Breeding system of *Embothrium coccineum* (Proteaceae) in two populations on different slopes of the Andes

Sistema reproductivo de *Embothrium coccineum* (Proteaceae) en dos poblaciones ubicadas en diferentes laderas de los Andes

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ABSTRACT

Plant breeding systems are considered to reflect species’ life history characteristics, selection due to biotic or abiotic factors, pollination conditions, or a combination of these. Reproductive systems may vary over ecological gradients. The breeding system of the ornithophilous *Embothrium coccineum* (Proteaceae) from temperate South America was studied by pollination treatments: manual self-pollination, manual cross-pollination, automatic self-pollination, and natural pollination. These treatments were conducted in a coastal western and an Andean eastern population. *Embothrium coccineum* was found to be self-incompatible and highly dependent on the pollinating agent at both sites. However, pollen limitations were greater in the coastal population, as breeding efficiency was lower. Populations have different floral visitors whose identity differentially affects reproductive efficiency and pollen flow in *E. coccineum*.

Key words: self-incompatibility, endemic species, temperate forest, Chile, Argentina.

RESUMEN

Los sistemas de compatibilidad reproductiva en las plantas son considerados una manifestación de la historia de vida, de la selección ante factores abióticos o bióticos, de las condiciones de polinización o una de combinación de esos factores. El sistema reproductivo de una especie puede variar a lo largo de un gradiente ecológico-ambiental. El sistema reproductivo de una especie puede variar a lo largo de un gradiente ecológico-ambiental. El sistema reproductivo de *Embothrium coccineum* (Proteaceae), un árbol ornitófilo endémico de los bosques templados de sur de Sudamérica, fue estudiado mediante experimentos de polinización: autopolinización manual, polinización manual cruzada, autopolinización automática y polinización natural en flores descubiertas. Este trabajo se realizó en una población costera al oeste de la cordillera de los Andes y una población andina ubicada al este de la cordillera de los Andes. En ambas poblaciones se encontró que *E. coccineum* es autoincompatible y altamente dependiente de los agentes polinizadores en ambos sitios. Sin embargo, la limitación por polen fue mayor en la población costera, donde la eficiencia reproductiva fue menor. Las poblaciones tienen diferentes visitantes florales cuya identidad afecta diferencialmente la eficiencia reproductiva y el flujo de polen en *E. coccineum*.

Palabras clave: rango geográfico, ensamble de polinizadores, eficiencia reproductiva, Chile, Argentina.

INTRODUCTION

Plant breeding systems include all events that operate to influence pollination and fertilisation as well morphological adaptations of floral structures and the temporal and spatial distribution of flowers on plants (Wyatt 1983, Brown 1990, Goldingay et al. 1998). Breeding compatibility systems can reflect species’ life history characteristics, natural selection regimes due to abiotic and/or biotic factors, pollination conditions, or a combination of these (Arroyo & Squeo 1990, Arroyo & Uslar 1993). As a result, the breeding system may vary over the geographical range of a given species (Dafni 1992). Therefore, knowledge of
the breeding system is particularly important in species of horticultural value inhabiting contrasting locations (Kearn & Inouye 1993). Field-based experiments on plant breeding systems are essential to estimate the extent of pollinator dependence for fruit and seed production, and to understand patterns of gene flow among populations (Bawa 1974, 1979, Dafni 1992).

Temperate forests from austral South America consist of an endemic flora mostly pollinated by biotic vectors (> 75 %) (Aizen & Ezcurra 1998, Aizen et al. 2002), yet investigations on reproductive systems of woody species are few (Arroyo & Uslar 1993, Humaña & Riveros 1994, Riveros et al. 1996, Arroyo & Humaña 1999). Furthermore, intraspecific variation of plant breeding systems along geographic ranges is limited (Arroyo & Squeo 1990). One of the species that has been the subject of breeding system studies in Chile is the monotypic *Embothrium coccineum* J.R. et G. Forster (Proteaceae) common name “notro” (Correa 1984). *E. coccineum* was assessed as self-incompatible in a mountain population at Puyehue (41° S, 72° W) (Riveros 1991) and also further south at 50° S (Arroyo & Squeo 1990). However, it was recorded as self-compatible at sea level in Valdivia (39° S, 73° W) and in low elevation areas of Puyehue (Riveros et al. 1996, M. Riveros personal communication).

Pollinators of *E. coccineum* also vary according to the location. In the agricultural landscape of Chiloé Island in southern Chile, two bird species, the passerine flycatcher *Elaenia albiceps* (Tyrannidae) and the hummingbird *Sephanoides sephaniodes* (Trichilidae) are the main pollinators (Smith-Ramírez & Armesto 1998). While these two species are found in Argentina, only the latter was recorded as pollinator of *E. coccineum* (Aizen et al. 2002). In this study, we investigate variation of the breeding system of *E. coccineum* at two locations on different slopes of the Andes. These two sites are at approximately equal latitudes but represent different environmental settings. We test the hypothesis that the breeding system and therefore reproductive efficiency of *E. coccineum* are influenced by the identity of the main pollinator and isolation mechanisms between studied locations. We aim to study fruit and seed production after different pollination treatments, breeding efficiency, and pollinator diversity in each population.

**MATERIAL AND METHODS**

**Study area**

This study was carried out on two populations within the Valdivian district of the Sub-Antarctic province (Dimitri 1977) on different slopes of the Andes. These two locations although they are at similar latitudes present different characteristics. One is a coastal population located at less than 50 m of altitude in northwest Chiloé Island in southern Chile (41° 53’ S, 73° 40’ W). It has a strong oceanic influence with 2,245 mm average annual rainfall. Mean annual temperature in Chiloé is 9.5 °C with maximum monthly temperatures of c. 20 °C during February (mid summer), and minimum temperatures of 3 °C during July and August (winter) (Di Castri & Hajek 1976). The other is a mountain population at 790 m of altitude in Puerto Blest, north-western Patagonia, Argentina (41° 01’ S, 71° 49’ W). Puerto Blest has 3,600 mm average annual rainfall and mean temperature is 9 °C. Maximum monthly temperatures are 14-16 °C in January, and average minimum temperatures are 2-4 °C in July and August (Dimitri 1972, Barros et al. 1983). The climate is temperate humid, but more continental than in Chiloé. In Chiloé *E. coccineum* typically occurs in agricultural landscapes where is restricted to forest edges. Forest remnants include emergent *Nothofagus nitida*, *N. dombeyi*, and *Eucryphia cordifolia* (Eucryphiaceae) and a lower canopy cover by *Amomyrtus meli*, *A. luma* (Myrtaceae), *Drimys winteri* (Winteraceae), and *Caldcluvia paniculata* (Cunoniaceae) (Díaz et al.1999). The mountain population at in Puerto Blest is a highly pristine forest within Nahuel Huapi National Park. *Embothrium coccineum* is found in open areas, and at forest and road edges. The forest is dominated by *Nothofagus dombeyi* (Nothofagaceae), *Saxegothaea conspicua* (Podocarpaceae), *Luma apiculata* (Myrtaceae), *Drimys winteri* (Winteraceae) and *Lomatia hirsuta* (Proteaceae) (Brion et al. 1988).

In agricultural landscapes of Chiloé Island main pollinators of *E. coccineum* are the
hummingbird *Sephanoides sephaniodes* and the passerine flycatcher *Elaenia albiceps* (Smith-Ramírez & Armesto 1998). Both are also present in Puerto Blest.

**The species**

*Embothrium coccineum* is a tree endemic to temperate forests of Argentina and Chile. It has a wide latitudinal range in Chile (35-55° S) and in Argentina (39-55° S) (Correa 1984, Romero et al. 1987). It is found from sea level to 1200 m of altitude. Flowers of *E. coccineum* are tubular, red and hermaphrodite (Smith-Ramírez 1993). They consist of four reflex tepals, each one with a sessile stamen in its apical concavity. The ovary has a column or basal stipe (Dimitri 1977). The flowers are protandrous (Humana & Riveros 1994), releasing pollen from the anthers before the stigma becomes receptive (Dafni 1992).

*Embothrium coccineum*, as most members of the Proteaceae, dispenses pollen not from the anthers, but from a specialised part of the gynoecium known as pollen presenter (Ladd 1994) which enhances cross-pollination. As a result, most taxa are strongly protandrous (Ladd et al. 1998). This reproductive mechanism within Proteaceae prevents autogamy increasing self-incompatibility efficiency.

Lifespan of *E. coccineum* flowers is at most four days. During this time they secrete large quantities of nectar (Smith-Ramírez & Armesto 1998). Flowering phenology *E. coccineum* varies over its geographical range. In Chiloé it flowers from September to the end of December (Smith-Ramírez & Armesto 1994), whereas in the Andean population at Puerto Blest, only flowers, from the end of October to the end of December (Brion et al. 1988).

**Sample scheme**

Sampling was conducted between October 2001 and March 2002 at the Senda Darwin Biological Station in Northwest Chiloé, and between November 2002 and March 2003 at Puerto Blest in Nahuel Huapi National Park. Ten breeding *E. coccineum* trees with low branches and plentiful flowers were selected at each location. At the beginning of the flowering season, 60 flower buds on each tree were isolated in fine mesh bags. Each treatment was performed on 15 buds per tree and therefore on a total of 150 buds. Pollination experiments followed protocols according to Dafni (1992). These included: manual self-pollination (SP), manual cross-pollination (CP), spontaneous or automatic self-pollination (AP), and natural pollination (NP). Treatments differed in their pollen-donor source except for spontaneous or automatic self-pollination (AP) and natural pollination (NP). Under manual self-pollination (SP), pollen from a different flower on the same individual was used. In natural pollination (NP) where flower buds were simply labelled, and flowers were permanently left exposed to their natural pollinators. For manual cross-pollination (CP), pollen from various individual donors located more than 500 m away from the receptor individual was used. For the automatic self-pollination (AP), flower buds were covered with bags in order to assess the species’ capacity to self-pollinate spontaneously or automatically. In all cases, pollinated flowers remained in bags until fruits ripened and seeds were released.

Manual pollination treatments (SP and CP) were performed when buds opened. Pollen removal was performed before pollination. Pollination was conducted every day during the four days of the flower’s life, during which the stigmas were completely covered with their own or foreign pollen, respectively, according to the treatment. The pollen used for the treatments was gathered from recently opened flowers. Agamospermy was excluded because rarely occurs within Proteaceae (Lamont et al. 1998). In addition, emasculation treatments are destructive particularly in species with pollen presenter such *E. coccineum*. Therefore negative results from those experiments may not necessarily indicate absence of apomixis (M. Aizen personal communication).

The breeding system was assessed by comparing fruiting percentages (number of fruits per number of marked flowers x 100) under the different pollination treatments. An index of self-incompatibility (ISI) was calculated (Ruiz & Arroyo 1978). This index is also known as self-compatibility index (SCI). It is calculated as the ratio between the percentages of fruits produced from manual self-pollination and those from cross
pollination experiments (%SP / %CP) (Ruiz & Arroyo 1978). Species with ratios < 0.2 are considered self-incompatible while higher values indicate that the plant is self-compatible.

Breeding efficiency was evaluated as the ratio between the percentage of fruiting from natural pollination (uncovered flowers) to manual cross pollination (%NP / %CP). This indicates the capacity of the plant to produce fruits under natural conditions (Ruiz & Arroyo 1978). Breeding success for each treatment was measured by the percentage of fruits and also by the number of seeds per ripe fruit (Dafni 1992, Burd 1994). Differences between treatments and locations were assessed by non-parametric Mann-Whitney and Kruskal-Wallis tests because data did not fit to normal distribution.

Pollinator diversity at each population was evaluated by observations of pollinators visiting flowers of *Embothrium coccineum* trees conducted at the peak of the flowering season. At Puerto Blest we selected groups of 2-4 individual trees growing contiguously. Total visits by birds and/or insects were recorded during 50 periods of 15-min. This information was compared to data gathered in Chiloé Island by a similar study (Smith-Ramírez & Armesto 2003).

RESULTS

Pollination experiments

At the Chiloé population, pollination treatments yielded significantly different fruiting percentages (Kruskal-Wallis test, H =19, P < 0.0001, and subsequent Student-Newman-Keuls test, P < 0.05). Cross pollination (CP) treatment produced the greatest number of fruits per flower, the free pollination treatment (NP) produced intermediate fruiting values, and the manual SP treatment produced a reduced number of fruits. In contrast, the spontaneous AP treatment produced no fruit (Table 1). *Embothrium coccineum* breeding success, measured as the number of seeds per fruit, was only assessed for the manual CP and NP treatments due to the small quantity of fruits produced by manual SP at this location. The manual CP treatment yielded significantly greater number of seeds per fruit and thus higher breeding success than the open CP (Mann-Whitney U-test, U = 641, P = 0.049). The self-incompatibility index for the Chiloé population was 0.035 and the efficiency of natural pollination was 0.464.

At the Puerto Blest site, fruit production differed among pollination treatments. Although fruiting percentages after NP and CP were similar (Mann-Whitney, U-test, U = 117, P = 0.384), no fruit developed from the spontaneous or self-pollination ones (Table 2). Breeding success estimated as the number of seeds per fruit, yielded no significant differences for the CP and NP treatments (Mann-Whitney U-test, U = 140, P = 0.622). Absence of fruits from the manual SP treatment resulted in a self-incompatibility index for this site of zero while the efficiency of NP for *E. coccineum* at Puerto Blest was 0.648.

The between-population comparison indicated that fruiting percentages of uncovered flowers from NP were twice as great in Chiloé (17.3 %) than in Puerto Blest (8.0 %) (Mann-Whitney U-test, U = 76, P = 0.027). Fruit production from manual CP was also significantly greater in Chiloé (37.3 %) than in Puerto Blest (12.3 %) (Mann-Whitney U-test, U = 69, P = 0.007). The breeding success as the mean number of seeds per fruit was greater for those obtained through CP on the coastal Chiloé population, but no significant difference was found in the number of seeds produced by the different treatments in the Puerto Blest area. In both locations self-incompatibility index were < 0.2. However, the average value for breeding efficiency was lower in Chiloé (0.464) than in Puerto Blest (0.648).

Pollinator diversity

In Chiloé, the main nectarivorous species is the Passerine *Elaenia albiceps* which visited 76.6 % of the flowers. The other pollinators are the Trochilidae *Sephanoides sephaniodes* (11.8 %), the Passeriformes *Carduelis barbatus* (Emberizidae) (5.6 %), *Phrygilus patagonicus* (Fringillidae) (5 %), and another five bird species in addition to the Hymenoptera *Diphaglossa gayi*, with less than 1 % visits each of them. In Puerto Blest, the main floral pollinators are the Trochilidae *Sephanoides sephaniodes*, which accounts for 86 % of the visits and Hymenoptera species *Bombus dahlbomii* (14 %).
BREEDING SYSTEM OF *EMBOTHRIUM COCCINEUM*

**DISCUSSION**

Our results indicate that *E. coccineum* is self-incompatible and therefore is highly dependent on pollination agents for sexual reproduction. The absence of fruits in the spontaneous pollination treatments shows that *E. coccineum* is unable to self-fertilise in absence of its pollinators. Manual self-pollination produced only two fruits, reflecting the high degree of incompatibility with pollen from the same plant. Therefore the species was found to be allogamous. Other woody species of the temperate forest of South America such as *Eucryphia cordifolia* (Eucryphiaceae), *Luma apiculata* (Myrtaceae) and *Drimys winteri* (Winteraceae) have also shown high self-incompatibility percentages (Riveros et al. 1996).

In this study we found no difference in the breeding system between the two geographical areas assessed. This result agrees with previous studies on *E. coccineum* on two locations east of the Puerto Blest population at 790 m of altitude (M. Aizen personal communication), and at a high-elevation population in Puyehue on the western slopes of the Andes (Riveros 1991). In contrast, two other populations on the western slopes, one at sea level in Valdivia and the other in low elevation areas (600 m of altitude) of Puyehue National Park, have been recorded as highly self-compatible (Riveros et
Nevertheless, we did find differences in the breeding efficiency of Embothrium between environments. The results show that fruit-forming capacity differs, and the Andean population at Puerto Blest is more efficient than the coastal population at Chiloé. Species richness of pollinators is often reduced in fragmented habitats relative to undisturbed continuous areas. Additionally species composition, abundance, and behaviour of pollinators are also affected by the degree of modification of the environment. Smith-Ramírez and Armesto (2003) found that pollinator visiting rates of Elaenia albiceps on E. coccineum were negatively correlated with forest patch area and were highest in isolated trees surrounded by pastures within fragmented landscapes in Chiloé.

Additionally, fruit percentage by natural pollination decrease along a West - East gradient. It varies from 17 % at the western most population in Chiloé (this work), 8 % at Blest (this work), 3.5 % at Cerro Otto (Aizen & Feinsinger 2003), and 1.5 % at the eastern most location near Bariloche City (Aizen & Feinsinger 2003). We believe that this is due both to intrinsic factors such as nectar composition, and to extrinsic factors, including the pollen vector agents in these environments. Main flower pollinators of E. coccineum differ between the coastal Chiloé and Puerto Blest (Fraga et al. 1997; Smith-Ramírez & Armesto 1998, this work). In Chiloé up to ten bird species were observed, whereas only two were recorded for the Puerto Blest area. About invertebrates, in both sites invertebrate-pollinated plants interact with an unexpectedly diverse assemblage of insect pollinators. There are similar number of pollinators in Chiloé (110 species) and Nahuel Huapi National Park (131 species). Species in common are Bombus dahlbomii and Cadeguala albopilosa. In contrast, Apis mellifera is more often found in Chiloé while Bombus ruderatus does so in Nahuel Huapi National Park (Aizen et al. 2003). Previous studies have shown that nectar composition of E. coccineum also differs between populations on each side of the Andes. In Chiloé, nectar is rich in hexose-type sugars as glucose and fructose (Smith-Ramírez & Armesto 1998), whereas in Puerto Blest it is rich in sucrose (Chalcoff 2001, M. Aizen & L. Galetto unpublished data). This different chemical composition of the nectar may influence visitor assemblages. In particular, the concentration of sucrose vs. hexose in the nectar of E. coccineum flowers seems to be directly related to the predominant type of pollinators present in each population. The flowers that produce nectar with greater sucrose content are visited mainly by Trochilidae as the Puerto Blest population, while those that produce hexose rich nectar are mainly pollinated by birds of the Order Passeriformes or insects (Smith-Ramírez & Armesto 1998) as the Chiloé population.

Study populations not only differed in the number of species that make up each floral pollinator assemblage, but also in pollinator behaviour. The main visitor in coastal Chiloé is the heavy bird E. albiceps, which is less mobile and pollinates few flowers at each visit (Smith-Ramírez & Armesto 2003). This may result in a pollen exchange among only a few nearby individuals. In contrast, S. sephaniodes, the main visitor at the Puerto Blest Andean population, is smaller and more agile in flight, which may visit several E. coccineum individuals. This difference in the main pollen-vector’s activity could directly affect pollen movement and therefore gene flow among E. coccineum individuals. Low pollinator mobility among plants may bring about high intra-individual pollen transfer, or transfer among few nearby individuals. Conversely, high mobility may favour greater pollen exchange among individuals within the population. In the case of E. coccineum, pollinators may have an effect on the probability of exogamy (i.e. the quality of pollen transferred between stigmas) and hence on the genetic makeup of the progeny. Preliminary results from a genetic study using isozymes suggest greater levels of within-population variation in Andean than extra-Andean (including coastal) populations of E. coccineum (C. Souto personal communication). This included increase heterozygosity, polymorphism, and number of alleles/locus in eastern populations which may reflect elevated gene flow at these populations as a result of pollinator activity. In fragmented landscapes of Chiloé, Mathiasen (2004) recorded greater overall genetic diversity in adults than progeny. Also moderate and significant genetic divergence exists among
adults and seedlings. Hence fragmentation may lessen genetic diversity while intensifying isolation in forthcoming generations. These results highlight the importance to conserve remnants of native forest in southern Chile to preserve genetic diversity and promote outcrossing (Mathiasen 2004). Limited gene flow among fragments is reinforced by reduced seed dispersal distances of *E. coccineum* (< 20 m) (Rovere & Premoli 2005).

This study shows that *E. coccineum* is predominantly self-incompatible and that pollinating agents are essential to its reproduction. We believe that differences in *E. coccineum* breeding efficiency on either side of the Andes Mountains is due not only to diversity of floral visitors, but also to their identity. We consider visitor identity to be important because of the morphological and behavioural differences between pollinators. Further studies should assess the specific efficiency of each pollinator experimentally.

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LITERATURE CITED


