THE EVOLUTION OF DISTYLY FROM TRISTYLY IN POPULATIONS OF Oxalis alpina (OXALIDACEAE) IN THE SKY ISLANDS OF THE SONORAN DESERT

Stephen G. Weller, César A. Domínguez, Francisco E. Molina-Freaner, Juan Fornoni, and Gretchen LeBuhn

2Department of Ecology and Evolutionary Biology, University of California, Irvine, California 92697 USA; 3Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, C.P. 04510, México D.F., México; 4Departamento de Ecología de la Biodiversidad, Estación Regional del Noroeste, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 1354, C.P. 83000, Hermosillo, Sonora, México; and 5Department of Geography, University of California, Irvine, California 92697 USA

The evolution of distyly from tristyle was investigated in populations of Oxalis alpina at high elevations throughout the Sky Islands of the Sonoran Desert. Incompatibility systems in tristyloous populations, where self-incompatible short-, mid-, and long-styled morphs occur in populations, vary from those typical of tristyloous species in which each morph is equally capable of fertilizing ovules of the other two morphs, to breeding systems in which incompatibility relationships are asymmetric. In these populations, selection against the allele controlling expression of the mid-styled morph is likely. The degree of modification of incompatibility in the short- and long-styled morphs in 10 populations was strongly associated with fewer mid-styled morphs, supporting models predicting the effect of these modifications of incompatibility on frequency of the mid-styled morph. Self-incompatibility of the mid-styled morph may be important for maintaining the frequency of this morph, depending on the level of self-pollination, self-fertilization, and the extent of inbreeding depression. Modifications of incompatibility in tristyloous populations and the distribution of distyloous populations of O. alpina in the Sky Island region have similar geographic components, indicating the potential importance of historical factors in the evolution of distyly from tristyle.

Key words: distyly; heterostyly; illegitimate crosses; legitimate crosses; Oxalis alpina; self-incompatibility; Sky Islands; tristyle.

Most flowering plant species are hermaphroditic, and many have the capacity for self-fertilization. Charles Darwin (1900) first demonstrated the deleterious consequences of self-fertilization and suggested that many reproductive systems in flowering plants have evolved to promote outcrossing (matings with other individuals in the same population). Understanding the evolutionary forces that underlie modifications of plant breeding systems, which themselves may influence genetic diversity, gene flow, population structure, and the potential for speciation, is a major challenge. Heterostyloous breeding systems have been useful model systems for addressing questions about breeding system evolution. In heterostyloous breeding systems, two or three floral morphs occur in a population (Barrett, 1992). Because of strong self-incompatibility, each floral morph is normally incapable of producing seeds. In tristyloous systems, three floral morphs occur in populations (Fig. 1A). Flowers have three levels at which organs can occur; stamens occupy two levels, while stigmas occur in the third. Morphs differ in the levels at which stigmas and anthers occur (Fig. 1A). Compatible matings are generally those occurring between anthers and stigmas located at the same level in the flower and were termed legitimate by Darwin (1877). Because there are three floral morphs, there are six categories of legitimate pollinations (L × l/S, L × l/M, etc.; see Fig. 1A for explanation of cross notation) that typically produce seeds. The numerous categories of illegitimate cross- and self-pollinations (L × m/S, L × s/M, etc.) normally fail to produce seeds.

The genetic system controlling tristyly, in those cases that have been investigated, usually consists of two linked or unlinked loci, each with two alleles (Weller, 1976b; Lewis and Jones, 1992). Theoretical studies have indicated that this system of genetic control leads to equal numbers of the three morphs in populations (Charlesworth, 1979; Heuch, 1979). In distyloous species, only two morphs occur in populations (Fig. 1B); as in the case of tristyly, fertilizations that are capable of leading to seed production are normally those occurring between anthers and stigmas at the same level. Distyly is found in approximately 25 families of flowering plants (Barrett, 1992). Tristyly is much less common and is found in only six flowering plants families (Barrett, 1993; Thompson et al., 1996). Distyly also occurs in four families with tristyly (Amaryllidaceae, Linaceae, Lythraceae, and Oxalidaceae) and is thought to be derived from tristyly in the Lythraceae and Oxalidaceae (Weller, 1992).

Theoretical analyses indicate that the most obvious cause for the loss of floral morphs in a tristyloous population is fitness differences among the morphs. Morph-specific fitness disadvantages may occur when pollinators discriminate among floral
morphs, when one of the morphs undergoes more self-fertilization and a reduction in fitness of offspring due to inbreeding depression (Charlesworth, 1979) or when the efficiency of pollen transfer is not symmetrical and one or two morphs suffer pollen limitation (e.g., Hodgins and Barrett, 2006). Similarly, modifications of heterostylous incompatibility allowing production of seeds following illegitimate crosses could lead to loss of a floral morph, assuming the occurrence of illegitimate pollen flow and pollen limitation (Charlesworth, 1979). A much less explored alternative is the loss of floral morphs in tristylistous populations independent of fitness differences among the morphs. These losses may occur if patterns of pollen flow and compatibility favor the propagation of one of the morph-determining alleles or if one of these alleles is more susceptible to drift (Eckert and Barrett, 1992).

We investigated *Oxalis alpina* to test whether modifications of tristylistous incompatibility relationships have resulted in the evolution of distyly in a number of populations of this species (Weller, 1976a; Charlesworth, 1979). The importance of these modifications was suggested by observations of incompatibility from populations of *O. alpina* in the Sky Islands (or Madrean Archipelago), isolated mountain ranges in the Sonoran Desert that have coniferous forest at higher elevations. In typical tristylistous species, including tristylistous species of *Oxalis* from southern Mexico (Weller, 1980), only legitimate pollinations (pollen transfer between stamens and stigmas occurring at the same level) lead to fertilization and seed set (Fig. 1A). In contrast, in the two populations investigated in the Sky Islands, incompatibility reactions were modified from the typical condition of southern Mexican species (Weller, 1976a, 1980). In the short- and long-styled morphs there was no differentiation of the incompatibility reactions of the two anther whorls, and the two morphs were completely intercompatible (for the long-styled morph, the L × m/S and L × l/S crosses had equivalent seed production; for the short-styled morph, the S × m/L and S × s/L crosses had equivalent seed production; Fig. 2).

Modifications of the L × m/S and S × m/L incompatibility reactions are likely to result in selection against the mid-morph for two reasons (Weller, 1976a). From the perspective of male fitness, pollen from two stamen whorls of the short-styled morph, but only a single stamen whorl of the mid-styled morph is capable of fertilizing ovules of long-styled morphs (Fig. 2). Similarly, pollen from two stamen whorls of the long-styled morph, but only a single stamen whorl of the mid-styled morph is capable of fertilizing ovules of short-styled morphs (Fig. 2). Unless the proportion of legitimate pollen flow (F × s/L and L × l/S crosses) is high relative to illegitimate but compatible pollen flow (S × m/L and L × m/S crosses), pollen from the short- and long-styled morphs should outcompete pollen from mid-styled morph (Weller, 1976a). From the perspective of female fitness, pollen from only two stamen whorls, the mid-stamen whorl of the short- and long-styled morphs, is capable of fertilizing mid-ovules, while pollen from three stamen whorls is capable of fertilizing ovules of both short- and long-styled morphs (Fig. 2), an approximate 50% advantage for the short- and long-styled morphs. Hence, the hypothesis predicts an alteration in the pattern of segregation within those populations where incompatibility reactions of the short- and long-styled morphs have been modified. In populations of *O. alpina* there is no evidence for reduced fecundity of the mid-styled morph (Weller, 1981a), presumably because pollinators transfer adequate pollen for full seed set. These results also indicate that it is unlikely that lower fecundity of the mid-styled morph has resulted in reduced frequency of this morph and consequent modifications of incompatibility in the short- and long-styled morphs.

The consequences of loss of incompatibility differentiation for the evolution of distyly were modeled by Deborah Charlesworth (1979). With relatively high levels of selfing and inbreeding depression, a modifier that resulted in loss of incompatibility differentiation usually resulted in loss of the mid-morph. Self-pollination of the mid-morph was considered more likely because stigmas are located between the two anther whorls (Charlesworth, 1979). Pollen limitation increased the likelihood of loss of the mid-morph. In contrast, when legitimate pollen flow was high (e.g., fewer L × m/S and S × mL pollinations), retention of the mid-morph was more likely. Charlesworth (1979) suggested that the ability of mid-level pollen to function in the L × m/S and S × mL crosses should be accompanied by a corresponding failure of M × m/S and M × mL crosses.

In this paper, we describe variation in incompatibility among 13 Sky Island populations of *O. alpina* and determine how these differences are associated with style-morph variation. In particular, we test the hypothesis that modifications of illegitimate crosses of the short- and long-styled morphs are
haploid chromosome number varies from 7 to 42, suggest that (Denton, 1973). This scapose species grows from a small bulb in response to widespread species ranging from Guatemala to the southwestern United States (Denton, 1976), and production of viable hybrid seed (Weller, 1978).

Monophyletic based on similar morphology, uniform tetraploidy (Weller and Denton, 1976a). We attempted to analyze incompatibility relationships in at least one population of O. alpina from each mountain range in the Sonoran Sky Islands. A few inaccessible populations or populations with inadequate population sizes (Sierra Los Pinitos, Sierra El Tigre, Sierra La Madera) were not included in the study. Bulbs were sampled throughout each population to minimize the chance that genets were represented more than once. Bulbs were planted in a soilless mix at the University of California Irvine greenhouse. All crosses were made during the summer months when O. alpina responds to water and grows actively. To assess the status of incompatibility in populations, we used approximately 10 individuals of each style morph as seed parents in crosses and an equal or larger number of plants as male parents. In each tristylos population, there were six legitimate crosses (Fig. 1A), 12 illegitimate crosses (crosses between individuals where anthers and stigmas occur at different levels), and six illegitimate self-pollinations (both anther and stigmatic parts of each morph used for self-pollinations). For each individual used as a maternal plant, two to five crosses per category were carried out, yielding a minimum of 750 crosses per tristylos population. Control flowers (tagged but not pollinated) were used to monitor any background pollination. There was virtually no evidence that potential insect pollinators gained access to the greenhouse, and data from control crosses are not presented. Capsules were collected 13–15 d following pollination, before explosive dehiscence. The average number of viable seeds per capsule was used as a measure of incompatibility following a cross.

**Incompatibility relationships in populations of O. alpina**—We collected bulbs from 13 tristylos and one distylos population in Arizona, New Mexico, and Sonora for investigation of incompatibility relationships. The distylos population was included in the crossing study to confirm incompatibility results obtained for other distylos populations throughout the range of O. alpina (Weller, 1976a). We attempted to analyze incompatibility relationships in at least one population of O. alpina from each mountain range in the Sonoran Sky Islands. A few inaccessible populations or populations with inadequate population sizes (Sierra Los Pinitos, Sierra El Tigre, Sierra La Madera) were not included in the study. Bulbs were sampled throughout each population to minimize the chance that genets were represented more than once. Bulbs were planted in a soilless mix at the University of California Irvine greenhouse. All crosses were made during the summer months when O. alpina responds to water and grows actively. To assess the status of incompatibility in populations, we used approximately 10 individuals of each style morph as seed parents in crosses and an equal or larger number of plants as male parents. In each tristylos population, there were six legitimate crosses (Fig. 1A), 12 illegitimate crosses (crosses between individuals where anthers and stigmas occur at different levels), and six illegitimate self-pollinations (both anther and stigmatic parts of each morph used for self-pollinations). For each individual used as a maternal plant, two to five crosses per category were carried out, yielding a minimum of 750 crosses per tristylos population. Control flowers (tagged but not pollinated) were used to monitor any background pollination. There was virtually no evidence that potential insect pollinators gained access to the greenhouse, and data from control crosses are not presented. Capsules were collected 13–15 d following pollination, before explosive dehiscence. The average number of viable seeds per capsule was used as a measure of incompatibility following a cross.

**Statistical analysis of crossing results**—Preplanned comparisons (SAS Institute, 1989) were used to test whether the S × mL and L × mS crosses had the same level of compatibility as legitimate crosses with short- and long-styled morphs as female parents (S × s/L, S × s/M, L × uS, L × uM). Loss of incompatibility differentiation between the mid- and long-anther whorls of the short-styled morph and the short- and mid-anther whorls of the long-styled morph was indicated when the preplanned contrast was not significant; a significant contrast indicated either partial or no modification of incompatibility differentiation. The contrast between these legitimate and illegitimate crosses is critical because of previous work indicating that high compatibility of the S × mL and L × mS crosses is likely to favor loss of the mid-styled morph (Weller, 1976a; Charlesworth, 1979). Preplanned contrasts were also used to determine whether the S × mL and L × mS crosses had greater seed set than the remaining illegitimate crosses (S × uM, S × uS, S × uL, M × uS, M × uL, M × s/M, M × l/M, L × s/M, L × mL, L × l/L). Lack of significance indicated that incompatibility of the S × mL and L × mS crosses was similar to the other illegitimate cross categories; a significant value for the contrast indicated some loss of incompatibility differentiation for the S × mL and L × mS crosses. Results from the two preplanned contrasts for each population were used to characterize tristylos incompatibility populations as having (1) retained ancestral tristylos incompatibility (contrast of four legitimate and two illegitimate crosses significant; contrast of S × mL and L × mS crosses with remaining 10 illegitimate crosses insignificant), (2) partial loss of incompatibility differentiation (contrast of four legitimate and two illegitimate crosses significant; contrast of S × mL and L × mS crosses with remaining 10 illegitimate crosses also significant), or (3) complete loss of incompatibility differentiation (contrast of four legitimate and two illegitimate crosses insignificant; contrast of S × mL and L × mS crosses with remaining 10 illegitimate crosses significant).

All legitimate crosses were compared using Tukey's post hoc tests (SAS Institute, 1989) to detect potential differences in viable seeds per capsule and especially modifications of mid-morph fecundity. Seed production following

![Diagram of modified incompatibility relationships in a population of Oxalis alpina from Morse Canyon in the Chiricahua Mts.](image)

**Fig. 2.** Modified incompatibility relationships in a population of Oxalis alpina from Morse Canyon in the Chiricahua Mts. (based on data (solid lines), led to seed production as high as those of the legitimate S × s/L and L × l/S crosses (dotted lines).**

**MATERIALS AND METHODS**

**Study species**—Oxalis alpina (Rose) Knuth (section Ionoxalis) is a widespread species ranging from Guatemala to the southwestern United States (Denton, 1973). This scapose species grows from a small bulb in response to summer rains. Sessile bulbs are formed in the leaf axils in populations from the Sky Island region. Cytogeographic studies, which have shown that the haploid chromosome number varies from 7 to 42, suggest that O. alpina is not monophyletic (Weller and Denton, 1976). In contrast, populations in the Sky Island region of Arizona, New Mexico, and Sonora are likely to be monophyletic based on similar morphology, uniform tetraploidy (Weller and Denton, 1976), and production of viable hybrid seed (Weller, 1978).

**Style-morph representation**—We surveyed 18 tristylos populations and six distylos populations for style-morph representation in 2001–2004. Populations were defined physically as an area where plants occurred in a spatially contiguous distribution, usually in moister canyons, separated from other populations by intervening unsuitable habitat (usually dry ridges). In the field, we defined flowering individuals by their physical separation from neighboring plants; spatially distinct flowering individuals may represent ramets of the same genet, although neighboring plants often had different style morphs, indicating that clonal growth in O. alpina may be limited. In several tristylos populations, style-morph frequencies were estimated from nonflowering bulbs collected in the field and grown in the greenhouse. Several populations sampled in 1977–1979 were also sampled in 2001, 2003, or 2004. We sampled a single population for style-morph frequencies in most mountain
self-fertilization was also compared using post hoc analyses to determine whether some morphs, particularly the mid-styled morph, have evolved detectable self-compatibility. Values of seed production for all cross categories were tested for deviation from zero using $t$ tests. Potential differences in mid self-compatibility were compared for populations where incompatibility differentiation was absent, partial, or complete with a one-way ANOVA on untransformed seed production data. All statistical analyses were carried out using SAS version 9.1 (SAS Institute, 2002–2003).

Loss of incompatibility differentiation and fecundity of the M × m/S and M × m/L crosses—We used regression to investigate the relationship between relative seed production of the legitimate M × m/S and M × m/L crosses to loss of incompatibility differentiation. Relative seed production of the M × m/S and M × m/L crosses was calculated by dividing the value for each of these crosses by the average value for seeds per capsule for the legitimate crosses with short- and long-styled morphs as seed parents. This analysis was carried out for all 13 tristyly populations.

Loss of incompatibility differentiation and frequency of the mid-morph—The potential effect of the degree of incompatibility differentiation in the short- and long-styled morphs on the frequency of the mid-styled morph in populations was investigated by dividing the mean number of seeds per capsule for the L × m/S cross by the mean number of seeds per capsule for the L × L/$\bar{S}$ cross. This ratio approaches 1 as the incompatibility between the L stigma and style and the m$/S$ pollen is lost. Similarly, S × m/L values were divided by S × s/L values. A value indicating the overall extent of incompatibility differentiation was obtained for each population by averaging values for the short- and long-styled morphs, with higher values indicating greater loss of incompatibility differentiation. Regression of mid-frequencies against these values was used to determine whether loss of incompatibility differentiation is associated with reduced mid-frequency. Only those populations with field surveys of style-morph frequencies were used in this analysis.

RESULTS

Style-morph frequencies—In the Sky Island region, distyly is apparently restricted to populations of *O. alpina* occurring in neighboring mountain ranges in Arizona (Table 1, Fig. 3) and the Chiricahua Mts., where distyly populations occur at the northern end of the range and tristyly populations at the southern end of the range (Weller, 1979). Distyly populations had equal numbers of short- and long-styled style morphs (defined as isoplethic by Heuch, 1979) in four of the six populations surveyed in 2001–2004 (Table 1). Among distyly populations, the greatest deviations from isoplethy occurred in the Sierra Ancha Mts., the most northern Arizona mountain range where *O. alpina* is found. In this mountain range style morphs were found in large patches, possibly because of clonal growth. While tristyly populations in Sonora were more likely to have equal numbers of short-, mid-, and long-styled morphs, style-morph frequencies deviated greatly from isoplethy in the more northern tristyly populations (Table 1). Populations in the Chiricahua Mts. (Morse Canyon, Fly Peak, South Fork Canyon, and Greenhouse Trail), near the Black River, and in the Pinos Altos Mts. had reduced frequencies of mid-styled morphs. Excesses of the long-styled morph were characteristic of anisoplethic populations (Table 1).

Distyly populations sampled across years had considerable heterogeneity in style-morph representation, despite the occurrence of isoplethy in most years. In two tristyly populations from the Huachuca Mts., the mid-styled morph remained at approximately the same frequency over a 23-yr period, although in one of these populations (Miller Canyon), the numbers of short- and long-styled morphs fluctuated substantially. In two of the four populations in the Chiricahua Mts., the frequency of the mid-styled morph dropped during this time interval (Table 1), while in the remaining two populations there was no significant heterogeneity across years. The frequency of the mid-morph appeared to decline in a population from the White Mts. sampled in 1977 and 2003.

Incompatibility relationships—Seed production following crosses within a distyly population from the Santa Catalina Mts. (voucher 706) was high for legitimate crosses (L × L/$\bar{S}$, L × m/S, S × s/L, and S × m/L in a distyly species; Fig. 1A) and very low for illegitimate crosses and selfs (Fig. 4A). In preplanned comparisons, there were no differences in seed production between the L × L/$\bar{S}$ and S × s/L crosses vs. the L × m/S and S × m/L crosses ($F = 0$, df = 1, $P = 0.99$). Post hoc comparisons indicated that short- and long-styled morphs produced similar numbers of seeds ($F = 1.76$, df = 3, 37, $P = 0.17$; Fig. 4A). Based on preplanned comparisons, seed production was much higher for the S × m/L and L × m/S crosses than for illegitimate crosses (S × m/S, S × L/$\bar{S}$, L × m/L and L × s/L in a distyly species), in which seed production did not deviate significantly from zero ($F = 16.63$, df = 1, $P < 0.0001$; Fig. 4A). Seed production following self-pollinations did not deviate significantly from zero.

Tristyly populations varied greatly in incompatibility relationships (Fig. 4B–N). Illegitimate crosses of the short- and long-styled morphs most likely to produce seeds were the S × m/L and L × m/S crosses. Illegitimate crosses of the mid-styled morph were more likely to have seed production significantly greater than zero (39% of all crosses across populations) than illegitimate crosses involving either the short- or long-styled morphs (10% of all crosses). Based on variation in seed production of the illegitimate S × m/L and L × m/S crosses, populations can be divided into three groups. The first group includes those populations, all in northern Sonora (Fig. 4H, Sierra San Luis, Sonora, 958; Fig. 4I, Sierra La Mariquita, Sonora, 960; and Fig. 4K, Sierra Azul, Sonora, 966) in which preplanned comparisons indicated that the four legitimate crosses using short-styled and long-styled morphs had significantly higher seed production than the two illegitimate S × m/L and L × m/S crosses (Table 2a). In these populations the S × m/L and L × m/S crosses also had the same low level of seed production as the remaining illegitimate crosses (preplanned comparisons, Table 2b).

In a second group that includes populations in Arizona and New Mexico and one population from northern Sonora, loss of incompatibility differentiation in the short- and long-styled morphs appears to be complete; the illegitimate S × m/L and L × m/S crosses have the same seed production as legitimate crosses with short- and long-styled morphs as seeds parents (Table 2a, Fig. 4C, Pinos Altos Mts., New Mexico, 971; Fig. 4D, Morse Canyon, Chiricahua Mts., Arizona, 727; Fig. 4E, Animas Mts., New Mexico, 973; and Fig. 4L, Sierra Los Ajos, Sonora, 967) and significantly greater seed production than the remaining illegitimate crosses (Table 2b). Incompatibility of the short- and long-styled morphs in these populations resembles incompatibility in distyly populations.

Partial loss of incompatibility differentiation occurs in a third group of populations scattered throughout the Sky Islands (Fig. 4B, White Mts., Arizona, 713; Fig. 4F, Miller Canyon, Huachuca Mts., Arizona, 702; Fig. 4G, Sierra San Jose, Sonora, 959; Fig. 4I, Sierra La Elenita, Sonora 956; Fig. 4M, Sierra Buenos Aires, Sonora, 961; Fig. 4N, Sierra La Purica, Sonora, 968). In these populations, the S × m/L and L × m/S
TABLE 1. Style-morph frequencies in distylous and tristylous populations of *Oxalis alpina* in Arizona and Mexico. For populations sampled across multiple years, probabilities for significant heterogeneity are presented. Tests for equality of style-morph representation (isoplethy) are also presented. Populations are ordered geographically starting with populations in northwestern Arizona (AZ) (all distylous) to populations located in eastern and southeastern Arizona, New Mexico (NM), and Sonora (SON), Mexico (see Fig. 3).

<table>
<thead>
<tr>
<th>Locality Voucher no.</th>
<th>Year of survey</th>
<th>Short</th>
<th>Mid</th>
<th>Long</th>
<th>N</th>
<th>Probability of heterogeneity</th>
<th>Test for isoplethy G (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distylous populations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sierra Ancha Mts., AZ</td>
<td>781</td>
<td>1977</td>
<td>9</td>
<td>91</td>
<td>116</td>
<td>&lt;0.0001</td>
<td>92.67 (0.0001)</td>
</tr>
<tr>
<td>Barfoot Park, Chiricahua Mts., AZ</td>
<td>785</td>
<td>1978</td>
<td>48</td>
<td>52</td>
<td>400</td>
<td>0.0112</td>
<td>0.49 (0.4839)</td>
</tr>
<tr>
<td>Santa Rita Mts., AZ</td>
<td>699</td>
<td>2001</td>
<td>50</td>
<td>50</td>
<td>212</td>
<td>0.00 (1.000)</td>
<td></td>
</tr>
<tr>
<td>Crest Trail, Chiricahua Mts., AZ</td>
<td>776</td>
<td>1977</td>
<td>51</td>
<td>49</td>
<td>200</td>
<td>0.7117</td>
<td>0.08 (0.7773)</td>
</tr>
<tr>
<td>Tristylous populations:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Mts., AZ</td>
<td>713</td>
<td>1977</td>
<td>15</td>
<td>36</td>
<td>49</td>
<td>204</td>
<td>0.0086</td>
</tr>
<tr>
<td>Pinos Altos Mts., NM</td>
<td>971</td>
<td>1977</td>
<td>40</td>
<td>11</td>
<td>49</td>
<td>204</td>
<td>0.2967</td>
</tr>
<tr>
<td>Morse Canyon, Chiricahua Mts., AZ</td>
<td>727</td>
<td>1978</td>
<td>23</td>
<td>30</td>
<td>47</td>
<td>200</td>
<td>0.0789</td>
</tr>
<tr>
<td>South Fork Canyon, Chiricahua Mts., AZ</td>
<td>779</td>
<td>1977</td>
<td>42</td>
<td>2</td>
<td>56</td>
<td>201</td>
<td>0.3745</td>
</tr>
<tr>
<td>Greenhouse Trail, Chiricahua Mts., AZ</td>
<td>789</td>
<td>1978</td>
<td>44</td>
<td>4</td>
<td>52</td>
<td>508</td>
<td>0.0863</td>
</tr>
<tr>
<td>Animas Mts., NM</td>
<td>973</td>
<td>2004</td>
<td>27</td>
<td>29</td>
<td>44</td>
<td>107</td>
<td>0.0027</td>
</tr>
<tr>
<td>Miller Canyon, Huachuca Mts., AZ</td>
<td>702</td>
<td>1978</td>
<td>26</td>
<td>24</td>
<td>50</td>
<td>796</td>
<td>0.0004</td>
</tr>
<tr>
<td>Carr Peak, Huachuca Mts., AZ</td>
<td>787</td>
<td>1978</td>
<td>15</td>
<td>31</td>
<td>54</td>
<td>474</td>
<td>0.0453</td>
</tr>
<tr>
<td>Sierra San Jose, SON</td>
<td>959</td>
<td>2004</td>
<td>22</td>
<td>24</td>
<td>53</td>
<td>92</td>
<td>0.15 (0.0001)</td>
</tr>
<tr>
<td>Sierra San Luis, SON</td>
<td>958</td>
<td>2001</td>
<td>46</td>
<td>21</td>
<td>34</td>
<td>111</td>
<td>0.10 (0.0001)</td>
</tr>
<tr>
<td>Sierra La Elenita, SON</td>
<td>956</td>
<td>2001</td>
<td>39</td>
<td>32</td>
<td>29</td>
<td>109</td>
<td>1.43 (0.4892)</td>
</tr>
<tr>
<td>Sierra La Mariquita, SON</td>
<td>960</td>
<td>2003</td>
<td>34</td>
<td>36</td>
<td>30</td>
<td>308</td>
<td>2.26 (0.3230)</td>
</tr>
<tr>
<td>Sierra Azul, SON</td>
<td>966</td>
<td>2003</td>
<td>33</td>
<td>34</td>
<td>33</td>
<td>123</td>
<td>0.05 (0.9753)</td>
</tr>
<tr>
<td>Sierra Los Ajos, SON</td>
<td>967</td>
<td>2003</td>
<td>34</td>
<td>33</td>
<td>33</td>
<td>259</td>
<td>0.05 (0.9753)</td>
</tr>
<tr>
<td>Sierra Buenos Aires, SON</td>
<td>961</td>
<td>2004</td>
<td>33</td>
<td>33</td>
<td>34</td>
<td>340</td>
<td>0.06 (0.9704)</td>
</tr>
<tr>
<td>Sierra La Purica, SON</td>
<td>968</td>
<td>2003</td>
<td>22</td>
<td>30</td>
<td>48</td>
<td>939</td>
<td>0.10 (0.0005)</td>
</tr>
</tbody>
</table>

Notes: Style-morph frequencies were sampled from plants flowering in the field with three exceptions (Animas Mts., Sierra La Elenita, and Sierra San Luis), where style-morph frequencies were based on plants grown in the greenhouse from bulbs collected in the field either before or after flowering. Tests for heterogeneity were first carried out for surveys in 1977–1979 (when multiple years were sampled) and then also including data from 2001–2003. All tests for isoplethy were significant after a sequential Bonferroni correction using morph ratios from the most recent population surveys.

Fig. 3. Distribution of tristylous and distylous populations of *Oxalis alpina* in the Sky Island region of the Sonoran Desert. Frequencies of short-, mid-, and long-styled morphs are based on the most recent surveys for each population. For several populations, limited field observations or herbarium records indicate the presence of tristylous (T) or distylous (D) populations. The distribution of oak woodland and coniferous forest follows Marshall (1957) and McLaughlin (1995), with modifications based on our field observations.
crosses produced significantly less seed than the legitimate crossing categories (Table 2a; contrasts of four legitimate vs. two illegitimate crosses were significant) but produced significantly more seed than the other categories of illegitimate crosses (Table 2b; contrasts of seed production for the S × m/L and L × m/S crosses vs. the remaining illegitimate cross categories were significant). The extent of modification varied widely among these populations with partially modified incompatibility differentiation. Populations with partially modified incompatibility located in the White Mts. and Sierra San Jose (Fig. 4B, 4G) closely resembled nearby populations where differentiation of incompatibility was complete (Pinos...
Fig. 4. Continued.
Fig. 4. Continued.

Cross type

Legitimate pollinations
Illegitimate intermorph pollinations
Illegitimate intramorph pollinations
Self-pollinations

G. Sierra San Jose, SON (959)
H. Sierra San Luis, SON (958)
I. Sierra La Elenita, SON (956)
J. Sierra La Mariquita, SON (960)
Fig. 4. Continued.
### Table 2. Summary of results from preplanned comparisons and post hoc contrasts following crosses within tristylos populations of *Oxalis alpina* from Arizona, New Mexico, and Sonora (localities listed in Table 1). Analyses include (A) preplanned contrasts of the four legitimate crosses (S × S, S × L, L × M, L × L) vs. the illegitimate S × mL and L × mS crosses, (B) preplanned contrasts of illegitimate S × mL and L × mS crosses with remaining illegitimate crosses, (C) post hoc comparisons among legitimate crosses using Tukey’s contrasts, and (D) post hoc comparisons of seed production following self-pollination using Tukey’s contrasts.

<table>
<thead>
<tr>
<th>Population, state, voucher no.</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morse, Chiricahua Mts., AZ 727</td>
<td>0.33, 0.5660</td>
<td>27.56, &lt;0.0001</td>
<td>2.81, 0.0225</td>
<td>8.61, &lt;0.0001</td>
<td>Complete modification</td>
</tr>
<tr>
<td>San Luis, SON 958</td>
<td>60.47, &lt;0.0001</td>
<td>0.81, 0.3714</td>
<td>0.73, 0.6033</td>
<td>0.60, 0.6966</td>
<td>No modification</td>
</tr>
<tr>
<td>La Elenta, SON 956</td>
<td>19.93, &lt;0.0001</td>
<td>6.86, 0.0100</td>
<td>1.44, 0.2180</td>
<td>1.31, 0.2713</td>
<td>Partial modification</td>
</tr>
<tr>
<td>Miller, Huachuca Mts., AZ 702</td>
<td>6.41, 0.0143</td>
<td>19.74, &lt;0.0001</td>
<td>0.69, 0.6314</td>
<td>0.60, 0.6966</td>
<td>Partial modification</td>
</tr>
<tr>
<td>San Jose, SON 959</td>
<td>17.79, &lt;0.0001</td>
<td>72.27, &lt;0.0001</td>
<td>11.41, &lt;0.0001</td>
<td>10.77, &lt;0.0001</td>
<td>Partial modification</td>
</tr>
<tr>
<td>La Purica, SON 968</td>
<td>40.13, &lt;0.0001</td>
<td>37.81, &lt;0.0001</td>
<td>1.17, 0.3301</td>
<td>2.97, 0.0184</td>
<td>Partial modification</td>
</tr>
<tr>
<td>Los Ajos, SON 967</td>
<td>2.62, 0.1099</td>
<td>47.57, &lt;0.0001</td>
<td>3.07, 0.0145</td>
<td>2.05, 0.0850</td>
<td>Complete modification</td>
</tr>
<tr>
<td>Azul, SON 966</td>
<td>54.57, &lt;0.0001</td>
<td>0.22, 0.6419</td>
<td>0.02, 0.9998</td>
<td>1.63, 0.1653</td>
<td>No modification</td>
</tr>
<tr>
<td>Buenos Aires, SON 961</td>
<td>6.73, &lt;0.0001</td>
<td>6.47, 0.0126</td>
<td>0.30, 0.9118</td>
<td>2.54, 0.0398</td>
<td>Partial modification</td>
</tr>
<tr>
<td>La Mariquita, SON 960</td>
<td>16.61, &lt;0.0001</td>
<td>0.06, 0.8098</td>
<td>0.81, 0.5455</td>
<td>0.49, 0.7792</td>
<td>No modification</td>
</tr>
<tr>
<td>Animas, NM 973</td>
<td>1.82, 0.1202</td>
<td>6.16, &lt;0.0001</td>
<td>4.49, 0.0014</td>
<td>1.42, 0.2397</td>
<td>Complete modification</td>
</tr>
<tr>
<td>White, AZ 713</td>
<td>9.24, 0.0035</td>
<td>110.64, &lt;0.0001</td>
<td>0.73, 0.6033</td>
<td>2.21, 0.0662</td>
<td>Partial modification</td>
</tr>
<tr>
<td>Pinos Altos, NM 971</td>
<td>1.62, 0.1655</td>
<td>19.35, &lt;0.0001</td>
<td>7.80, &lt;0.0001</td>
<td>9.03, &lt;0.0001</td>
<td>Complete modification</td>
</tr>
</tbody>
</table>

**Notes:** Retention of typical tristylos incompatibility relationships is indicated when the S × mL and L × mS crosses have low seed production relative to the legitimate crosses with short- and long-styled morphs as seed parents (significant F values for preplanned comparisons in column A), and when the S × mL and L × mS crosses have similar levels of seed production compared to the remaining illegitimate crosses (nonsignificant F values for preplanned comparisons in column B). Evolution of incompatibility relationships resembling distyly is indicated by insignificant F values in column A and significant F values for column B. Partial loss of incompatibility differentiation is indicated when F values are significant for (A) and (B). Although a significant F value was obtained for the Buenos Aires population when seed production following self-pollination was compared, the post hoc comparison was not significant.

Variation in self-incompatibility in populations of *O. alpina*—Self-compatibility varied substantially among populations of *O. alpina*, with a tendency for mid-styled morphs to have greater self-compatibility than the short- or long-styled morphs, especially in those populations with partial or complete loss of incompatibility differentiation (Table 2d; Fig. 4A–N). Significant post hoc contrasts indicated higher self-compatibility of the mid-styled vs. short- and long-styled morphs for Morse Canyon, Chiricahua Mts. (727), Sierra San Jose (959), and the Pinos Altos Mts. (971), populations where modification of incompatibility differentiation is either complete (727, 971) or nearly complete (959). Mid-styled morphs from all four populations with complete loss of incompatibility differentiation had significant seed production following selfing (Fig. 4C–E and 4L). Three of six populations with partial loss of incompatibility differentiation had significant self-compatibility in the mid-styled morph (Fig. 4B, 4G, and 4M), and one of three populations possessing full incompatibility differentiation had significant seed production following selfing of mid-styled morphs (Fig. 4K). When self-compatibility of the mid-styled morph, measured as the average of seed production for the M × l/M and M × m/S self-fertilizations, was compared for populations with unmodified, partially modified, and fully modified incompatibility, there were no significant differences (F = 0.30; df = 2, 10, P = 0.7464). There appeared to be little correlation between seed production of the M × l/M and M × m/S self-pollinations within mid-styled individuals (Fig. 4).

Modification of fecundity of the M × m/S and M × m/L crosses—Loss of incompatibility differentiation was associated with modification of seed production of the legitimate M × m/S cross, as indicated by a significant regression of relative fecundity of the M × m/S cross (seed production of M × m/S divided by the average fecundity of legitimate crosses of the short- and long-styled morphs) against a measure of loss of incompatibility differentiation (F = 8.26; df = 1, 11; P = 0.015; Fig. 5). Populations with the most pronounced loss of incompatibility differentiation of the short- and long-styled morphs had the lowest relative seed production for the M × m/S cross (Table 2c). This trend was most apparent in populations from the White, Pinos Altos, Chiricahua (Morse Canyon), and Animas Mts. The relationship of M × m/L relative fecundity to...
incompatibility modification was not significant ($F = 1.96$, df = 1, 11; $P = 0.189$).

**Relationship between incompatibility and style morph frequencies**—Complete loss of incompatibility differentiation in the short- and long-styled morphs in the Chiricahua (Morse Canyon), Los Ajos, Animas, and Pinos Altos populations was associated with reduced frequencies of the mid-styled morph (average mid-frequency = 20.5%; $N = 4$, using a mean value of mid-styled morph frequency in the Chiricahua Mts. of 7.5% averaged over all tristylos populations) relative to those populations with partial loss of incompatibility differentiation (average mid-styled morph frequency = 28.2%; $N = 6$), or unmodified incompatibility differentiation (average mid-styled morph frequency = 35%; $N = 3$). There was a strong negative relationship between loss of incompatibility differentiation of the short- and long-styled morphs and the frequency of the mid-styled morph (Fig. 6; $F = 12.7$; df = 1, 8; $P = 0.0074$).

**DISCUSSION**

**Variation in incompatibility relationships in O. alpina and selection against the mid-allele**—Incompatibility relationships in *O. alpina* show remarkable diversity over short geographic distances, and differences among populations in the extent of incompatibility differentiation between the stamen whorls of the short- and long-styled morphs were strongly related to the frequency of mid-styled morphs. Loss of incompatibility differentiation is expected to result in selection against the mid allele (Fig. 2) (Weller, 1976a, 1986; Charlesworth, 1979), and in populations of *O. alpina* from the Sky Islands, the mid-styled morph occurs in significantly lower frequencies in populations with greater seed production for $S \times m/L$ and $L \times m/S$ crosses (Fig. 4). Reduction in frequency of the mid-styled morph most likely results from modifications of incompatibility in the short- and long-styled morphs, rather than from reduced fitness of the mid-styled morph because in previous studies (Weller, 1981a), no differences in seed production were detected among morphs in the field. In most populations with modified incompatibility, the $S \times m/L$ and $L \times m/S$ crosses had similar seed production, although in several cases (e.g., Sierras Los Ajos, Buenos Aires, and La Purica; Fig. 4D) the fecundity was substantially greater for the $L \times m/S$ cross than for the $S \times m/L$ cross. The regression of mid-morph frequency against incompatibility modification assumes that each population represents an independent data point, although we do not know the phylogeographic history of these populations.

**Geographic patterns to breeding system modification in O. alpina**—*Oxalis alpina* was probably continuously distributed throughout the Sky Island region during the Pleistocene pluvial period because packrat–midden studies confirm that coniferous forests occurred at lower elevations during this period (Van Devender, 1977; Van Devender and Spaulding, 1979). Isolation of *O. alpina* to separate mountain ranges probably occurred following the Pleistocene, when drying caused the retreat of forests and species like *O. alpina* to higher elevations. The current distribution of distylos and tristylos populations could have been established before isolation of *O. alpina* populations or after populations became disjunct on isolated mountain ranges at the end of the pluvial period. The restriction of distylos populations of *O. alpina* to mountain ranges in the northwestern part of the Sky Islands (Fig. 3) may indicate that a single transition to distyly occurred in this region, prior to isolation of populations by post-Pleistocene drying. Populations throughout the Sky Islands have probably been
genetically isolated from one another because drying occurred at the end of the Pleistocene pluvial period. Several studies of other organisms in this region (e.g., Aquilegia, Strand et al., 1996; jumping spiders, Masta, 2000) have used phylogeographic approaches and concluded that gene flow is limited between these isolated mountain ranges. Phylogeographic information would be useful for determining how many transitions to distyly have occurred in populations of O. alpina in the Sky Islands region.

The concentration of distylous populations in the northwestern part of the Sky Islands suggests that environmental differences may be important in explaining modifications of heterostyly in O. alpina. Despite the geographic pattern of breeding system variation, however, no obvious environmental gradients or differences in pollinators are associated with the transition to distyly (Weller, 1981b; F. Baena [Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México], C. Domínguez, J. Fornoni, F. Molina-Freaner, S. Weller, unpublished observations). At present, we do not know why modifications of incompatibility are more common in the northern Sky Island populations of O. alpina.

Changes in style-morph frequencies over time—Comparisons of style-morph frequencies over a 24–26 yr period indicate apparent stability in the frequency of the mid-morph, with the exception of several populations from the Chiricahua Mts., as well as a population from the White Mts., where the frequency of the mid-morph declined significantly. Full or partial loss of incompatibility differentiation is characteristic of these populations, indicating that modified incompatibility relationships may have resulted in decline of the mid-morph. Distylous populations fluctuated less in style-morph frequencies over the sampling interval, with the exception of the Sierra Ancha population, where clonal growth appears to predominate and leads to substantial differences in style-morph representation, depending on the area sampled (S. Weller, C. Domínguez, and F. Molina-Freaner, unpublished observations).

Fitness of the mid-morph—Charlesworth (1979) suggested that in populations with modified incompatibility of the short- and long-styled morphs, the ability of mid-level pollen from these morphs to function on long and short stigmas would be inversely associated with function on mid stigmas. Reduction in fertility of the $M \times m/S$ cross in populations of O. alpina with loss of incompatibility differentiation is consistent with previous observations (Weller, 1976a), although there is no apparent loss of function of mid-level pollen from the long-styled morph on mid stigmas. High levels of natural pollination (Weller, 1981a), as well as excesses of the long-styled morph serving as pollen donor to the mid-styled morph, may explain the absence of differences in seed production between the morphs of O. alpina despite low seed production following $M \times m/S$ crosses.

Differences in seed production of the $M \times m/S$ and $M \times m/L$ crosses also explain the results of direct tests for the effects of loss of incompatibility modification on mid-styled morph fertility. Progeny raised from naturally pollinated plants in Chiricahua populations were expected to show deficits of the mid-styled morph because of pollen competition that favored the short- and long-styled morphs (Weller, 1986). Contrary to this prediction, mid-styled morph excesses were found, particularly in the progeny of mid-styled morphs, which produced almost no short-styled morphs in their offspring (Weller, 1986). This unexpected result can now be understood in light of reduced seed production following the $M \times m/S$ cross in the Morse Canyon population in the Chiricahua Mts. (Fig. 4). Because the $S$ allele is epistatic to the $M$ allele (Weller, 1976b), if the $M \times m/S$ cross produces few seeds, most mid-styled offspring will result from $M \times m/L$ crosses, and the $M$ allele is more likely to be expressed in these progeny. Of the four populations with complete loss of incompatibility differentiation (Chiricahua, Los Ajos, Animas, and Finos Altos), the $M \times m/S$ cross had low seed production in all but the Los Ajos population. Because rejection of pollen from short-styled morph parents would increase the frequency of mid-styled morphs in the progeny of mid-styled morphs, this mechanism may have been favored in mid-styled morphs that are under negative selection as a result of incompatibility modifications in short- and long-styled morphs.

Significant self-fertility was observed for a number of populations, and half of these cases involved self-fertility of the mid-styled morph. Self-fertility of the mid-styled morph might result in selection against this morph, depending on the selfing rate and the extent of inbreeding depression (Charlesworth, 1979). Alternatively, self-fertility of the mid-styled morph might be advantageous in the face of modifications of incompatibility in short-styled and long-styled morphs that place mid-styled morphs at a disadvantage during outcrossing. Assuming that inbreeding depression was minimal, selfing would favor the mid-styled morph because of the occurrence of only mid- and long-styled morphs among progeny derived from selfing. Preliminary data suggest that self-pollination of mid-styled morphs is substantial (F. Baena, C. Domínguez, J. Fornoni, F. Molina-Freaner, S. Weller, unpublished observations), although the degree of natural self-fertilization of mid-styled morphs is unknown. We are investigating selfing rates and the extent of inbreeding depression in populations of O. alpina.

Distribution of breeding systems in Oxalis section Ionoxalis—The distribution of tristylos and distylous populations in the Sky Island mirrors the overall distribution of heterostyly in section Ionoxalis, with tristylos species found in southern Mexico and distylous species found more commonly in northern Mexico and the United States. Within section Ionoxalis, most tristylos species are diploid and have restricted ranges, while distylous species have broader distributions and higher ploidy levels, an overall pattern suggesting that distyly is derived from tristyly (Weller and Denton, 1976). Within O. alpina, which occurs from Guatemala to New Mexico, ploidy levels are diverse, but they are not correlated with the distribution of distylos and tristylos breeding systems. In the Sky Islands region, all populations have the same ploidy level ($n = 14$). The closest populations outside of Sonora and Chihuahua with the same chromosome number as the Sky Islands populations occur in distylous populations in central Mexico. North of the Sky Islands region in New Mexico, populations of O. alpina are hexaploid ($n = 21$) and distylous. At present, the evolutionary relationships among populations of O. alpina throughout its range are poorly understood, although breeding-system lability within O. alpina is largely confined to the Sky Islands region. Additional studies, particularly those that model the effects of all modifications of self-incompatibility (J. Fornoni, C. Domínguez, F. Molina-Freaner, and S. Weller, unpublished
data) and investigate the extent of self-pollination, self-fertilization, and inbreeding depression in progeny of mid-styled morphs, will be particularly useful for understanding the dynamics of style-morph representation in populations of *O. alpina*.

**LITERATURE CITED**


