

Cytogenetics of *Hibiscadelphus* (Malvaceae): A Meiotic Analysis of Hybrids in Hawaii Volcanoes National Park¹

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ABSTRACT: The chromosome number of $2n = 20_{11}$ reported here is the first determination for *Hibiscadelphus giffardianus* Rock and *H. distans* Bishop & Herbst. An earlier report of the same number for *H. hualalaiensis* Rock is substantiated.

Cytogenetic analysis of *H. × puakuahiwi* Baker & Allen, a hybrid cross between *H. giffardianus* and *H. hualalaiensis* suggests that chromosome pairing and microsporogenesis are not affected in the first generation. However, although chromosome pairing is normal at diakinesis in the seven F_2 s analysed, subsequent stages of meiosis and microsporogenesis are severely disturbed and result in the formation of a high percentage of abnormal meiospores in some of the plants. Nevertheless, the apparent lack of hybrid breakdown in two of the F_2 plants and the presence of probable F_3 s in one area suggest that gene flow between the two taxa is possible, and, therefore, must be taken into consideration if these two endangered species are to be maintained as distinct entities.

Hibiscadelphus has been collected on the islands of Kauai, Hawaii, and Maui, but, according to Bishop and Herbst (1973), all known wild populations of the five species recognized by them are now extinct or imminently threatened. Fortunately, *Hibiscadelphus* can be cultivated and has been established in various localities including Kipuka Puaulu and Kipuka Ki in Hawaii Volcanoes National Park.

One result of cultivation was the spontaneous production of hybrid seeds where *H. giffardianus* Rock and *H. hualalaiensis* Rock were planted close together in Kipuka Puaulu. Park personnel unwittingly collected some of these hybrid seeds from *H. giffardianus* and planted the propagated hybrid seedlings in various areas of the park with the intention of increasing the numbers of *H.*

giffardianus. Subsequently, park personnel collected seeds from F_1 trees with the same intention of propagating more *H. giffardianus*. Accordingly, F_2 s were unknowingly cultivated until the existence of the hybrids was detected in 1973 and described by Baker and Allen (1976).

The occurrence of these cultivated hybrids (cf. Figure 1A) stimulated the present investigation in which it was our intent to characterize the nature and degree of cytogenetic differentiation between *H. giffardianus* and *H. hualalaiensis*.

MATERIALS AND METHODS

Floral bud material for meiotic analysis was fixed in a mixture of chloroform, absolute ethanol, and glacial acetic acid (6:3:1). Anthers were squashed in acetocarmine and mounted according to Beeks' permanent squash method (Beeks 1955). Slides were examined and photographed with the aid of phase contrast optics.

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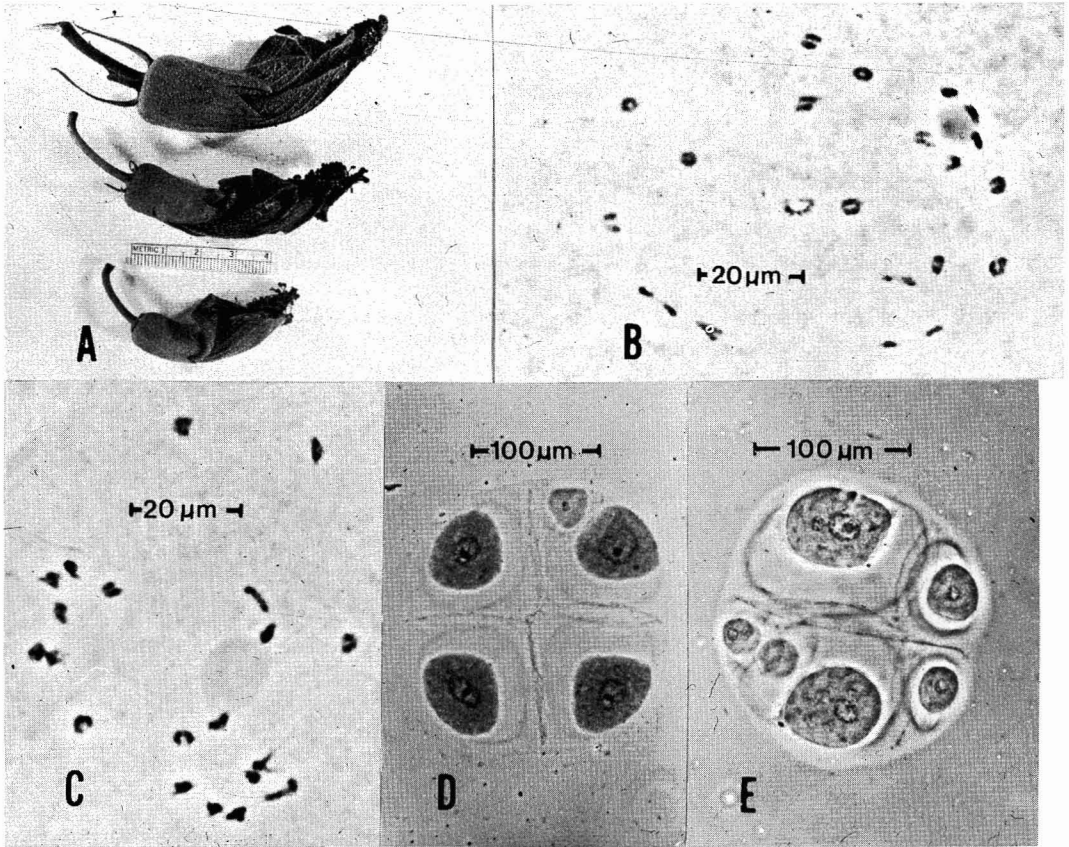


FIGURE 1. *Hibiscadelphus* flowers and microsporogenesis. *A*, above—*H. giffardianus*, center—*H. x puakuahiwi*, below—*H. hualalaiensis*; *B*, diakinesis in *H. hualalaiensis*, $2n = 20_{11}$; *C*, diakinesis in KKHXF₂-1, $2n = 20_{11}$; *D*, polyad from KPHXF₂-11, five microspores; *E*, polyad from KKHXF₂-1, six microspores.

TABLE 1
CHROMOSOME NUMBERS OF *Hibiscadelphus*

SPECIES	CHROMOSOME NUMBER	SOURCE
<i>H. distans</i>	$2n = 20_{11}$	Planting, Waimea Arboretum, Haleiwa, Hawaii—seed from holotype, <i>Bishop & Herbst 1318</i>
<i>H. giffardianus</i>	$2n = 20_{11}$	Planting, Kipuka Puauu, Hawaii Volcanoes National Park
<i>H. hualalaiensis</i>	$2n = 20_{11}$	Planting, Kipuka Puauu, Hawaii Volcanoes National Park

RESULTS

Meiotic prophase in *Hibiscadelphus giffardianus*, *H. hualalaiensis*, and *H. distans* Bishop & Herbst is characterized by regular bivalent formation, and all three species have 20 pairs of chromosomes (Table 1, Figure 1*B*). Later stages of meiosis and microspore formation in these species are also normal.

Likewise, an F_1 hybrid between *H. giffardianus* and *H. hualalaiensis* (*H. x puakuahiwi* Baker & Allen) exhibited regular bivalent formation during meiotic prophase prior to normal chromosome disjunction and microspore formation (Table 2).

In all seven F_2 plants analysed, chromosome pairing was essentially normal at diakinesis and each had 20 pairs of chromo-

somes (Figure 1C), although in a few instances one of the larger pairs seemed loosely associated. One of these plants (KPHXF₂-5, Table 2) also exhibited normal microspore development. However, in five other F₂ plants microsporogenesis resulted in the formation of various frequencies (1–13.1 percent, Table 2) of abnormal polyads along with apparently normal tetrads. Most of the abnormal polyads in these plants contain five or six microspores of two size classes rather than four equal microspores. Four of these are generally large and almost equal in size and one or two are much smaller (Figure 1D). A seventh F₂ plant (KKHXF₂-1) produces a very high frequency of abnormal polyads (53.8 percent) along with apparently normal tetrads. These abnormal polyads usually contain two to eight microspores of various sizes (Figure 1E).

Meiosis in all F₂ plants appears to be normal through anaphase I. However, during prophase preceding metaphase II, some of the sister chromatids separate precociously in some microsporocytes, especially in those of KKHXF₂-1. In this plant a high percentage of microsporocytes exhibit two groups of 20 chromatids each at a stage corresponding to metaphase II. Whether this condition results from disruption of the spindle or because of a timing problem is not yet clear. In any case, chromatid movement to the poles is somewhat haphazard, producing a number of deficient nuclei that give rise to abnormal microspores of various sizes.

DISCUSSION

The uniform chromosome number of $2n = 20_{II}$ reported here for *Hibiscadelphus distans*, *H. giffardianus*, and *H. hualalaiensis* agrees with earlier reports in the genus (*H. hualalaiensis*, Niimoto 1966 and personal communication; *Hibiscadelphus* sp., Skovsted 1941). Although $2n = 20_{II}$ has also been reported for species of *Hibiscus* (Skovsted 1941), this number has not yet been detected in Hawaiian members of the genus. Thus, together with other factors, the uniformly unique chromosome number of *Hibiscadelphus* among Hawaiian Malvaceae supports

TABLE 2
FREQUENCY OF ABNORMAL MEIOTIC POLYADS IN
Hibiscadelphus HYBRIDS

HYBRID	FREQUENCY OF ABNORMAL POLYADS (%)
<i>H. × puakuahiwi</i> —KKHX-1*	0.4†
KPHXF ₂ -5	0.3
KPHXF ₂ -4	1.0
KKHX1F ₂ -1	4.6
KPHXF ₂ -8	6.5
KPHXF ₂ -11	12.5‡
KPHXF ₂ -14	13.1
KKHXF ₂ -1	53.8

* Permanent tag designation.

† Levels of abnormality this high were detected in the parental species.

‡ Based on observation of 176 polyads; all others based on more than 300 polyads.

its status as a genus apart from *Hibiscus* and lessens the significance of Hutchinson's indication that the two genera should be united (Hutchinson 1967).

Many other examples of interspecific F₁ hybrids with normal chromosome pairing and fertility have been described (e.g., Stebbins 1950: 234) that resemble the present case of *Hibiscadelphus*. Likewise, reduction of vigor and/or fertility of second-generation hybrids whose F₁s are normal has been noted in several instances (Stebbins 1950: 227) and has been termed F₂ or hybrid breakdown. This phenomenon is due ostensibly to formation of disharmonious gene combinations that result from meiotic recombination subsequent to the F₁ generation. Presumably, the more divergent the parental gene pools, the more intense this problem is likely to be.

Most of the examples of F₂ breakdown appear to involve reduction of vigor in the second generation rather than reduction in fertility only, as seems to be the case in *Hibiscadelphus*. One very similar situation is that described by Avers (1953) who found that certain interspecific *Aster* F₁ hybrids apparently were fully fertile, but that F₂ and backcross generations exhibited reduced fertility and thus constituted a barrier to gene exchange between the two species involved.

Of the seven second-generation *Hibiscadelphus* hybrids discussed here, two seem little affected by hybrid breakdown, four are affected to a moderate degree, and one suffers

from severe meiotic perturbations. These meiotic abnormalities are probably caused by a timing problem and perhaps by abnormalities of the spindle apparatus. Similar but more completely documented instances of meiotic abnormalities resulting from genic imbalance or other genetic factors include multiple spindle formation (Vasek 1962), discontinuous or incompact spindle (Darlington and Thomas 1937), and inactivity of the spindle after the first telophase (Janaki-Ammal 1941). All of the foregoing meiotic maladies may result in formation of abnormal meiospores such as those seen in Figure 1E.

However, it may be recalled that not all of the *Hibiscadelphus* hybrids have disturbed meiosis. In view of the apparent high fertility of some of the second generation hybrids and the probable occurrence of F_3 s, it seems highly likely that gene flow between *H. giffardianus* and *H. hualalaiensis* is possible. These factors should be carefully weighed in any program designed to protect the genetic integrity of these highly endangered species.

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