outplanting site. Error bars ± 1 SE.

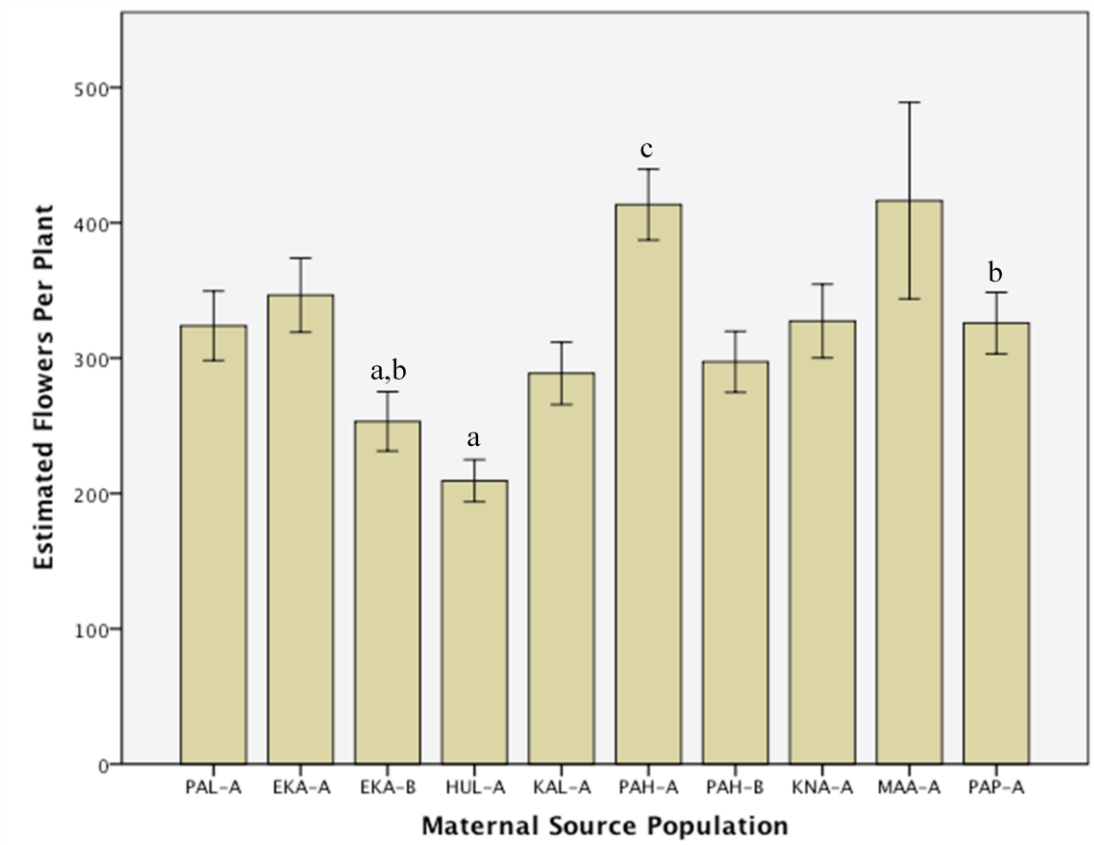

Fig. 4.21. Mean estimated number of flowers per plant by maternal source population. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Error bars ± 1 SE. Values labeled different letters are significantly different from each other.

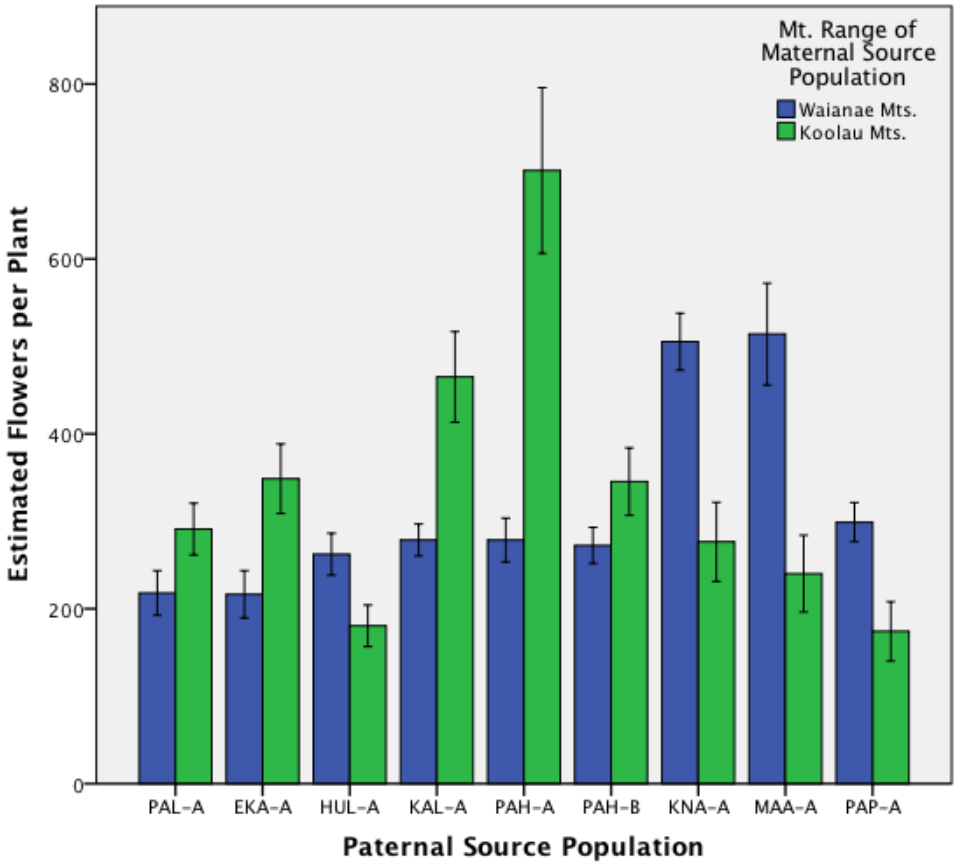

Fig. 4.22. Mean estimated number of flowers per plant by outplanting site and paternal source population. Populations arranged from most southern Waiʻanae population (6 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu. Error bars ± 1 SE. Refer to text for significant differences between populations.

Plants at the ʻĒkahanui outplanting site produced more fruit per inflorescence than plants from the Hakipuʻu outplanting site (Fig. 4.23; Mann-Whitney U = 16783, z = 10.800, $p < 0.001$). The mountain range of maternal source did not affect the number of fruits an inflorescence produced (Mann-Whitney U = 30699, z = 0.529, $p = 0.597$). Maternal source population did not affect the amount of fruit produced per inflorescence (Kruskal-Wallis, $\chi^2 = 10.926$, df = 9, $p = 0.281$). The proximity of parent populations affected the number of fruit per inflorescence (Fig. 4.23; Kruskal-Wallis, $\chi^2 = 24.951$, df = 4, $p < 0.001$). Plants from the selfed treatment had fewer fruit per inflorescence than

plants from all the crosses between populations (400-5000m, 5000-20000m, and > 20000m apart; Mann-Whitney U = 1938, z = 2.899, $p = 0.004$; Mann-Whitney U = 2038, z = 4.139, $p < 0.001$; Mann-Whitney U = 3323, z = 4.809, $p < 0.001$, respectively). Paternal source population did not affect the amount of fruit produced per inflorescence (Kruskal-Wallis, $\chi^2 = 10.570$, df = 8, $p = 0.227$).

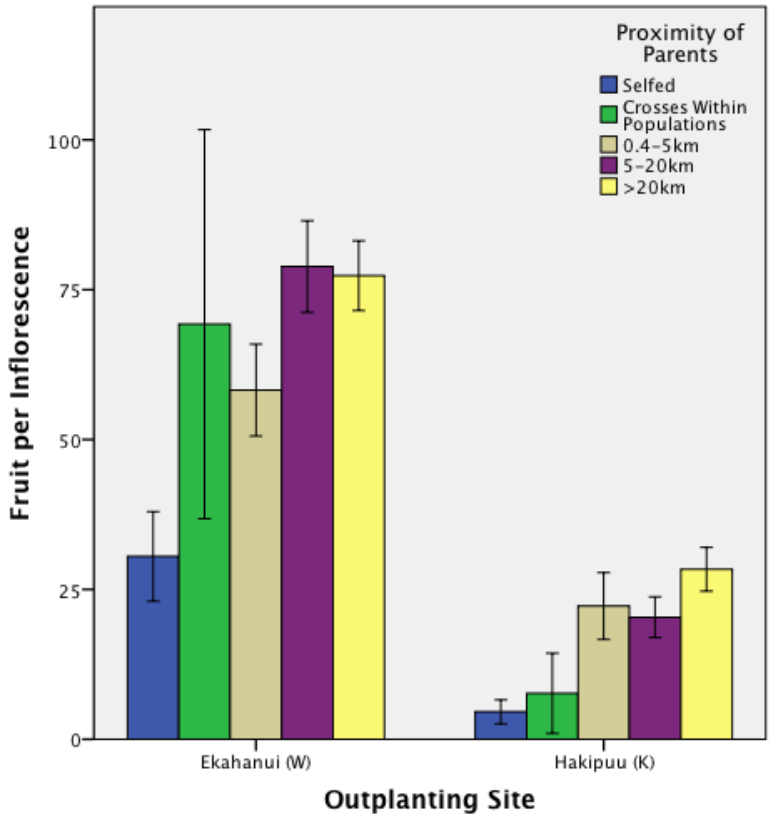

Fig. 4.23. Mean number of fruit per inflorescence outplanting site and the geographic distance between parent populations. Error bars ± 1SE.

There were more seeds per fruit on plants at the ʻĒkahanui outplanting than at the Hakipuʻu outplanting site (Fig. 4.24; GLM3, $F = 74.033$, df = 1, $p < 0.001$). Neither mountain range of the maternal source population nor its interaction with the outplanting site affected number of seeds per fruit ($F = 0.041$, df = 1, $p = 0.840$; $F = 0.033$, df = 1, $p = 0.856$). The proximity of the parent populations to each other and the maternal source

population both affected seeds per fruit (GLM3, $F = 2.995$, df = 3, $p = 0.031$; $F = 3.766$, df = 9, $p < 0.001$, respectively), but their interaction did not ($F = 1.414$, df = 24, $p = 0.094$). Plants from the selfed treatment produced fewer seeds per fruit than plants from crosses between all parent populations (Fig. 4.24; 400-5000m, 5000-20000m, > 20000m; Helmert Contrast, $F = 2.880$, df = 3, $p = 0.036$, contrast estimate = 1.492 ± 0.606, $p = 0.014$). Plants from the ʻĒkahanui-A maternal source population produced more seed per fruit than the plants from the Huliwai, Kaluaʻa, and Kahana populations (Hochberg's GT2 mean diff. = 2.501 ± 0.568, $p = 0.001$; 2.658 ± 0.636, $p = 0.002$; 2.686 ± 0.565, $p < 0.001$, respectively). Neither the paternal source population nor its interaction with the maternal source population affected the amount of seeds produced per fruit (GLM3, $F = 1.960$, df = 8, $p = 0.050$; $F = 0.846$, df = 55, $p = 0.774$, respectively).

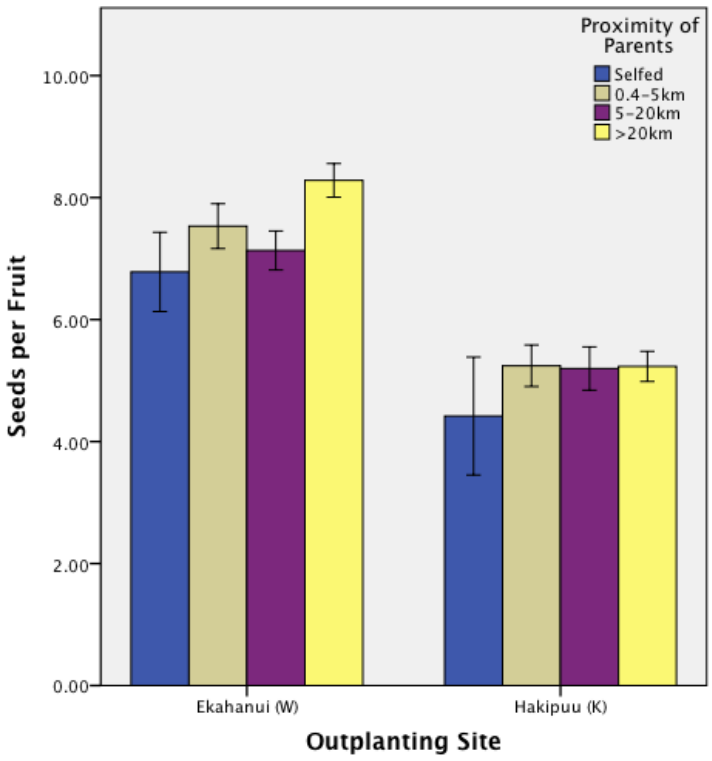

Fig. 4.24. Mean number of seeds per fruit produced by plants by proximity of parents and outplanting site. Error bars ± 1 SE.

Plants at the ʻĒkahanui site had more seeds per plant than the plants at the Hakipuʻu site (Fig. 4.25; Mann-Whitney U = 66710, z = 22.168, $p < 0.001$). Maternal and paternal sources affected the estimated number of seeds produced per plant (Fig. 4.25 & 4.26; Kruskal-Wallis, $\chi^2 = 44.714$, df = 9, $p < 0.001$; $\chi^2 = 44.187$, df = 8, $p < 0.001$, respectively). Plants from the ʻĒkahanui-A and ʻĒkahanui-B maternal populations produced fewer seeds per fruit than the plants from the Kahana and Maʻakua maternal sources (ʻĒkahanui-A: Mann-Whitney U = 8007, z = 3.928 (Kahana); Mann-Whitney U = 4005, z = 4.332 (Maʻakua); ʻĒkahanui-B: Mann-Whitney U = 7928, z = 4.692 (Kahana); Mann-Whitney U = 3791, z = 5.226; $p < 0.001$). Plants from the ʻĒkahanui-A, Huliwai, and Pālāwai paternal source populations produced fewer seeds per plants than the plants from the Maʻakua and Pahole-A paternal sources (ʻĒkahanui-A: Mann-Whitney U = 8075, z = 3.638 (Pahole-A); Mann-Whitney U = 3985, z = 3.632 (Maʻakua); Huliwai: Mann-Whitney U = 8694, z = 4.506 (Pahole-A); Mann-Whitney U = 4003, z = 4.664 (Maʻakua); Pālāwai: Mann-Whitney U = 5515, z = 3.752 (Pahole-A); Mann-Whitney U = 2558, z = 4.045 (Maʻakua); $p < 0.001$).

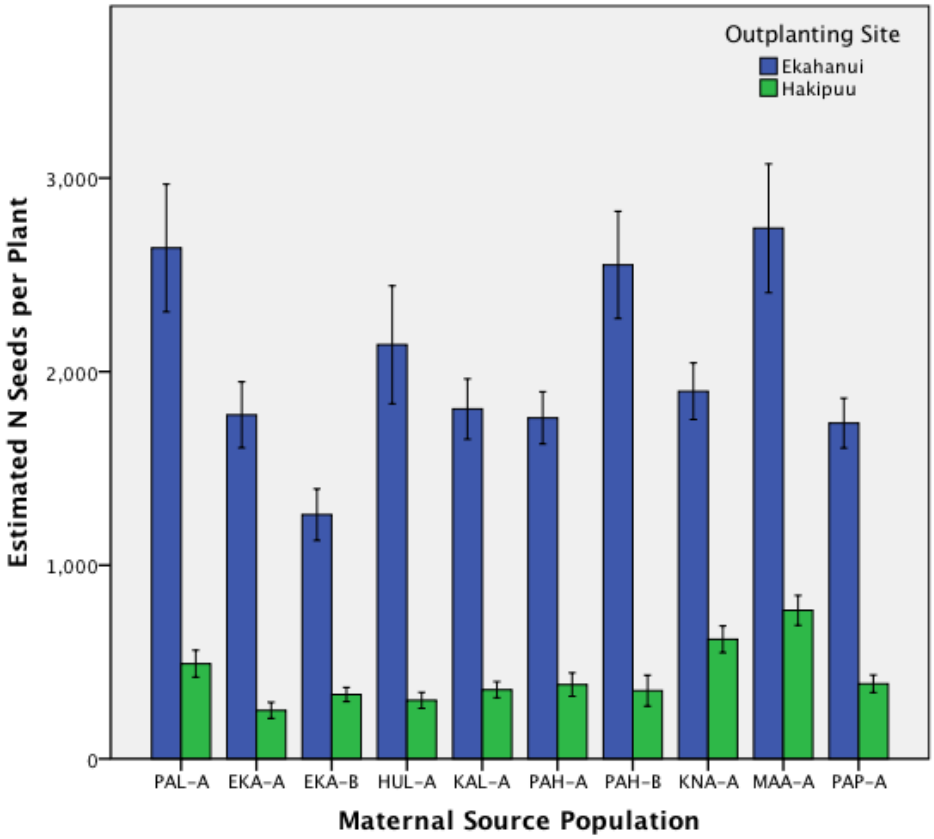

Fig. 4.25. Mean estimated number of seeds produced per plant by outplanting site and maternal source population. Error bars ± 1 SE. Populations arranged from most southern Waiʻanae population (7 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, EKA-B = ʻĒkahanui-B, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu.

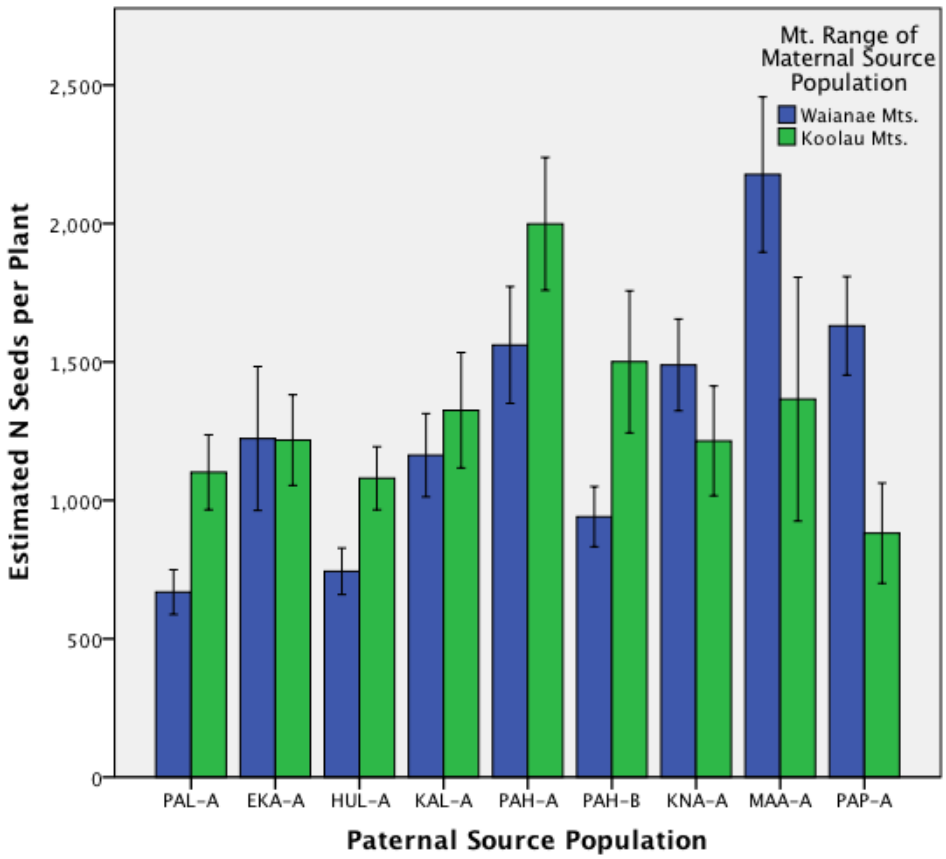

Fig. 4.26. Mean estimated number of seeds produced per plant by mountain range of the maternal source population and paternal source population. Error bars ± 1 SE. Populations arranged from most southern Waiʻanae population (6 populations on left) to most northern Koʻolau population (3 populations on right). PAL-A = Pālāwai, EKA-A = ʻĒkahanui-A, HUL-A = Huliwai, KAL-A = Kaluaʻa, PAH-A = Pahole-A, PAH-B = Pahole-B, KNA-A = Kahana, MAA-A = Maʻakua, PAP-A = Kaipapaʻu.

Plants with a maternal source from the Waiʻanae Mountains had fewer seeds per plant than plants with a maternal source from the Koʻolau Mountains (Fig. 4.27; Mann-Whitney U = 156693, z = 4.616, $p < 0.001$). The proximity of the parent populations affected the number of seeds per plant (Fig. 4.27; Kruskal-Wallis, $\chi^2 = 132.826$, df = 4, $p < 0.001$). Plants from the selfed treatment had fewer seeds per plants than plants from the crosses 400-5000m apart, 5000-20000m apart, and > 20000m apart (Mann-Whitney U = 10225, z = 4.691, $p < 0.001$; U = 10483, z = 7.354, $p < 0.001$; U = 12455, z = 9.700, $p < 0.001$, respectively). Plants from crosses between parent populations 400-5000m apart had fewer seeds produced per plant than plants crosses between parent populations 5000-

20000m apart and >20000m apart (Mann-Whitney U = 46347, z = 3.645, $p < 0.001$; U = 59669, z = 7.502, $p < 0.001$, respectively). Lastly, plants from crosses between parent populations that are 5000-20000m apart had fewer seeds produced than plants from crosses between mountain ranges (>20000m; Mann-Whitney U = 97010, z = 4.283, $p < 0.001$).

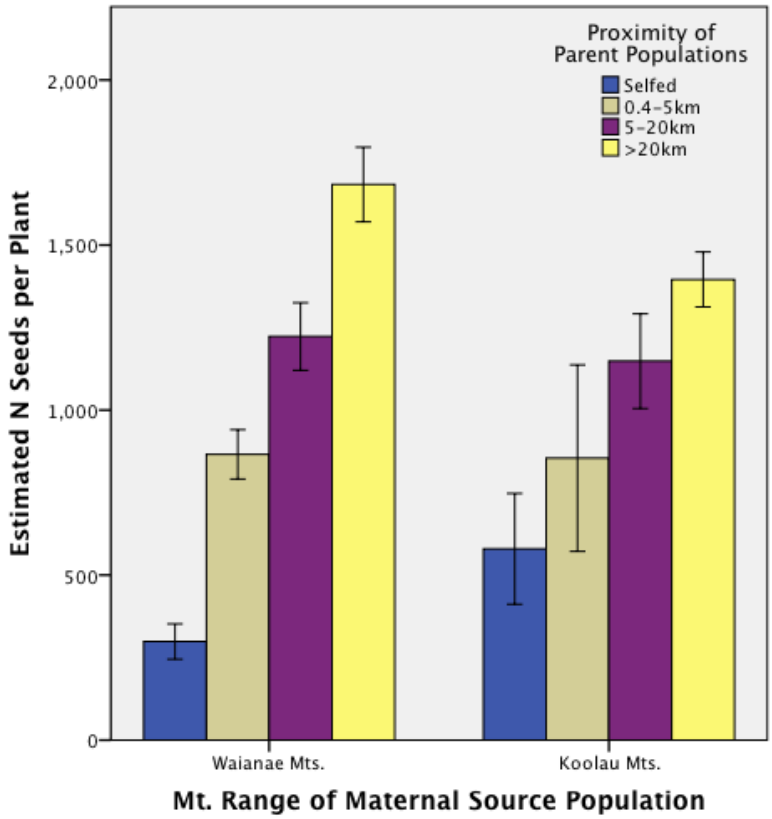

Fig. 4.27. Mean estimated number of seeds produced per plant by mountain range of the maternal source population and the geographic distance between parent populations. Error bars ± 1 SE.

Relative Performance (*RP*) and Cumulative Fitness: Cumulative fitness was higher at the ʻĒkahanui outplanting than the Hakipuʻu outplanting (Fig. 4.28; Mann-Whitney U = 171, z = 2.410, $p = 0.016$). A cumulative fitness value was calculated for each population (Table 4.4; Fig. 4.29). There were no differences among the maternal populations with regard to cumulative fitness (Kruskal-Wallis Test; $\chi^2 = 5.046$, df = 9, $p = 0.830$), though

the populations with the lowest cumulative fitness values (Kaluaʻa and Kahana), often had the lowest values at individual stages. Cross treatment affected the cumulative fitness (Kruskal-Wallis Test; $\chi^2 = 27.103$, df = 2, $p < 0.001$), as crosses between populations had higher cumulative fitness than both the selfed treatment (Mann-Whitney U = 33, z = 4.529, $p < 0.001$) and the crosses within populations (Fig. 4.29; Mann-Whitney U = 1, z = 4.019, $p < 0.001$).

Table 4.4. Cumulative fitness at each outplanting site (seeds per pollination (GH) * survival * N inflorescences* N fruit per inflorescence * N seeds per fruit. Values are means per population for progeny obtained through self fertilization (S), progeny of crosses within populations (Xw), and progeny of crosses between populations (Xb).

Maternal Population	Cross Treatment	Cumulative Fitness	Cumulative Fitness
		ʻĒkahanui	Hakipuʻu
ʻĒkahanui-A	S	1770.52	93.81
ʻĒkahanui-A	Xw	396.26	1.48
ʻĒkahanui-A	Xb	5446.18	1191.17
ʻĒkahanui-B	S	116.32	0.00
ʻĒkahanui-B	Xb	6386.64	1278.85
Huliwai	S	2287.23	17.55
Huliwai	Xb	7851.18	1096.62
Kaluaʻa	S	0.00	0.00
Kaluaʻa	Xb	1740.21	336.64
Kahana	S	64.43	0.00
Kahana	Xw	0.49	208.79
Kahana	Xb	2968.74	851.73
Maʻakua	S	0.00	0.00
Maʻakua	Xw	0.40	0.40
Maʻakua	Xb	7353.38	1996.58
Pahole-A	S	237.12	143.50
Pahole-A	Xb	4980.15	976.35
Pahole-B	S	731.20	16.29
Pahole-B	Xb	6015.30	891.10
Pālāwai	S	27.54	0.00
Pālāwai	Xb	5319.66	850.94
Kaipapaʻu	S	5230.05	163.87
Kaipapaʻu	Xw	0.00	0.00
Kaipapaʻu	Xb	13729.89	2588.45

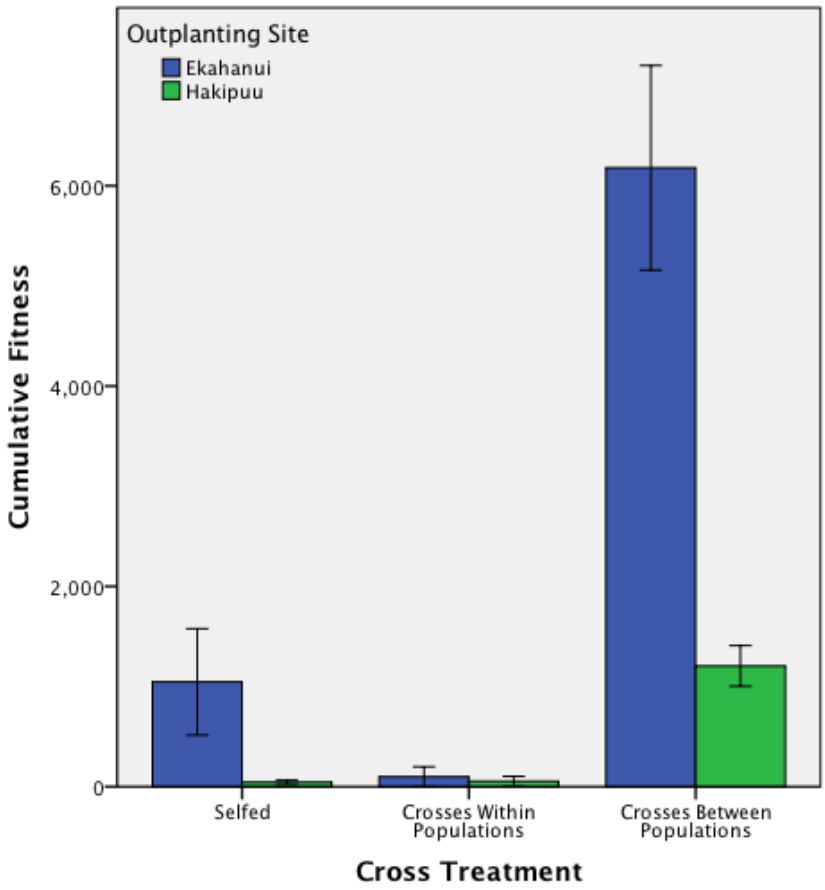

Fig. 4.28. Cumulative fitness by cross treatment and outplanting site. Error bars ± 1 SE. Cumulative fitness is the product of N seeds per pollination, survival, N inflorescences, N fruit per inflorescence, and N seeds per fruit.

The relative performance of crosstypes (*RP*) was calculated for several of the measured variables as well as cumulative fitness (Appendix 2b). Heterosis (crosses within populations/crosses between population) was detected in the number of seeds per pollination, the earliest life stage measured (RP_h; $t = 6.344$, df = 7, $p < 0.001$), and cumulative fitness (RP_h; $t = 31.319$, df = 7, $p < 0.001$). There was neither inbreeding nor outbreeding depression at any stage for the RP_i cross type (selfed/crosses within populations). The RP_b crosstype (selfed/crosses between populations) was significantly higher than zero, indicating inbreeding depression and/or heterosis, for all stages, including cumulative fitness. Since the majority of populations only have one founder,

the RP_b crosstype was the only estimate of relative performance for six of the ten populations. Since the RP_i for cumulative fitness was close to zero and negative, there was no support for inbreeding depression. Heterosis was the more likely mechanism for higher fitness values following crosses between populations, although differences among the three crosstypes were not significantly different (Fig. 4.29; GLM1, Welch's F = 2.094, df = 2, p = 0.180). There was variation in the RP_b values among the different stages (Table 4.5, Fig. 4.30; GLM1, F = 4.861, df = 4, p = 0.001), as RP_b was higher in the number of fruit per inflorescence in comparison to survival (Games-Howell mean diff. = 0.5365 ± 0.1221, p < 0.001). Maternal population also affected RP_b values (Fig. 4.31; GLM1, F = 4.495, df = 9, p < 0.001), as progeny from the Kaluaʻa population had higher values than progeny from the ʻĒkahanui -A (Games-Howell mean diff. = 0.7313 ± 0.0917, p = 0.001), suggesting higher levels of heterosis in progeny from Kaluaʻa compared to ʻĒkahanui –A, the largest extant population.

Table 4.5. *T*-test results for relative performance in each stage used to calculate cumulative fitness and including cumulative fitness. Alpha significance at $p < 0.05$.

Stage - *RP*	*t*	df	*p*	Mean Difference
Seed/Pollination - RP_i	-1.631	7	0.147	-0.3335
Seed/Pollination – RP_b	6.106	19	< 0.001	0.4845
Seed/Pollination - RP_h	6.344	7	< 0.001	0.6673
Survival - RP_i	0.658	4	0.546	0.1767
Survival – RP_b	2.428	19	0.025	0.2211
Survival - RP_h	0.701	4	0.522	0.0963
N Inflorescences - RP_i	0.214	3	0.844	0.0819
N Inflorescences – RP_b	6.529	19	< 0.001	0.5000
N Inflorescences - RP_h	-0.416	3	0.705	-0.1212
Fruit/Inflorescence - RP_i	0.198	2	0.861	0.1039
Fruit/Inflorescence – RP_b	10.862	18	< 0.001	0.7577
Fruit/Inflorescence - RP_h	1.177	2	0.36	0.3743
Seeds/Fruit – RP_b	5.092	19	< 0.001	0.5201
Cumulative Fitness - RP_i	-0.612	7	0.56	-0.2191
Cumulative Fitness – RP_b	34.757	19	< 0.001	0.9233
Cumulative Fitness - RP_h	31.319	7	< 0.001	0.9600

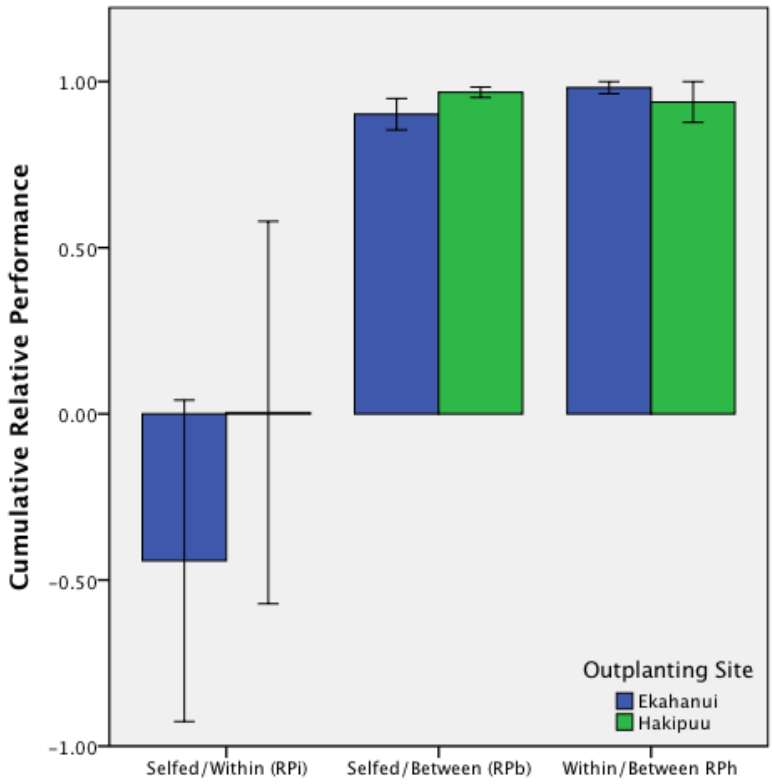

Fig. 4.29. Cumulative relative performance for all three crosstypes. Error bars ± 1 SE.

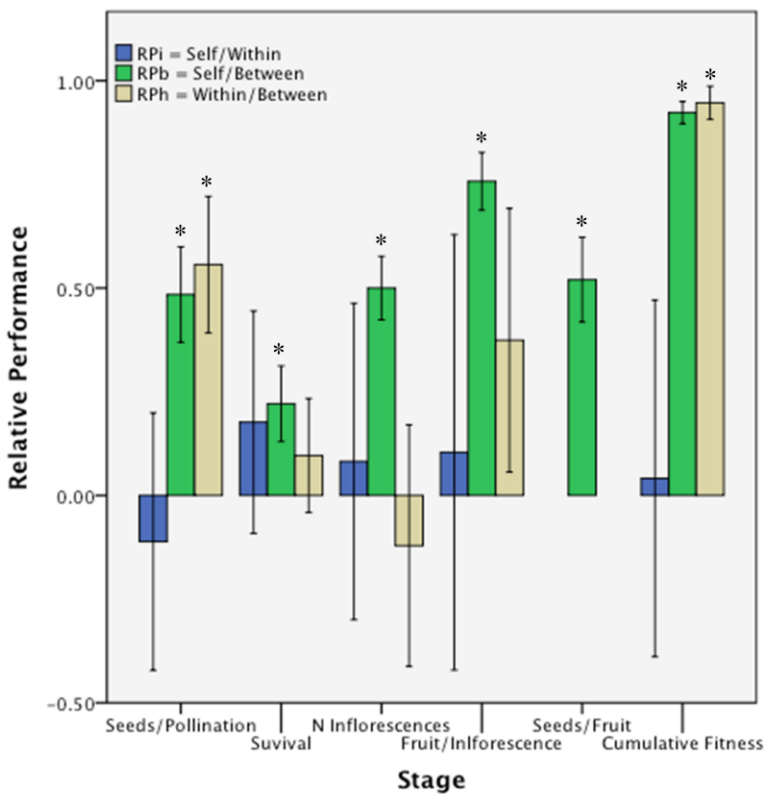

Fig. 4.30. Mean *RP* by cross type and stage. RP_i = selfed progeny / progeny from crosses within populations; RP_b = selfed progeny / progeny of crosses between populations; RP_h = progeny from crosses within populations / crosses between populations. Error bars ± 1 SE. * *RP* is not zero ($p < 0.05$).

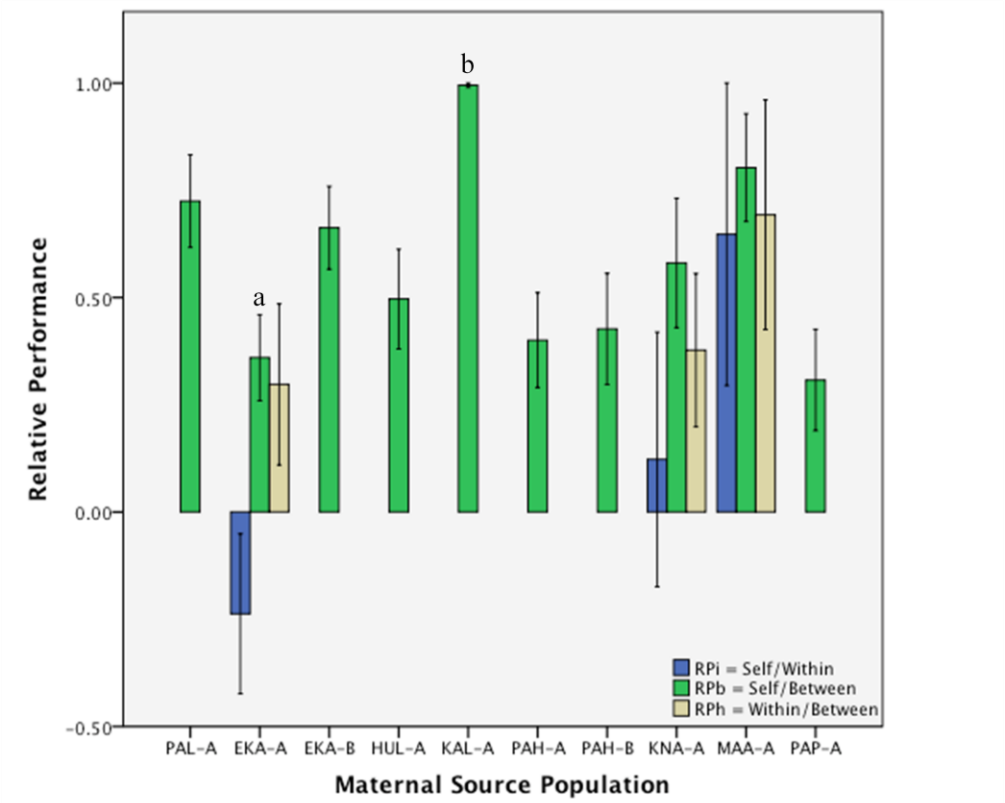

Fig. 4.31. Mean *RP* (of all stage means) by cross type and maternal source population. RP_i = selfed progeny / progeny from crosses within populations; RP_b = selfed progeny / progeny of crosses between populations; RP_h = progeny from crosses within populations / crosses between populations. Error bars ± 1 SE. Only PAH-C, LEH-B, and LEH-A had RP_i and RP_h values. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha. RP_b values labeled a and b are significantly different from each other (p = 0.001) within the given maternal source population.

Discussion

The most noticeable difference among the cross treatments was the increase in fitness (male, female, cumulative, and at individual stages) with increasing geographic distance between the parent source populations. Heterosis was the strongest and only detectable force among the progeny though inbreeding depression was difficult to assess in this species due to the low number of populations with more than one individual available to cross. The optimal distance for outcrossing distance in this study was the furthest distance, which was between the mountain ranges. The majority of populations

consisted of only one founding individual, and so many of the differences are attributed to variation among individuals as opposed to one population being more fit than another population.

Population Differences: Most fitness stages were affected by the source of the plants used as maternal and paternal parents in crosses. Many of the maternal population differences were between mountain ranges. Seeds from Koʻolau Mt. plants (produced in the nursery hand via hand-pollinations) had lower viability than seeds from Waiʻanae Mt. populations. Other life stages in the outplanted progeny showed a different trend. Plants with maternal populations found in the Koʻolau Mountains grew larger, were more likely to mature within the first year following outplanting, and produced more inflorecences and seeds. Plants from the Koʻolau Mountains may produce more seeds, but a larger percentage of them may be inviable compared to seeds from Waiʻanae Mt. plants. Differences among paternal source populations were only detected in the early life stages (fruit and seeds per pollination) and in the number of inflorescences and seeds per plant. Plants with maternal sources from the Koʻolau Mountains did not have higher cumulative fitness than plants from the Waiʻanae Mountains, though plants from Kaipapaʻu (Koʻolau Mountains) had the highest cumulative fitness (though not significantly so). Huliwai parentage (Waiʻanae Mountains; all from one *in situ* indvidual) led to higher early life stage fitness variables in the nursery (fruits and seeds per pollinations), but lower male and female fitness estimates in the field study, and had the second highest cumulative fitness estimate. If larger populations existed, the variation among individuals within populations may have reduced the strong variation between populations as seen here, since the majority of between "populations" for this species is actually between individuals.

Benefits of Outcrossing: Contrary to expectations, inbreeding depression was not detected at any life stage; no fitness differences were present between the selfed treatment and crosses within populations. Detection of inbreeding depression was hindered by the small number of populations that had more than one plant to use for within-population crosses. Based on inflorescence morphology and pollen:ovule ratios (Cruden 1977, Weller et al. 1998) that suggest that *S. kaalae* is a predominantly

outcrossing species, inbreeding depression was predicted. Additionally, control flowers of plants in pollinator-free enclosures produced fewer fruits than hand-pollinated flowers (and emasculated flowers did not produce fruit), indicating that flowers are able to self in the absence of pollinators and outcrossing. The lack of inbreeding depression may also suggest that selfing rates may have increased for long enough to purge mutational loads (Lande and Schemske 1985). The high levels of heterosis, along with historically small populations and a likely lifespan of over 10 years, makes it likely that *S. kaalae* has the evolutionary stable strategy of high selfing rates and low inbreeding depression (Lande and Schemske 1985). Futhermore, the gradual decrease in population sizes is more likely due to habitat disturbance and unmitigated threats (such as invasive plant species and possibly slugs; MIT 2003) to *S. kaalae*, rather than inbreeding depresison.

Heterosis was detected at almost every stage. Differences between selfed treatments and crosses between populations were likely due to heterosis because inbreeding depression was never detected. Only seed mass and viability, growth, and number of flowers per inflorescence did not show signs of heterosis. Outcrossed progeny fitness also increased as the distance between parents source populations increased at both outplanting sites. This may be due to an increase in heterozygosity and decrease in the number of fixed deleterious alleles, and possibly also as the amount of genetic variation between the populations increases with geographic distance (Betran et al. 2003, Charlesworth and Willis 2009). Outbreeding depression was not present in any crosses between populations, including between the mountain ranges.

Local Adaptation: The outplanting site at ʻĒkahanui produced more fecund plants, regardless of provenance. Plants at ʻĒkahanui had a better chance of survival, produced more flowers per inflorescence and plant, more fruit per inflorescence, and more seeds per fruit and plant. The two outplanting sites were designed to determine if plants with parents from the Waiʻanae Mountains would be more relatively fit at an outplanting in the Waiʻanae Mountains in comparison to the Koʻolau Mountains, and vice versa for Koʻolau Mt. plants. If stock from either mountain produced progeny that were more fit in the corresponding mountain range, it could be indicative of local adaptation, and a preference for "home-site" habitat characteristics. More plants from the Waiʻanae

Mountains died at the Koʻolau Mt. outplanting site (Hakipuʻu) than expected. This reduced chance of survival of Waiʻanae Mt. stock planted in the Koʻolau Mountains (in Hakipuʻu gulch) suggests that there is a home-site advantage. As described earlier, populations of *S. kaalae* located in the Koʻolau Mountains are found in wetter habitats than the populations located in the Waiʻanae Mts (Table 1). Many of the plants from the Waiʻanae Mountains growing at Hakipuʻu had roots that rotted directly below the base of the plant in (pers. observation). Plants from the Waiʻanae Mt. may be poorly adapated to the wetter Ko`olau habitats. This trend has not been detected for any other life history stages, and this was the only indication that there may be local adaptation in this species.

Management Recommendations: Management suggestions have been developed for reintroductions efforts for this species. Edmands (2002, 2007) suggests that populations should not be mixed unless inbreeding depression has been detected. Frankham et al. (2011) has developed a key for determining the likelihood of outbreeding depression and, if the chance of outbreeding depression is low, gene flow should be re-established between populations. Due to the distance between the mountain ranges, it was assumed that there had not been gene flow between the ranges within the last 500 years. There are also environmental differences between the mountain ranges. Following the key, we would not mix plants between mountain ranges. The benefit from heterosis, however, should also influence management suggestions. Heterotic individuals may be the most successful in new populations (Lande and Schemske 1985), and consequently contribute to the success of the reintroduction, especially when there is genetic representation from a very low number of wild plants and several unmitigated threats to the survival of the outplants. The benefit of outplanting heterotic individuals likely outweighs any local adaptaion of Waiʻanae Mt. plants to their own mountian range. Current recommendations are as follows:

1. Koʻolau Mt. reintroductions would benefit from the influx of Waiʻanae Mt. plants. Reintroductions should be balanced among all Koʻolau Mt. founders so that all maternal sources of the outplants should be from the Koʻolau Mountains. As maternal lines in the nursery, Koʻolau Mt. founders should receive two pollination treatments. One would be mixed pollen loads from all Koʻolau

founders and the second would be mixed pollen loads from all Waiʻanae founders. Outplants should be monitored and, if recruitment varies between these two pollination treatments, the less successful treatment should be discontinued.

2. Plants from Koʻolau Mt. populations did not decrease in fitness when planted in the Waiʻanae Mountains, and plants from Koʻolau Mt. populations had higher female fitness than plants from Waiʻanae Mt. populations. Plants from Koʻolau Mt. populations can be planted with Waiʻanae Mt. populations in the Waiʻanae Mountains. Continued monitoring of survival at the ʻĒkahanui study site should continue to confirm this recommendation. Outplants should be outcrossed progeny from nursery hand-pollinations. All founders should receive the two treatments described in Recommendation #1.
3. The only current outplanting site where floral visitors were observed was the ʻĒkahanui outplanting site in this study, with over 600 more plants than any other *in situ* or reintroduced site. If indeed population size and pollinator attraction are correlated in this species, outplantings should be initiated with large numbers of plants and at high densities. If high outcrossing rates could persist in the reintroductions of heteroic plants, it is possible to carry the benefits of heterosis for future generations.

APPENDIX A. Means, SE, and sample size (N) for stages by maternal source population and cross treatment for *Schiedea obovata*. Cross treatments for controls, selfed progeny (Selfed), progeny from crosses within populations (Within), and progeny from crosses between populations (Between). Only PAH-C, LEH-B, and LEH-A had intrapopulation crosses. MMR-A = Kahanahāiki, PAH-C = Pahole, PAH-A = Pahole Rim, PIL-A = Keawapilau, LEH-B = NW Makaleha, LEH-A = W Makaleha. Fruit/Pollination, Seeds/Pollination, Seeds/Fruit (GH), Seed Mass and Viability stages all from nursery hand-pollinations.

Stage	Maternal Population	Cross Treatment	Mean	SE	N
Fruit/Pollination	MMR-A	Control (calyx)	0.707	0.193	3
Fruit/Pollination	MMR-A	Control (no calyx)	0.546	0.193	3
Fruit/Pollination	MMR-A	Selfed	0.340	0.168	4
Fruit/Pollination	MMR-A	Between	0.134	0.087	15
Fruit/Pollination	PAH-C	Control (calyx)	0.714	0.137	6
Fruit/Pollination	PAH-C	Control (no calyx)	0.228	0.150	5
Fruit/Pollination	PAH-C	Selfed	0.424	0.168	4
Fruit/Pollination	PAH-C	Within	0.775	0.127	7
Fruit/Pollination	PAH-C	Between	0.433	0.063	28
Fruit/Pollination	PAH-A	Control (calyx)	0.500	0.168	4
Fruit/Pollination	PAH-A	Control (no calyx)	0.600	0.150	5
Fruit/Pollination	PAH-A	Selfed	0.547	0.150	5
Fruit/Pollination	PAH-A	Between	0.603	0.060	31
Fruit/Pollination	PIL-A	Control (calyx)	0.702	0.127	7
Fruit/Pollination	PIL-A	Control (no calyx)	0.478	0.137	6
Fruit/Pollination	PIL-A	Selfed	0.484	0.101	11
Fruit/Pollination	PIL-A	Between	0.322	0.060	31
Fruit/Pollination	LEH-B	Control (calyx)	0.572	0.137	6
Fruit/Pollination	LEH-B	Control (no calyx)	0.473	0.150	5
Fruit/Pollination	LEH-B	Selfed	0.811	0.150	5
Fruit/Pollination	LEH-B	Within	0.660	0.127	7
Fruit/Pollination	LEH-B	Between	0.560	0.067	25
Fruit/Pollination	LEH-A	Control (calyx)	0.463	0.168	4
Fruit/Pollination	LEH-A	Control (no calyx)	0.029	0.150	5
Fruit/Pollination	LEH-A	Selfed	0.273	0.150	5
Fruit/Pollination	LEH-A	Within	0.525	0.150	5
Fruit/Pollination	LEH-A	Between	0.492	0.061	30
Seeds/Pollination	MMR-A	Control (calyx)	48.262	14.350	3
Seeds/Pollination	MMR-A	Control (no calyx)	18.095	14.350	3
Seeds/Pollination	MMR-A	Selfed	5.781	12.427	4
Seeds/Pollination	MMR-A	Between	4.211	6.417	15

Seeds/Pollination	PAH-C	Control (calyx)	62.976	10.147	6
Seeds/Pollination	PAH-C	Control (no calyx)	13.736	11.115	5
Seeds/Pollination	PAH-C	Selfed	38.298	14.350	3
Seeds/Pollination	PAH-C	Within	51.570	9.394	7
Seeds/Pollination	PAH-C	Between	28.363	4.697	28
Seeds/Pollination	PAH-A	Control (calyx)	33.031	12.427	4
Seeds/Pollination	PAH-A	Control (no calyx)	42.800	11.115	5
Seeds/Pollination	PAH-A	Selfed	17.067	11.115	5
Seeds/Pollination	PAH-A	Between	27.500	4.464	31
Seeds/Pollination	PIL-A	Control (calyx)	85.208	9.394	7
Seeds/Pollination	PIL-A	Control (no calyx)	31.511	10.147	6
Seeds/Pollination	PIL-A	Selfed	23.029	7.494	11
Seeds/Pollination	PIL-A	Between	15.822	4.464	31
Seeds/Pollination	LEH-B	Control (calyx)	30.035	10.147	6
Seeds/Pollination	LEH-B	Control (no calyx)	12.547	11.115	5
Seeds/Pollination	LEH-B	Selfed	23.294	11.115	5
Seeds/Pollination	LEH-B	Within	19.906	9.394	7
Seeds/Pollination	LEH-B	Between	11.036	4.971	25
Seeds/Pollination	LEH-A	Control (calyx)	19.863	12.427	4
Seeds/Pollination	LEH-A	Control (no calyx)	0.200	11.115	5
Seeds/Pollination	LEH-A	Selfed	8.183	11.115	5
Seeds/Pollination	LEH-A	Within	12.500	11.115	5
Seeds/Pollination	LEH-A	Between	19.070	4.538	30
Seeds / Fruit (GH)	MMR-A	Control (calyx)	63.833	38.981	3
Seeds / Fruit (GH)	MMR-A	Control (no calyx)	27.413	13.359	3
Seeds / Fruit (GH)	MMR-A	Selfed	16.542	19.202	4
Seeds / Fruit (GH)	MMR-A	Between	33.917	35.319	10
Seeds / Fruit (GH)	PAH-C	Control (calyx)	94.432	47.305	5
Seeds / Fruit (GH)	PAH-C	Control (no calyx)	61.900	57.069	3
Seeds / Fruit (GH)	PAH-C	Selfed	77.931	28.063	3
Seeds / Fruit (GH)	PAH-C	Within	67.833	31.084	7
Seeds / Fruit (GH)	PAH-C	Between	66.630	38.364	20
Seeds / Fruit (GH)	PAH-A	Control (calyx)	66.063	36.151	2
Seeds / Fruit (GH)	PAH-A	Control (no calyx)	62.556	19.483	3
Seeds / Fruit (GH)	PAH-A	Selfed	28.208	20.819	4
Seeds / Fruit (GH)	PAH-A	Between	51.472	52.296	27
Seeds / Fruit (GH)	PIL-A	Control (calyx)	103.231	56.132	7
Seeds / Fruit (GH)	PIL-A	Control (no calyx)	45.333	33.405	6
Seeds / Fruit (GH)	PIL-A	Selfed	45.517	22.474	10
Seeds / Fruit (GH)	PIL-A	Between	52.978	30.336	22

Seeds / Fruit (GH)	LEH-B	Control (calyx)	50.408	22.549	4
Seeds / Fruit (GH)	LEH-B	Control (no calyx)	28.917	20.635	4
Seeds / Fruit (GH)	LEH-B	Selfed	22.960	16.135	5
Seeds / Fruit (GH)	LEH-B	Within	25.476	22.936	7
Seeds / Fruit (GH)	LEH-B	Between	18.035	11.243	21
Seeds / Fruit (GH)	LEH-A	Control (calyx)	47.375	18.714	4
Seeds / Fruit (GH)	LEH-A	Control (no calyx)	7.000	.	1
Seeds / Fruit (GH)	LEH-A	Selfed	28.000	20.573	3
Seeds / Fruit (GH)	LEH-A	Within	25.917	8.681	4
Seeds / Fruit (GH)	LEH-A	Between	36.074	23.188	26
Seed Mass	MMR-A	Control (calyx)	0.662	0.553	3
Seed Mass	MMR-A	Control (no calyx)	0.441	0.332	3
Seed Mass	MMR-A	Selfed	0.537	0.429	3
Seed Mass	MMR-A	Between	0.447	0.030	10
Seed Mass	PAH-C	Control (calyx)	0.681	0.043	5
Seed Mass	PAH-C	Control (no calyx)	0.408	0.055	3
Seed Mass	PAH-C	Selfed	0.469	0.055	3
Seed Mass	PAH-C	Within	0.410	0.036	7
Seed Mass	PAH-C	Between	0.478	0.021	20
Seed Mass	PAH-A	Control (calyx)	0.570	0.067	2
Seed Mass	PAH-A	Control (no calyx)	0.454	0.055	3
Seed Mass	PAH-A	Selfed	0.460	0.048	4
Seed Mass	PAH-A	Between	0.452	0.018	27
Seed Mass	PIL-A	Control (calyx)	0.609	0.036	7
Seed Mass	PIL-A	Control (no calyx)	0.493	0.039	6
Seed Mass	PIL-A	Selfed	0.444	0.030	10
Seed Mass	PIL-A	Between	0.438	0.020	22
Seed Mass	LEH-B	Control (calyx)	0.811	0.048	4
Seed Mass	LEH-B	Control (no calyx)	0.651	0.048	4
Seed Mass	LEH-B	Selfed	0.681	0.043	5
Seed Mass	LEH-B	Within	0.661	0.036	7
Seed Mass	LEH-B	Between	0.690	0.021	21
Seed Mass	LEH-A	Control (calyx)	0.599	0.048	4
Seed Mass	LEH-A	Control (no calyx)	0.521	0.095	1
Seed Mass	LEH-A	Selfed	0.524	0.055	3
Seed Mass	LEH-A	Within	0.401	0.048	4
Seed Mass	LEH-A	Between	0.528	0.019	25
Seed Viability	MMR-A	Selfed	0.851	0.099	6
Seed Viability	MMR-A	Between	0.569	0.067	13
Seed Viability	MMR-A	Control (no calyx)	0.681	0.099	6

Seed Viability	MMR-A	Control (no calyx)	0.770	0.081	9
Seed Viability	PAH-C	Selfed	0.860	0.086	8
Seed Viability	PAH-C	Within	0.830	0.059	17
Seed Viability	PAH-C	Between	0.850	0.037	43
Seed Viability	PAH-C	Control (no calyx)	0.808	0.099	6
Seed Viability	PAH-C	Control (no calyx)	0.819	0.086	8
Seed Viability	PAH-A	Selfed	0.699	0.092	7
Seed Viability	PAH-A	Between	0.839	0.033	53
Seed Viability	PAH-A	Control (no calyx)	0.697	0.109	5
Seed Viability	PAH-A	Control (no calyx)	0.773	0.109	5
Seed Viability	PIL-A	Selfed	0.708	0.065	14
Seed Viability	PIL-A	Between	0.876	0.041	35
Seed Viability	PIL-A	Control (no calyx)	0.784	0.081	9
Seed Viability	PIL-A	Control (no calyx)	0.977	0.070	12
Seed Viability	LEH-B	Selfed	0.831	0.067	13
Seed Viability	LEH-B	Within	0.886	0.056	19
Seed Viability	LEH-B	Between	0.895	0.042	33
Seed Viability	LEH-B	Control (no calyx)	0.769	0.122	4
Seed Viability	LEH-B	Control (no calyx)	0.937	0.073	11
Seed Viability	LEH-A	Selfed	0.979	0.099	6
Seed Viability	LEH-A	Within	0.833	0.099	6
Seed Viability	LEH-A	Between	0.909	0.040	37
Seed Viability	LEH-A	Control (no calyx)	1.000	0.243	1
Seed Viability	LEH-A	Control (no calyx)	0.901	0.122	4
Growth	MMR-A	Selfed	0.761	0.109	15
Growth	MMR-A	Between	0.846	0.050	71
Growth	PAH-C	Selfed	0.573	0.117	13
Growth	PAH-C	Within	0.275	0.113	14
Growth	PAH-C	Between	0.681	0.050	72
Growth	PAH-A	Selfed	0.732	0.117	13
Growth	PAH-A	Between	0.746	0.052	67
Growth	PIL-A	Selfed	0.388	0.117	13
Growth	PIL-A	Between	0.611	0.049	73
Growth	LEH-B	Selfed	0.676	0.117	13
Growth	LEH-B	Within	0.265	0.117	13
Growth	LEH-B	Between	0.808	0.051	70
Growth	LEH-A	Selfed	0.358	0.117	13
Growth	LEH-A	Within	0.187	0.117	13
Growth	LEH-A	Between	0.502	0.051	68
N Inflorescences	MMR-A	Selfed	1.400	0.374	15

N Inflorescences	MMR-A	Between	1.500	0.171	72
N Inflorescences	PAH-C	Selfed	1.467	0.374	15
N Inflorescences	PAH-C	Within	1.200	0.374	15
N Inflorescences	PAH-C	Between	1.973	0.167	75
N Inflorescences	PAH-A	Selfed	1.133	0.374	15
N Inflorescences	PAH-A	Between	1.480	0.167	75
N Inflorescences	PIL-A	Selfed	1.733	0.265	30
N Inflorescences	PIL-A	Between	1.573	0.167	75
N Inflorescences	LEH-B	Selfed	1.333	0.374	15
N Inflorescences	LEH-B	Within	1.267	0.374	15
N Inflorescences	LEH-B	Between	1.720	0.167	75
N Inflorescences	LEH-A	Selfed	0.533	0.374	15
N Inflorescences	LEH-A	Within	1.133	0.374	15
N Inflorescences	LEH-A	Between	1.533	0.167	75
Flw / Inflorescence	MMR-A	Selfed	21.200	5.853	5
Flw / Inflorescence	MMR-A	Between	20.765	3.175	17
Flw / Inflorescence	PAH-C	Selfed	23.667	7.557	3
Flw / Inflorescence	PAH-C	Within	16.333	7.557	3
Flw / Inflorescence	PAH-C	Between	20.250	2.672	24
Flw / Inflorescence	PAH-A	Selfed	8.250	6.544	4
Flw / Inflorescence	PAH-A	Between	22.167	3.085	18
Flw / Inflorescence	PIL-A	Selfed	13.667	4.363	9
Flw / Inflorescence	PIL-A	Between	21.700	2.927	20
Flw / Inflorescence	LEH-B	Selfed	22.667	7.557	3
Flw / Inflorescence	LEH-B	Within	22.000	7.557	3
Flw / Inflorescence	LEH-B	Between	20.714	2.856	21
Flw / Inflorescence	LEH-A	Selfed	18.667	7.557	3
Flw / Inflorescence	LEH-A	Within	6.667	7.557	3
Flw / Inflorescence	LEH-A	Between	19.933	3.380	15
Flower / Plant	MMR-A	Selfed	29.680	8.149	15
Flower / Plant	MMR-A	Between	30.978	3.719	72
Flower / Plant	PAH-C	Selfed	34.716	8.149	15
Flower / Plant	PAH-C	Within	19.596	8.149	15
Flower / Plant	PAH-C	Between	40.469	3.644	75
Flower / Plant	PAH-A	Selfed	9.350	8.149	15
Flower / Plant	PAH-A	Between	32.096	3.644	75
Flower / Plant	PIL-A	Selfed	23.695	5.762	30
Flower / Plant	PIL-A	Between	34.962	3.644	75
Flower / Plant	LEH-B	Selfed	30.227	8.149	15
Flower / Plant	LEH-B	Within	27.867	8.149	15

Flower / Plant	LEH-B	Between	37.383	3.644	75
Flower / Plant	LEH-A	Selfed	9.957	8.149	15
Flower / Plant	LEH-A	Within	7.559	8.149	15
Flower / Plant	LEH-A	Between	29.404	3.644	75
Frt / Inflorescence	MMR-A	Selfed	12.500	3.936	4
Frt / Inflorescence	MMR-A	Between	13.500	1.678	22
Frt / Inflorescence	PAH-C	Selfed	9.750	3.936	4
Frt / Inflorescence	PAH-C	Within	8.000	3.520	5
Frt / Inflorescence	PAH-C	Between	12.952	1.718	21
Frt / Inflorescence	PAH-A	Selfed	12.000	4.544	3
Frt / Inflorescence	PAH-A	Between	11.818	1.678	22
Frt / Inflorescence	PIL-A	Selfed	4.600	2.489	10
Frt / Inflorescence	PIL-A	Between	9.300	1.760	20
Frt / Inflorescence	LEH-B	Selfed	13.000	2.783	8
Frt / Inflorescence	LEH-B	Within	9.750	3.936	4
Frt / Inflorescence	LEH-B	Between	14.286	1.718	21
Frt / Inflorescence	LEH-A	Selfed	6.333	4.544	3
Frt / Inflorescence	LEH-A	Within	5.571	2.975	7
Frt / Inflorescence	LEH-A	Between	10.450	1.760	20
Seeds / Fruit	MMR-A	Selfed	124.483	20.003	5
Seeds / Fruit	MMR-A	Between	116.811	9.536	22
Seeds / Fruit	PAH-C	Selfed	115.333	16.906	7
Seeds / Fruit	PAH-C	Within	92.163	22.364	4
Seeds / Fruit	PAH-C	Between	111.649	8.453	28
Seeds / Fruit	PAH-A	Selfed	92.333	25.824	3
Seeds / Fruit	PAH-A	Between	101.408	8.608	27
Seeds / Fruit	PIL-A	Selfed	66.510	16.906	7
Seeds / Fruit	PIL-A	Between	105.541	9.536	22
Seeds / Fruit	LEH-B	Selfed	35.917	25.824	3
Seeds / Fruit	LEH-B	Within	38.489	25.824	3
Seeds / Fruit	LEH-B	Between	78.110	8.453	28
Seeds / Fruit	LEH-A	Selfed	102.000	44.729	1
Seeds / Fruit	LEH-A	Within	67.333	31.628	2
Seeds / Fruit	LEH-A	Between	93.009	10.848	17
Seeds / Plant	MMR-A	Selfed	2178.458	482.369	15
Seeds / Plant	MMR-A	Between	2285.980	220.170	72
Seeds / Plant	PAH-C	Selfed	1649.266	482.369	15
Seeds / Plant	PAH-C	Within	884.760	482.369	15
Seeds / Plant	PAH-C	Between	2945.344	215.722	75
Seeds / Plant	PAH-A	Selfed	1255.733	482.369	15

Seeds / Plant	PAH-A	Between	1893.106	215.722	75
Seeds / Plant	PIL-A	Selfed	720.955	341.086	30
Seeds / Plant	PIL-A	Between	1553.821	215.722	75
Seeds / Plant	LEH-B	Selfed	622.555	482.369	15
Seeds / Plant	LEH-B	Within	475.337	482.369	15
Seeds / Plant	LEH-B	Between	1766.870	215.722	75
Seeds / Plant	LEH-A	Within	474.743	482.369	15
Seeds / Plant	LEH-A	Between	1413.375	215.722	75
Survival	LEH-A	Selfed	0.870		15
Survival	LEH-A	Within	0.870		15
Survival	LEH-A	Between	0.920		75
Survival	LEH-A	Total	0.900		105
Survival	LEH-B	Selfed	0.870		15
Survival	LEH-B	Within	0.870		15
Survival	LEH-B	Between	0.930		75
Survival	LEH-B	Total	0.910		105
Survival	MMR-A	Selfed	1.000		15
Survival	MMR-A	Between	0.990		72
Survival	MMR-A	Total	0.990		87
Survival	PAH-A	Selfed	0.870		15
Survival	PAH-A	Between	0.890		75
Survival	PAH-A	Total	0.890		90
Survival	PAH-C	Selfed	0.930		15
Survival	PAH-C	Within	0.930		15
Survival	PAH-C	Between	0.960		75
Survival	PAH-C	Total	0.950		105
Survival	PIL-A	Selfed	0.870		15
Survival	PIL-A	Between	0.970		75
Survival	PIL-A	Total	0.960		90
Survival	Total	Selfed	0.900		90
Survival	Total	Within	0.890		45
Survival	Total	Between	0.940		447
Survival	Total	Total	0.930		582
Maturity	LEH-A	Selfed	0.470		15
Maturity	LEH-A	Within	0.800		15
Maturity	LEH-A	Between	0.750		75
Maturity	LEH-A	Total	0.710		105
Maturity	LEH-B	Selfed	0.800		15
Maturity	LEH-B	Within	0.600		15
Maturity	LEH-B	Between	0.730		75

Maturity	LEH-B	Total	0.720		105
Maturity	MMR-A	Selfed	0.930		15
Maturity	MMR-A	Between	0.740		72
Maturity	MMR-A	Total	0.770		87
Maturity	PAH-A	Selfed	0.800		15
Maturity	PAH-A	Between	0.630		75
Maturity	PAH-A	Total	0.660		90
Maturity	PAH-C	Selfed	0.730		15
Maturity	PAH-C	Within	0.670		15
Maturity	PAH-C	Between	0.830		75
Maturity	PAH-C	Total	0.790		105
Maturity	PIL-A	Selfed	0.770		30
Maturity	PIL-A	Between	0.750		75
Maturity	PIL-A	Total	0.750		105
Maturity	Total	Selfed	0.750		105
Maturity	Total	Within	0.690		45
Maturity	Total	Between	0.740		447
Maturity	Total	Total	0.740		597
Cumulative Fitness	MMR-A	Selfed	12594.210		
Cumulative Fitness	MMR-A	Between	9861.190		
Cumulative Fitness	PAH-C	Selfed	58741.570		
Cumulative Fitness	PAH-C	Within	42432.950		
Cumulative Fitness	PAH-C	Between	77701.830		
Cumulative Fitness	PAH-A	Selfed	18644.980		
Cumulative Fitness	PAH-A	Between	43411.320		
Cumulative Fitness	PIL-A	Selfed	10624.910		
Cumulative Fitness	PIL-A	Between	23699.880		
Cumulative Fitness	LEH-B	Selfed	12616.810		
Cumulative Fitness	LEH-B	Within	8231.910		
Cumulative Fitness	LEH-B	Between	19698.460		
Cumulative Fitness	LEH-A	Selfed	2452.900		
Cumulative Fitness	LEH-A	Within	4623.630		
Cumulative Fitness	LEH-A	Between	26146.590		
Cumulative Fitness	Total	Selfed	19407.850		
Cumulative Fitness	Total	Within	12191.730		
Cumulative Fitness	Total	Between	35630.930		

APPENDIX B. Mean, SE and N for all stages by outplanting site, mountain range of maternal parent, and geographic distance between parent populations of *S. kaalae*

Variable	Out-planting Site	Mountain Range	Proximity of Parents	Mean	Std. Error	N
Frt/Flw (GH)		Waiʻanae Mts.	Control	0.000	0.151	5
Frt/Flw (GH)		Waiʻanae Mts.	Selfed	0.504	0.072	22
Frt/Flw (GH)		Waiʻanae Mts.	Crosses W/in Pops.	0.470	0.102	11
Frt/Flw (GH)		Waiʻanae Mts.	400-5000m	0.564	0.044	58
Frt/Flw (GH)		Waiʻanae Mts.	5000-20000m	0.631	0.045	57
Frt/Flw (GH)		Waiʻanae Mts.	>20000m	0.642	0.048	49
Frt/Flw (GH)		Koʻolau Mts.	Control	0.146	0.169	4
Frt/Flw (GH)		Koʻolau Mts.	Selfed	0.339	0.113	9
Frt/Flw (GH)		Koʻolau Mts.	Crosses W/in Pops.	0.247	0.113	9
Frt/Flw (GH)		Koʻolau Mts.	400-5000m	1.000	0.239	2
Frt/Flw (GH)		Koʻolau Mts.	5000-20000m	0.639	0.098	12
Frt/Flw (GH)		Koʻolau Mts.	>20000m	0.561	0.049	48
Seeds/Flw (GH)		Waiʻanae Mts.	Control	0.000	1.326	5
Seeds/Flw (GH)		Waiʻanae Mts.	Selfed	2.679	0.632	22
Seeds/Flw (GH)		Waiʻanae Mts.	Crosses W/in Pops.	2.121	0.894	11
Seeds/Flw (GH)		Waiʻanae Mts.	400-5000m	2.818	0.389	58
Seeds/Flw (GH)		Waiʻanae Mts.	5000-20000m	3.238	0.393	57
Seeds/Flw (GH)		Waiʻanae Mts.	>20000m	3.836	0.424	49
Seeds/Flw (GH)		Koʻolau Mts.	Control	0.208	1.483	4
Seeds/Flw (GH)		Koʻolau Mts.	Selfed	1.403	0.989	9
Seeds/Flw (GH)		Koʻolau Mts.	Crosses W/in Pops.	0.825	0.989	9
Seeds/Flw (GH)		Koʻolau Mts.	400-5000m	7.167	2.097	2
Seeds/Flw (GH)		Koʻolau Mts.	5000-20000m	3.754	0.856	12
Seeds/Flw (GH)		Koʻolau Mts.	>20000m	2.962	0.428	48
Seed Mass (mg)		Waiʻanae Mts.	Selfed	0.365	0.022	20
Seed Mass (mg)		Waiʻanae Mts.	Crosses W/in Pops.	0.397	0.035	8
Seed Mass (mg)		Waiʻanae Mts.	400-5000m	0.417	0.013	53
Seed Mass (mg)		Waiʻanae Mts.	5000-20000m	0.389	0.014	50
Seed Mass (mg)		Waiʻanae Mts.	>20000m	0.422	0.014	47
Seed Mass (mg)		Koʻolau Mts.	Selfed	0.434	0.037	7
Seed Mass (mg)		Koʻolau Mts.	Crosses W/in Pops.	0.355	0.049	4
Seed Mass (mg)		Koʻolau Mts.	400-5000m	0.317	0.069	2
Seed Mass (mg)		Koʻolau Mts.	5000-20000m	0.422	0.030	11
Seed Mass (mg)		Koʻolau Mts.	>20000m	0.442	0.015	44
Seed Viability (%)		Waiʻanae Mts.	Selfed	0.786	0.047	45
Seed Viability (%)		Waiʻanae Mts.	Crosses W/in Pops.	0.825	0.112	8

Seed Viability (%)		Waiʻanae Mts.	400-5000m	0.862	0.031	103
Seed Viability (%)		Waiʻanae Mts.	5000-20000m	0.834	0.030	113
Seed Viability (%)		Waiʻanae Mts.	>20000m	0.775	0.033	94
Seed Viability (%)		Koʻolau Mts.	Selfed	0.485	0.095	11
Seed Viability (%)		Koʻolau Mts.	Crosses W/in Pops.	0.323	0.158	4
Seed Viability (%)		Koʻolau Mts.	400-5000m	0.500	0.158	4
Seed Viability (%)		Koʻolau Mts.	5000-20000m	0.753	0.066	23
Seed Viability (%)		Koʻolau Mts.	>20000m	0.717	0.030	113
Growth		Waiʻanae Mts.	Selfed	0.523	0.029	69
Growth		Waiʻanae Mts.	Crosses W/in Pops.	0.706	0.061	16
Growth		Waiʻanae Mts.	400-5000m	0.658	0.016	220
Growth		Waiʻanae Mts.	5000-20000m	0.657	0.015	272
Growth		Waiʻanae Mts.	>20000m	0.780	0.014	305
Growth		Koʻolau Mts.	Selfed	0.832	0.050	24
Growth		Koʻolau Mts.	Crosses W/in Pops.	0.805	0.141	3
Growth		Koʻolau Mts.	400-5000m	0.825	0.122	4
Growth		Koʻolau Mts.	5000-20000m	0.826	0.031	62
Growth		Koʻolau Mts.	>20000m	0.723	0.016	230
N Inflors.	ʻĒkahanui	Waiʻanae Mts.	selfed	1.820	0.300	50
N Inflors.	ʻĒkahanui	Waiʻanae Mts.	Crosses W/in Pops.	3.600	0.670	10
N Inflors.	ʻĒkahanui	Waiʻanae Mts.	400-5000m	3.061	0.175	147
N Inflors.	ʻĒkahanui	Waiʻanae Mts.	5000-20000m	3.044	0.167	161
N Inflors.	ʻĒkahanui	Waiʻanae Mts.	>20000m	4.064	0.162	172
N Inflors.	ʻĒkahanui	Koʻolau Mts.	selfed	2.571	0.566	14
N Inflors.	ʻĒkahanui	Koʻolau Mts.	Crosses W/in Pops.	1.333	1.223	3
N Inflors.	ʻĒkahanui	Koʻolau Mts.	400-5000m	2.667	1.223	3
N Inflors.	ʻĒkahanui	Koʻolau Mts.	5000-20000m	4.250	0.353	36
N Inflors.	ʻĒkahanui	Koʻolau Mts.	>20000m	3.679	0.183	134
N Inflors.	Hakipuʻu	Waiʻanae Mts.	selfed	1.816	0.303	49
N Inflors.	Hakipuʻu	Waiʻanae Mts.	Crosses W/in Pops.	1.333	0.706	9
N Inflors.	Hakipuʻu	Waiʻanae Mts.	400-5000m	2.461	0.179	141
N Inflors.	Hakipuʻu	Waiʻanae Mts.	5000-20000m	3.142	0.167	162
N Inflors.	Hakipuʻu	Waiʻanae Mts.	>20000m	3.928	0.164	167
N Inflors.	Hakipuʻu	Koʻolau Mts.	selfed	2.083	0.612	12
N Inflors.	Hakipuʻu	Koʻolau Mts.	Crosses W/in Pops.	9.000	2.119	1
N Inflors.	Hakipuʻu	Koʻolau Mts.	400-5000m	1.000	1.223	3
N Inflors.	Hakipuʻu	Koʻolau Mts.	5000-20000m	3.750	0.353	36
N Inflors.	Hakipuʻu	Koʻolau Mts.	>20000m	3.786	0.185	131
N Flws/Inflor.	ʻĒkahanui	Waiʻanae Mts.	selfed	103.765	22.076	17
N Flws/Inflor.	ʻĒkahanui	Waiʻanae Mts.	Crosses W/in Pops.	68.000	45.511	4

N Flws/Inflor.	ʻĒkahanui	Waiʻanae Mts.	400-5000m	111.833	12.387	54
N Flws/Inflor.	ʻĒkahanui	Waiʻanae Mts.	5000-20000m	95.828	11.952	58
N Flws/Inflor.	ʻĒkahanui	Waiʻanae Mts.	>20000m	117.649	10.373	77
N Flws/Inflor.	ʻĒkahanui	Koʻolau Mts.	selfed	27.500	45.511	4
N Flws/Inflor.	ʻĒkahanui	Koʻolau Mts.	Crosses W/in Pops.	17.000	91.022	1
N Flws/Inflor.	ʻĒkahanui	Koʻolau Mts.	400-5000m	130.500	64.362	2
N Flws/Inflor.	ʻĒkahanui	Koʻolau Mts.	5000-20000m	80.556	21.454	18
N Flws/Inflor.	ʻĒkahanui	Koʻolau Mts.	>20000m	129.167	12.387	54
N Flws/Inflor.	Hakipuʻu	Waiʻanae Mts.	selfed	52.083	26.276	12
N Flws/Inflor.	Hakipuʻu	Waiʻanae Mts.	Crosses W/in Pops.	0.000	64.362	2
N Flws/Inflor.	Hakipuʻu	Waiʻanae Mts.	400-5000m	45.085	13.277	47
N Flws/Inflor.	Hakipuʻu	Waiʻanae Mts.	5000-20000m	80.273	12.273	55
N Flws/Inflor.	Hakipuʻu	Waiʻanae Mts.	>20000m	78.452	11.560	62
N Flws/Inflor.	Hakipuʻu	Koʻolau Mts.	selfed	3.600	40.706	5
N Flws/Inflor.	Hakipuʻu	Koʻolau Mts.	Crosses W/in Pops.	12.000	91.022	1
N Flws/Inflor.	Hakipuʻu	Koʻolau Mts.	400-5000m	25.000	91.022	1
N Flws/Inflor.	Hakipuʻu	Koʻolau Mts.	5000-20000m	30.933	23.502	15
N Flws/Inflor.	Hakipuʻu	Koʻolau Mts.	>20000m	69.192	12.623	52
N Frt/Inflor.	ʻĒkahanui	Waiʻanae Mts.	selfed	30.389	12.003	18
N Frt/Inflor.	ʻĒkahanui	Waiʻanae Mts.	Crosses W/in Pops.	69.250	25.461	4
N Frt/Inflor.	ʻĒkahanui	Waiʻanae Mts.	400-5000m	59.033	6.520	61
N Frt/Inflor.	ʻĒkahanui	Waiʻanae Mts.	5000-20000m	84.296	6.930	54
N Frt/Inflor.	ʻĒkahanui	Waiʻanae Mts.	>20000m	76.296	6.043	71
N Frt/Inflor.	ʻĒkahanui	Koʻolau Mts.	selfed	30.833	20.789	6
N Frt/Inflor.	ʻĒkahanui	Koʻolau Mts.	400-5000m	42.333	29.400	3
N Frt/Inflor.	ʻĒkahanui	Koʻolau Mts.	5000-20000m	57.929	13.610	14
N Frt/Inflor.	ʻĒkahanui	Koʻolau Mts.	>20000m	78.704	6.930	54
N Frt/Inflor.	Hakipuʻu	Waiʻanae Mts.	selfed	5.471	12.351	17
N Frt/Inflor.	Hakipuʻu	Waiʻanae Mts.	Crosses W/in Pops.	1.000	36.008	2
N Frt/Inflor.	Hakipuʻu	Waiʻanae Mts.	400-5000m	22.458	7.350	48
N Frt/Inflor.	Hakipuʻu	Waiʻanae Mts.	5000-20000m	21.895	6.745	57
N Frt/Inflor.	Hakipuʻu	Waiʻanae Mts.	>20000m	26.111	6.416	63
N Frt/Inflor.	Hakipuʻu	Koʻolau Mts.	selfed	2.429	19.247	7
N Frt/Inflor.	Hakipuʻu	Koʻolau Mts.	Crosses W/in Pops.	21.000	50.923	1
N Frt/Inflor.	Hakipuʻu	Koʻolau Mts.	400-5000m	11.000	50.923	1
N Frt/Inflor.	Hakipuʻu	Koʻolau Mts.	5000-20000m	14.875	12.731	16
N Frt/Inflor.	Hakipuʻu	Koʻolau Mts.	>20000m	30.776	6.687	58
N Flws/Plant	ʻĒkahanui	Waiʻanae Mts.	selfed	203.903	41.931	50
N Flws/Plant	ʻĒkahanui	Waiʻanae Mts.	Crosses W/in Pops.	244.800	93.761	10
N Flws/Plant	ʻĒkahanui	Waiʻanae Mts.	400-5000m	351.017	24.538	146

N Flws/Plant	ʻĒkahanui	Waiʻanae Mts.	5000-20000m	287.128	23.367	161
N Flws/Plant	ʻĒkahanui	Waiʻanae Mts.	>20000m	501.294	22.608	172
N Flws/Plant	ʻĒkahanui	Koʻolau Mts.	selfed	63.188	85.591	12
N Flws/Plant	ʻĒkahanui	Koʻolau Mts.	Crosses W/in Pops.	0.000	209.655	2
N Flws/Plant	ʻĒkahanui	Koʻolau Mts.	400-5000m	386.773	171.182	3
N Flws/Plant	ʻĒkahanui	Koʻolau Mts.	5000-20000m	374.606	49.416	36
N Flws/Plant	ʻĒkahanui	Koʻolau Mts.	>20000m	522.309	25.710	133
N Flws/Plant	Hakipuʻu	Waiʻanae Mts.	selfed	128.458	44.699	44
N Flws/Plant	Hakipuʻu	Waiʻanae Mts.	Crosses W/in Pops.	0.000	148.248	4
N Flws/Plant	Hakipuʻu	Waiʻanae Mts.	400-5000m	115.588	25.518	135
N Flws/Plant	Hakipuʻu	Waiʻanae Mts.	5000-20000m	267.546	23.295	162
N Flws/Plant	Hakipuʻu	Waiʻanae Mts.	>20000m	340.298	23.013	166
N Flws/Plant	Hakipuʻu	Koʻolau Mts.	selfed	13.200	93.761	10
N Flws/Plant	Hakipuʻu	Koʻolau Mts.	400-5000m	0.000	296.497	1
N Flws/Plant	Hakipuʻu	Koʻolau Mts.	5000-20000m	96.594	49.416	36
N Flws/Plant	Hakipuʻu	Koʻolau Mts.	>20000m	282.400	26.005	130
N Seeds/Fruit	ʻĒkahanui	Waiʻanae Mts.	selfed	6.589	0.590	22
N Seeds/Fruit	ʻĒkahanui	Waiʻanae Mts.	400-5000m	7.526	0.377	54
N Seeds/Fruit	ʻĒkahanui	Waiʻanae Mts.	5000-20000m	6.987	0.357	60
N Seeds/Fruit	ʻĒkahanui	Waiʻanae Mts.	>20000m	8.891	0.349	63
N Seeds/Fruit	ʻĒkahanui	Koʻolau Mts.	selfed	7.386	1.046	7
N Seeds/Fruit	ʻĒkahanui	Koʻolau Mts.	400-5000m	7.700	1.956	2
N Seeds/Fruit	ʻĒkahanui	Koʻolau Mts.	5000-20000m	7.861	0.799	12
N Seeds/Fruit	ʻĒkahanui	Koʻolau Mts.	>20000m	7.483	0.399	48
N Seeds/Fruit	Hakipuʻu	Waiʻanae Mts.	selfed	4.071	1.046	7
N Seeds/Fruit	Hakipuʻu	Waiʻanae Mts.	400-5000m	5.243	0.449	38
N Seeds/Fruit	Hakipuʻu	Waiʻanae Mts.	5000-20000m	5.248	0.399	48
N Seeds/Fruit	Hakipuʻu	Waiʻanae Mts.	>20000m	5.200	0.338	67
N Seeds/Fruit	Hakipuʻu	Koʻolau Mts.	selfed	5.633	1.956	2
N Seeds/Fruit	Hakipuʻu	Koʻolau Mts.	5000-20000m	5.021	0.739	14
N Seeds/Fruit	Hakipuʻu	Koʻolau Mts.	>20000m	5.276	0.391	50
N Seeds/Plant	ʻĒkahanui	Waiʻanae Mts.	selfed	435.820	192.456	50
N Seeds/Plant	ʻĒkahanui	Waiʻanae Mts.	Crosses W/in Pops.	1263.120	430.345	10
N Seeds/Plant	ʻĒkahanui	Waiʻanae Mts.	400-5000m	1457.425	112.626	146
N Seeds/Plant	ʻĒkahanui	Waiʻanae Mts.	5000-20000m	2028.615	107.252	161
N Seeds/Plant	ʻĒkahanui	Waiʻanae Mts.	>20000m	2839.270	103.765	172
N Seeds/Plant	ʻĒkahanui	Koʻolau Mts.	selfed	963.912	363.708	14
N Seeds/Plant	ʻĒkahanui	Koʻolau Mts.	400-5000m	854.564	785.699	3
N Seeds/Plant	ʻĒkahanui	Koʻolau Mts.	5000-20000m	1938.215	226.812	36
N Seeds/Plant	ʻĒkahanui	Koʻolau Mts.	>20000m	2153.630	118.449	132

N Seeds/Plant	Hakipuʻu	Waiʻanae Mts.	selfed	62.178	252.707	29
N Seeds/Plant	Hakipuʻu	Waiʻanae Mts.	Crosses W/in Pops.	37.608	453.623	9
N Seeds/Plant	Hakipuʻu	Waiʻanae Mts.	400-5000m	244.430	115.428	139
N Seeds/Plant	Hakipuʻu	Waiʻanae Mts.	5000-20000m	369.514	110.381	152
N Seeds/Plant	Hakipuʻu	Waiʻanae Mts.	>20000m	486.208	105.624	166
N Seeds/Plant	Hakipuʻu	Koʻolau Mts.	selfed	41.914	430.345	10
N Seeds/Plant	Hakipuʻu	Koʻolau Mts.	5000-20000m	358.297	226.812	36
N Seeds/Plant	Hakipuʻu	Koʻolau Mts.	>20000m	631.955	118.900	131
Survivorship	Ekahanui	Waianae Mts.	selfed	0.820	0.050	50
Survivorship	Ekahanui	Waianae Mts.	Crosses W/in Pops.	1.000	0.111	10
Survivorship	Ekahanui	Waianae Mts.	400-5000m	0.900	0.029	147
Survivorship	Ekahanui	Waianae Mts.	5000-20000m	0.910	0.028	162
Survivorship	Ekahanui	Waianae Mts.	>20000m	0.940	0.027	173
Survivorship	Ekahanui	Koolau Mts.	selfed	0.860	0.094	14
Survivorship	Ekahanui	Koolau Mts.	Crosses W/in Pops.	0.500	0.175	4
Survivorship	Ekahanui	Koolau Mts.	400-5000m	1.000	0.175	4
Survivorship	Ekahanui	Koolau Mts.	5000-20000m	0.860	0.058	36
Survivorship	Ekahanui	Koolau Mts.	>20000m	0.900	0.030	134
Survivorship	Hakipuu	Waianae Mts.	selfed	0.590	0.050	49
Survivorship	Hakipuu	Waianae Mts.	Crosses W/in Pops.	0.670	0.117	9
Survivorship	Hakipuu	Waianae Mts.	400-5000m	0.640	0.030	141
Survivorship	Hakipuu	Waianae Mts.	5000-20000m	0.790	0.028	162
Survivorship	Hakipuu	Waianae Mts.	>20000m	0.890	0.027	167
Survivorship	Hakipuu	Koolau Mts.	selfed	0.920	0.097	13
Survivorship	Hakipuu	Koolau Mts.	Crosses W/in Pops.	1.000	0.351	1
Survivorship	Hakipuu	Koolau Mts.	400-5000m	0.670	0.203	3
Survivorship	Hakipuu	Koolau Mts.	5000-20000m	0.890	0.058	36
Survivorship	Hakipuu	Koolau Mts.	>20000m	0.840	0.031	132
Maturity	Ekahanui	Waianae Mts.	selfed	0.840	0.037	50
Maturity	Ekahanui	Waianae Mts.	Crosses W/in Pops.	0.900	0.083	10
Maturity	Ekahanui	Waianae Mts.	400-5000m	0.952	0.022	147
Maturity	Ekahanui	Waianae Mts.	5000-20000m	0.957	0.021	161
Maturity	Ekahanui	Waianae Mts.	>20000m	0.988	0.020	172
Maturity	Ekahanui	Koolau Mts.	selfed	0.786	0.071	14
Maturity	Ekahanui	Koolau Mts.	Crosses W/in Pops.	0.333	0.152	3
Maturity	Ekahanui	Koolau Mts.	400-5000m	1.000	0.152	3
Maturity	Ekahanui	Koolau Mts.	5000-20000m	0.972	0.044	36
Maturity	Ekahanui	Koolau Mts.	>20000m	0.978	0.023	134
Maturity	Hakipuu	Waianae Mts.	selfed	0.633	0.038	49
Maturity	Hakipuu	Waianae Mts.	Crosses W/in Pops.	0.556	0.088	9

Maturity	Hakipuu	Waianae Mts.	400-5000m	0.787	0.022	141
Maturity	Hakipuu	Waianae Mts.	5000-20000m	0.895	0.021	162
Maturity	Hakipuu	Waianae Mts.	>20000m	0.958	0.020	167
Maturity	Hakipuu	Koolau Mts.	selfed	0.833	0.076	12
Maturity	Hakipuu	Koolau Mts.	Crosses W/in Pops.	1.000	0.264	1
Maturity	Hakipuu	Koolau Mts.	400-5000m	0.667	0.152	3
Maturity	Hakipuu	Koolau Mts.	5000-20000m	0.944	0.044	36
Maturity	Hakipuu	Koolau Mts.	>20000m	0.954	0.023	131

APPENDIX C. Relative performance of crosstypes for progeny: RP_i (s/w = selfed/crosses within populations); RP_b (s/b = selfed/crosses between populations); RP_h (w/b = crosses within populations/crosses between populations). Positive s/w indicates inbreeding depression, negative s/w indicates outbreeding depression, positive w/b indicates heterosis, and negative w/b indicates outbreeding depression.

Variable	Outplanting Site	Maternal Population	RP_i (s/w)	RP_b (s/b)	RP_h (w/b)
Seeds/Pollination		Pālāwai		0.7136	
Seeds/Pollination		ʻĒkahanui-A	-0.4331	0.0407	0.4561
Seeds/Pollination		ʻĒkahanui-B		0.6128	
Seeds/Pollination		Huliwai		-0.1278	
Seeds/Pollination		Kaluaʻa		0.9434	
Seeds/Pollination		Pahole-A		0.6635	
Seeds/Pollination		Pahole-B		0.0583	
Seeds/Pollination		Kahana	0.5091	0.6737	0.3353
Seeds/Pollination		Maʻakua	-0.4099	0.7928	0.8777
Seeds/Pollination		Kaipapaʻu	-1.0000	0.4738	1.0000
Survival	ʻĒkahanui	Pālāwai		-0.0429	
Survival	ʻĒkahanui	ʻĒkahanui-A	0.3000	0.2548	-0.0606
Survival	ʻĒkahanui	ʻĒkahanui-B		0.3154	
Survival	ʻĒkahanui	Huliwai		-0.1045	
Survival	ʻĒkahanui	Kaluaʻa		1.0000	
Survival	ʻĒkahanui	Pahole-A		0.0571	
Survival	ʻĒkahanui	Pahole-B		0.0169	
Survival	ʻĒkahanui	Kahana	-0.6667	-0.1231	0.6199
Survival	ʻĒkahanui	Maʻakua	1.0000	1.0000	-0.1053
Survival	ʻĒkahanui	Kaipapaʻu		-0.0923	
Survival	Hakipuʻu	ʻĒkahanui-A	0.2500	0.3431	0.1242
Survival	Hakipuʻu	ʻĒkahanui-B		0.2500	
Survival	Hakipuʻu	Huliwai		0.6192	
Survival	Hakipuʻu	Kaluaʻa		1.0000	
Survival	Hakipuʻu	Kahana	0.0000	-0.0968	-0.0968
Survival	Hakipuʻu	Maʻakua		-0.2051	
Survival	Hakipuʻu	Pahole-A		-0.1364	
Survival	Hakipuʻu	Pahole-B		-0.0720	
Survival	Hakipuʻu	Pālāwai		0.5278	
Survival	Hakipuʻu	Kaipapaʻu		-0.0886	
N Inflorescences	ʻĒkahanui	Pālāwai		0.2734	
N Inflorescences	ʻĒkahanui	ʻĒkahanui-A	0.5413	0.5286	-0.0269
N Inflorescences	ʻĒkahanui	ʻĒkahanui-B		0.4962	

N Inflorescences	ʻĒkahanui	Huliwai		0.5456	
N Inflorescences	ʻĒkahanui	Kaluaʻa		1.0000	
N Inflorescences	ʻĒkahanui	Pahole-A		0.3512	
N Inflorescences	ʻĒkahanui	Pahole-B		0.4192	
N Inflorescences	ʻĒkahanui	Kahana	-0.7576	-0.2727	0.6667
N Inflorescences	ʻĒkahanui	Maʻakua		1.0000	
N Inflorescences	ʻĒkahanui	Kaipapaʻu		0.3243	
N Inflorescences	Hakipuʻu	Pālāwai		1.0000	
N Inflorescences	Hakipuʻu	ʻĒkahanui-A	-0.3450	0.3309	-0.5618
N Inflorescences	Hakipuʻu	ʻĒkahanui-B		0.6178	
N Inflorescences	Hakipuʻu	Huliwai		0.6586	
N Inflorescences	Hakipuʻu	Kaluaʻa		1.0000	
N Inflorescences	Hakipuʻu	Pahole-A		0.1418	
N Inflorescences	Hakipuʻu	Pahole-B		0.0038	
N Inflorescences	Hakipuʻu	Kahana	0.8889	0.7459	-0.5627
N Inflorescences	Hakipuʻu	Maʻakua		0.4412	
N Inflorescences	Hakipuʻu	Kaipapaʻu		0.3937	
Frt/ Inflorescence	ʻĒkahanui	Pālāwai		0.9458	
Frt/ Inflorescence	ʻĒkahanui	ʻĒkahanui-A	0.1300	-0.1362	-0.1362
Frt/ Inflorescence	ʻĒkahanui	ʻĒkahanui-B		0.8331	
Frt/ Inflorescence	ʻĒkahanui	Huliwai		0.4166	
Frt/ Inflorescence	ʻĒkahanui	Kaluaʻa		1.0000	
Frt/ Inflorescence	ʻĒkahanui	Pahole-A		0.6528	
Frt/ Inflorescence	ʻĒkahanui	Pahole-B		0.7690	
Frt/ Inflorescence	ʻĒkahanui	Kahana		0.8986	
Frt/ Inflorescence	ʻĒkahanui	Kaipapaʻu		0.2711	
Frt/ Inflorescence	Hakipuʻu	Pālāwai		1.0000	
Frt/ Inflorescence	Hakipuʻu	ʻĒkahanui-A	-0.8182	0.7704	0.9583
Frt/ Inflorescence	Hakipuʻu	ʻĒkahanui-B		1.0000	
Frt/ Inflorescence	Hakipuʻu	Huliwai		0.6297	
Frt/ Inflorescence	Hakipuʻu	Kaluaʻa		1.0000	
Frt/ Inflorescence	Hakipuʻu	Pahole-A		0.5781	
Frt/ Inflorescence	Hakipuʻu	Pahole-B		0.9444	
Frt/ Inflorescence	Hakipuʻu	Kahana	1.0000	1.0000	0.3008
Frt/ Inflorescence	Hakipuʻu	Maʻakua		1.0000	
Frt/ Inflorescence	Hakipuʻu	Kaipapaʻu		0.8223	
Seeds/ Fruit	ʻĒkahanui	Pālāwai		0.5608	
Seeds/ Fruit	ʻĒkahanui	ʻĒkahanui-A		0.0422	
Seeds/ Fruit	ʻĒkahanui	ʻĒkahanui-B		0.1829	
Seeds/ Fruit	ʻĒkahanui	Huliwai		0.1322	

Seeds/ Fruit	ʻĒkahanui	Kaluaʻa		1.0000	
Seeds/ Fruit	ʻĒkahanui	Pahole-A		0.3338	
Seeds/ Fruit	ʻĒkahanui	Pahole-B		0.0214	
Seeds/ Fruit	ʻĒkahanui	Kahana		0.5818	
Seeds/ Fruit	ʻĒkahanui	Maʻakua		1.0000	
Seeds/ Fruit	ʻĒkahanui	Kaipapaʻu		-0.2568	
Seeds/ Fruit	Hakipuʻu	Pālāwai		1.0000	
Seeds/ Fruit	Hakipuʻu	ʻĒkahanui-A		0.1866	
Seeds/ Fruit	Hakipuʻu	ʻĒkahanui-B		1.0000	
Seeds/ Fruit	Hakipuʻu	Huliwai		1.0000	
Seeds/ Fruit	Hakipuʻu	Kaluaʻa		1.0000	
Seeds/ Fruit	Hakipuʻu	Pahole-A		-0.0402	
Seeds/ Fruit	Hakipuʻu	Pahole-B		0.6745	
Seeds/ Fruit	Hakipuʻu	Kahana		1.0000	
Seeds/ Fruit	Hakipuʻu	Maʻakua		1.0000	
Seeds/ Fruit	Hakipuʻu	Kaipapaʻu		-0.0172	
Cumulat. Fitness	ʻĒkahanui	Pālāwai		0.9948	
Cumulat. Fitness	ʻĒkahanui	ʻĒkahanui-A	-0.7762	0.6749	0.9272
Cumulat. Fitness	ʻĒkahanui	ʻĒkahanui-B		0.9818	
Cumulat. Fitness	ʻĒkahanui	Huliwai		0.7087	
Cumulat. Fitness	ʻĒkahanui	Kaluaʻa		1.0000	
Cumulat. Fitness	ʻĒkahanui	Pahole-A		0.9524	
Cumulat. Fitness	ʻĒkahanui	Pahole-B		0.8784	
Cumulat. Fitness	ʻĒkahanui	Kahana	-0.9924	0.9783	0.9998
Cumulat. Fitness	ʻĒkahanui	Maʻakua	1.0000	1.0000	0.9999
Cumulat. Fitness	ʻĒkahanui	Kaipapaʻu	-1.0000	0.6191	1.0000
Cumulat. Fitness	Hakipuʻu	Pālāwai		1.0000	
Cumulat. Fitness	Hakipuʻu	ʻĒkahanui-A	-0.9842	0.9212	0.9988
Cumulat. Fitness	Hakipuʻu	ʻĒkahanui-B		1.0000	
Cumulat. Fitness	Hakipuʻu	Huliwai		0.9840	
Cumulat. Fitness	Hakipuʻu	Kaluaʻa		1.0000	
Cumulat. Fitness	Hakipuʻu	Pahole-A		0.8530	
Cumulat. Fitness	Hakipuʻu	Pahole-B		0.9817	
Cumulat. Fitness	Hakipuʻu	Kahana	1.0000	1.0000	0.7549
Cumulat. Fitness	Hakipuʻu	Maʻakua	1.0000	1.0000	0.9998
Cumulat. Fitness	Hakipuʻu	Kaipapaʻu	-1.0000	0.9367	1.0000

APPENDIX D: FLORAL VISITOR OBSERVATIONS

Presumed modes of pollination (bird, insect, or wind) are often described for the Hawaiian flora, though detailed knowledge of relationships between effective pollinators and plant taxa is limited (Wagner et al. 1999). Determining what is effectively pollinating a species is difficult, but observing which birds and insects visit flowers can help estimate current levels of outcrossing within a plant population and determine if the habitat is host to appropriate floral visitors for a particular plant taxon. For rare plant species, historic plant-pollinator interactions may be unknown and current populations and habitats may be reduced and altered so that both the floral and faunal communities may be modified (Kolb 2008). For these species where natural habitats have been disturbed, populations have been extirpated, or population sizes have substantially declined, reintroductions may be necessary to create new, healthy populations (Guerrant 1996, Guerrant and Kaye 2007, Menges 2008). Understanding the mating system, including levels of selfing and outcrossing, and the dependence of the mating system on pollinators, will benefit recovery efforts and reintroduction designs (Dudash and Murren 2008).

If effects of selfing and outcrossing on the relative fitness of plants are known, and outcrossing is beneficial to the population, reintroduction sites with appropriate floral visitors may be more successful than reintroduction sites where potential pollinators are absent. Examining plant-pollinator relationships before selecting reintroduction locations may be important to prevent reintroductions in sites devoid of necessary pollinators (Kearns et al. 1998). The success of reintroductions may be dependent on outcrossing levels of a species. Outcrossing may maintain genetic variation and potentially prevent the expression of inbreeding depression in species harboring recessive deleterious alleles (Lande and Schemske 1985, Ellstrand and Elam 1993). If the plants are animal pollinated, an appropriate pollinator must be first present, and second, interact with the reproductive parts of flowers to promote cross-pollination and prevent the expression of inbreeding depression.

Species in the endemic Hawaiian genus *Schiedea* (Caryophyllaceae), in which much information on the mating and breeding systems is known, may benefit from observations of interactions with potential pollinators. This information could help

determine if the current habitats can support healthy populations of *Schiedea*. For rare species in this genus, this information could help guide the determination of suitable reintroduction locations. *Schiedea* is one of the largest adaptive radiations of vascular plants in the Hawaiian Islands, comprised of 34 species that evolved from a single hermaphroditic ancestor (Wagner et al. 1999, Willyard et al. 2011). Research on *Schiedea* has focused on the phylogeny, genetic diversity, and evolution of dimorphic breeding systems (Weller and Sakai 1990, Weller et al. 1995, Weller et al. 1996, Weller et al. 1998, Weller et al. 2001, Wagner et al. 2005, Sakai et al. 2006). Mating systems have been quantified for several species. Six species have shown low levels of selfing in hermaphroditic species, with high levels of inbreeding depression, or high levels of selfing in hermaphrodites in dimorphic species (Sakai et al. 1989, Norman et al. 1995, Sakai et al. 1997, Culley et al. 1999, Rankin et al. 2002, Wagner et al. 2005, Weller and Sakai 2005, Sakai et al. 2008). A seventh species showed high levels of selfing and low levels of inbreeding depression (Weller et al. 2005). Prior studies have indicated that native Pyralid moths could be potential pollinators for *S. lydgatei* (Norman et al. 1997), a hermaphroditic species in the section *Schiedea*. Four hermaphroditic species are considered potentially bird-pollinated (due to morphology and nectar volume and concentration; Weller et al. 1998), but no observations of interactions between these taxa are known. Several other hermaphroditic species have evolved facultative or obligate autogamy, and all dimorphic species are wind-pollinated (Weller et al. 1998, Wagner et al. 2005).

Fifteen species (44%) in the genus *Schiedea* are federally listed as threatened or endangered (Wagner et al. 2005). Reintroduction efforts and designs are underway for three (all hermaphroditic) of these listed species by natural resource managers on O‘ahu (Keir and Weisenberger 2011). Two species, *S. nuttallii* Hooker and *S. kaalae* Wawra, are sister species in the section *Mononeura*, and both are predicted to be predominantly outcrossing species, though the latter is predicted to have higher rates of outcrossing than the former (Weller et al. 1998). *S. nuttallii* is relatively shorter-lived and known to have recently sustained large population crashes, while *S. kaalae* has mostly been known from small populations dispersed throughout many locations on the island (Keir and Weisenberger 2011). The third species, *S. obovata* (Sherff) W.L. Wagner & Weller, is in

section *Alsinidendron*, and is predicted to be a predominantly selfing species, but is also one of the four species suspected of bird-pollination (Weller et al. 1998). The goal of this study was to observe floral visitors, estimate *in situ* levels of potential outcrossing, and determine if some locations are more suitable for reintroductions of *S. nuttallii, S. kaalae*, and *S. obovata* than others because of pollinator availability.

Methods: Floral visitor observations were conducted throughout the flowering period at established reintroduction sites (and one research study site; Chapter 4) of all three taxa on Oʻahu (Table 1). Locations were throughout the Waiʻanae Mts. except for one reintroduction in the Koʻolau Mts. of *S. kaalae*. The Oʻahu Army Natural Resources Program (University of Hawaiʻi – Pacific Cooperative Studies Unit) manages the reintroduction sites, located in the following gulches: Kahanahaiki and W Makaleha (*S. obovata*), Pahole (*S. nuttallii*), and Makaua (Koʻolau Mts.), ʻĒkahanui (augmentation and study site) and Kaluaʻa (*S. kaalae*). Observations consisted of recording the taxa that visited a plant within a ten or thirty minute block of time. Locations for observations were chosen based on the differences in the number of reproductive plants at each site, to determine if the amount of pollinator observations was correlated with the number of mature plants in a population (Kolb 2008). Observations were made during daylight hours (dawn to dusk). Plants were haphazardly selected, with only one plant observed within each block of time. A camera (Moultrie Feeders Infrared Game Spy Model #MFH-I65, EBSCO Industries, Inc.) took ten seconds of video once an hour for one night at the ʻĒkahanui outplanting (study) site and the Makaua reintroduction for *S. kaalae*. Visitor taxa, length of visit per flower, behavior of visitor, number of flowers visited, number of flowers on the observed plant and the number of mature plants were recorded at each observation (excluding video). Floral morphology suggests that *S. nuttallii* and *S. kaalae* are potentially insect-pollinated, and observations were made at close distance (ca. 1m). Floral morphology and nectar volume and composition of *S. obovata* suggest possible bird pollination (Weller et al. 1998). Observations on this taxon were made at a distance of 10m with binoculars in silence and without moving. A five-minute buffer, where the observer remained in position, quiet and still, prefaced every 30-minute observation (as not to disturb birds immediately prior to observing).

Results: There were very few floral visitors; 0.86% of the total observation time (40 out of 4215 minutes) was spent actually viewing visitors on flowers (Table 1). There were not enough observations of visitors to detect correlations with the number of flowers available at a site or on a plant. Two non-native flies visited *S. nuttallii* and *S. obovata* each for short durations (10 and 1.17 minutes, respectively). Contrary to the lack of observations on *S. nuttallii* and *S. obovata*, moths of the endemic genus *Mestolobes* (Butler 1882; Crambidae) visited the flowers of *S. kaalae*. The moth observations on *S. kaalae* at the ʻĒkahanui outplanting site were the only substantial visitation recorded. While no visitation was seen (out of 780 minutes) during daylight observations of this species, 23.2% (29 out of 125 minutes) of the time spent during dusk documented moths visiting flowers. At dusk, dozens of moths were observed around the flowering plants at the ʻĒkahanui outplanting study site. For the first five to ten minutes, moths flew around plants and hovered around flowers, but only for a few seconds before moving away. After this time, moths began to land on flowers, where they could remain for several minutes at a time. Moths were observed in video recordings between 6:00pm to 8:00pm. Anthers on flowers of *S. kaalae* typically dehisced around 4pm (pers. observations) and nectar was seen during dusk observations gathered at the tips of the nectar shafts. The timing of flower visitation and release of pollen and floral rewards, indicate a crepuscular association with pollinators.

Discussion: Visitation, or lack there of, has important implications in understanding the mating systems of these species. Based on the low visitation rates, it can be assumed that selfing rates are high for the populations of *S. nuttallii* and *S. obovata* observed in this study. Visitation by moths to *S. kaalae* may be an important interaction that maintains outcrossing in this species. Moths, however, were only observed visiting flowers during dusk at the ʻĒkahanui study site, and not at the Makaua reintroduction site (the only other *S. kaalae* location with dusk observations). The number of mature plants was 58 times more at the ʻĒkahanui site than at the Makaua site, and the relative abundance of flowers may have influenced visitation rates. Further dusk observations at all locations are necessary to determine if population size is correlated with floral visitation, and if this species of *Mestolobes* has limited distribution on Oʻahu.

Levels of inbreeding depression and outbreeding depression have been quantified in these species (Chapters 2-4). The lack of inbreeding depression and predicted high levels of selfing in *S. nuttallii* and *S. obovata* suggest that hermaphroditism is stable in these species (Lande and Schemske 1985). Furthermore, the success of a reintroduction is likely not dependent on the faunal community present at the location of an outplanting for these species. On the contrary, *S. kaalae* may have higher outcrossing rates, and the presence of moths may be a critical factor in determining appropriate habitat for the reintroduction of this species. No inbreeding depression was detected, though substantial heterosis was present upon outcrossing distant populations. Despite the fact that species with high levels of outcrossing are predicted to express inbreeding depression upon selfing (Lande and Schemske 1985), most wild locations of *S. kaalae* only have one to two plants. It is likely that selfing rates are much higher at these sites than at the large ʻĒkahanui study site. Deleterious alleles may have been purged at the wild populations, limiting the amount of detectable inbreeding depression (Husband and Schemske 1996). *S. kaalae* populations seem able to withstand high levels of selfing in the absence of floral visitation and yet benefit greatly from outcrossing. Mixed-source reintroductions have been proposed for this species, along with outplanting heterotic individuals (Chapter 4). If the observed levels of visitation are present at future mixed-source reintroductions, levels of outcrossing may maintain heterotic individuals for future generations. Since the floral visitation was observed at a recent outplanting site, and the number of wild plants is so low (28 plants remain total at six locations) it is likely that the visitors are generalists; visiting more taxa than just *S. kaalae*. *Mestolobes* may also possibly visit a population of *S. hookeri* located near the study site, and visitor observations should be conducted on this taxon as well. The presence of *Mestolobes* and other crepuscular microlepidoptera taxa should be investigated in areas where future reintroductions are planned.

Table 1. Floral Visitor Observations. Visitor-Flower contact = contact with reproductive parts. *2 flies, crawled into flowers, many flies in area and on plants. **1 fly, sat on top of several flowers. *** Dozens of moths on flowers, several for several minutes. *B. dorsalis* and *A. obliqua* identified by David Preston (Bishop Museum). *Mestolobes* identified by William Haines (Univ. of Hawai‘i).

Species	Location	Date	Obser-vation Time (hr)	Pop. Size (Mature Plants Only)	Visitor-Flower Contact (hr.)	N Visits	N Plants Observed	Time @ Flowers (hrs)/ Visits	Mean Visits/ Flower/ Hr	Visitor Species	Order: Family
S. obovata	Kahanahaiki	Jun 2009	2	101	0.01945	2*	3	0.010	0.1333± 0.00	*Bactrocera dorsalis*	Diptera: Tephrittidae
S. obovata	Kahanahaiki	Mar 2010	3	143	0	0	6	0	0	NA	
S. obovata	W Makaleha	Apr 2009	4	76	0	0	4	0	0	NA	
S. obovata	W Makaleha	May 2009	7	76	0	0	10	0	0	NA	
S. nuttallii	Pahole	Apr 2009	9	55	0	0	6	0	0	NA	
S. nuttallii	Pahole	Apr 2009	9.33	55	0	0	17	0	0	NA	
S. nuttallii	Pahole	May 2009	7	55	0.167	2**	13	0.083	0.0382± 0.008	*Allograpta obliqua*	Diptera: Syrphidae
S. kaalae	Kaluaa	May 2009	4	147	0	0	4	0	0	NA	
S. kaalae	Makaua	Apr 2009	1.83	12	0	0	3	0	0	NA	
S. kaalae	Makaua	Mar 2010	7	12	0	0	6	0	0	NA	
S. kaalae	Ekahanui (augmentation)	Apr 2009	0.5	696	0	0	2	0	0	NA	
S. kaalae	Ekahanui (study site)	Mar 2010	15.083	696	0.519	20***	30	0.0396 ± 0.0299	2.132± 2.003	*Mestolobes* sp.	Lepidoptera: Crambidae

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