Revta brasil. Bot., São Paulo, V.23, n.4, p.395-400, dez. 2000

Late-acting self-incompatibility in *Capparis retusa* (Capparaceae), a species of Chaco woodland in NE Argentina

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(received: December 8, 1999; accepted: July 12, 2000)

ABSTRACT - (Late-acting self-incompatibility in *Capparis retusa*, a species of the Chaco woodland in NE Argentina). The reproductive biology of *Capparis retusa* was studied by means of controlled pollination experiments and fluorescence microscopy observations of post-pollination events. Self-pollinated flowers mostly failed to form fruits despite the fact that self-pollen tubes grew to the ovary and penetrated ovules within 24 h. Since embryo development to globular stage was observed in some self-fertilized ovules it was concluded that control of self-fertilization in this species occurs by some kind of post-zygotic mechanism.

RESUMO - (Auto-incompatibilidade de ação tardia em *Capparis retusa*, uma espécie da mata chaquenha do NE da Argentina). A biologia reprodutiva da espécie *Capparis retusa* foi estudada por polinizações manuais e estudos de microscopia de fluorescência dos acontecimentos pós-polinização no pistilo. Flores auto-polinizadas, em sua maioria, não formaram frutos apesar do crescimento dos tubos até o ovário e penetração nos óvulos dentro de 24 h. Visto que também o desenvolvimento de embriões até a fase globular foi observado em alguns óvulos, concluiu-se que o controle de autofecundação nesta espécie atua por meio de algum tipo de mecanismo pós-zigótico.

Key words - Breeding system, ovarian sterility

Introduction

Chaco is the name applied to the vegetation covering the vast plains of north-central Argentina, W Paraguay and SE Bolivia between latitudes 15-35°. In general the Chaco is divided into an eastern sector, with a more humid climate, and a western, drier sector, whilst the submeridional lowlands include a large depression between these two areas (Lewis 1991). Forest and savanna vegetation occupy the E - W sectors whilst the submeridional lowlands are dominated by *Spartina argentinensis* grasslands. To our knowledge, there are relatively very few studies on the breeding systems of species of Chaco woodland (e.g. Aizen & Feinsinger 1994, Gibbs & Bianchi 1999 and Bianchi et al. 2000).

The genus *Capparis* (Capparaceae), with some 250 species (Mabberly 1989) of shrubs and trees, is a very characteristic evergreen component of dry neotropical forests (Gentry 1995). Prado (1991) listed five *Capparis* species for the Chaco: *C. atamisquea* O. Kuntze., *C. retusa* Grisenb., *C. salicifolia* Grisenb., *C. speciosa* Grisenb. and *C. tweediana* Eichl. Breeding system data is available for five species of this genus: an early report of apomixis in *Capparis frondosa* (Mauritzon 1935) is the source of citations of apomixis for the '*Capparis* spp.' in Frankel & Galun (1977) and Richards (1986). Ruiz & Arroyo (1978), in studies in Venezuela, reported *C. flexuosa* as self-compatible and *C. verrucosa* as self-incompatible (SI), and the latter species was also reported as SI in Mexico by Bullock (1985). Likewise, *C. pittieri* from Costa Rica (Bawa et al. 1985) and *C. atamisquea* (treated as *Atamisquea emarginata* Miers) from Argentina (Aizen & Feinsinger 1994) were also reported as SI. *C. pittieri* was the only species which was also studied for pollen tube growth following hand pollinations, and self pollen tubes were observed to grow to the ovary (Bawa et al. 1985). Here we report on controlled pollination studies with *C. retusa*. Our objectives were to determine the breeding system of this species, and if self-incompatibility was indicated, to ascertain whether self tubes grow to the ovary.

Materials and methods

Field studies were carried out in 1994 and 1997 in an area of Chaco woodland at Las Gamas, near Vera (29°30'S and

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60°10'W), Santa Fe province. This locality is in an area within the Eastern Chaco known as the Santa Fe Wedge which is situated between the submeridional lowlands and the River Paraná (Lewis 1991) with a 'Quebrachal' woodland (Prado 1993) characterized by various mimosoid legumes (*Acacia* and *Prosopis* spp.) together with *Aspidosperma quebracho-blanco*, *Capparis retusa*, *Geoffroea decorticans*, *Myrcianthes cisplatensis*, *Schinopsis balansae*, *Tabebuia nodosa*, *Zizyphus mistol*, amongst other tree species. Despite repeated flooding episodes in this woodland each summer, cacti and bromeliads predominate in the understorey, the latter sometimes forming impenetrable spiny-leaved thickets.

Trees of the studied species, *Capparis retusa*, were common in our area but mostly did not exceed 6 m and some shrubby individuals intergraded with the understorey stratum. Flowering was of the 'cornucopia' type (Gentry 1974) over a three to four week period from early-December to early-January. Flowers are of the 'brush' type with four short, around 11 mm petals and many (over 40) stamens with long, 30 mm filaments, and with the ca 20-ovuled pistil borne on a slender gynophore such that the sessile stigma is more or less level with the anthers (figure 1).

Clusters of mature flower buds on a sample of 14 trees were enclosed in paper bags during the late afternoon and hand pollinations were effected between 21.00-22.00 h. Flowers were selfed with pollen from the same flower or other flowers of the same plant. For crosses, flowers were emasculated by removing the undehisced anthers and pollen from trees some 10 m distant was used. Pollinated flowers were tagged with labelled cotton thread and the bags were replaced for 24 h so as to exclude the possibility of contaminant pollination by diurnal visitors. Some flowers were left bagged with no further manipulation to check for automatic selfing. A sample of untreated flowers was tagged to assess fruiting success from natural pollination. Fruit-set was censused after 25 days.

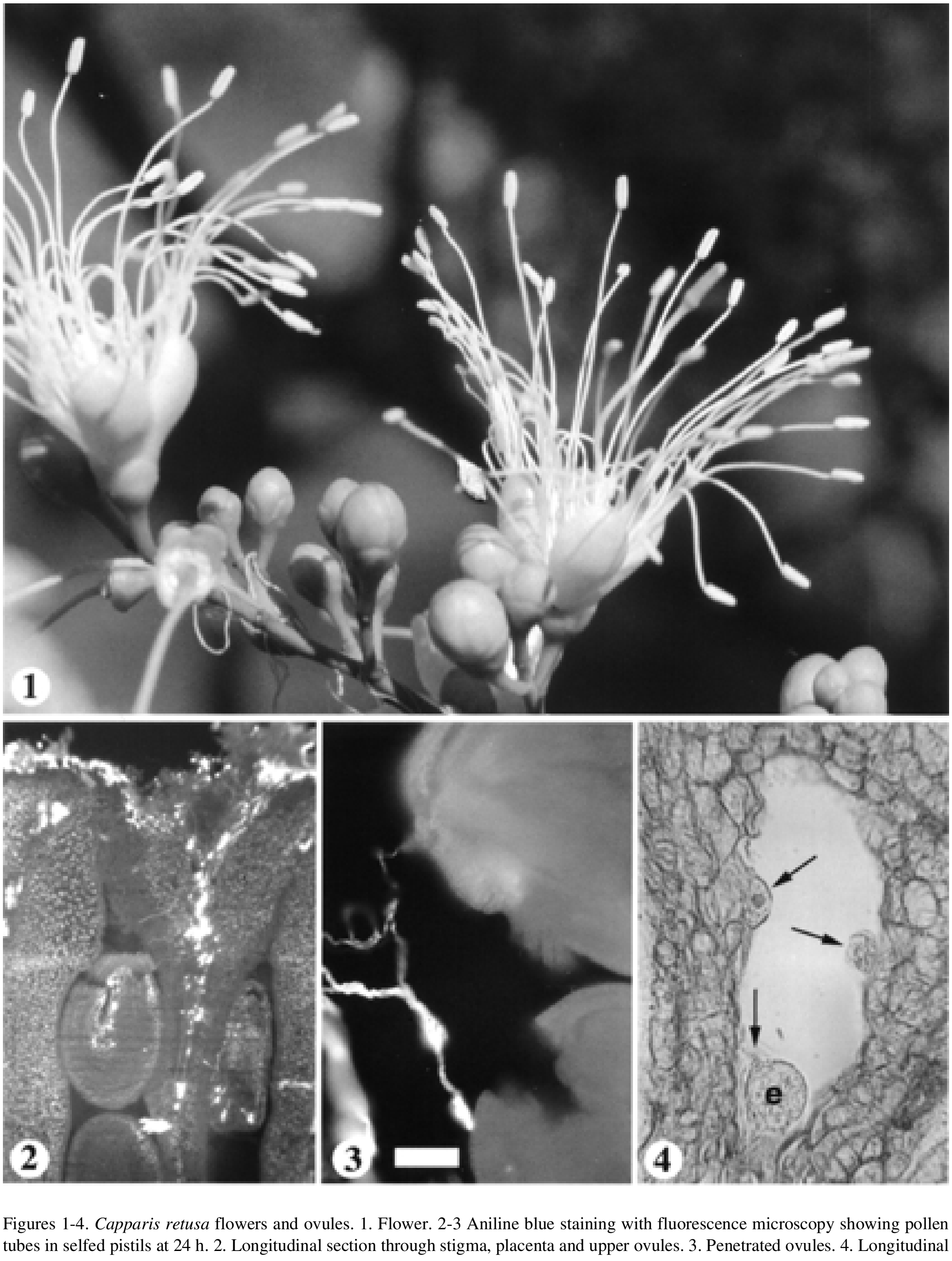
A subsample of hand-pollinated pistils, together with some buds at anthesis, were fixed at 24-72 h and also 7 days post pollination in FAA (ethanol 70% - glacial acetic acid - formaldehyde 9:1:1). Pollinated pistils were studied for pollen tube growth using leucoaniline blue staining and fluorescence microscopy (Martin 1959). Squash preparations were made by pre-softening pistils with 8M NaOH at 60 °C for 10 min, teasing out ovules from the dissected ovary in a droplet of stain, and then checking for pollen tube 'tails' at the micropyle. Other pistils were embedded in wax following tertiary butanol (2-methylpropan-2-ol) dehydration - infiltration (Johansen 1940), sectioned at 14 µm, and stained with aniline blue or alcian blue and safranin (Sass 1955). For some pistils at anthesis, and also some selfed and crossed pistils fixed at 24-96 h post-pollination, ovule size was measured using a microscope with an ocular micrometer.

Results

Anthesis is nocturnal and the flowers were visited by sphingid moths. Flowers began to open at dusk (ca. 18.00 h), initially signalled by a slight relaxation of the petals to create an opening at the bud apex through which first the stigma, and subsequently the entire pistil were exserted, with the convoluted gynophore and stamens still enclosed within the petals. Over a period of an hour or so the petals folded back and the mass of stamens together with the gynophore became fully turgid and erect. Since the stigma was receptive to pollen (tested by hand pollinations) from the time of initial exsertion, whereas the anthers did not begin to dehisce until the filaments were fully expanded, flowers of *C. retusa* are protogynous, although flower visitation was not observed to occur until flowers were fully opened. Flowers have a one night longevity and by mid-morning of the following day the petals and stamens have wilted. Longitudinal sections through the pistil show that the 'dry-type' (Heslop-Harrison & Shivanna 1977) non- papillate stigmatic 'cushion' is located directly on the pistil with only some 0.5 mm of transmitting tissue separating it from the first ovule (figure 2).

Results of fruit set from hand, control and natural pollinations are given in table 1. Most trees set no fruits from selfed flowers, and the ISI = 0.08 (index of self-incompatibility: ratio of selfed to crossed fruit set, cf. Bawa 1974, Bullock 1985) indicates a self-incompatible species. Most selfed pistils abscissed by 8-9 d. However, five trees produced some selfed fruits and one in particular, tree no. 4, produced 22% selfed fruits from manual pollinations and this was also the only tree to produce some selfed fruits by automatic selfing (25%). Selfed fruits which survived to census were not obviously malformed but contained significantly (Student t test) fewer seeds than cross fruits (table 1).

Selfed pistils at 24 h post-pollination had prolific growth of pollen tubes into the central placenta of the ovary (figure 2), as with crosses, and preparations of ovules from dissected selfed pistils at 24 h showed many ovule penetrations (figure 3). Sections of selfed pistils at 7 days showed some ovules with a globular stage embryo and endosperm nuclei (figure 4), but such observations were infrequent with the limited fixed material available and it was not possible to make quantitative studies. Consequently, it is not known whether fertilization and initial embryo and endosperm formation occurred in most or all selfed ovules. Ovule size in selfed pistils increased over 48-96 h (table 2), but this increase in size lagged significantly behind that of crossed pistils over this time interval (ANOVA with Scheffé pairwise comparison).



Figures 1-4. *Capparis retusa* flowers and ovules. 1. Flower. 2-3 Aniline blue staining with fluorescence microscopy showing pollen tubes in selfed pistils at 24 h. 2. Longitudinal section through stigma, placenta and upper ovules. 3. Penetrated ovules. 4. Longitudinal section through embryo-sac of selfed ovule at 7 d showing embryo (e) and endosperm nuclei (arrowed). Scale bar: (1) = 5 mm, (2) = 160 µm, (3) = 50 µm, (4) = 25 µm.

Table 1. Fruit- and seed-set from controlled and natural pollinations in *Capparis retusa*.

	Selfs	Automatic selfs	Crosses	Natural
Fruit-set (fruit/flowers)	9/274 (3.3%)[a]	2/50 (4.0%)[b]	67/164 (40.8%)[c]	53/245 (21.6%)[d]
Mean seed-set (range)	1.4 (1-3)	1.5 (1-2)	6 (1-12)	3 (1-7)

[a]n = 13 trees, five of which produced some selfed fruits, with plant no. 4 having the largest percentage of fruits (2/9:22%); [b]n = 5, all fruits occurred on plant no. 4; [c]n = 11; [d]n = 8. Mean seed-set from hand-crosses was significantly different from selfs (t = 8.1, p = 0.001) and natural pollination (t = 7.3, p = 0.001), but not between hand selfs and automatic selfs (t = 0.14).

Tabela 2. Ovule length (µm) following cross- and self-pollinations in *Capparis retusa* (mean/standard error).

	Non-pollinated	Cross-pollinated	Self pollinated
Anthesis	401.5 (2.22)[a]		
48 h	-	584.4 (5.27)[b]	
72 h	-	-	445.8 (5.80)[c]
96 h	-	583.8 (5.95)[b]	479.0 (6.92)[d]

Values with different letters are significantly different at p = 0.05.

Discussion

The results of the hand pollination experiments indicate that *Capparis retusa* is apparently self-incompatible, although with some 'leakiness' in the SI mechanism since five of 13 trees produced some selfed fruits. Moreover, tree no. 4 produced 22% and 25% fruits from manual and automatic selfing respectively, whilst mean self fruit-set in the other four trees was 4.4%, indicating some variability in selfing ability between individuals in the population. Variable expression of selfing ability, as found in *C. retusa*, was also observed in *C. verrucosa* in Venezuela by Ruiz & Arroyo (1978). Whilst overall, *C. verrucosa* scored an ISI = 0.09, some individuals produced 15% selfed fruits. However, only 3% self fruit-set was recorded for this species in Mexico by Bullock (1985), and for *Capparis pittieri* Bawa et al. (1985) reported no fruit-set from selfed flowers (71 flowers from 10 individuals).

The 'self-incompatibility' in *C. retusa* occurs despite the fact that self pollen tubes grow throughout the ovarian placenta and penetrate many ovules. The lack of a style in *Capparis* species might seem to favour some kind of delayed variant of homomorphic gametophytic self-incompatibility (GSI) since it is usually within the transmitting tract of the style that rejection of self-pollen tubes occurs in this widespread SI mechanism. However, species of the genus *Papaver* which have GSI also lack a style but in this case self pollen either does not germinate or self pollen tubes are rejected before entering the ovary (Lawrence 1975). Likewise, in species of Cistaceae which lack a style and which are also self-incompatible, e.g. *Cistus ladanifer* (Talavera et al. 1993), self pollen tubes cease growth before entering the ovary. It is unlikely, therefore, that the lack of a style is an important factor in the self-sterility found in *C. retusa* or, given the absence of any manifestation of rejection (short growth, swollen tips etc.) of any self pollen tubes, that this is merely a variant of GSI. Rather some kind of 'late-acting self-incompatibility' (LSI) or ovarian sterility (OS), (see reviews in Seavey & Bawa 1986, Sage et al. 1994) seems to be present in this species.

LSI or OS, in which plants are self-sterile (or at least have markedly constrained selfing ability) despite apparently successful growth of self pollen tubes to the ovules, is a still poorly understood phenomenon despite having been reported in some 35 species from diverse families (Gibbs & Bianchi 1999). It is likely that LSI or OS phenomena encompass a range of diverse mechanisms. Some authors, e.g. Klekowski (1988) have favoured an early inbreeding depression explanation, with deleterious alleles present in homozygous condition responsible for embryo deaths provoking selfed pistil/fruit abscission, e.g. as in *Epilobium obcordatum* (Seavey & Carter 1994, 1996) and *Gomidesia* spp. (Nic Lughadha 1998).

However, the possibility that in some taxa self-sterility may be due to some form of as yet uncharacterized late-acting self-incompatibility mechanisms (Seavey & Bawa 1986, Gibbs & Bianchi 1993) which are independent of the action of lethal alleles, acting on embryo development cannot be discounted. This is particularly the case in species where most selfed pistils are uniformly abscised over a relatively brief time interval. Some early workers, e.g. Sears (1937) suggested that adverse signals from self-pollen tubes growing in the style might affect ovule development deleteriously despite subsequent penetration. Sage et al. (1999) have provided good evidence in *Narcissus triandrus* of 'long distance' signalling from self pollen tubes growing in the style causing deleterious effects on ovules such that in selfed pistils a proportion of ovules which are still immature at anthesis do not develop further, and a proportion of mature ovules also degenerate before the arrival of pollen tubes in the ovary. In this species, therefore, the effect of self pollen signalling is to increase the number of ovules which are sterile and so cannot be successfully fertilized.

For *Capparis*, as for most species reported with LSI - OS, such detailed histological studies of ovules at both pre- and post penetration stages are lacking. The very limited observations of post-pollination events reported here simply indicate that at least some selfed ovules initiate embryo development. However, it would seem that all selfed ovules show very early malfunctioning since although selfed ovules did increase in size over 72-96 h, they lagged behind the size increase shown by crossed ovules over this period. Whether this reflects the action of very early acting deleterious recessive alleles or whether the abortion of most selfed ovules is triggered by other events remains to be determined.

Capparis pittieri (Bawa et al. 1985) is the only species studied, other than the present one, in which pollen tube growth was observed, and self pollen tubes were reported as growing to the ovules. It is not known whether the 'self-incompatibility' reported for *C. verrucosa* (Ruiz & Arroyo 1978, Bullock 1985) and *C. atamisquea* (Aizen & Feinsinger 1994) also involves ovule penetration by self pollen tubes, but it is of interest that there are now two species of the genus known to have LSI type self-sterility. Gibbs & Bianchi (1999) have drawn attention to the fact that based on 35 species cited in the literature, LSI - OS type self-sterility tends to be clustered in certain families, e.g. Asclepiadaceae, Bombacaceae - Sterculiaceae, Leguminosae etc. and including the Capparaceae. Such familial clustering lends some support for a genetically based late-acting rejection mechanism, perhaps mediated by signals from self pollen tubes, since one would expect self-sterility due to accumulated recessive lethals in the population to have a taxonomically random distribution in perennial plants.

Acknowledgements - Field assistance by D. Prado and J. Vesprini is gratefully acknowledged. PEG thanks the Wallace Genetic Foundation for travel funding.

References

AIZEN, M.A. & FEINSINGER, P. 1994. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75:330-351.

BAWA, K.S. 1974. Breeding systems of tree species in a tropical lowland community. Evolution 28:85-92.

BAWA, K.S., PERRY, D.R. & BEACH, J.H. 1985. Reproductive biology of tropical lowland rain forest trees. I. Sexual systems and incompatibility mechanisms. American Journal of Botany 72:331-345.

BIANCHI, M.B., GIBBS, P.E., PRADO, D.E. & VESPRINI, J.L. 2000. Studies on the breeding systems of understorey species of a Chaco woodland in NE Argentina. Flora (in press).

BULLOCK, S.H. 1985. Breeding systems in the flora of a tropical deciduous forest in Mexico. Biotropica 17:287-301.

FRANKEL, R. & GALUN, E. 1977. Pollination mechanisms, reproduction and plant breeding. Springer-Verlag. Berlin, Heidelberg & New York.

GENTRY, A.H. 1974. Flowering phenology and diversity in tropical Bignoniaceae. Biotropica 6:64-68.

GENTRY, A.H. 1995. Diversity and floristic composition of neotropical dry forests. In Seasonally dry tropical forests (S.H. Bullock, H.A. Mooney & E. Medina, eds.) Cambridge University Press, Cambridge, p.146-194.

GIBBS, P.E. & BIANCHI, M.B. 1993. Post-pollination events in species of *Chorisia* (Bombacaceae) and *Tabebuia* (Bignoniaceae) with late-acting self-incompatibility. Botanica Acta 106:64-71.

GIBBS, P.E. & BIANCHI, M.B. 1999. Does late-acting self-incompatibility (LSI) show family clustering? Two more species of Bignoniaceae with LSI: *Dolichandra cynanchoides* and *Tabebuia nodosa*. Annals of Botany 84:449-457.

HESLOP-HARRISON, Y. & SHIVANNA, K.R. 1977. The receptive surface of the angiosperm stigma. Annals of Botany 41:1233-1258.

JOHANSEN, D.A. 1940. Plant microtechnique. McGraw-Hill, New York.

KLEKOWSKI, E.J. 1988. Mutation, developmental selection and plant evaluation. Columbia University Press, New York.

LAWRENCE, M.J. 1975. The genetics of self-incompatibility in *Papaver rhoeas*. Proceedings of the Royal Society, London, series B, 188:275-285.

LEWIS, J.P. 1991. Three levels of floristical variation in the forests of Chaco, Argentina. Journal of Vegetation Science 2:125-130.

MABBERLY, D.J. 1989. The Plant Book. Cambridge University Press, Cambridge.

MARTIN, F.W. 1959. Staining and observing pollen tubes by means of fluorescence. Stain Technology 34:125-128.

MAURITZON, J. 1935. Die embryologie einiger Capparidaceen sowie von *Tovaria pendula*. Arkiv för Botanik, Häfte 4, Band 26A, no. 15:1-14

NIC LUGHADHA, E. 1998. Preferential outcrossing in *Gomidesia* (Myrtaceae) is maintained by a post-zygotic mechanism. In Reproductive biology in systematics, conservation and economic botany (S.J. Owens & P.J. Rudall eds.). Royal Botanic Gardens, Kew, p.363-379.

PRADO, D.E. 1991. A critical evaluation of the floristic links between Chaco and Caatinga vegetation in South America. PhD. thesis. University of St Andrews.

PRADO, D.E. 1993. What is the Gran Chaco vegetation of South America? I.A review. Contribution to the study of the flora and vegetation of the Chaco V. Candollea 48:145-172.

RICHARDS, A.J. 1986. Plant breeding systems. Allen & Unwin, London.

RUIZ, T.Z. & ARROYO, M.T.K. 1978. Plant reproductive biology of a secondary deciduous forest in Venezuela. Biotropica 10:221-230.

SAGE, T.L., BERTIN, R.J., WILLIAMS, E.G. 1994. Ovarian and other late-acting self-incompatibility systems. In Genetic control of self-incompatibility and reproductive development in flowering plants. (E.G. Williams, A.E. Clarke & R.B. Knox, eds.). Dordrecht, Kluwer Academic, p.116-140.

SAGE, T.L., STRUMAS, F., COLE, W.W. & BARRETT, S.C.H. 1999. Differential ovule development following self- and cross-pollination: the basis of self-sterility in *Narcissus triandrus* (Amaryllidaceae). American Journal of Botany 86:855-870.

SASS, J.E. 1955. Botanical microtechnique. Iowa State University Press. Ames Iowa.

SEARS, E.R. 1937. Self-sterility in plants. Genetics 22:130-181.

SEAVEY, S.R. & BAWA, K.S. 1986. Late-acting self-incompatibility. Botanical Review 52:196-217.

SEAVEY, S.R, CARTER, S.K. 1994. Self-sterility in *Epilobium obcordatum* (Onagraceae). American Journal of Botany 81:331-338.

SEAVEY, S.R, CARTER, S.K. 1996. Ovule fates in *Epilobium obcordatum* (Onagraceae). American Journal of Botany 83:316-325.

TALAVERA, S., GIBBS, P.E. & HERRERA, J. 1993. Reproductive biology of *Cistus ladanifer* L. (Cistaceae). Plant Systematics and Evolution 186:123-134.