VULNERABILITY AND DETERMINANTS OF
REPRODUCTIVE SUCCESS IN THE NARROW ENDEMIC
ANTIRRHINUM MICROPHYLLUM (SCROPHULARIACEAE)\(^1\)

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The breeding system and flowering phenology of the narrow endemic Antirrhinum microphyllum (Scrophulariaceae) were studied in order to assess the main factors affecting female reproductive success and to identify existing or potential threats to the viability of its populations. Hand-pollination experiments showed that A. microphyllum is an allogamous self-incompatible species. In both populations studied, the flowering season was 4 mo long and mean flowering duration per plant was about 1 mo. Peak flower production took place between mid-April and mid-May and overlapped with the period of activity of the main pollinator, Rhodanthidium sticticum (Megachilidae). Estimated mean number of seeds produced per plant was 9391, showing that population viability is not presently limited by seed output. The study of the direct and indirect effects of plant size, phenological traits (first flowering date, flowering duration, and flowering synchrony), and distance to neighbor plants on reproductive success was performed using structural equation modeling (SEM). In both populations, number of flowers and plant size were the main factors that determined the total number of fruits produced by a plant. First flowering date and flowering synchrony also affected fruit production. Multisample comparison of path coefficients for the two populations rejected the possibility that reproductive patterns could be described by one single model.

Key words: Antirrhinum microphyllum; breeding system; conservation; endangered species; flowering phenology; Scrophulariaceae; self-incompatibility; structural equation modeling.

Numerous studies show that narrow endemics are susceptible to extinction for a variety of reasons, including habitat destruction, biotic interactions, and genetic collapse (Schemske et al., 1994). Many of these factors increase the vulnerability of the species by lowering reproductive success. Consequently, the study of the factors that critically affect reproductive success needs to be considered in the design of conservation strategies (Godt and Hamrick, 1995).

Female reproductive success depends on numerous factors that control the process of flower, fruit, and seed production (Primack, 1987). Among these factors, it has been shown that plant size (Bishop and Schemske, 1998) and phenological traits (Sobrevila, 1988; English-Loeb and Karban, 1992; Gómez, 1993; Kudo, 1993) have a relevant effect in many plant species. Moreover, female reproductive success is also conditioned by the breeding system of the species (Byers and Meagher, 1992; DeMauro, 1993), which also affects the structure of genetic diversity (Loveless and Hamrick, 1984; Hamrick and Godt, 1990; Les, Reinartz, and Esselman, 1991) and the fitness of individuals (Kittelson and Maron, 2000).

Most studies of the relationships between flowering phenology, plant size, and reproductive success have analyzed the effect of each variable by correlation or multiple regression (Schmitt, 1983; Farris and Lechowicz, 1990; Dieringer, 1991). However, these approaches do not take into account the simultaneous effect of all factors, or the interdependence relationships among factors, which makes it difficult to assess the main factors that determine fruit production.

Structural equation modeling (SEM) is a powerful alternative tool in exploring and contrasting complex hypotheses on causal relationships among variables using observational data (Mitchell, 1992). It is especially useful in conservation studies of small populations for which the implementation of specific treatments may have negative effects on population viability (Albert, Escudero, and Iriondo, 2001). The most noticeable advantages of SEM are the global perspective provided in the study of complex problems, the ability to discern the essential from the accessory, and the possibility of evaluating one’s own hypotheses (Batista and Coenders, 2000).

In this study we used SEM to assess the reproductive success of Antirrhinum microphyllum Rothm. (Scrophulariaceae), a perennial snapdragon that grows in the cracks of vertical dolomitic cliffs. The only four populations presently known (Entrepenás, Bolarque, Buendía, and Anguix) are located in the north of Sierra de Altomira, Guadalajara, Central Spain (Fig. 1). The extent of its total known range is an area of approximately 30 km\(^2\). This species has been classified as “vulnerable” according to IUCN criteria (VV.AA., 2000) and is protected by the regional legislation of Junta de Comunidades de Castilla—La Mancha (Anonymous, 1998).

The aim of this study was to find answers to the following questions: (1) What is the breeding system of the species? (2) What are the current main features of flowering phenology and female reproductive success? (3) What are the main factors that affect reproductive success? (4) Which factors are susceptible to change and increase the vulnerability of the species? (5) What are the implications for the management and conservation of the species?

This paper is part of a research project in which the genetic structure of the populations of A. microphyllum was also studied as well as the main ecological features of their habitats. The general purpose of the project was to obtain an accurate diagnosis of the status of the species through an integrated approach and to establish the main factors that determine the viability of the populations.
MATERIALS AND METHODS

Breeding system—To determine the breeding system of *A. microphyllum* we performed 650 hand pollinations under greenhouse conditions. We used 200 plants obtained from seeds collected at the Buendía population. Before the onset of flowering, anthers were removed and all flowers were bagged to prevent floral visitors. The treatments were (1) active autogamy, in which pollen from a flower was applied to its own stigma; (2) geitonogamy, in which pollen from one flower was applied to other flowers of the same plant; and (3) xenogamy, in which pollen from plants growing in the greenhouse and/or in the field (Bolarque and Entrepeñas populations) was applied to flowers from different plants. All pollinations were made with fresh pollen and when the stigmas were receptive. In addition, we bagged 100 flowers in the field without any treatment to test for passive autogamy. A G test (Sokal and Rohlf, 1995) was used to compare the fruit set of the different pollination treatments.

Flowering phenology—The monitoring of flowering phenology was conducted in the Bolarque and Entrepeñas populations in 1997. Before anthesis, we randomly tagged 50 individuals at Bolarque and 100 individuals at Entrepeñas and measured the size of each plant. The distance to the three nearest *A. microphyllum* plants was also measured. On each census day, we recorded the total number of individuals in flower and the number of open flowers and fruits on each individual.

We summarized individual phenologies using the following four variables: (1) first flowering date, (2) flowering peak date, (3) flowering duration, and (4) flowering synchrony. We calculated first flowering date for each individual as the number of days between 1 January and the day that its first flower was produced. Similarly, flowering peak date was defined as the number of days between 1 January and the day that the maximum number of open flowers was reached. Flowering duration was estimated as the number of days the plant remained in bloom. Finally, for flowering synchrony, we estimated the number of days that the flowering of one individual overlapped with the flowering of the rest of the plants in the sample (Gómez, 1993). This variable was calculated as follows:

\[
S = \frac{1}{n} - \frac{1}{n} \sum_{i,j} e_{ij}
\]

where \(e_{ij}\) is the number of days in which both individuals \(i\) and \(j\) flower synchronously, \(e_i\) is the number of days individual \(i\) is in flower, and \(n\) is the number of plants in the population. \(S\) ranges between 0 and 1; \(S = 0\) when there is no overlap in flowering among individuals, and \(S = 1\) when the overlap is complete. The synchrony of the population was defined as:

\[
S = \frac{1}{n} \sum_{i,j} S_{ij}
\]

where \(S_{ij}\) is the number of days in which both individuals \(i\) and \(j\) flower synchronously and \(e_{ij}\) is the number of days individual \(i\) is in flower and \(n\) is the number of plants in the population.

Normality of all phenological variables was examined by Kolmogorov tests. Means between populations were compared by t tests or the Mann-Whitney test when necessary. Skewness of the curves of percentage of flowering plants and number of flowers per day was computed as \(g_i\) (Sokal and Rohlf, 1995).

To study the effect of climatic conditions on flowering, we analyzed data of mean temperatures and rainfall at Bolarque daily from 1 January to 31 July. We also compared the mean monthly temperature and total rainfall with the mean of these variables for the period 1967–1997. An ombrothermic climatic diagram (Emberger et al., 1963) was also made to assess the periods of water deficit.

Female reproductive success—As previously mentioned, the number of open flowers and the number of developed fruits were counted for every plant sampled on each census day. The percentage of flowers producing fruits (fruit set) was calculated for each plant as an estimator of fruiting efficiency. To estimate female reproductive success we calculated total seed production per plant by multiplying the mean number of fruits per plant by the mean number of seeds per fruit (Dafni, 1992). The number of seeds per fruit was determined by taking a random sample of 100 fruits (1 fruit per plant) and counting the number of seeds per fruit. The fruits were collected in both populations before they were open.

Structural equation modeling analysis—The potential relationships among individual plant size, phenological traits, distance to other plants, and reproductive success were investigated using structural equation modeling. The structure of these relationships is illustrated in the path diagram of Fig. 2, in which an arrow indicates the causal effect of one variable on another. There are six dependent variables (number of flowers, first flowering date, flowering duration, flowering synchrony, fruit set, and number of fruits) and two independent variables (plant size and mean nearest-neighbor distance). The hypotheses of causal relationships between variables were formulated according to our previous experience with the species and existing literature on the reproductive biology of other species.

We hypothesized that larger plants would have a higher number of flowers because they would have more resources dedicated to reproduction (Schmitt, 1983; Garwood and Horvitz, 1985; Dieringer, 1991). Furthermore, if the onset of flowering in *A. microphyllum* depends on reaching a resource threshold (Lacey, 1986), larger plants would also have an earlier flowering date and a longer flowering duration (Schmitt, 1983). Flower production is also likely to be influenced by external environmental factors, such as temperature or moisture availability, and internal physiological factors, such as a specific balance of growth regulators. So, the sooner the appropriate external and internal conditions for flowering were reached (first flowering date), the more flowers the plant would have a chance to produce in the season.

We expected plants with a greater flower production to have a greater fruit set since they could be more attractive to pollinators. However, intensive flowering could also favor geitonogamy and reduce the rate at which flowers produce fruits if it were a self-incompatible species (Augspurger, 1980; Klinkhamer, De Jong, and De Bruyn, 1989; De Jong et al., 1992; Díaz-Lifante, 1996). In addition to number of flowers, both flowering synchrony and flowering duration would influence fruit set, because the number of days that the flowering of one individual overlaps with the flowering of the rest of the
plants and the number of days that it is in bloom condition the probability of being visited by pollinators. Another factor that may affect fruit set is the mean distance to the nearest neighbors. This variable is relevant in allogamous species, especially in the case of self-incompatible plants where pollination with pollen from a different plant is needed for a successful fruit set.

Finally, we considered that the total number of fruits developed by a plant would strictly depend on the total number of flowers produced by the plant and the efficiency with which flowers are changed into fruits (fruit set).

Structural equation modeling analysis was performed with the CALIS procedure of the SAS statistical software package (SAS Institute, 1990). The model was evaluated separately in each population. We estimated standardized partial regression coefficients (path coefficients) for each independent variable using the maximum likelihood method. Previously, we calculated variance inflation factors (VIFs) to detect if there was collinearity between predictor variables (Petraitis, Dunham, and Niewiarowski, 1996). All VIFs were less than ten, a value suggested by Myers (1990) as the limit to maintain a variable. We also tested variables for deviation from normality. Only flowering synchrony had a normal distribution. Flowering duration and number of flowers and fruits were log transformed \((t = \log(x + 1))\). Since no transformations were able to normalize the distribution of plant size, first flowering date, fruit set, and mean distance to the nearest neighbors, these variables were left unaltered. A matrix of correlation coefficients between all variables included in SEM analysis is available at the American Journal of Botany’s website (http://ajb.succ.botany.org/v89/torres.doc).

We did not allow estimation of the path coefficients for the effect of fruit set on number fruit but instead fixed their values to their standardized partial regression coefficients. This was necessary because fruit set was not measured independently, but calculated from number of flowers and number of fruits, and allowing these path coefficients to be freely estimated would artificially increase the model fit (Loehlin, 1987).

We used the multisample analysis procedure to find out whether the relationships between variables in both populations could be explained by a single model. The analysis was carried out by imposing cross-group constraints on the path models, in which the path coefficients were constrained to be equal in both groups (Bishop and Schemske, 1998). Next, a Lagrange multiplier test was used to identify the set of constraints that, simultaneously released, would result in a significantly better model (Bentler, 1989).

The goodness of fit of the model was contrasted through three different statistics (1) \(\chi^2\) goodness of fit; (2) Bentler-Bonett normed fit index (NFI) (Bentler and Bonett, 1980); and (3) goodness of fit index (GFI) (Jöreskog and Sörbom, 1985). The significance of individual path coefficients was assessed by a multivariate Wald test \((P < 0.05)\).

**RESULTS**

**Breeding system**—Fruit set for each of the pollination treatments is shown in Table 1. Nonmanipulated bagged flowers produced no fruits, and only 2.2% of self-pollinated flowers developed fruits, compared to 90% of cross-pollinated flowers. No significant differences were found in fruit production between flowers pollinated with pollen from the same population (89.4%) or from a different population (93.2%) \((G = 0.967, P > 0.05)\).

<table>
<thead>
<tr>
<th>Pollen resource</th>
<th>Pollination treatment</th>
<th>Fruit set (%)</th>
<th>Total number of crossings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Passive autogamy</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>Active autogamy</td>
<td>2.2</td>
<td>183</td>
</tr>
<tr>
<td>3</td>
<td>Geitonogamy</td>
<td>3.1</td>
<td>129</td>
</tr>
<tr>
<td>4</td>
<td>Xenogamy</td>
<td>89.4</td>
<td>265</td>
</tr>
<tr>
<td>6</td>
<td>Xenogamy</td>
<td>93.2</td>
<td>73</td>
</tr>
</tbody>
</table>

*1 = pollen from same flower; 2 = pollen from different flowers but same plant; 3 = pollen from different plant; and 4 = pollen from flowers collected at Entrepenas and Bolarque.*

**Flowering phenology**—The flowering season extended from mid-March to mid-July, and 145 of the 150 tagged plants (96.7%) flowered in 1997. Both populations had a peak of flower production between mid-April and mid-May (Fig. 3). Within this period, 50% of plants sampled were in bloom and 90% of total flowers were produced. The pattern of the percentage of flowering plants through time was symmetrical in both populations (Entrepenas: \(g_1 = 0.49, t_s = 1.59, ns\); and Bolarque: \(g_1 = 0.28, t_s = 0.77, ns\), whereas flower production was significantly skewed to the right (Entrepenas: \(g_1 = 1.99, t_s = 7.59, P < 0.001\) and Bolarque: \(g_1 = 1.54, t_s = 4.27, P < 0.001\)). Thus, there was a sudden increase in flower production at the beginning of the flowering period until the peak was reached and a slow descent until the end of the flowering period.

The mean flowering duration was 33.8 ± 18.6 d (mean ± SD) with a range from 7 to 104 d (Table 2). Flower production was not always constant and, thus, two different patterns were observed in plants: (1) a unimodal flowering pattern, followed by 80% of the individuals, in which the flowering peak was reached in the first third of the total flowering period; and (2)
Flowering phenology of *Antirrhinum microphyllum* in Entrepenas (a) and Bolarque (b) populations in 1997. Closed circles represent percentage of open flowers; open circles represent percentage of flowering plants.

A bimodal flowering, in which there was a first interval of intense flowering from which over 70% of the flowers were produced, a subsequent period with no flowers, and a final period of lower flower production in mid-June.

The average flowering synchrony was 0.46 at Entrepenas and 0.51 at Bolarque (Table 2). The flowering synchrony between populations was 0.45. No significant differences were detected in first flowering date and flowering duration between Bolarque and Entrepenas. However, Entrepenas had an earlier flowering peak and a lower flowering synchrony than Bolarque.

### Climatic conditions during flowering interval

The patterns of mean daily temperature and precipitation at Bolarque during the first half of 1997 are shown in Fig. 4a and b, respectively. Spring 1997 was quite typical in terms of rainfall, with a total precipitation of 102.3 mm and 17 rainy days in April and May, at the peak of flower production. These values were similar to the average of these variables for the previous 30 yr (1967–1997: 102.8 mm and 18 d). According to the ombrothermic diagram (Fig. 4c), there was no water deficit during the flowering season.

### Female reproductive success

The mean numbers of flowers and fruits produced per plant in each population are shown in Table 3. No significant differences between populations were found in terms of flower (U = 1759.5, P > 0.05) and fruit production (U = 1765.0, P > 0.05) per plant. However, fruit set was significantly higher at Bolarque (90.6%) than at Entrepenas (81.5%) (U = 1281.1, P < 0.05). The number of seeds per fruit was 199 ± 68.9 (N = 100), with a range from 37 to 395. Thus, the estimated mean seed production per plant was 9391 seeds.

### Structural equation modeling analysis

The model obtained for Entrepenas had GFI and NFI values of 0.90 and 0.93, respectively. However, significant differences were detected with the \( \chi^2 \) test (\( \chi^2 = 39.27, \text{df} = 14, P < 0.001 \)). Similarly, the model of Bolarque had GFI and NFI values of 0.92 and 0.96, respectively, and significant differences were also detected with the \( \chi^2 \) test (\( \chi^2 = 15.02, \text{df} = 15, P < 0.001 \)). The path diagrams in Fig. 2 illustrate the direction and magnitude of direct effects for the Entrepenas and Bolarque populations.

More than 97% of the variation in the total number of fruits was explained by the model in Entrepenas. Both number of flowers and fruit set had a significant direct effect on fruit production, with number of flowers being the variable with the greatest path coefficient. The indirect effects of plant size (0.62) and first flowering date (−0.58) were also relevant. In Bolarque, 99% of the variation in the total number of fruits was explained by the model. Number of flowers was the most important variable, followed by plant size (Table 4).

The multisample analysis rejected the hypothesis that the two populations could be described by identical models. The Lagrange multiplier test rejected 9 of 24 possible constraints.

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**Table 2.** Mean and standard deviation of phenological variables of *Antirrhinum microphyllum* in Entrepenas and Bolarque populations. Maximum and minimum values are shown in parentheses. \( N = \) sample size.

<table>
<thead>
<tr>
<th>Phenological variables</th>
<th>Entrepenas ( (N = 84) )</th>
<th>Bolarque ( (N = 43) )</th>
<th>Total ( (N = 127) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>First flowering date (d)</td>
<td>90.8 ± 21.3(^a) (68–172)</td>
<td>94.9 ± 15.6(^a) (63–142)</td>
<td>92.2 ± 19.6</td>
</tr>
<tr>
<td>Flowering peak date (d)</td>
<td>101.1 ± 20.1(^a) (74–176)</td>
<td>108.9 ± 15.0(^b) (83–153)</td>
<td>103.7 ± 18.8</td>
</tr>
<tr>
<td>Flowering duration (d)</td>
<td>31.9 ± 19.7(^a) (7–104)</td>
<td>36.8 ± 15.5(^a) (15–76)</td>
<td>33.8 ± 18.6</td>
</tr>
<tr>
<td>Flowering synchrony</td>
<td>0.46 ± 0.12(^a) (0.12–0.64)</td>
<td>0.51 ± 0.09(^b) (0.29–0.7)</td>
<td>0.47 ± 0.11</td>
</tr>
</tbody>
</table>

\(^{a,b}\) In each row, mean values not sharing the same letter are significantly different (t tests or Mann-Whitney tests; \( P < 0.05 \)).
self-incompatible and compatible species (Xue et al., 1996) and early studies showed that self-incompatibility in *Antirrhinum* is determined by a multiallelic $S$ locus with gametophytic control of pollen compatibility (Gruber, 1932; Sherman, 1939). Self-incompatibility mechanisms have arisen in evolution as a way of promoting within-population diversity by enhancing cross-pollination events against self-pollination (Richards, 1986). As expected in an allogamous breeding system, genetic studies performed on *A. microphyllum* populations through isozyme and RAPD (random amplified polymorphic DNA) markers have shown a high level of within-population genetic diversity (Torres, 1999).

Reproductive success may be reduced in self-incompatible species with small populations (Les, Reinartz, and Esselman, 1991; Byers and Meagher, 1992), as self-incompatibility restricts mate availability and conditions fruit set to pollinator activity. Nevertheless, fruit set values in both Bolarque and Entrepen Áas populations were very high (Table 3) and similar to those obtained in the greenhouse from hand cross-pollinations, suggesting that reproductive success of *A. microphyllum* is not limited by pollinator activity or by the number of self-incompatibility alleles.

Although outbreeding depression has been observed in several species where genetic differentiation between populations was significant (Fischer and Matthies, 1997; Montalvo and Ellstrand, 2001), it was not detected in *A. microphyllum*, where the experimental crossings with pollen originating from different populations did not yield a lower fruit set (Table 1).

**Flowering phenology**—The extended blooming period observed in *A. microphyllum* increases the individual’s chance of having a large number of mates both as pollen donor and recipient. Furthermore, it reduces the risk of reproductive failure resulting from bad weather or lack of pollinators (Bawa, 1983), which may be adaptive under continental Mediterranean climate conditions, with extreme temperatures and low and erratic precipitation during the reproductive season (Fig. 4). On the other hand, the flowering peak overlaps with the period of activity of *Rhodanthidium sticticum*, a solitary bee that is the main pollinator of *A. microphyllum* (Torres et al., 2001).

The symmetric curve of plants in flower through time, along with the skewness to the right in the flower production curve, indicates that early-flowering individuals produce more flowers than late-flowering individuals. This phenological pattern has also been documented in other studies (Dieringer, 1991), and it has been suggested that it may be an adaptive response

<table>
<thead>
<tr>
<th>Reproductive variables</th>
<th>Entrepen Áas ($N = 84$)</th>
<th>Bolarque ($N = 43$)</th>
<th>Total ($N = 127$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers</td>
<td>56.25 ± 59.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.91 ± 53.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.44 ± 56.52</td>
</tr>
<tr>
<td>(1–316)</td>
<td>(2–265)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits</td>
<td>47.19 ± 49.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.67 ± 48.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.19 ± 49.46</td>
</tr>
<tr>
<td>(0–263)</td>
<td>(1–226)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit set (%)</td>
<td>81.46 ± 23.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.60 ± 13.84&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.53 ± 21.37</td>
</tr>
<tr>
<td>(0–100)</td>
<td>(38.46–100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b</sup> In each row mean values not sharing the same letter are significantly different (Mann-Whitney test, $P < 0.05$).
to attract pollinators that usually visit other species (Thomson, 1980). The onlyrupicolous species that flowers at the same time as A. microphyllum is Sarcocapnos enneaphylla and we occasionally observed R. sticticum collecting nectar of S. enneaphylla flowers. This flowering pattern may be partially mediated by plant size. The negative effect of plant size on first flowering date and the positive effect of plant size on number of flowers observed in both populations (Fig. 2) promote the formation of an asymmetric and positively skewed flower production curve.

Flowering synchrony values obtained in both populations mean that on an average day each plant can exchange genes with only one half of the population. According to Rathcke and Lacey (1985) some degree of asynchronous flowering in a population has the benefit of promoting outcrossing by forcing pollinators to move between individuals. The matching degrees of synchrony within and between populations suggest that the sites have similar habitat conditions and that pollen-mediated gene flow is feasible between the populations. However, in practice, gene flow is probably very low as the existing geographic distance (15 km) is too long when compared with the average flight distance of the main pollinator.

**Female reproductive success**—Production of high number of seeds has also been reported in other Antirrhinum species (Juan, Pastor, and Fernández, 1996). This strategy is especially advantageous in a rupicolous habitat, like in A. microphyllum, where ecological niche availability is limited and a great proportion of seeds do not find a suitable habitat after dispersal. Reproductive output can decline considerably when climatic conditions are not appropriate. A large number of cloudy or rainy days during the flowering season may limit pollination since R. sticticum is not active under these climatic conditions. Nevertheless, limitations to seed production in any single year are unlikely to lead to bottlenecks in the populations, given that most individuals are long lived (80% of the individuals sampled in 1997 were still present in 1999). Moreover, the large proportion of flowering plants observed (96.7%) means that the adult stage is achieved in a short time, a feature that favors the viability of the populations. This conclusion is supported by experimental studies that have shown that seeds sown in fall produce plants that bear flowers in the following spring (M. Gris, Universidad Politécnica de Madrid, unpublished data).

### Table 4. Direct, indirect, and total effects of each variable on number of fruits in the Entrepeñas and Bolarque populations. Direct effects correspond to standardized path coefficients, indirect effects are calculated by multiplying path coefficients involved in the indirect effect, and total effects is the sum of direct and indirect components.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Direct effects</th>
<th>Indirect effects</th>
<th>Total effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entrepeñas</td>
<td>Bolarque</td>
<td>Entrepeñas</td>
</tr>
<tr>
<td>Plant size</td>
<td>0</td>
<td>0</td>
<td>0.613</td>
</tr>
<tr>
<td>First flowering date</td>
<td>0</td>
<td>0</td>
<td>−0.575</td>
</tr>
<tr>
<td>Flowering duration</td>
<td>0</td>
<td>0</td>
<td>0.048</td>
</tr>
<tr>
<td>Flowering synchrony</td>
<td>0</td>
<td>0</td>
<td>0.086</td>
</tr>
<tr>
<td>Mean nearest neighbor distance</td>
<td>0</td>
<td>0</td>
<td>0.078</td>
</tr>
<tr>
<td>Number of flowers</td>
<td>0.863</td>
<td>0.954</td>
<td>−0.021</td>
</tr>
<tr>
<td>Fruit set</td>
<td>0.280</td>
<td>0.154</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 5. Multisample comparison of path coefficients for the Entrepeñas and Bolarque populations. Entrepeñas → Bolarque represents the model constrained by Bolarque path coefficients fitted to Entrepeñas plant data. Bolarque → Entrepeñas represents the model constrained by Entrepeñas path coefficients fitted to Bolarque plant data. Paths with significant $\chi^2$ differ between populations. $\chi^2$ is the increment in model $\chi^2$ caused by forcing that path coefficient to be constrained to the opposite population data. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, assessed by Lagrange multiplier test index.

<table>
<thead>
<tr>
<th>Path</th>
<th>Independent variable</th>
<th>Dependent variable</th>
<th>Entrepeñas → Bolarque</th>
<th>Bolarque → Entrepeñas</th>
<th>$\chi^2$ comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean nearest neighbor distance</td>
<td>Fruit set</td>
<td></td>
<td>10.870***</td>
<td>4.865*</td>
<td></td>
</tr>
<tr>
<td>Flowering synchrony</td>
<td>Fruit set</td>
<td></td>
<td>0.076</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>First flowering date</td>
<td>Fruit set</td>
<td></td>
<td>0.242</td>
<td>0.561</td>
<td></td>
</tr>
<tr>
<td>Number of flowers</td>
<td>Fruit set</td>
<td></td>
<td>1.389</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Flowering duration</td>
<td>Fruit set</td>
<td></td>
<td>0.530</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>First flowering date</td>
<td>Flowering synchrony</td>
<td></td>
<td>16.444***</td>
<td>6.461*</td>
<td></td>
</tr>
<tr>
<td>Plant size</td>
<td></td>
<td>First flowering date</td>
<td>0.034</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td>Plant size</td>
<td></td>
<td>Number of flowers</td>
<td>0.902</td>
<td>0.420</td>
<td></td>
</tr>
<tr>
<td>First flowering date</td>
<td></td>
<td>Number of flowers</td>
<td>8.171***</td>
<td>3.349</td>
<td></td>
</tr>
<tr>
<td>Fruit set</td>
<td></td>
<td>Number of fruits</td>
<td>19.991***</td>
<td>17.856***</td>
<td></td>
</tr>
<tr>
<td>Number of flowers</td>
<td></td>
<td>Number of fruits</td>
<td>5.312*</td>
<td>9.964**</td>
<td></td>
</tr>
<tr>
<td>Plant size</td>
<td></td>
<td>Flowering duration</td>
<td>0.064</td>
<td>0.031</td>
<td></td>
</tr>
</tbody>
</table>
the $\chi^2$ test, we consider that the results obtained are due to a departure from the required assumptions. Therefore, we conclude that the models are acceptable.

Both Entrepeñas and Bolarque models have a similar general framework in which number of flowers and plant size are the main factors that affect fruit production, followed by first flowering date and fruit set (Table 4). The number of flowers and the efficiency by which flowers develop into fruits through pollination and fecundation (fruit set) are components of the reproductive process that lead to fruit production and obviously have an impact on this variable. In both populations, variation in the number of flowers had a much greater effect than variation in fruit set on final fruit production.

Positive relationships between plant size and fecundity have also been observed in many other species (Schmitt, 1983; Ferris and Lechowicz, 1990; Dieringer, 1991). If plant size is a measure of stored resources available for reproduction (Weiner, 1988), large plants should produce more flowers and fruits than small plants because they have accumulated more nutrients. A similar argument can be used to explain the positive effect of plant size on flowering duration (Widén, 1991). Finally, the negative effect of plant size on first flowering date may indicate that a critical size and a threshold of nutrients must be reached by the plant before it starts flowering. Nevertheless, plant size is only partially responsible for variation in flowering onset ($R^2 = 0.04–0.06$). Other factors not included in the model, such as genetic differences, soil moisture, temperature and light, must play a more significant role (Jackson, 1966; Edwards and Goldenberg, 1976; Widén, 1991; Tarasjev, 1997).

The effect of first flowering date was much more relevant than the effects of flowering synchrony and flowering duration. The negative path coefficients from first flowering date to number of flowers and fruit set indicate that *A. microphyllum* plants that flower at the end of the season have a lower fruit production than plants that flower earlier. This effect of seasonality on flower and fruit production is consistent with other studies (Sobrevila, 1988; Dieringer, 1991) and can be explained by the variation of environmental factors (rainfall and temperature) through the flowering season as well as the presence of adequate pollinators. Early-flowering plants start blooming in April and May, which correspond with the months of maximum precipitation and to the peak of activity of *R. sticticum*. However, late-flowering plants start blooming in June, when water stress limits flower and fruit production and pollinator activity is less intense.

The models also show a positive effect of flowering synchrony on fruit set. This was expected in a strictly self-incompatible plant since under these circumstances fruit set critically depends on successful pollination between two plants that flower simultaneously. However, the effect of this factor was low because most plants had synchrony values that did not limit the availability of compatible mates.

Finally, the mean nearest neighbor distance had a significant positive effect on fruit set at Entrepeñas but not at Bolarque. Why should fruit set values improve when the mean distance to the nearest neighbors increase? A possible answer lies in the self-incompatible system and the existence of genetic neighborhoods in the spatial structure of *A. microphyllum*, probably as a consequence of the lack of specialized seed dispersal mechanisms and the territorial behavior of *R. sticticum* (Torres, 1999). Thus, when the distance to the nearest neighbors is short, it is more likely that most pollen transport is between plants that are genetically related and share self-incompatibility alleles. Under some circumstances (e.g., low flowering synchrony or lack of genetically distinct individuals in the neighborhood), this may significantly affect fruit set. In addition to the self-incompatibility system, early postfertilization abortion due to biparental inbreeding depression could also affect fruit set. Reduction of reproductive fitness components due to inbreeding in genetically structured populations has been observed in other allogamous species (Oostermeijer, Altenburg, and Den Nijs, 1995). The lack of a significant effect of mean nearest neighbor distance on fruit set in Bolarque may be due to the lower statistical power of the data set in Bolarque or to differences in the spatial structure of individuals between the two populations.

**Differences between populations**—Significant differences in flowering peak date, flowering synchrony, and fruit set were detected between populations. In Bolarque, flowering synchrony and fruit set were higher, whereas the flowering peak was later than in Entrepeñas (Table 2 and Table 3).

Although most relationships between the variables considered for the study seem to follow similar schemes in both populations, several differences between models were detected. In Entrepeñas, significant path coefficients from plant size to first flowering date, from first flowering date to flowering synchrony and to fruit set, and from mean nearest neighbor distance to fruit set were obtained (Fig. 2a), whereas these path coefficients were not significant in Bolarque (Fig. 2b). It can be argued that these differences are due to the lower statistical power of the Bolarque data set as it has fewer observations. Nevertheless, multisample analyses showed that five constrained paths produced a significant increase in the $\chi^2$ when the Entrepeñas data was used in the Bolarque model. Four of these paths also produced a significant increase in the $\chi^2$ when the Bolarque data was used in the Entrepeñas model (Table 5). In addition to the paths from mean nearest neighbor distance to fruit set and first flowering date to flowering synchrony already mentioned, these analyses detected significant departures between models in the paths from number of flowers and fruit set to number of fruits.

In Entrepeñas, the spatial genetic structure of the population may be indirectly affecting fruit set values through incompatibility reactions and/or inbreeding depression mechanisms. Differences in fruit set can also be consequence of a lower pollinator abundance in this population, at least at the end of flowering season. The lower effect of fruit set on the number of fruits observed in Bolarque can probably be explained by its higher and more uniform fruit set values.

**Vulnerability of the species and implications for conservation and management**—The viability of the studied populations of *A. microphyllum* is not presently limited by the flowering or fruiting process. However, this taxon is self-incompatible and depends on the availability of compatible mates for reproduction. Any incidence that drastically reduces density or size of the populations may have an important effect on reproductive success. This fact must be taken into account in conservation and management plans for the species, being especially critical in any possible future reintroduction or reinforcement scenarios. In such cases, individuals should be genetically unrelated to prevent biparental inbreeding and should be distributed at distances that guarantee interplant movements. Moreover, the perdurence of the main pollinator,
Rhodanthidium sticticum, is essential for the reproductive success of A. microphyllum, and conservation strategies should include actions to protect these pollinators from human impact. Since phenological factors also play an important role as determinants of reproductive success, long-term changes in climatic conditions may indirectly influence the reproduction of this species through changes in its phenological features and those of its pollinators.

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