Hybridization in the Catalina Island Mountain Mahogany (*Cercocarpus traskiae*): RAPD Evidence

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Introduction

The Catalina Island mountain mahogany is one of 10 species of shrubs and small trees (Lis 1992) comprising the genus *Cercocarpus* H. B. K. (Rosaceae). Species in the genus are widely distributed throughout western North America and are delimited primarily on the basis of leaf characteristics (Mortenson 1973; Lis 1992). The Catalina Island mountain mahogany (*C. traskiae* Eastwood) is one of the most distinctive species in the genus due to its thick, coriaceous leaves and woolly pubescence on the leaf undersurface. The species is extremely rare, restricted to Wild Boar Gully on the southwest side of Santa Catalina Island in the Channel Islands off the coast of California (Fig. 1). When the population was first discovered in 1897, it consisted of over 40 individuals (Thorne 1967). Due to overgrazing by introduced mammalian herbivores (Coblentz 1980; Hobbs 1980), however, the population has been greatly reduced (Rieseberg et al. 1989). *Cercocarpus traskiae* is listed as endangered by the state of California and thus is afforded protection by California law (Smith & Berg 1988). Inexplicably, the species is not listed as endangered or threatened by the U.S. Fish and Wildlife Service, although it is under consideration for listing.

In the late 1970s a detailed inventory of the Wild Boar Gully population was undertaken, and a total of eight *Cercocarpus* trees were discovered and tagged (Martin 1984). In addition, two of the trees (A, B; Fig. 1) were fenced to protect them from herbivores, and in 1985 the Catalina Conservancy constructed a larger fence around an area that included both trees, as well as about an acre of good habitat. The fencing effort was extremely successful, and by 1988 approximately 70 seedlings were observed within the fenced area (Rieseberg et al. 1989).

Paper submitted April 1994; revised manuscript accepted July 15, 1994.

Unfortunately, several of the adult plants resembled the island mountain mahogany, *Cercocarpus betuloides* Torrey & A. Gray var. *blancheae* (C. Schneider) Little, in key morphological features such as leaf pubescence and leaf thickness or were immediate for these characters (descriptions of both species are given by R. Lis in Hickman [1993]). *Cercocarpus betuloides* var. *blancheae* is only slightly more widespread than *C. traskiae,* occurring on all of the Channel Islands except San Clemente. But the species is much more abundant than *C. traskiae* on Santa Catalina and is known to occur in adjacent canyons, so the possibility of hybridization between the two species could not be dismissed. Furthermore, it is suspected that some of the seedlings might be of hybrid origin (T. Martin, personal communication).

In an attempt to clarify the identity of morphologically ambiguous individuals, Rieseberg et al. (1989) undertook a study of allozymic variation and leaf anatomy of all the adult mahogany trees discovered in Wild Boar Gully. Of the eight adult trees in the Gully, three were anatomically intermediate between *C. traskiae* and *C. betuloides* var. *blancheae,* suggestive of hybridization. Analysis of 17 isozyme loci in *C. traskiae,* as well as a nearby population of *C. betuloides* var. *blancheae* (Fig. 1), revealed one diagnostic isozyme polymorphism differentiating the two species. Based on this single isozyme polymorphism, five of the eight trees were diagnosed as *C. traskiae,* one as *C. betuloides* var. *blancheae,* and two as hybrids. However, isozyme analyses of the 28 seedlings large enough to survive the loss of a single leaf revealed all to be "pure" *C. traskiae.*

The results from this study were not entirely satisfactory, however, because the classification of individuals as parental versus hybrid was based on a single locus. The use of a single diagnostic locus can ensure the correct identification of first-generation hybrids, but F_2- or later-generation hybrid or backcross progeny could easily be misdiagnosed as belonging to one of the parental classes. Thus, it was not surprising that two of the plants lacking a hybrid enzyme genotype were anatomically

Conservation Biology, Pages 199–203
Volume 9, No. 1, February 1995

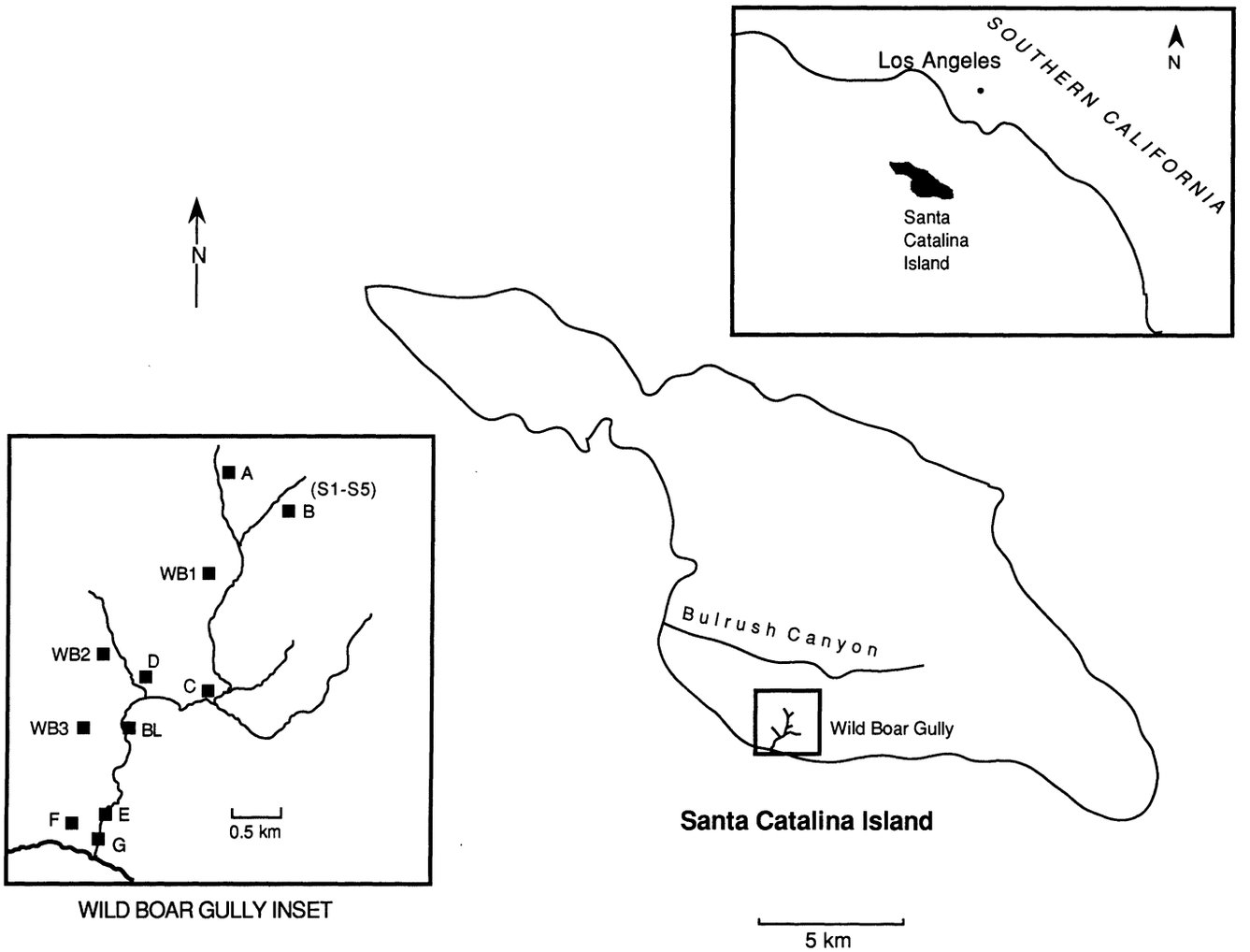

Figure 1. Map of Santa Catalina Island showing distribution of Cercocarpus *in Wild Boar Gully (map has been updated and corrected from Rieseberg et al. 1989). Trees* A-D, WB1-WB2 *are true* C. traskiae; *trees* E-G, BL, WB3 *and seedlings* S1-S5 *are hybrids between* C. traskiae *and* C. betuloides *var.* blancheae.

intermediate. Rieseberg et al. (1989) were unable to distinguish between phenotypic plasticity and hybridization as explanations for the discordance between the anatomical and isozyme data sets.

In the five years following the publication of the paper by Rieseberg et al. (1989), three additional *Cercocarpus* trees (for a current total of 11) were discovered in Wild Boar Gully (M. Gay, personal communication). In addition, five seedlings were found in the fenced area that were morphologically intermediate between *C. traskiae* and *C. betuloides* var. *blancheae.* The discovery of the three additional trees, the possibility that several of the seedlings are of hybrid origin, and the uncertainty regarding the correct classification of several trees from the previous study, prompted a more detailed genetic analysis of the *C. traskiae* situation using random amplified polymorphic DNAs (RAPDs; Williams et al. 1990). In addition, the diagnostic isozyme locus *6pgdl* employed by Rieseberg et al. (1989) was assayed in the newly discovered trees.

Methods

Leaves for DNA and isozyme analyses were collected from each of the 11 adult *Cercocarpus* trees in Wild Boar Gully (Fig. 1) and from the five morphologically intermediate seedlings. For *C. betuloides* var. *blancheae,* frozen leaf tissue from the 23 individuals analyzed by Rieseberg et al. (1989) was employed for DNA isolations.

Genomic DNA was isolated following Swensen et al. (1995), which is a modification of Doyle and Doyle (1987) CTAB method. To achieve reproducible amplifications, the DNA was further purified using the ELUQUICK™ DNA Purification Kit (Schleicher & Schuell

Co.), quantitated by fluorometry (Hoefer Co.), and standardized to 20 ng/μl.

For analysis of RAPD variation, DNA from true *C. traskiae* (trees *A* and *B*) and from three individuals of *C. betuloides* var. *blancheae* were surveyed for the presence of potential species-specific RAPD polymorphisms using 135 arbitrary 10-mer oligonucleotide primers known to amplify strongly in other flowering plants (Fritsch et al. 1993). Eighteen of these displayed promising patterns of variation and were assayed in all 39 individuals: University of British Columbia Biotechnology Center primers 101, 155, 173, 188, 199, 222, 287, 290, 308, 437, 450, 455, 458, 470, 489; Operon Technology primers C6, C8, B17. Of these, eight primers amplified fragments that were informative and reproducible (Table 1). Amplification parameters and electrophoretic conditions followed Rieseberg et al. (1994).

To eliminate inadvertent scoring of artifactual RAPD variation (Ellsworth et al. 1993; Muralidharan & Wakeland 1993), primer and DNA template concentrations were varied 25% in both directions relative to the initial concentration for the potentially informative primers (Rieseberg et al. 1994). Only polymorphisms that were stable under all six different reaction conditions were scored (Table 1).

Electrophoresis and detection of 6-phosphogluconate dehydrogenase (6PGD) followed Rieseberg et al. (1989).

Results and Discussion

A total of 10 RAPD fragments were scored (Table 1). Of these, three were specific to individuals with the *C. traskiae* phenotype and three were exclusive to *C. betuloides* var. *blancheae*. The remaining four loci were not taxon-specific, but they were polymorphic in the adult trees in Wild Boar Gully and thus potentially informative regarding the parentage of the seedlings. The isozyme locus *6pgdl* was diagnostic for the two species, with *6pgdl-a* specific to *C. betuloides* var. *blancheae* and 6pgdl-b exclusive to *C. traskiae*. Individuals that combined RAPD fragments or allozymes from both parental species were classified as hybrids (below).

Classification of Adult Trees

Analysis of the RAPD and isozyme data (Table 1) generated the following classification of hybrid and parental *Cercocarpus* trees. First, six of the 11 adult mahogany trees in Wild Boar Gully appear to be pure *C. traskieae* (trees *A-D, WB1-WB2*). Second, the remaining five adult trees in Wild Boar Gully appear to be of hybrid origin. Of these, trees *E, BL,* and *WB3* appear to represent later generation of hybrids. Trees *F* and *G* may be later-generation hybrids as well, but the possibility that they represent F_1 hybrids cannot be ruled out. These results accord well with morphological and anatomical observations (Rieseberg et al. 1989). The only remaining discrepancy between molecular and structural data sets is the classification of tree *E,* which is indistinguishable anatomically from *C. betuloides* var. *blancheae* but is clearly a later-generation hybrid based on the molecular data (Table 1).

Classification of Seedlings

RAPD and isozyme analysis of the five seedlings of putative hybrid origin revealed that at least one of the

Table 1. RAPD and isozyme data for *Cercocarpus traskiae* and *C. betuloides* var. *blancheae*.*

Taxon/ Individual — *Primer: Fragment (kb):*	*450 0.55*	*450 0.70*	*470 0.80†*	*290 0.80*	*290 0.95*	*188 1.4*	*458 1.0*	*437 0.7†*	*173 0.9†*	*155 0.7†*	*6pgdl*
blancheae (frequencies)	0.71	0.82	0.35	0	0	0.73	0	0.10	0.15	0.55	aa
traskiae											
A	−	−	+	+	+	−	+	+	+	+	bb
B	−	−	+	+	+	−	+	−	+	+	bb
C	−	−	−	−	−	−	+	−	+	+	bb
D	−	−	−	−	−	−	+	+	+	+	bb
WB1	−	−	+	+	+	−	+	+	+	+	bb
WB2	−	−	+	+	+	−	+	+	+	+	bb
Hybrid Trees											
BL	−	−	−	+	+	−	−	+	+	+	aa
E	−	+	−	−	−	−	−	−	+	−	bb
F	+	+	−	−	−	+	−	+	+	−	ab
G	+	+	+	+	+	−	−	+	+	−	ab
WB3	−	−	−	−	−	−	+	−	+	+	aa
Seedlings											
S1	+	−	−	+	+	+	+	−	−	+	bb
S2	−	−	+	+	+	−	−	−	+	+	ab
S3	−	−	+	+	+	−	+	−	+	+	ab
S4	−	−	−	+	+	−	+	−	+	+	ab
S5	−	+	+	+	+	+	+	−	+	+	bb

* + = *presence of fragments;* − = *absence of fragments.*
† *Fragments that are not specific to either parental taxon but are potentially informative regarding the parentage of the seedlings.*

parents of each seedling must have been a hybrid tree (Table 1). If we assume that tree *B* is the maternal parent of the seedlings (a reasonable assumption given that the seedlings occur beside tree *B;* Fig. 1), then the probable father(s) of each seedling can be determined (Table 1): *S1* and *S5* must have been sired by tree *F; S2-S4* could have been sired by trees F, G, BL, or *WB3*. It is noteworthy that two of the seedlings have the *bb* genotype for *6pgdl* and thus are indistinguishable at this locus from the 28 seedlings examined in the 1989 study. This raises the possibility that some of the seedlings classified as pure *C. traskiae* in 1989 also have at least one hybrid parent.

Conservation Implications

Hybridization between rare and common species has two potentially harmful consequences for the conservation of biological diversity. If the F_1- or later-generation hybrids are partly sterile or have reduced vigor, then the rare species may be endangered by outbreeding depression (Price & Waser 1979; Templeton 1986; Leberg 1993). That is, rare populations may have reduced fitness due to gamete wastage in the formation of unfit hybrid individuals. Because only a small fraction of the pollen produced by plants is actually required to fertilize ovules, the primary cost of outbreeding depression in plants appears to be reduced seed set by the maternal parent (Ellstrand & Elam 1993). For example, seed abortion rates of over 50% and 90% have been reported for interspecific matings in *Gilia* (Grant 1964) and *Helianthus* (Heiser et al. 1969), respectively. Nonetheless, outbreeding depression is unlikely to be a problem in long-lived perennial plants, such as *Cercocarpus,* where the number of seeds produced greatly exceeds the number of seedlings that can become established.

On the other hand, if the hybrids are fertile and vigorous, hybridization may lead to the genetic assimilation of the rare taxon by a numerically larger one (see Cade 1983; Rieseberg et al. 1989; O'Brien et al. 1990; Wayne & Jenks 1991; Leary et al. 1993). Island plants are particularly susceptible to genetic assimilation through hybridization because of small population size, the general lack of strong genic or chromosomal sterility barriers, the invasion and colonization of islands by closely related exotics, and the increasing loss and disturbance of habitat due to human activities (Rieseberg 1991). Habitat disturbance tends to increase the frequency of hybrid matings and establishment (Anderson 1949), particularly in island systems where ecological barriers to hybridization are significant. For example, surveys of the hybrid flora of Hawaii (Ellstrand & Rieseberg, unpublished data) revealed the occurrence of hybridization in close to 40 genera and 23 plant families. The majority of the hybrid combinations involved endemic, often rare, species of *Cyrtandra* (67 hybrid combinations), *Dubautia* (24), *Bidens* (10), and *Clermontia* (8). Hybridization also occurs frequently on the California Channel Islands, often involving endangered taxa such as the federally listed San Clemente island endemic *Lotus scoparius* ssp. *traskiae* (Liston et al. 1990) or *C. traskiae.* We suspect that habitat disturbance due to the introduction of goats and pigs has been the critical factor leading to hybridization in most of these instances. Nonetheless, it must be recognized that only in situations where the frequency of interspecific mating is high and the population at risk is numerically smaller than its congener does hybridization pose a significant threat (Ellstrand & Elam 1993).

Where these conditions occur, such as in *C. traskiae,* there are two possible management solutions: elimination of the less-desired species from the area of hybridization and/or transplantation of the rare population to a remote location where the other hybridizing taxon does not occur (Rieseberg 1991). In some instances it may also be necessary to eliminate all hybrid and introgressive individuals as well as the less-desired species (see Allendorf & Leary 1988). However, none of the *Cercocarpus* in Wild Boar Gully are now classified as *C. betuloides* var. *blancheae* (Table 1). Furthermore, close to one-half of the global genetic diversity in the *C. traskiae* would be lost by the removal of hybrid adult and juvenile individuals. Thus, there appears to be little gain and much to lose by eliminating hybrid individuals from Wild Boar Gully.

The other potential solution recommended by Rieseberg et al. (1989) involves the propagation and transplanting of cuttings of "true" *C. traskiae* individuals to other locations where hybridization is unlikely. This approach is currently being implemented by the Catalina Conservancy (J. Takara, personal communication), and a total of 16 trees have been propagated and returned to test plots on the island (J. Takara, personal communication). In addition, five plants remain at the Catalina Island nursery. Survival rates of outplanted trees have been remarkably high (close to 90%). A number of the trees are producing viable fruit, and seedlings have been observed at one of the sites. Potential problems (which are recognized by the Conservancy) include the low number of individuals (1–5) planted at each site, the lack of herbivore protection at most sites (only the Women's grove site has a perimeter fence), the dissimilarity between the habitat of the test plot sites and the original population, and the loss of identification tags from many of the trees. Nonetheless, the existence of these test populations has clearly reduced the short-term extinction potential of the species and may contribute to its long-term preservation.

Acknowledgments

We wish to thank M. Gay and J. Takara for assistance in locating and collecting *Cercocarpus* specimens from

Catalina Island, providing information on the Catalina Conservancy transplantation program, and correcting mistakes on the original map of Wild Boar Gully. We also thank Oscar Dorado and Jeanette Whitton for constructive comments on an earlier version of the manuscript. This research was supported by NSF 9019872 to L. H. Riesberg.

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