EXPERIMENTAL HYBRIDIZATIONS IN HAWAIIAN *METROSIDEROS*

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The genus *Metrosideros* of the family Myrtaceae is native, but not endemic to Hawai'i. The genus occurs scattered from eastern Australia through the high islands of the Pacific Ocean, with the largest number of species located in New Zealand. If New Zealand is considered to be the probable center of origin for the genus because it has the largest number of extant species, then the high islands of the Pacific to the east and north could have served as "stepping stones" in the migration of the genus to Hawai'i by long distance dispersal (Corn 1972*b*). The most common species occurring on oceanic islands of the Pacific is *M. collina* (J. R. & G. Forst.) Gray, whose distribution ranges from the New Hebrides on the west to Pitcairn Island on the east to the Hawaiian Islands on the north. The species is fairly uniform in the western part of its range (i.e., Tahiti and Marquesas) and reaches its greatest variability in the Hawaiian Islands (Smith 1973). This species in Hawai'i is presently called *M. polymorpha* Gaud. (St. John 1979).

The genus is abundant in the relatively undisturbed portions of the six largest Hawaiian Islands. It occurs in diverse edaphic, topographic, and climatic habitats as either a tree or shrub. The plants are variable in height, shape, vegetative and floral characteristics. Taxonomic treatments of the genus (Hillebrand 1888; Rock 1917; Skottsberg 1944; Porter 1972; St. John 1979) are difficult since the taxon may be best described as a polymorphic group of plants. Sastrapradja and Lamoureux (1969) found no distinct patterns of variation in wood variation. Anatomical and morphological evidence of leaf variation in mature plants along an altitudinal and rainfall transect on Mauna Loa, Hawai'i, often suggests clinal patterns of variation commonly found among outbreeding forest trees (Corn 1979).

This paper includes information from observations and field hybridizations of seven varieties of *M. polymorpha* present on Mauna Loa, Hawai'i. A basic number of $n = 11$ chromosomes was described by Niimoto (1950), Skottsberg (1955), and Carr (1978), which corresponds to the basic count for New Zealand material by Mousel (1965). However, Skottsberg also noted counts of $n = 12$ and $n = 13$ in material from the Island of Hawai'i for varieties *incana* and *glabrifolia*, respectively. Carpenter (1976) in a paper on plant-pollinator interactions described two yellow-flowered plants as self-compatible, but the red-flowered plants as being partially self-incompatible.

The objective of this paper is to ascertain if hybridization can occur between plants that are morphologically different.

METHODS

Pollen samples were obtained by two methods. The first method involved coating glass slides with either vasoline or scotch tape and placing them around and under blooming *Metrosideros* trees for four days and nights to see if *Metrosideros* pollen was wind-dispersed. The second method was devised to see if birds were transmitting *Metrosideros* pollen between trees at 1220 to 2012 m elevation in Hawaii Volcanoes National Park and Kilauea Forest Reserve. Birds were caught in mist nets, their head feathers around their beaks were sampled with scotch tape, and the scotch tape was fastened to microscope slides and viewed for *Metrosideros* pollen.

Experimental field hybridizations were prepared by removing all but five to seven buds in an inflorescence and emasculating the remaining buds. A wire frame was erected around the prepared inflorescence which was covered by an organdy bag, so that the bag did not touch the elongating styles during windy weather. The bag was secured by cotton and string at its base to prevent ants and other insects from crawling into the bag. A small plastic umbrella was erected above the inflorescences used in nectar production studies to prevent rain from diluting the nectar. Some of the prepared bags with emasculated flowers served as controls which were not crossed. Other bagged inflorescences were crossed with pollen from select plants about 10 days after emasculation. Pollination bags were again secured after the flowers were crossed and labelled. Approximately four to seven months later when the capsules were mature, the labelled bags were clipped off the female parent plant and brought into the laboratory for analysis.

Each bag containing mature capsules was air-dried until the capsules opened. One hundred seeds from each cross were placed into petri dishes, given light and water for germination trials. After one month the percent seed germination for each cross was tallied. The seedlings were then planted into soil and grown.

RESULTS

Metrosideros grows as a tree or shrub which bears conspicuous flower clusters at the terminal portions of its branches. Although it flowers most commonly during the spring and summer, sporadic blooms may be seen on a few trees throughout the year. The inflorescence is composed of a flower cluster that may vary in number from several to about 30, but normally between 18 and 24 flowers.

Flowers normally have no scent and are red in color. However, their color on different trees may vary from deep red to various shades of red, salmon, orange, yellow, and very rarely white. The flowers are perfect with five small petals and numerous protruding stamens which are the showiest portion of the flower. The flowers open with the modified floral cup facing upward which allows the secreted nectar to be retained within the cup. The height of the stamens in relation to the style varies, as does the space between the row of stamens and the central style.

Nectar production begins as the petals and stamens unfold, and is greatest several days later when the anthers begin to dehisce. It ceases three to five days later when the anthers and petals abscise. The receptive period of the stigma may vary from one to two days after the stamens begin to exert (Carpenter 1976) to several days after the anthers begin to dehisce (Corn 1972a). Within an inflorescence it is common to have flowers in all stages of the blooming cycle.

Nectar is 10 to 15% solid (by refractometer measurements) when flowers are enclosed within a plastic bag. However, these same flowers when exposed to wind, low humidity, and no nectar-gathering animals, may have nectar concentrations of > 60% (Corn 1979). Analysis of nectar yields low protein (or histidine) content.

The stamens produce abundant sticky pollen which attract various hymenopterans, including native and introduced bees and wasps. These hymenopterans may also obtain nectar on sunny, hot days. Other insects seen on the flowers are nocturnal caterpillars that live in the flower buds during the day and emerge at night. They feed on the anthers and young succulent portions of the flower buds. Moths, crickets, ants, and even centipedes have been seen on the blossoms. No wind-dispersed *Metrosideros* pollen was obtained using sticky slides placed under and around blooming trees.

Although the flowers are open and relatively unspecialized, their dimensions and position on the branch are probably best suited to bird pollination. Various native and introduced birds visit the flowers for nectar and/or insects. The pollen adheres to the feathers and beaks of birds visiting the flowers. Twenty-three of 27 sampled birds had *Metrosideros* pollen (Table 1). Species carrying pollen include: 'Amakihi (*Loxops virens*), 'Apapane (*Himatione sanguinea*), Hawaiian Creeper (*Loxops maculatus mana*), Japanese White-eye (*Zosterops japonica*), House Finch (*Carpodacus mexicanus frontalis*), and House Sparrow (*Passer domesticus*). Since the birds commonly flit from tree to tree visiting the flowers, they can serve as active and efficient cross-pollinators. Carpenter (1976) found more capsules produced on trees that birds were visiting the blossoms, than on trees where insects but no birds were visiting the blossoms.

Selective field hybridizations yielded mixed results. None of the uncrossed emasculated flowers produced seed or mature capsules. Since apomixes is not usually associated with diploidy, it is probably safe to assume that apomixes plays no role in seed production.

Hybridizations were made between trees within one site and between varieties at various sites. In some cases successful crosses were made in one direction between two plants, but the reciprocal cross was not successful. Carpenter (1976) suggested a partial self-incompatibility system was present in red-flowering plants. Of nine self-pollinated red-flowering plants, four of these (36%) set no seed. When these same plants were outcrossed, five of 36 crosses (or 14%) produced no seed. Seed germination from selfed individuals commonly yielded few seedlings.

Crosses were attempted among the seven varieties found on Mauna Loa (Fig. 1). All but one possible combination was attempted. Of the 20 combinations tried, seven of them did not produce capsules and seed. Many factors could have contributed to these failures. For example, some bags were broken off the trees between the time of pollination and capsule maturity; sometimes the style was injured during emasculation; the timing of the cross was poor; the pollen too old; or climatic factors unfavorable. Before self-incompatibilities are attributed to these failures, additional trials and cytological studies need to be made.

Not all successful crosses diagramed in Figure 1 had their capsules collected at the proper time. Some capsules had dehisced and the seeds exposed to rain. When this happened the seeds became darker in color and did not germinate.

A subset of data shown in Figure 1 (Fig. 2) illustrates those instances where seed germination could be tabulated. Some of the 55 crosses that were tried on 16 trees failed to produce capsules. Where capsules were produced, germination varied from <1% to 42%. Additional crosses may result in higher seed germination, due to various factors that affect crossability.

DISCUSSION

Certain varieties occur more commonly in certain localities (Corn 1979). M. polymorpha varieties imbricata, incana, and polymorpha occur together in seasonally dry sites. At higher elevations var. imbricata is absent, but var. nuda is found in association with var. polymorpha and var. incana. In rain forests at mid-elevations the other three varieties, newellii, macrophylla, and glaberrima, are common, although some var. incana, var. polymorpha, and var. imbricata may also exist in these sites.

Therefore, the occurrence of these varieties in certain habitats may limit their chances of crossing with other varieties that are not present in the immediate area. However, these varieties may still be able to hybridize if given the opportunity. A good example of this can be seen in a cross I made two years ago between a plant from the Marquesas Islands and a plant from O'ahu. The Marquesas Island plant which is growing at Lyon Arboretum, is distinctly different in appearance from the O'ahu plant. Although no seed was produced using the O'ahu tree as the female parent, 22% seed germination was obtained using the Marquesas Islands tree as the female parent.

In summary, various crosses have been attempted among the seven recognized varieties found on Mauna Loa, Hawai'i. Some of these crosses produce viable seeds with the hybrids now being grown for future analysis and crosses. Other crosses did not result in seed set. The reasons for these failures are not known. Additional work needs to be done to verify if these crosses are genetically incompatible.

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TABLE 1. The amount of *Metrosideros* pollen found on bird species at three localities on the Island of Hawai'i [1].

Bird Species	Hawaii Volcanoes National Park Residential Area 1220 m	Kīlauea Forest Reserve 1615 m	Mauna Loa Strip Road 2012 m
House Finch (*Carpodacus mexicanus frontalis*)	3, 2		
House Sparrow (*Passer domesticus*)	3, 3		
Japanese White-eye (*Zosterops japonicus*)	0, 1, 1, 1, 1, 3		1, 2
'Amakihi (*Loxops virens*)	0, 3, 1		1, 0, 1, 1
'Apapane (*Himatione sanguinea*)	2		1, 2
Hawaiian Creeper (*Loxops maculatus mana*)			3, 2, 2, 1
Hawaiian Thrush (*Phaeornis obscurus*)		0	

[1] Amount of *Metrosideros* pollen present: 0 = no pollen grains/slide; 1 = 1 to 10 pollen grains/slide; 2 = 11 to 50 pollen grains/slide; 3 = 51 or more pollen grains/slide.

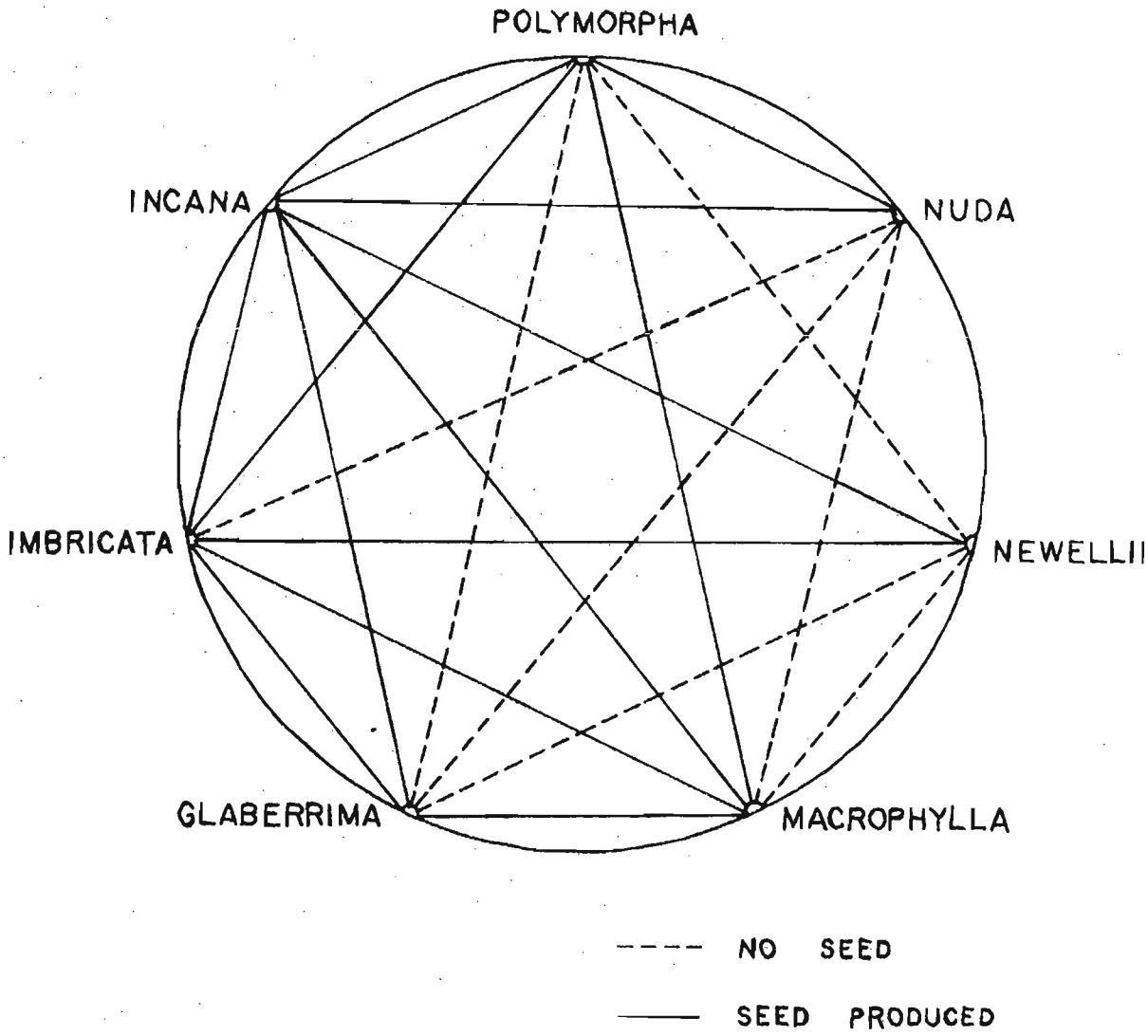

Figure 1. Field hybridizations attempted between *Metrosideros polymorpha* varieties.

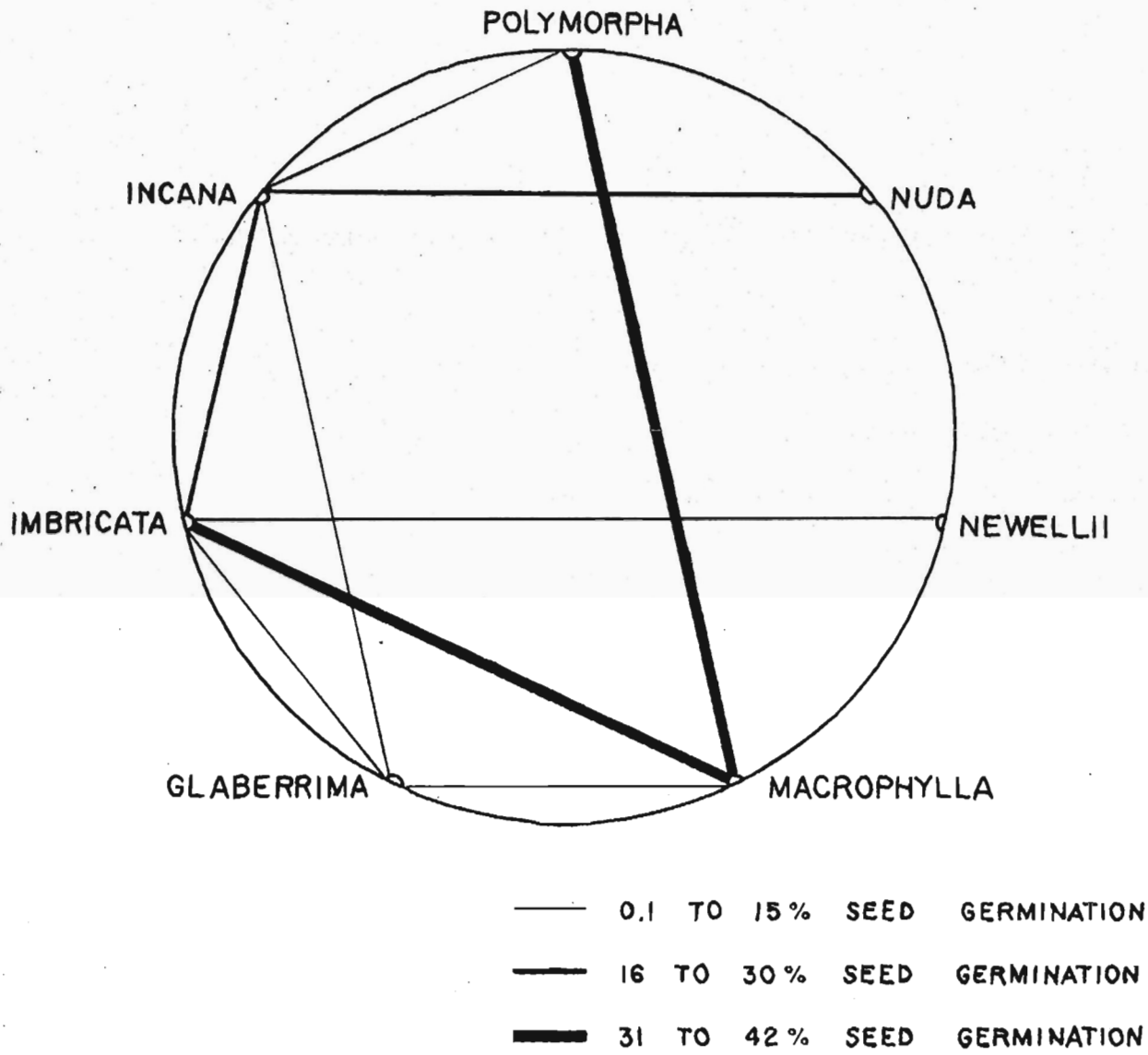

Figure 2. Seed germination for a subset of field hybridizations shown in Figure 1 of <u>Metrosideros</u> polymorpha varieties.