

Hybridization between *Malacothrix polycephala* and *M. incana* (Asteraceae) on San Nicolas Island, California

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Abstract – Natural hybridization between *Malacothrix polycephala* W. Davis (ined.), an annual, and *M. incana* (Nutt.) Torrey & A. Gray, a perennial, on San Nicolas Island, California was examined using herbarium studies, studies in the field, and studies of plants grown in growth chambers. *Malacothrix incana* has apparently been on San Nicolas Island for less than 25 yr, but *M. polycephala* is a long-time resident. The two species differ morphologically, physiologically, and in their banding patterns for the enzyme aspartate aminotransferase. No strong isolating mechanisms have been found between the two species and extensive hybridization is occurring on San Nicolas Island. Studies of growth chamber progenies indicate that natural hybridization has produced many combinations of the morphological and physiological features of the parental species. The evolutionary potential of hybridization between *M. polycephala* and *M. incana* appears to be considerable.

Introduction

For the last 6 yr we have been studying natural hybridization between *Malacothrix incana* (Nutt.) Torrey & A. Gray, and *M. polycephala* W. Davis (ined.) on San Nicolas Island, California. Natural hybridization between *M. incana* and *M. polycephala* is of special evolutionary and systematic interest for several reasons. First, *M. incana* is a perennial species and *M. polycephala* is an annual species; published studies of natural hybridization between annual and perennial taxa are few. Second, both species are self-compatible. Evidence from a detailed study of the nature

and extent of natural hybridization between *M. incana* and *M. polycephala* can be used to assess the efficiency of autogamy in its role as an isolating mechanism (e.g., see Levin 1978). Third, it is likely that hybridization between *M. incana* and *M. polycephala* has been going on for less than 20 yr; evidence from a multifaceted study can be used to assess the rate at which variation can be generated by hybridization. Finally, the findings from a study of hybridization between *M. incana* and *M. polycephala* may be of help in understanding the evolution of other taxonomically confusing annual species of *Malacothrix*, endemic to the California Islands (Munz 1974:207), that appear to be closely related to *M. incana* and *M. polycephala* (Davis 1980).

Malacothrix polycephala is a small annual species endemic to San Nicolas Island. It is most closely related to *M. foliosa* A. Gray, and early collections were generally referred to that species or to another insular endemic, *M. indecora* E. Greene. The first documented collection of *M. polycephala* from San Nicolas Island was that of Blanche Trask in 1897 who noted that the species covered large areas on ridges (Eastwood 1898). Since that time, *M. polycephala* has regularly been collected by other visitors to the island. A historical summary of botanical exploration on San Nicolas Island is to be found in Foreman (1967).

Malacothrix incana is a sand dune endemic that occurs on mainland California in Santa Barbara and San Luis Obispo Counties, and on four of the Southern California Islands. The holotype of *M. incana* was collected in the 1800's at San Diego, California, but it has not been found in that area since then.

The first documented collection of *Malacothrix incana* from San Nicolas Island is

that of Hesselden, 6 June 1977 (UCSB). Foreman visited San Nicolas Island in July 1965, December 1965, and April 1966 in the course of producing a flora of the island. He found no *M. incana*, and in his flora (Foreman 1967) specifically excluded Dunkle's report (1950) of *M. incana* on the island; we have examined Dunkle's specimen and it is *Malacothrix saxatilis* var. *implicata* (Eastw.) H.M. Hall. During a visit to San Nicolas Island in April 1966, Raven and Thompson recorded no *M. incana* during an extensive reconnaissance of the island. At the present time, *M. incana* is abundant on San Nicolas Island and occurs in dense colonies on unstabilized or stabilized sand dunes, most commonly along the northern coast.

In this paper we will report the results of herbarium studies, field studies, and studies of plants of *Malacothrix incana*, *M. polycephala* and putative hybrids in cultivation. Evidence from studies of progenies grown from wild achenes or from achenes produced via an extensive hybridization program in growth chambers at the University of Louisville has been particularly useful in characterizing variation in the parental species and in hybrids. Growth chamber studies are nearly completed; evidence from such studies will be used for comparative purposes during future, more intensive field studies.

Materials and Methods

Field studies were conducted during 2-3 day visits to San Nicolas Island in 1984, 1985, 1986, 1987, and 1988; the general distribution of *Malacothrix incana* and *M. polycephala* was noted, and putative hybrid swarms were identified. Non-quantitative judgements were made regarding vegetation patterns and edaphic factors. A limited number of field measurements were taken from flowers; leaves and flower heads were collected and preserved in 70% alcohol for later study. The most intensive field studies have been conducted in the vicinity of Corral Harbor where a large hybrid swarm was found in 1984.

Beginning in 1984, progenies were grown in growth chambers at the University of Louisville from wild achenes of *Malacothrix incana*, *M. polycephala*, and putative hybrids collected on San Nicolas Island. Methods used are described in Davis & Philbrick (1986). Among the progenies were representatives of *M. polycephala* collected on San Nicolas Island by others prior to 1970, and representatives of *M. incana* from San Miguel Island, Santa Rosa Island, and mainland California. An extensive hybridization program was conducted in cultivation; unopened heads were bagged with plastic wrap, and self- and cross-pollinations were performed by rubbing heads together after they had opened. The number of filled and unfilled achenes was recorded for each cross; the total constitutes the number of ovules per head. More than 15 progenies have been grown from achenes produced in the hybridization program.

During studies of breeding system in growth chambers, plants were considered to be self-incompatible if filled achenes were produced following cross-pollinations, but not following self-pollinations or in undisturbed heads.

In cultivation, floral measurements were taken from two mature heads from each plant using a Bausch and Lomb measuring magnifier (metric scale; lines 0.1 mm apart). Plant height was measured every 5 days after bolting had occurred until the first flower head opened. Leaves and flower heads were collected in 70% ethanol for later study. Immature heads were collected in 3:1 v:v absolute ethanol, glacial acetic acid for meiotic studies.

Standard acetocarmine-squash techniques were used for studies of meiosis in pollen mother cells (15 cells from each plant), and mitotic chromosomes were studied in root tips of 5-day-old seedlings (10 cells from each root tip) using the Feulgen technique following 4 hr of treatment with 0.002 M 8-hydroxyquinoline.

The methods of Soltis & co-authors (1983) were used for starch gel electrophoresis of enzymes.

Herbarium specimens from the following herbaria were used in our studies: CAS, DHL, DS, GH, LAM, LL, MO, ND, POM, RSA, SBBG, SBM, SD, UC, UCSB, UNM, US (standard herbarium abbreviations are from Holmgren *et al.* 1981).

Achenes for scanning electron microscopy (SEM) were mounted on stubs, coated with 9 nm gold, and viewed with either a Cambridge or an SPI Model 40 SEM.

Voucher specimens of field collections and from growth chamber studies are housed in the Davies Herbarium (DHL), Department of Biology, University of Louisville, Louisville, KY 40292.

Results

Studies of *Malacothrix incana* and *M. polycephala*: Observations from herbarium studies, growth chamber studies, and field studies indicate that *Malacothrix incana* and *M. polycephala* are phenetically very distinct. Some of the distinguishing features found in growth chamber plants are listed in Table 1.

Stems of *Malacothrix polycephala* are glabrous; stems of *M. incana* are generally densely hairy, but some plants have glabrous stems. Perennial growth habit is first expressed in growth chamber plants of *M. incana* by an expansion in the width of the root just below the soil line. After a year of growth this area eventually reaches a diameter approximately twice that of the stem immediately above. Roots of *M. polycephala* remain nearly the same diameter as the stems throughout the life of the plant.

Plants of both species flower in the first year of growth in growth chambers. Flowering in *Malacothrix polycephala* occurs 12-30 wk after germination; an increase in photoperiod is not required for initiation. Flowering in *M. incana* occurs 30-40 wk after germination and requires a lengthening of photoperiod from 9 hr to 11 hr. In nature, *M. polycephala* begins to flower before *M. incana*, but flowering periods overlap.

Meiotic behavior was normal in 15 growth chamber plants of *Malacothrix incana*, and 24

growth chamber plants of *M. polycephala*; all had a chromosome number of $2n = 7_{II}$. Stainable pollen was more than 80% in 90% of growth chamber plants of *M. incana* and *M. polycephala*. Chromosome number in seedling root tips was $2n = 14$.

All growth chamber plants of *Malacothrix polycephala* grown from wild achenes and from achenes produced following self-pollinations or intraspecific crosses were self-compatible; 90% produced filled achenes in undisturbed heads. All of the growth chamber plants of *M. incana* grown from wild achenes and from achenes produced from intraspecific crosses were self-compatible, but filled achenes generally were not found in undisturbed heads.

In 1986 and 1987 a limited survey of enzyme variation was conducted using leaves of *Malacothrix incana* and *M. polycephala* from growth chamber plants. Identical enzyme banding patterns were obtained from leaves of *M. incana* and *M. polycephala* for the following enzyme systems: esterase, leucine aminopeptidase, peroxidase, and acid phosphatase. The two species differ consistently, however, in their electrophoretic banding patterns for aspartate aminotransferase (AAT).

On San Nicolas Island, the distribution of *Malacothrix incana* parallels the distribution of sand dunes on San Nicolas Island (for distribution of soil types on San Nicolas Island see Estrada *et al.* 1985; place names in this report are those indicated on the map of San Nicolas Island distributed with Menke & Miller 1975).

Along the north coast of San Nicolas Island, the distribution of *Malacothrix incana* is essentially continuous on sand dunes from the western tip to a point 1 km west of the eastern tip where one plant was found in 1988 on a newly formed, unstabilized dune. Between Red Eye Beach and Vizcaino Point numerous mature plants of *M. incana* occur along the north-facing, 45° slope of a 50 m tall, densely vegetated, stabilized sand dune approximately 200 m in length. Along the south shore the distribution of *M. incana* also parallels the

Table 1. A comparison of features of growth chamber plants of *Malacothrix incana*, *M. polycephala* and F₁ hybrids. (\bar{x} = mean, SD = standard deviation, *n* = number of plants analyzed).

Species	Root Width (mm)	Stem Width (mm)	Involucre Height (mm)	Ligule Exsertion (mm)	Corolla Length (mm)	Head Width (mm)	Achene Length (u)*	Number of Ovules per Head	Growth Rate (mm/day)
<i>M. incana</i>									
\bar{x}	5.7	3.7	11.2	5.7	13.4	4.9	46.8	63.5	1.6
SD	1.2	0.5	1.8	0.7	1.1	0.7	4.2	21.1	0.7
range	4.3-7.8	3.0-4.7	8.2-11.9	5.0-7.1	11.3-14.0	4.2-6.2	38-54	27-106	0.5-3.0
<i>n</i>	15	15	15	15	12	14	26	33	20
<i>M. polycephala</i>									
\bar{x}	2.4	2.4	5.9	3.3	7.4	2.7	28.1	38.8	8.2
SD	0.6	0.6	0.6	0.4	0.6	0.4	1.6	10.1	2.0
range	1.3-3.6	1.4-3.9	4.9-7.8	2.5-4.0	6.0-8.5	2.0-3.4	24-31	21-57	3.7-13.0
<i>n</i>	23	23	23	23	23	23	35	19	16
F ₁ Hybrids									
\bar{x}	3.2	2.5	7.7	4.9	10.4	3.7	39.5	35.8	6.0
SD	0.5	0.3	0.9	0.8	0.9	0.5	2.8	8.5	2.6
range	2.4-4.0	1.8-3.1	7.0-9.5	3.2-6.0	8.0-11.8	2.7-4.7	35-47	15-57	0.7-9.8
<i>n</i>	23	22	23	23	23	23	23	23	20

*50u=1.67mm

occurrence of sand dunes near the ocean; the eastern limit of distribution is a sand dune area above Elephant Seal Beach. Dense colonies of *M. incana* also occur on sand that has accumulated on flats in the middle-western portion of the island. *Malacothrix polycephala* is most common along the north coast of San Nicolas Island; many populations occur on the flats and gentle slopes between the ocean and the steep escarpments to the south. Numerous populations also occur on the flattened mesa top of the island. A particularly large population, consisting of thousands of plants, occurs along the north side of the airfield runway. Along the south coast, *M. polycephala* is restricted to the slopes of the steep escarpments, where its abundance varies from year to year. There is no record of *M. polycephala* from the extensive sand dune areas at the northwestern tip of the island, and we have not found it there during our visits.

Studies of Hybrids: A limited number of F₁ progenies from artificial crosses between *Malacothrix incana* and *M. polycephala* have been grown in cultivation. As many as 94% of the

filled achenes from heads of *M. polycephala* cross-pollinated with *M. incana* produced hybrid plants; less than 20% of the filled achenes from heads of *M. incana* that had been cross-pollinated by *M. polycephala* produced hybrids. Twenty-three F₁ hybrids reached maturity in cultivation; all had more than 80% stainable pollen, were self-compatible, and produced filled achenes in undisturbed heads. Meiosis in 10 plants was analysed; regular pairing and normal segregation of chromosomes at anaphase I and anaphase II was observed. In most morphological characteristics, artificial F₁ hybrids were intermediate between the parental species (Table 1); all were annual in growth habit.

Evidence from progenies propagated from wild achenes collected in the field from plants identified as *Malacothrix polycephala* or *M. incana* indicates that cross-fertilization is occurring between the two species in both directions in nature. Hybrid plants regularly appeared in progenies grown from achenes collected from *M. polycephala* that were growing together with *M. incana* in hybrid areas. Furthermore, one hybrid plant appeared in a

Table 2. A comparison of features in plants in a growth chamber progeny grown from achenes collected from a perennial hybrid plant (D85-II-10) growing in the hybrid swarm at Corral Harbor, San Nicolas Island. Arranged by descending order of corolla length.

Propagation Number	Root Width (mm)	Stem Width (mm)	Involucre Height (mm)	Ligule Exsertion (mm)	Corolla Length (mm)	Head Width (mm)	Achene Length (u)*	Growth Rate (mm/day)
363-21	4.9	2.5	9.4	5.2	13.1	3.2	—	5.1
363-15	5.1	4.8	10.0	5.2	12.9	5.2	43.5	2.7
363-12	6.4	3.2	12.5	4.5	12.1	4.5	39.6	6.7
363-27	4.5	3.6	10.0	5.9	12.1	4.7	36.3	10.0
363-8a	2.7	2.3	9.0	5.4	12.0	4.3	37.8	—
363-14	5.5	3.4	8.2	6.0	11.6	3.2	41.1	6.5
363-22	4.3	4.5	7.9	4.3	11.1	3.7	41.0	2.1
363-23	6.9	3.6	10.2	6.0	11.0	4.2	47.3	3.1
363-7	5.9	3.6	8.8	4.8	11.0	4.6	36.9	4.5
363-2	4.7	4.5	10.0	4.2	11.0	4.0	43.1	4.5
363-3	4.6	3.7	9.0	4.9	10.5	4.5	37.5	6.1
363-19	4.6	4.0	7.0	4.7	10.0	3.7	35.2	8.7
363-17	4.8	3.5	7.5	5.0	9.9	3.2	38.1	6.0
363-24	2.8	2.8	7.5	5.3	9.9	3.2	34.8	7.3
363-11	3.3	2.6	7.6	4.4	9.9	4.4	34.4	5.9
363-26	3.6	2.9	6.0	5.0	9.8	3.5	33.1	6.6
363-18a	4.5	3.1	8.0	4.2	9.8	4.0	—	5.3
363-4	4.4	3.4	7.7	4.5	9.7	3.6	36.5	14.0
363-6	3.3	2.3	7.2	4.2	9.5	3.1	36.8	13.0
363-16	5.0	3.9	7.4	4.9	9.5	3.6	36.8	6.7
363-25a	2.5	2.8	7.0	5.0	9.5	3.5	—	14.0
363-20	5.3	3.7	7.0	5.0	9.4	3.4	35.0	7.4
363-8b	5.5	3.6	6.5	4.8	9.1	3.1	36.3	6.0
363-5	3.5	3.2	7.2	4.0	8.7	2.7	35.3	11.0
363-9	3.7	3.6	7.0	4.4	8.6	3.1	36.6	6.4
363-10	4.4	3.3	6.1	4.0	8.5	2.9	36.3	9.0
363-1b	4.0	3.6	7.0	0.6	5.6	3.2	—	5.6

*50u = 1.67mm

progeny of 14 plants produced from achenes collected from a mature *M. incana* that was growing in an area where *M. polycephala* and hybrid plants were growing. The plant was identified as a hybrid in the rosette stage by its banding pattern for the enzyme AAT, and soon developed a number of other hybrid features.

A wide range of morphological and physiological variation occurs in hybrid populations in nature and in progenies grown from wild achenes collected from hybrids in the field; at least 15 different combinations of parental features have been identified in growth chamber progenies grown from wild achenes. In nature, the morphological continuum in some hybrid swarms makes

difficult the unequivocal identification of particular morphotypes as hybrid. The most useful marker for positive identification of hybrids in the field is stem indumentum. Stems of most *Malacothrix incana* on San Nicolas Island are tomentose; in contrast, stems of *M. polycephala* are always glabrous. Thus, plants that are otherwise like *M. polycephala* can be identified as hybrids on the basis of the presence of stem hair.

In order to judge the general potential of hybridization between *Malacothrix incana* and *M. polycephala* for generating variation, and to gather evidence for future studies of character coherence, several progenies were propagated from achenes collected from plants identified in

the field as hybrid. Variation in several features of plants in a progeny grown from achenes taken from a perennial hybrid (D85-H-10) in the hybrid swarm at Corral Harbor is summarized in Table 2. Pollen stainability in this progeny and in other progenies ranged from 0-100%; 45% of 109 hybrid plants had less than 80% stainable pollen. Abnormalities in microspore development were indicated by lightly stained gametophytes, empty microspores, microspores of different sizes, and microspores with cytoplasm pulled away from the wall. Eighty-five percent of plants in all of the hybrid growth chamber progenies were self-compatible; a majority of self-compatible plants produced filled achenes in undisturbed heads.

Hybridization between *Malacotbrix incana* and *M. polycephala* on San Nicolas Island is occurring wherever sympatric populations are found; most hybrid swarms have been found along the north shore. Commonly, *M. incana* and *M. polycephala* are intermixed with numerous hybrid plants in what appears to be a random pattern; no discrete zone of hybridization is discernible. Hybrid populations of this kind have been studied at Corral Harbor, and along Tufts Road, west of its junction with Shannon Road.

A different kind of hybridization pattern was found in 1988 along the north shore of San Nicolas Island, approximately 100 m south of Tranquility Beach. In this area, fingers of unstabilized sand dunes covered with *Malacotbrix incana* extend into areas where dense populations of *M. polycephala* occur. Hybrid plants are relatively infrequent and occur in groups of several individuals in the flats downwind of single plants of *M. incana* that are surrounded by *M. polycephala*.

Not all hybrid plants in nature have been found in hybrid swarms. In 1988, a single mature, perennial hybrid was found along the south coast near the Rock Crusher in the midst of a large population of *Malacotbrix incana*. The size and branching pattern of this hybrid indicates that its age was 4-5 years. A search of the area disclosed no other hybrids.

Discussion

Many authors have discussed the role of hybridization in the evolution of flowering plants (e.g., Stebbins 1974). Most have emphasized the importance of hybridization as a means of enriching gene pools, and point out that this effect is restricted when hybrid products are partially sterile. Self-compatibility does not appear to be an effective isolating mechanism between *Malacotbrix incana* and *M. polycephala* on San Nicolas Island, and there is little evidence for other restrictions to gene flow between the two species. The lack of strong interspecific isolating mechanisms, and the apparent structural homology of genomes suggests that the evolutionary potential of natural hybridization between the two species is considerable. Gene flow between the two species in nature has become generally more frequent as flowering periods of hybrid plants have overlapped those of the parental species. Evidence from the study, so far, indicates that hybridization has produced many combinations of the morphological, and physiological features of the two species. Perennial hybrids in nature provide, through self-pollination and cross-pollination, a long term source of variable offspring on which natural selection can act.

Many questions remain to be answered concerning the details of hybridization between *Malacotbrix incana* and *M. polycephala* on San Nicolas Island. To provide answers for some of these questions, additional studies are planned. For example, an analysis of enzyme banding patterns in *M. incana*, *M. polycephala*, and hybrids will be carried out in order to provide evidence that can help us identify hybrids in nature, and to better understand the extent of mixing of parental genotypes in hybrids. Information from the enzyme study can be used, together with morphological evidence, during more intensive field studies designed to determine the ecological factors correlated with the distribution of *M. incana*, *M. polycephala* and hybrid populations on San Nicolas Island.

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