

INFLUENCES OF ANIMAL POLLINATION AND SEED DISPERSAL ON WINTER FLOWERING IN A TEMPERATE MISTLETOE

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Abstract. The evolution of flowering time can be influenced directly by pollinators and indirectly by seed dispersers. In temperate latitudes, interactions with climate, especially temperature, may affect both plants and their animal mutualists. Winter-flowering plants allow assessment of biotic influences on the evolution of flowering time because temperature is unlikely to select directly for this uncommon reproductive behavior. In northwestern Patagonia, the hemiparasitic mistletoe *Tristerix corymbosus* flowers from early fall, through the cool winter, to late spring. This species is pollinated by the hummingbird *Sephanoides galeritus*, and its seeds are dispersed by the marsupial *Dromiciops australis*. In two populations during two years, I analyzed seasonal variation in pollination, fruit production, and fruit removal. Hummingbird visitation was lowest in winter and late spring, and flowers opening during those periods showed reduced pollination and fruit set, partly due to pollinator limitation, compared to flowers that opened during fall or early spring. Fruits that ripened during summer (January–March) had a higher chance of being removed than fruits ripening during either spring or fall, due to their overlap with the period of maximum disperser activity. Timing of flower opening was strongly associated with fruit maturation time and with fruit removal rates. Thus, even though flowers that opened during winter exhibited only moderate fruit production, their fruits benefited from high fruit removal and seed dispersal in mid to late summer. These results suggest that the activity period of this plant's disperser, in combination with a lack of strong pollination constraints, probably governed the evolution of flowering phenology in this mistletoe. However, the proximate influence of temperature on flower and fruit development may prevent a fine-tuned match between flowering phenology and the period of maximum mutualist activity.

Key words: flowering phenology; fruit removal; fruit set; hummingbird pollination; marsupial dispersal; mistletoe; pollen limitation; seed dispersal; *Tristerix corymbosus*; winter flowering.

INTRODUCTION

The evolution of flowering time, its duration, and synchrony may be affected by the interactions of plants with their pollinators and seed dispersers (Stiles 1977, Wheelwright 1985, Bronstein 1995, Stone et al. 1998). However, these two types of mutualisms influence the evolution of flowering phenology through different paths. Most obviously, patterns of pollinator activity may directly promote a particular time and duration of flowering. On the other hand, the influence of seed dispersers on flowering phenology is always indirect and mediated by their effect on fruiting time.

Pollinator abundance typically fluctuates over the potential flowering season. Thus, plants can enhance their reproductive success by opening flowers during periods of high pollinator activity, which will ensure high pollen removal and export to other flowers as well as high pollen import (Mazer et al. 1989, Harder and Wilson 1994, Aizen 2001). Such direct selection could be particularly strong for plant species engaged in very

specific pollination mutualisms for which reproductive fate depends largely on the temporal overlap between flowering and the activity period of one or a few pollinators (Rathcke and Lacey 1985, Bronstein 1995). In plant species sharing pollinators, individuals can also increase their reproductive success by flowering when plants of other species do not, thus avoiding competition for pollinator service (Rathcke 1988, Feinsinger et al. 1991, Stone et al. 1998).

Fruiting follows flowering and, at least in some species, the duration of the fruit ripening period may be genetically fixed or constrained by fruit size (Rathcke and Lacey 1985, Primack 1987). Hence selection for fruit maturation during periods of high disperser availability may indirectly influence flowering phenology and even relegate flower opening to a period of sub-optimal climatic conditions for pollinator activity (Wheelwright 1985).

The direct or indirect influences of plant–animal mutualisms on flowering phenology may be most apparent in tropical latitudes (Stiles 1977, Wheelwright 1985, Stone et al. 1998). While such influences can also occur in temperate regions, they may be more difficult to disentangle from abiotic factors. In particular, milder temperatures during spring and summer can be favor-

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able for both flower development (e.g., Johnson 1992, Lechowicz 1995, Diekmann 1996) and the activity of animal mutualists (Rathcke and Lacey 1985). Thus, winter-flowering plants are particularly suitable biological models to test hypotheses about the role of biotic interactions in shaping flowering phenology in the temperate zone, because climate (and temperature in particular) is unlikely to be the primary selective factor for such an unusual reproductive behavior. Despite their taxonomic rarity, plant species that flower during winter may play a critical ecological role in maintaining resident populations of key pollinators and frugivores (Hopper 1981, Smith-Ramírez 1993, Armesto et al. 1996). In turn, availability of pollinators and efficient seed dispersers could strongly influence the timing of reproductive events in such plants (Rathcke and Lacey 1985, Primack 1987).

In this paper, I assess the possible influences of plant–animal mutualisms (i.e., pollination and seed dispersal) on the evolution of flowering phenology in a winter-flowering plant. In particular, I investigated the dependence of pollination, fruit set, and fruit removal on the month of flower opening in *Tristerix corymbosus* (Loranthaceae), a hummingbird-pollinated and marsupial-dispersed mistletoe native to the temperate forest of southern South America. This plant flowers from March (austral fall) to November (austral spring), during the cold and snowy winters that characterize the southern Andean part of its geographic range.

The temperate forest of southern South America hosts a rich hummingbird-pollinated flora comparable to that of many Neotropical forests (Aizen and Ezcurra 1998, Aizen et al. 2002). Unlike hummingbird floras from lower latitudes, this temperate ornithophilous flora is pollinated principally by a single species, *Sephanoides galeritus* (Cocucci 1991, Smith-Ramírez 1993, Armesto et al. 1996). Therefore, *T. corymbosus* may benefit from opening flowers during winter, when no other plants bloom, by monopolizing the foraging activity of its hummingbird pollinator.

In this temperate forest, seeds of *T. corymbosus* are dispersed exclusively and efficiently by the endemic arboreal marsupial *Dromiciops australis* (Amico and Aizen 2000). Therefore, even though winter flowering could expose *T. corymbosus* to poor pollination due to severe weather, it could also promote dispersal success if flowers that open during winter mature fruit during summer when disperser abundance peaks (Rau et al. 1995, Amico 2000). This proposition assumes that either selection on fruit development time is weak or that this trait is highly canalized and unlikely to respond to selection.

In this study, I took advantage of the extended phenology of *T. corymbosus* to address the following hypotheses: (H1) Winter flowering increases pollination and seed production (“pollinator-availability” hypothesis). Because no other hummingbird-pollinated plant flowers during winter in the temperate forest of South

America, I predicted higher hummingbird visitation to *T. corymbosus* flowers that open during winter when no alternative nectar resources are available. Elevated visitation should induce higher pollination and seed output for flowers opening during winter than either in fall or spring. (H2) Winter flowering favors seed dispersal (“disperser-availability” hypothesis). Because the abundance of seed dispersers varies seasonally, I predicted higher dispersal of fruits ripening from winter flowers than from either fall or spring flowers, if winter flowers mature fruit when dispersers are most abundant. Implicit in this hypothesis is a strong correlation between date of fruit maturation and date of flower opening.

MATERIALS AND METHODS

The plant species and study area

Tristerix corymbosus (quintral) is a shrubby mistletoe distributed along the Pacific rim of South America from 32° to 42° S (Kuijt 1988). Reproductive plants can produce several hundred flower buds that differentiate and develop during summer. The tubular flowers are large (~5 cm long), red and radially symmetrical, and clustered in inflorescences with 4–14 flowers. The filaments and style, which are yellow during anthesis, turn red when a flower senesces (Tadey and Aizen 2001). Controlled pollination showed that this mistletoe is self-compatible, but animal visitation is needed to achieve full reproductive output (M. A. Aizen, unpublished data). Flowers mature into 1 × 0.5-cm green pseudoberries, each containing one “naked” seed 0.7 cm long surrounded by a sweet viscous pulp (Amico and Aizen 2000). After a few weeks and if not removed, fruits senesce on the plant. Senescent fruits are characterized by a wrinkled, black pericarp and a bitter pulp.

The study was conducted in areas adjacent to Nahuel Huapi National Park, in the eastern foothills of the Patagonian Andes in southern Argentina. At 785 m, the altitude of Lake Nahuel Huapi, mean July and January temperatures are 2.2°C and 14.1°C, respectively (1914–1996 temperature record at Bariloche airport). A strong W–E precipitation gradient ranges from >3500 to <700 mm in less than 50 km from the continental divide. On average, only 12% of the annual precipitation falls during the summer months (December–February). Snowfalls are common during winter (Barros et al. 1983).

I studied flowering phenology, animal visitation to flowers, pollination, fruit set, and fruit removal during two complete flowering–fruiting periods (1997–1998 and 1998–1999) in two populations situated 10 km apart and ~25 km west of the city of San Carlos de Bariloche (41°8′ S, 71°19′ W). One population is in the Llao-Llao forest reserve (hereafter Llao-Llao), a mesic old-growth forest dominated by *Nothofagus dombeyi* (mean annual precipitation ~1900 mm), and the other is in the Península de San Pedro (hereafter

Península) on the southern margin of Lake Nahuel Huapi (mean annual precipitation ~1400 mm). At Llao-Llao, *T. corymbosus* mostly parasitizes branches of the shrub *Aristotelia chilensis*, whereas at Península it occurs on the tree *Maytenus boaria*.

Field sampling

During the 1997 flowering season (March–December) and subsequent 1997/1998 fruiting season (October–April), I sampled flowers and fruits in 20 individuals of *T. corymbosus* in each of the two populations. At weekly intervals, I counted the number of open flowers on three tagged branches per plant. Also, I marked 3–5 swollen flower buds per plant per week using numbered jewelry tags. Thereafter, I checked the status of each tagged bud at weekly intervals and classified it according to one of the following categories: bud, open flower, senescent flower, swollen ovary, developing fruit, mature fruit, and senescent fruit. On each sampling date, I also recorded the number of hummingbirds or other flower visitors observed foraging on *T. corymbosus* while I was in the field. For even-numbered plants (i.e., 10 individuals per population), I hand-pollinated half of all tagged flowers at anthesis using pollen collected from 3–5 other individuals to test for pollen limitation of fruit set.

To assess pollination, I collected styles from approximately half of all tagged flowers (either open- or hand-pollinated) ~2–3 wk after flowers were first recorded as senescent. Styles were kept individually in microcentrifuge tubes containing formalin:acetic acid:ethyl alcohol (FAA, 5:5:90). In the laboratory, styles were cleared overnight in 10 mol/L NaOH, stained with 0.1% aniline blue in 0.1 mol/L K_3PO_4 , squashed, and examined with an epifluorescence microscope (Martin 1959). I later counted the number of pollen tubes penetrating the stigma (i.e., just below the stigma surface).

Fruits of *T. corymbosus* remain green when ripe. Therefore, I considered a fruit to be mature when it was turgid, but soft to the touch. Approximately 10% of all fruits that developed from tagged flowers were bagged with nylon mesh just before maturation to estimate maximum retention time. Based on observations from bagged fruits, mature fruits rarely fall naturally (~2%). Thus I considered a fruit to have been removed by a disperser when an exposed fruit that had been classified in a given census as mature was absent in the following census. The marsupial disperser of *T. corymbosus* does not remove or consume senescent fruits (*personal observation*), so that when a senescent fruit disappeared between censuses it was considered as fallen.

I sampled flowers and fruits from the same individuals during the following flowering and fruiting season (i.e., 1998 and 1998/1999), except that one plant in Llao-Llao and six plants in Península died and were replaced. Sample sizes during this second year totaled 23 plants (Llao-Llao) and 21 plants (Península). In ad-

dition, I hand-pollinated half of all tagged flowers on all sampled plants rather than on a subset of plants. Results of comparing plants with and without hand-pollinated flowers from the previous year showed that open-pollinated flowers have the same probability of setting fruit, independent of the presence of hand-pollinated flowers (one-way ANOVA; $F_{1,16} = 0.16$, $P = 0.69$ and $F_{1,13} = 0.05$, $P = 0.83$ for fruit set at Llao-Llao and Península, respectively). Thus, an increase in fruit set among hand-pollinated *T. corymbosus* flowers can be attributed to pollen limitation rather than translocation of resources from lightly to heavily pollinated flowers (see Fox 1992). Over both years, I acquired data on flowering, pollination, and fruiting for ~10 000 flowers.

Data analysis

To address how pollination and seed dispersal might have influenced the evolution of flowering phenology in *T. corymbosus*, I used a flower-cohort approach rather than the more common phenotypic-selection approach (e.g., Widén 1991, Gómez 1993, O'Neil 1999), because the flowering phenology of this mistletoe varies little among plants (see *Results*). Although this approach does not permit estimation of the strength of natural selection, it allows assessment of the reproductive benefits associated with flowers opening at different times and identification of potential factors influencing the evolution of flowering phenology (e.g., Pico and Retana 2000, Wolfe and Burns 2001). Here, I classified and averaged data by month of either flower opening (i.e., March, April, May, etc.) or fruit maturation (i.e., October, November, December, etc.).

Flowering phenology.—I described flowering phenology as the proportion of all flowers counted on tagged branches that were open during a given flowering month. Monthly values were then averaged across individuals. However, because the number of open flowers reflects both flower opening rate and flower longevity (Primack 1985), flower counts may not measure accurately the number of flowers opening during a period if flower longevity varies seasonally. Therefore, for each individual I estimated monthly means of flower longevity (i.e., time a flower remained open) in weeks (individual flowers last 0–4 wk). Then I estimated the proportion of all flowers coming into anthesis in a given month by weighting the number of open flowers by the inverse of mean flower longevity for that month. Resulting values were restandardized (thus the sum of this variable over flowering months equaled one) and then averaged across individuals.

The pollinator-availability hypothesis.—I computed a relative visitation index as the number of hummingbirds (or other flower visitors) observed foraging during a given sampling date divided by the product of number of hours I spent in the field and the number of open flowers on tagged branches for that date (i.e., number of hummingbirds per hour per flower). I av-

eraged visitation index values by month for each site and year. Although this index cannot be used to compare visitation to flowers between sites or years because I counted flowers in a subset of branches and plants occurring in each population, it should provide an unbiased estimate of the seasonal change in pollinator activity at *T. corymbosus* flowers.

For each plant and month of flowering, mean number of pollen tubes and fruit set were calculated for open and hand-pollinated flowers separately. I assessed seasonal trends in pollination and fruit set and temporal changes in pollen limitation using ANOVA with pollination treatment (i.e., open vs. supplemental hand-pollination) and month of flowering as crossed main factors and individual plant as a blocking factor. According to the pollinator-availability hypothesis (H1), differences in pollination and fruit set between open and hand-pollinated flowers should be smaller during winter than either fall or spring months.

Flower-to-fruit time interval and fruit phenology.—From date of individual flower opening and fruit maturation, I estimated the time from flower anthesis to fruit. I also calculated the relative proportion of fruits that matured during a given month from different flower cohorts. For a given site and year, I used a likelihood ratio test (*G* test) to compare the proportion of fruits that ripened each month originating from flowers that opened in the fall (March–May), winter (June–August), and spring (September–December).

For each plant, I estimated the proportion of all fruits that ripened during month *m* (i.e., fr_m) as

$$fr_m = \sum_i fl_i fr_i p_{im} \quad (1)$$

where fl_i is the proportion of all flowers opening during month *i*, fr_i is the proportion of those flowers setting fruit, and p_{im} the proportion of those fruits that ripened during month *m*. Resulting values were then averaged across individuals from a given population and year.

The disperser-availability hypothesis.—For each plant, I estimated probabilities of fruit removal by month of fruit ripening and by month of flower opening based on the proportion of fruits that ripened in a given month (or alternatively produced by flowers that opened in a given month) that I considered as removed by an animal vector. Fruit removal can be used as a surrogate for seed dispersal for *T. corymbosus* because the marsupial *Dromiciops australis* effectively disperses mistletoe seeds and is the main frugivore that removes and ingests fruits in the study populations of *T. corymbosus* (see *Discussion*).

I also computed monthly means of total fruit retention time for exposed fruits (i.e., available for removal) and bagged fruits separately. I estimated retention times of individual fruits as the number of weeks between the time a fruit was first considered mature and the time it was recorded as absent (either “removed” or “fallen”).

I analyzed seasonal trends in fruit removal using ANOVA with month of fruit ripening (or alternatively month of flower opening) as the main factor and individual plant as a blocking factor. I also used ANOVA to assess the effects of fruit bagging, month of fruit ripening (or alternatively month of flower opening), and the bagging treatment \times month interaction on mean fruit retention time. According to the disperser availability hypothesis (H2), fruit removal should increase and fruit retention time should decrease among fruits set by flowers opening during winter months.

Per-flower contribution to fitness.—I estimated the cumulative success of a flower opening in a given month by integrating pollination and seed dispersal into a single measure. I used fruit set as a measure of pollination success and fruit removal as a surrogate of seed dispersal. Therefore, the benefits of interacting with mutualists for a flower opening in month *i* (F_i) can be summarized as

$$F_i = fr_i rm_i \quad (2)$$

where fr_i and rm_i are the fruit set and removal rate, respectively, exhibited by a flower opening during month *i*. This measure represents the probability that a flower opening in a given month sets a fruit that is removed and weighs the relative influences of pollination and seed dispersal on a flower's contribution to plant fitness. For each population and year, I analyzed seasonal variation in F_i using ANOVA with month of flowering as the main factor and individual plant as the blocking factor.

RESULTS

Flowering phenology

In the study populations, flowering extended from March to December in 1997 and from March to November in 1998 (Fig. 1). Individual plants flowered throughout most of the flowering period for their population. Nineteen of 20 surviving plants at Llao-Llao and 17 of 17 at Península flowered uninterruptedly for eight or more months in the 1997 season. Comparable figures for 1998 were 17 of 23 and 15 of 20 individuals. The onset of flowering was highly synchronous. During 1997 all sampled individuals started flowering in March. During 1998, at Península, 19 individuals started flowering in March and one in April; at Llao-Llao 14 individuals started flowering during March, five during April, and four during May. The number of open flowers peaked during May–June in 1997 at both sites and in 1998 at Península. Flowering at Llao-Llao in 1998 peaked during August (Fig. 1). I counted 45–48% of all flowers on tagged branches during winter (June–August).

Flower longevity averaged slightly more than 1 wk (1.14 ± 0.016 wk, Llao-Llao 1997; 1.04 ± 0.016 wk, Península 1997; 1.15 ± 0.015 wk, Llao-Llao 1998; and 1.05 ± 0.015 wk, Península 1998; means ± 1 SE), but

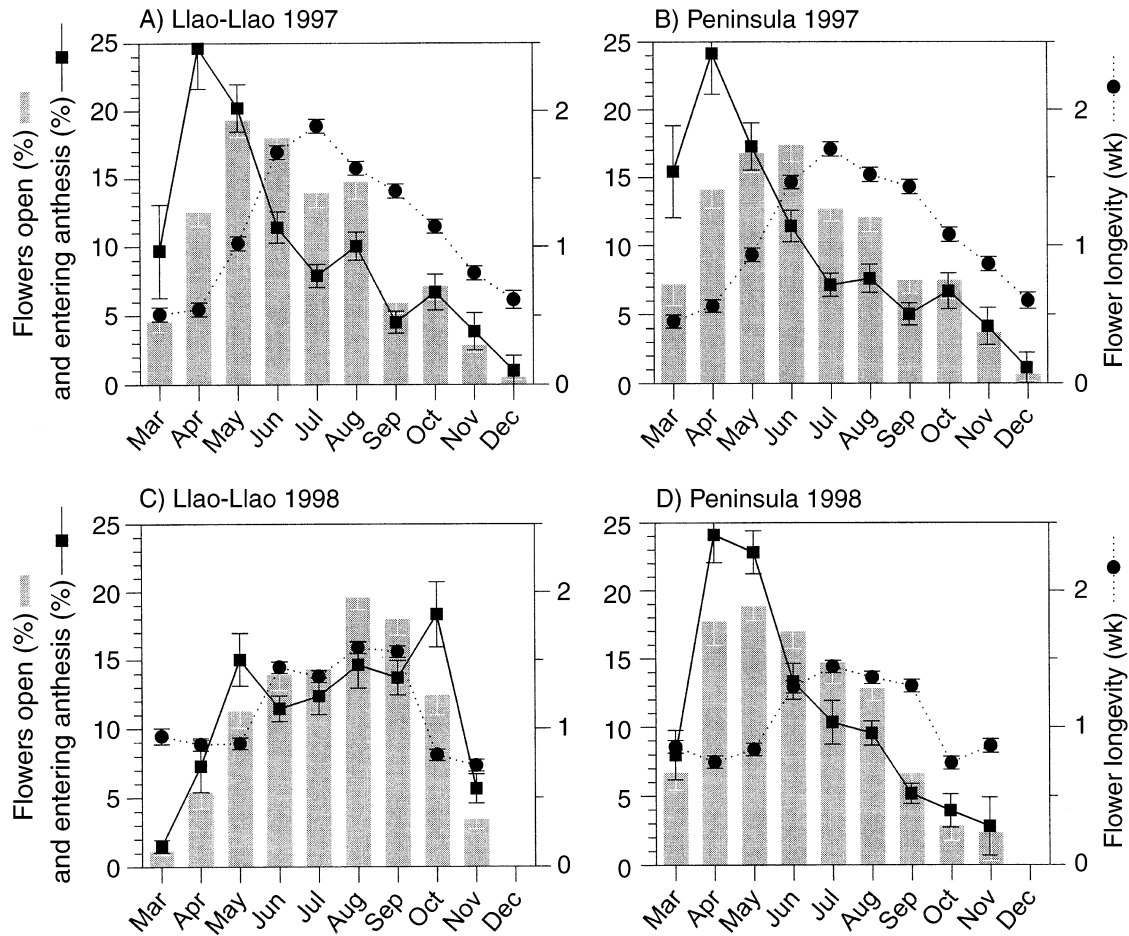


FIG. 1. Flowering phenology of *Tristerix corymbosus* in the Llao-Llao and Península sites in areas adjacent to Nahuel Huapi National Park, in the eastern foothills of the Patagonian Andes in southern Argentina during 1997 and 1998. Bars depict the percentage of all flowers observed open in a given month (means \pm 1 SE). Dotted lines depict estimates of flower longevity. Solid lines indicate the percentage of all flowers coming into anthesis in a given month.

increased during winter (Fig. 1). The mean flower opening in July lasted 2–3 times longer than flowers opening in March or November. Seasonal differences in flower longevity were more marked in 1997 (Fig. 1).

Because of the seasonal change in flower longevity, the phenological patterns in counts of open flowers differed from the patterns in date of flower opening: the proportion of flowers entering anthesis in a given month peaked earlier (April) in both populations in 1997 and at Península in 1998 (Fig. 1). Only 29% and 26% of all flowers produced during 1997 and 38% and 33% during 1998 at Llao-Llao and Península, respectively, entered anthesis during winter months. Thus, the full-bloom winter appearance of this mistletoe is accentuated by flower persistence.

Flower visitation, pollination, and fruit set

The hummingbird *Sephanoides galeritus* was the most frequent flower visitor, accounting for >85% of all individuals observed visiting flowers of *T. corymbosus* (Fig. 2). Queens of *Bombus dahlbomii* also vis-

ited mistletoe flowers during early fall at Península and during late spring at Llao-Llao in both years. Hummingbirds visited flowers throughout the flowering season with maximum visitation in early fall and declining to a minimum during late spring. A secondary peak in hummingbird visitation occurred in September.

In both populations and years, the number of pollen tubes per style showed significant seasonal variation (Appendix A). In open-pollinated flowers, pollination peaked at the beginning of the flowering season (March) and again during September 1997 and July–August 1998 (Fig. 3). With one exception (Península 1997), May- and June-opening flowers had lower numbers of pollen tubes in open-pollinated flowers. In both sites and years, pollination levels in open-pollinated flowers declined strongly by the end of the flowering season (October–December). Styles of open-pollinated flowers had, on average, significantly fewer pollen tubes than hand-pollinated flowers (Appendices A and B).

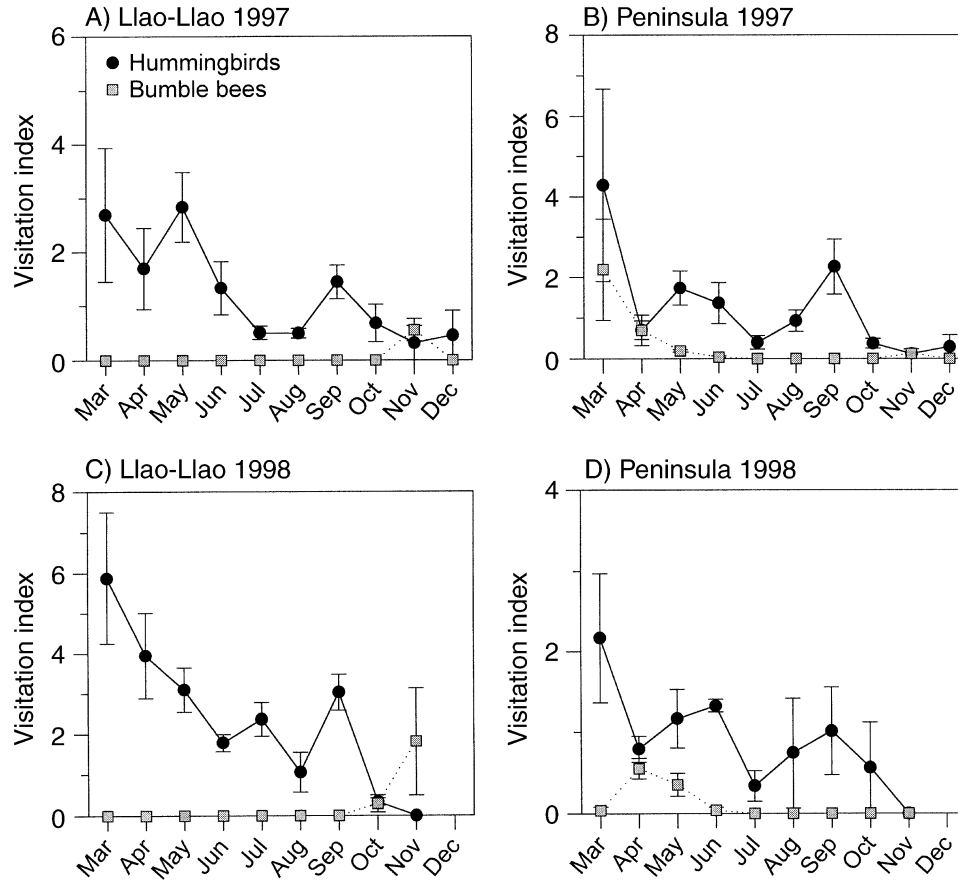


FIG. 2. Seasonal changes in relative visitation rates (visitation index) to the flowers of *Tristerix corymbosus* in Llao-Llao and Península over 1997 and 1998 by hummingbirds and bumble bees. Values are means ± 1 SE.

With one exception (Península 1998), there were no significant interactions between pollination treatment and month of flower opening (Appendix A). However, examination of Fig. 3 shows some consistent differences in the seasonal variation exhibited by open- and hand-pollinated flowers, particularly pronounced in flowers opening during May 1998 (both populations) and during July 1998 (Llao-Llao). The decline in pollination in open-pollinated flowers entering anthesis at the end of the flowering season was not observed in hand-pollinated flowers and thus, differences in pollination between treatments was greater for flowers that opened in late spring in both years and populations (Fig. 3).

Fruit set showed highly significant seasonal variation (Appendix A). In parallel with pollination (Fig. 3), fruit set from open-pollinated flowers was highest for flowers opening at the beginning of the flowering season (March–April) and in September (Fig. 4), whereas flowers opening during early to mid-winter (June–July) showed lower fruit set. Lower fruit set was also observed among open-pollinated flowers opening after September (Fig. 4). In both populations and years,

hand-pollinated flowers set significantly more fruit than open-pollinated flowers (Appendices A and B).

Although interacting effects of month and treatment on fruit set were significant and marginally significant only in Llao-Llao 1997 and Península 1998 (Appendix A), pollination limitation was more common during some months than others (Fig. 4). With some exceptions, fruit production was more pollen-limited in flowers that opened in late fall to mid-winter (May–July) and late in the flowering season (October–December).

Flower-to-fruit time interval and fruit phenology

Fruit development time showed strong seasonal patterns. The period from flower anthesis to fruit maturation decreased over flowering at a rate of ~ 2 wk/mo from >30 wk for flowers opening early in the season (March–April) to <20 wk (November–December) at the end of the flowering season (Fig. 5). Despite decreasing maturation times, early flowers matured early fruits. Season of flower opening was strongly associated with month of fruit ripening (G test, $P < 0.0001$ for both populations and years). Most fruits developing from fall flowers matured in spring (October–Decem-

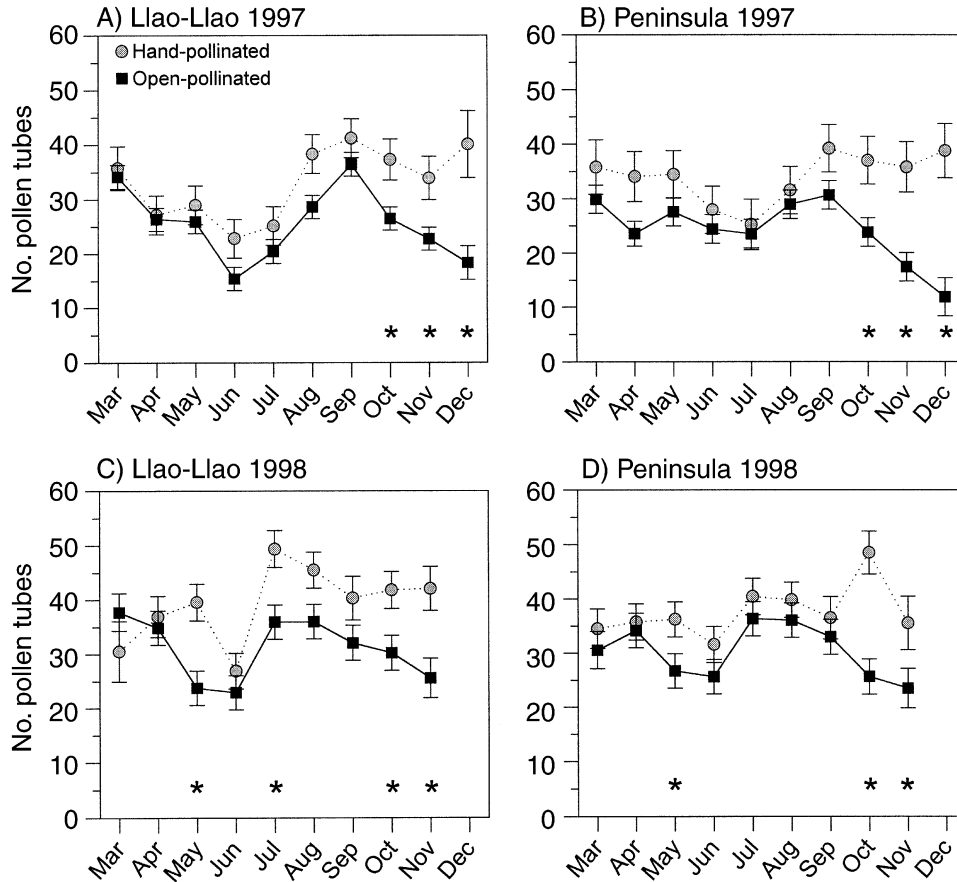


FIG. 3. Seasonal changes in pollination of open- and hand-pollinated flowers at Llao-Llao and Península during the 1997 and 1998 flowering periods (means \pm 1 SE). Asterisks indicate individual months showing significant differences (paired *t* test, $P < 0.05$) in number of pollen tubes between open- and hand-pollinated flowers after application of Bonferroni's correction for multiple tests (Rice 1989). See Appendix A for statistical analyses.

ber), fruits from winter flowers matured in summer (January–February), and those from spring flowers matured in late summer–early fall (March–May; Fig. 6). Fruits developed more slowly during 1997, particularly for flowers opening during the first half of the flowering season (March–July; Fig. 5). As a result, a higher proportion of fruits set from fall flowers delayed ripening until the following summer during 1997 than during 1998. Whereas 1997 fall flowers contributed 40.3% (Llao-Llao) and 24.7% (Península) of all fruits ripening during the following January, these contributions decreased to 11.3% and 10.9% for fruits set by the 1998 fall flowers (Fig. 6). Fruit ripening (Eq. 1) peaked in December, except at Llao-Llao in 1998/1999, which peaked in February (Fig. 6).

Fruit removal and retention time

Frugivores removed a large proportion of fruits (0.79 ± 0.022 , Llao-Llao 1997; 0.65 ± 0.026 , Península 1997; 0.61 ± 0.018 , Llao-Llao 1998; and 0.58 ± 0.023 , Península 1998). Exposed fruits were retained on the plants for a shorter time than bagged fruits (Appendices

B and C). However, the proportion of fruits removed and the duration of fruit retention on the plants varied strongly according to month of fruit ripening (Fig. 7, Appendix C). In all cases, fruits maturing during summer (January–March) had the highest probabilities of removal and shortest retention times (Fig. 7). Fruits maturing early in the season (October–November) showed low removal rates (<40%), and their mean retention time did not differ from that of isolated, bagged fruits. Also, fruit removal declined by the end of the fruiting season (April–May), particularly in 1997/1998 (Fig. 7).

Because of the relationship between date of anthesis and date of fruit ripening, fruits developing from winter-opening flowers (June–September) had higher removal rates (>70% and up to ~90%) and lower retention times (<3 wk) than fruits maturing from flowers that open at the beginning and end of the flowering season (Appendix D, Fig. 8). Fruits set by flowers opening at the tails of the flowering phenology exhibited removal rates as low as ~20% and their retention times did not differ significantly from bagged fruits.

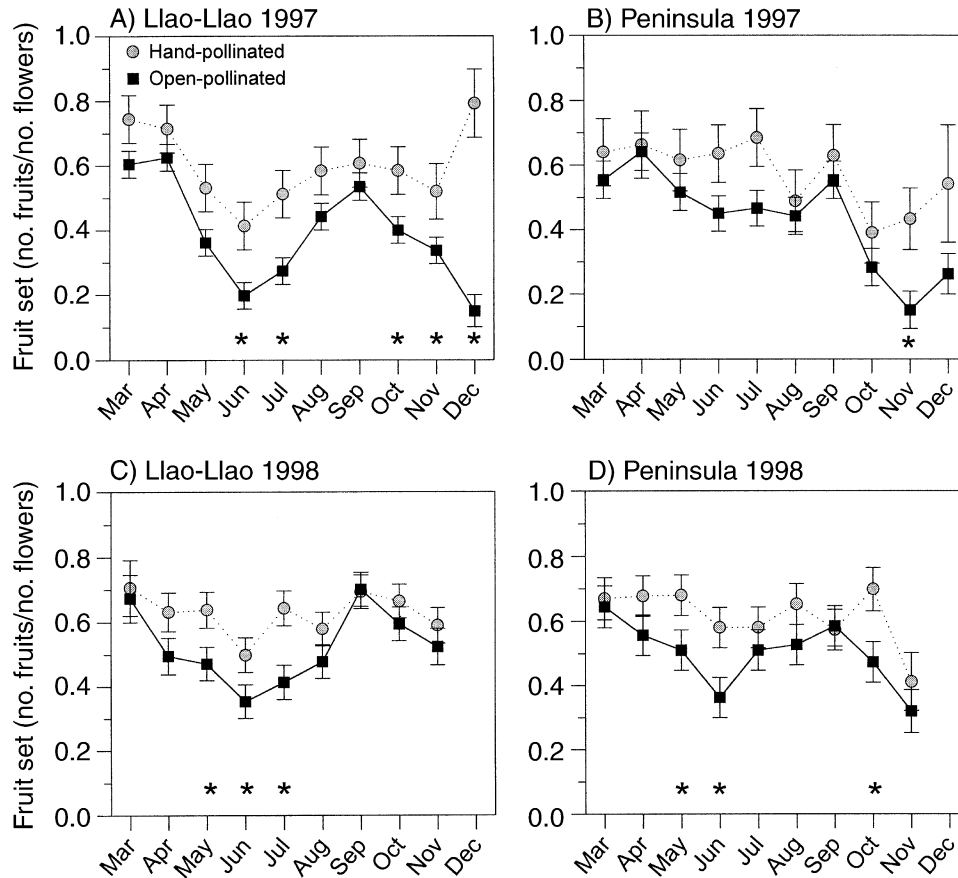


FIG. 4. Fruit set of open- and hand-pollinated flowers in relation to month of flower opening at Llao-Llao and Península during the 1997 and 1998 flowering phenologies (means \pm 1 SE). Asterisks indicate individual months showing significant differences (paired *t* test, $P < 0.05$) in fruit set between open- and hand-pollinated flowers after application of Bonferroni's correction for multiple tests (Rice 1989). See Appendix A for statistical analyses.

Per-flower contribution to fitness

Monthly estimates of per-flower contribution to fitness (Eq. 2) varied over flowering season consistently across years and populations (Fig. 9). The probability of a flower setting a removed fruit increased over flowering period, peaking during late winter (August–September) and decreasing abruptly afterwards. This pattern resulted because flowers opening during late winter exhibited both high fruit set (Fig. 4) and a high chance of fruit removal when ripe (Fig. 8). Fall flowers (March–May) had a lower individual contribution to overall plant fitness despite high fruit set because of poor removal. Flowers opening during mid- to late spring (October–December) contributed little to fitness because of both poor fruit set and removal.

DISCUSSION

Winter flowering and pollination

Sephanoides galeritus was the most important, if not exclusive, pollinator of *T. corymbosus*, interacting with this mistletoe throughout its flowering phenology. Hummingbirds visited flowers even during heavy

snowfalls (*personal observation*) and are effective pollinators (Smith-Ramírez 1993, Tadey and Aizen 2001). In comparison, queens of *Bombus dahlbomii* visited flowers of *T. corymbosus* less frequently, mostly under relatively benign climatic conditions at either end of the flowering period (Fig. 2), often missing contact with anthers and stigmas (*personal observation*).

The relatively poor pollination of winter flowers strongly suggests that pollination advantages cannot selectively promote the odd flowering phenology exhibited by this mistletoe. According to the pollinator-availability hypothesis (H1), winter flowers of *T. corymbosus* would monopolize the hummingbird's attention in the absence of alternative floral resources, leading to higher pollination and seed set for winter flowers. Overall, I did not find support for this hypothesis. Contrary to expectation, hummingbird visitation was highest during early fall, at the beginning of the flowering period of *T. corymbosus*, and declined strongly in winter with a secondary peak during early spring. Accordingly, flowers opening during early winter (June) and mid- to late spring (October–December) had lower pollination and fruit set than those that opened during early fall (March) or early spring

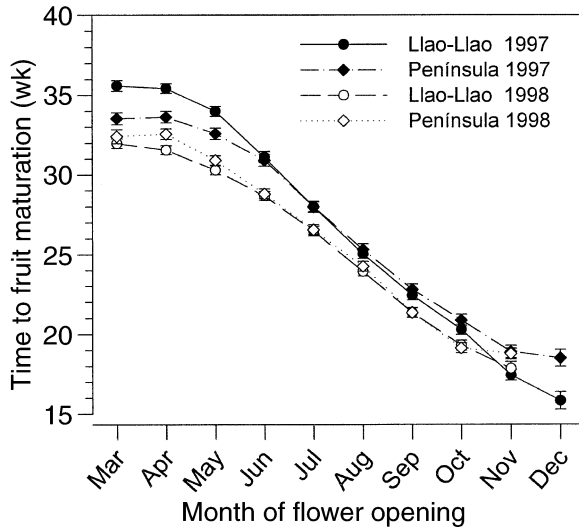


FIG. 5. Period from flower anthesis to fruit maturation vs. month of flower opening (means \pm 1 SE). Observed curves were fitted by the following linear regression equations: $y = 44.6 - 2.44x$ ($r^2 = 0.98$, $n = 10$) for Llao-Llao 1997; $y = 41.2 - 2.08x$ ($r^2 = 0.97$, $n = 10$) for Península 1997; $y = 39.8 - 1.94x$ ($r^2 = 0.98$, $n = 9$) for Llao-Llao 1998; and $y = 39.3 - 1.93x$ ($r^2 = 0.97$, $n = 9$) for Península 1998. March was month 3 of the year. In all instances, $P < 0.0001$.

(September). Reduced fruit set from flowers opening during winter and mid- to late spring could be ascribed in part to limited pollen deposition. Consistent seasonal patterns of variation in pollinator limitation over a species' flowering phenology has been reported for at least two other plant taxa (Ramsey 1995, O'Neil 1999). However, the lack of pollen limitation among winter flowers in the Península population during 1997 and late-spring flowers of the Llao-Llao population during 1998 (Figs. 3 and 4) indicates that the seasonal outcome of this mistletoe-pollinator interaction varies among years and populations.

The observed pattern in pollinator visitation to the flowers of *T. corymbosus* probably reflects seasonal changes of hummingbird abundance at a larger spatial scale, rather than changes in visitation rates associated with variation in flower nectar production (Rathcke 1992). Hummingbird visitation to an unlimited source of artificial nectar over a 4-yr period (Appendix E) exhibited a seasonal trend similar to that found for flowers of *T. corymbosus*. Low winter abundance of hummingbirds may result from partial emigration westwards and northwards and a shift towards a more insectivorous diet while breeding during October–November (C. Smith-Ramírez, *personal communication*).

Although pollination peaked in March–April and again in August–September, in parallel with hummingbird activity, pollination and fruit set did not show the same decreasing trend throughout the flowering period. Two aspects of the reproductive biology of *T. corymbosus* could buffer sexual reproduction against low

rates of hummingbird visitation. First, despite the requirement of hummingbird visits for maximum seed set, this plant exhibits a limited capacity for autonomous self-pollination (about one-third of full seed set; M. A. Aizen, *unpublished data*). Autonomous within-flower self-pollination is a common feature of the breeding system of many winter and early-spring flowering plants (Schemske et al. 1978, Motten 1982, Herrera et al. 2001). Second, particularly for flowers opening during winter, prolonged flower longevity could partially offset dwindling hummingbird visitation rates (cf. Primack 1985, Totland 1994).

Winter temperatures may also affect pollination and fruit set, independent of pollinator activity or other buffering factors. In this study, evidence of environmental stress on plant sexual reproduction can be inferred from the decline in the number of pollen tubes and fruit set in hand-pollinated flowers during one or more winter months (Figs. 3 and 4). Pollen grain germination, tube growth, and fruit development are all susceptible to low temperatures (Primack 1987, Stephenson et al. 1992, Totland 1994). In addition, although flowers of *T. corymbosus* can withstand subzero temperatures, they can be frost-damaged when exposed to rapid temperature fluctuations (*personal observation*).

Winter flowering and seed dispersal

Because of its aerial parasitic lifestyle, seed dispersal is critical for mistletoe survival. Dispersal of mistletoe seeds involves not only the transport of seeds away from the maternal plant, but also their placement on the living branches of an appropriate host. Given these stringent requirements, it is not surprising that mistletoe-disperser interactions are among the most specialized and efficient plant–frugivore interactions known (Reid 1991).

Dromiciops australis is highly efficient in dispersing *T. corymbosus* seeds. Almost all of the seeds it ingests are defecated undamaged, and >80% of them are placed on branches or trunks of host plants. Furthermore, the passage of the seeds through the marsupial's gut is critical for germination and host infection (Amico and Aizen 2000). Therefore, behavior of this mammal could exert strong selection on reproductive traits of *T. corymbosus*, including phenology. In particular, winter flowering should be favored if it enhances seed dispersal by increasing the overlap between fruiting and the activity period of the marsupial; i.e., the disperser-availability hypothesis (H2). I found strong support for this hypothesis.

Fruit removal varied strongly over the 6–7 mo fruiting period of *T. corymbosus*, with fruits ripening during mid- to late summer experiencing the highest removal (Fig. 7). During summer, *D. australis* becomes the dominant arboreal mammal in the forests studied. *Dromiciops australis* is also one of the few mammals in southern South America with confirmed hibernation

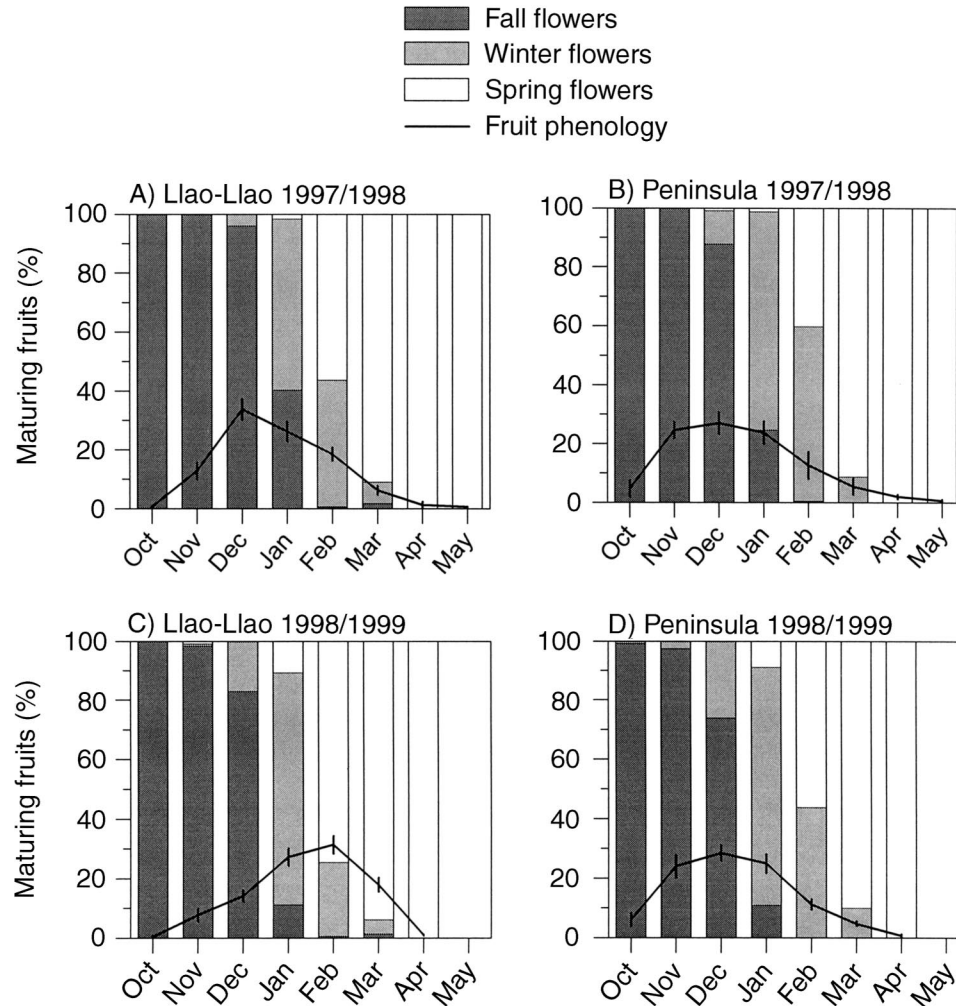


FIG. 6. Percentage of fruits that matured during different months that derived from fall (March–May), winter (June–August), and spring (September–December) flowers at Llao-Llao and Peninsula (bars). Fruit phenology (solid line, means ± 1 SE) is indicated as the percentage of all fruits produced that ripened during a given month (Eq. 1). The 1997/1998 fruiting period corresponds to the 1997 flowering period, whereas the 1998/1999 fruiting period corresponds to the 1998 flowering period.

(Jaksic 1997) and is mostly inactive from late fall to early spring, at least in the Andean part of its range (Kelt 1994, Rau et al. 1995). Amico (2000) captured individuals of *D. australis* beginning in December at Llao-Llao. Abundance and proportion of juveniles increased significantly through summer until March and decreased markedly afterwards. Thus, the summer peak in fruit removal is correlated with the seasonal activity pattern of the marsupial. Also, seasonal patterns of marsupial activity and abundance closely reflect temporal variation in rates of seed deposition on branches of host plants (Amico 2000). An ongoing study assessing spatial variation in mistletoe dispersal across six *T. corymbosus* populations showed that local abundance of *D. australis* is a good predictor ($r^2 > 0.75$) of the rates of mistletoe fruit removal, and fruit removal is a good predictor of both seed deposition on branches

and number of established seedlings per host (M. Rodríguez-Cabal, *unpublished data*). These observations indicate that fruit removal rates satisfactorily depict the outcome of this mistletoe–marsupial dispersal interaction from the plant's perspective.

Even with a strong association between dispersal rates and fruit phenology, the seasonal activity pattern of an efficient disperser can selectively influence the timing of flowering only if flower-to-fruit developmental time is genetically fixed or under weak selection (cf. Endler 1986). Flower-to-fruit time intervals in *T. corymbosus* responded to temperature (see Ratchcke and Lacey 1985, Primack 1987); flowers opening during fall produce fruit that remains unripe through winter, exhibiting a development period twice as long as that observed in spring flowers. In addition, at both study populations fruit development was, on average,

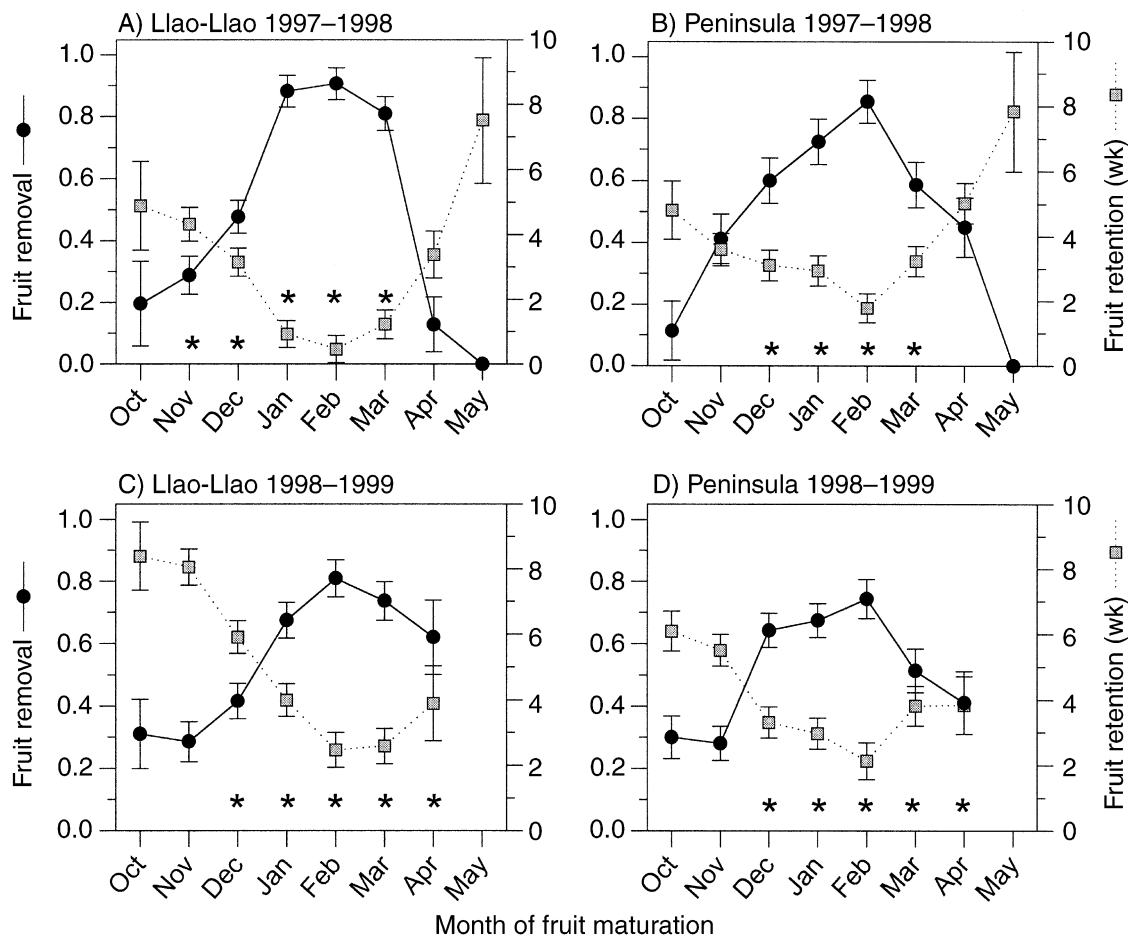


FIG. 7. Proportion of fruits removed (circles) and retention time (squares) of exposed fruits in relation to month of fruit maturation in the Llao-Llao and Península populations during the 1997/1998 (October–May) and 1998/1999 (October–April) fruiting periods (means \pm 1 SE). Stars indicate individual months showing significant differences (paired t test, $P < 0.05$) in retention time between exposed and bagged fruits (not shown) after application of Bonferroni's correction for multiple tests (Rice 1989). See Appendix C for statistical analyses.

1–2 wk shorter for flowers opening during the unusually warm winter of 1998 than during the winter of 1997 (Fig. 5, Appendix E). On the other hand, I found low among-plant variability in mean fruit maturation times ($cvs \sim 5\%$), and a strong coupling between flower and fruit phenology arising because differences in the date of flower anthesis were not offset by reductions in flower-to-fruit maturation times of the same magnitude (Fig. 5). These findings might indicate some developmental canalization and low genetic variation underlying this trait in *T. corymbosus* (cf. Endler 1986).

The unimodal association between removal rate and date of fruit ripening (Fig. 7) translated into an equivalent humped relationship between fruit removal and date of flower anthesis (Fig. 8). Therefore, high dispersal during summer clearly favors winter flowering in *T. corymbosus*. I do not know of comparable studies assessing the consequences of flowering phenology for animal seed dispersal (or fruit removal) within a plant species. Other studies have found that seed predators

may also constitute indirect agents of selection on flowering phenology (English-Loeb and Karban 1992, Brody 1997, Picó and Retana 2000). However, those study species have short periods from flower to seed and, in many instances, eggs of seed-eating larvae are laid directly on the flowers.

Adaptation, constraints, and evolutionary scenarios

This study provides clear evidence that winter flowering enhances plant fitness in *T. corymbosus* principally by increasing the number of removed and eventually dispersed seeds during summer. However, pollination and fruit production also influence variation in the contribution of individual flowers to plant fitness over the flowering season. In particular, high fruit set determines a peak associated with flowers that open during late rather than early winter. This seasonal pattern was similar among years and populations (Fig. 9) suggesting that plant–animal mutualisms exert consistent selection on flowering phenology over space and

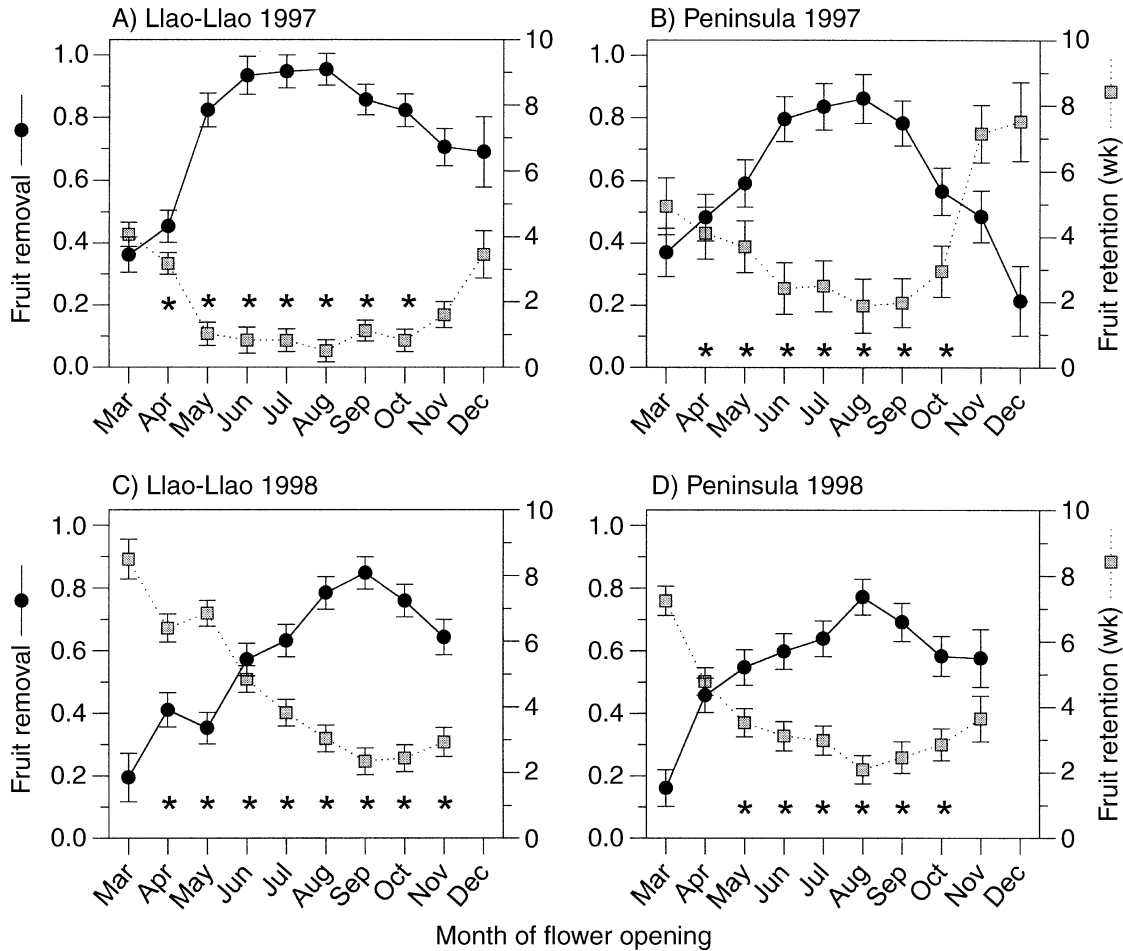


FIG. 8. Proportion of fruits removed (circles) and retention time (squares) of exposed fruits in relation to month of flower opening at Llao-Llao and Península (means \pm 1 SE). Stars indicate individual months showing significant differences (paired t test, $P < 0.05$) in retention time between exposed and bagged fruits (not shown) after application of Bonferroni's correction for multiple tests (Rice 1989). See Appendix D for statistical analyses.

time in *T. corymbosus*, a prerequisite for these interactions to result in evolutionary change (Herrera 1988, Horvitz and Schemske 1990, Willson and Whelan 1993, Waser et al. 1996).

This study does not consider seedling establishment, which can affect the shape of the curves depicted in Fig. 9 (see also Picó and Retana 2000). Seeds of *T. corymbosus* lack dormancy and are very prone to desiccation (*personal observation*; see also Calder [1983], Kuijt [1988]). Germinating immediately after dispersal, they infect the host within 1–2 wk. While a mean of 26% of all effectively dispersed seeds germinate, infect the host, and survive the first year until producing the first true leaves (Amico 2000), the rate of seedling establishment is not seasonally uniform. The proportion of dispersed seeds reaching the seedling stage increases by \sim 20% for fruits ripening during late February and March, at the end of the dry summer season (G. Amico, *unpublished data*). Because these mid- to

late-summer fruits are set mainly by late-winter and spring flowers, seedling establishment is likely to accentuate the contribution of winter flowers to plant fitness.

Results of this study could explain why *T. corymbosus* does not flower during the main course of the summer, despite relatively high abundance of hummingbirds in the forest (Amico 2000; Appendix E). Extrapolating from Fig. 5, I estimated that fruits developing from summer flowers would ripen during winter when dispersal would be limited because the marsupial is inactive. However, peak flowering during fall as recorded in both study populations in 1997 and in Península in 1998 may be somewhat maladaptive since resulting fruit maturation peaks during December, 2 mo before maximum fruit removal (Fig. 7). On the other hand, a higher proportion of flowers opened during August–September in Llao-Llao in 1998, resulting in a summer peak in fruit maturation that overlapped the

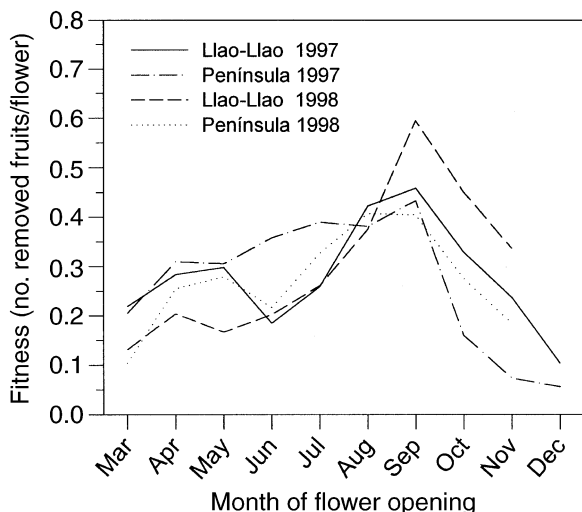


FIG. 9. Variation in per-flower contribution to fitness over the flowering period of *Tristerix corymbosus* (Eq. 2). Seasonal variation in mean per-flower contribution to fitness was statistically significant for the two study populations over the two flowering phenologies: $F_{9,122} = 5.16$, $P < 0.0001$ for Llao-Llao 1997; $F_{9,113} = 5.61$, $P < 0.0001$ for Península 1997; $F_{9,149} = 19.07$, $P < 0.0001$ for Llao-Llao 1998; and $F_{8,120} = 4.41$, $P = 0.0001$ for Península 1998. Standard errors are not shown for the sake of clarity.

period of maximum disperser activity. Therefore, I propose the existence of strong selection for late-winter flowering in *T. corymbosus*.

Why does *T. corymbosus* not start flowering later or concentrate flowering during the last months of winter? At this time, I can only suggest some possible answers. For example, use of temperature as an environmental cue triggering flowering may constrain evolutionary possibilities. If "temperature sum" is the proximate trigger for flowering in this mistletoe (e.g., Diekmann 1996), low winter temperatures would not be as effective in triggering flowering. In addition, the strong, direct influence of temperature on flower longevity and rate of flower opening may produce a flowering peak during fall.

Another possibility is that I underestimated the long-term fitness contribution of fall flowers because the 1997 and 1998 winters were unusually warm. In years with cooler winters, slower fruit development and delayed ripening may increase the removal success for fruits derived from fall flowers. However, the relative invariance observed in the flower-level fitness curves between 1997 and 1998 (Fig. 9), years showing an $\sim 2^{\circ}\text{C}$ difference in mean July temperature, suggests that the overall phenology of fitness is basically unaltered over a wide range of winter temperature.

A third explanation is that fall flowers produced outside the optimum time are of little cost and represent a bet-hedging strategy against complete reproductive failure in very severe winters (see also Picó et al. [2002]). However, this hypothesis cannot explain why

the peak in flower opening occurs more commonly in the fall than in the winter. Lastly, fall-opening flowers could boost the pollination of winter flowers by encouraging ongoing presence of this plant's hummingbird pollinator. However, this should lead to a highly asynchronous (and selectively unstable) onset of flowering because individuals starting flowering much later in the winter could increase their fitness at the expense of early-flowering individuals.

One key question concerns the extent to which flowering time, duration, or intensity can change over evolutionary time (Rathcke and Lacey 1985, Fox and Kelly 1993, Wright and Calderon 1995). Responsiveness to selection on flowering phenology seems likely in the Loranthaceae, since southern South American species show a wide range of flowering phenologies (Hoffman 1982, 1995, Smith-Ramírez 1993, Rivera et al. 1996), suggesting that the family lacks intrinsic limitation on response to selection. The phenological behavior of *T. corymbosus* even varies over its geographical range. Although south-Andean populations do not flower during summer (see Riveros and Smith-Ramírez [1996]), Chilean populations at a similar latitude near the Pacific coast flower year-around (Smith-Ramírez 1993). This difference in phenological behavior can be consistent with the disperser-availability hypothesis if marsupial dispersers remain active during the milder coastal winter. Comparative studies on the relationship between flowering phenology and seed dispersal in *T. corymbosus* among these contrasting geographical localities should be enlightening (see also Aizen et al. [2002]).

This study supports the hypothesis that efficient animal seed dispersers can become important agents of natural selection on flowering phenologies of temperate plant species. Flowers may open during suboptimal periods for pollination if this flowering behavior increases seed dispersal. However, the proximate effect of temperature and other environmental factors on flower and fruit development might curtail fine adjustment between flowering phenology and the seasonal activity of animal mutualists.

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APPENDIX A

A table presenting results of ANOVA testing of the effects of supplemental hand- vs. open pollination (treatment) and month of flower opening on the mean number of pollen tubes per style and fruit set is available in ESA's Electronic Data Archive: *Ecological Archives* E084-068-A1.

APPENDIX B

A table presenting the number (means \pm 1 SE) of pollen tubes and fruit set of open- and hand-pollinated flowers and fruit retention time of exposed vs. bagged fruits is available in ESA's Electronic Data Archive: *Ecological Archives* E084-068-A2.

APPENDIX C

A table presenting results of ANOVA testing of the effects of month of fruit maturation on the proportion of fruits removed and bagging treatment (i.e., exposed vs. bagged fruits) and month of flower opening on fruit retention is available in ESA's Electronic Data Archive: *Ecological Archives* E084-068-A3.

APPENDIX D

A table presenting results of ANOVA testing of the effects of month of flower opening on the proportion of fruits that were removed by animals and bagging treatment (i.e., exposed vs. bagged fruits) and month of flower opening on fruit retention time is available in ESA's Electronic Data Archive: *Ecological Archives* E084-068-A4.

APPENDIX E

A figure depicting seasonal changes in hummingbird visitation to an artificial feeder and weather over a 4-yr period is available in ESA's Electronic Data Archive: *Ecological Archives* E084-068-A5.