

SELF-INCOMPATIBILITY AND MALE FERTILIZATION SUCCESS IN *PHILLYREA ANGUSTIFOLIA* (OLEACEAE)

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Androdioecy is a rare breeding system in which low male frequency is expected in populations because males require a strong increase in their fertility to be maintained by selection. *Phillyrea angustifolia* L. has previously been reported as possibly functionally androdioecious. However, 1 : 1 sex ratios have been reported and suggest functional dioecy. In this article, we compared both pollen tube growth and siring success of male and hermaphrodite pollen in two single-donor pollination experiments. We verified at both pre- and postzygotic levels that hermaphrodites produce functional pollen. Self-incompatibility was also clearly established. However, pollen from hermaphrodites was less efficient than male pollen. The probability of a pollen tube growing through the style was higher for male than for hermaphrodite pollen donors, and males sired twice as many fruits as hermaphrodites. The twofold male advantage in relative fecundity was mainly because of lower pollen fertility of hermaphrodites and possible cross-incompatibility among hermaphrodites.

Keywords: androdioecy, male reproductive success, Oleaceae, *Phillyrea angustifolia*, pollen tube growth, self-incompatibility.

Introduction

Within the great diversity of breeding systems observed in the Angiosperms, the co-occurrence of male and hermaphrodite individuals within the same population, termed “androdioecy,” is the most rare (Darwin 1877; Yampolsky and Yampolsky 1922). According to theoretical models based on nuclear inheritance of sex, the extreme rarity of functional androdioecy (where hermaphrodites are male-fertile) is probably mainly a result of (1) the large fitness gain required for male plants to be maintained by selection and (2) the fact that androdioecy does not provide an outbreeding advantage, as opposed to gynodioecy (Lloyd 1975). To be maintained in natural populations, male fertility of the male plants must be at least double that of hermaphrodites, and this increase must be infinite for a 1 : 1 sex ratio to occur, corresponding to functional dioecy (i.e., where morphological hermaphrodites are male-sterile). An even greater advantage is required in partially self-fertilizing populations if there is no inbreeding depression, because fewer ovules will be available for outcrossing (Lloyd 1975; Charlesworth and Charlesworth 1978; Charlesworth 1984; Pannell 1997a). Functionally androdioecious species are thus expected to exhibit low male frequencies in populations, self-incompatibility (SI) or at least a low selfing rate, and a large male advantage.

At least three androdioecious species exhibit all three traits predicted by theoretical models. In *Datisca glomerata* (Datisceae), a wind-pollinated perennial herb, the male frequency

is consistently <27% in natural populations (Liston et al. 1990). In this species, hermaphrodites exhibit low selfing rates (Fritsch and Rieseberg 1992), selfed progeny suffer from inbreeding depression (Rieseberg et al. 1993), and males produce 3.8 times more pollen than hermaphrodites (Philbrick and Rieseberg 1994). In *Fraxinus lanuginosa* (Oleaceae), a wind- and insect-pollinated tree, male frequencies in populations range from 10% to 50%, hermaphrodites exhibit strong self-sterility (Ishida and Hiura 1998), and the male advantage is threefold in terms of flower production (Hiura and Ishida 1994) and 2.7-fold in terms of seed production (Ishida and Hiura 1998). In androdioecious populations of *Mercurialis annua* (Euphorbiaceae), male frequencies are always <30% (Pannell 1997b), hermaphrodites are self-fertile but protogyny effectively favors outcrossing (Pannell 1997d), and males produce four to 10 times more pollen than hermaphrodites (Pannell 1997c). Nevertheless, labile males can bear pistillate flowers under certain environmental conditions (Pannell 1997b).

Other species are functionally androdioecious but depart from model expectations. For example, although male frequencies are <28% in natural populations of *Schizopepon bryoniaefolius* (Cucurbitaceae), an entomophilous annual herb, hermaphrodites are self-compatible, and populations are characterized by an autogamous mating system (Akimoto et al. 1999). The male advantage has not yet been measured in this species. However, interestingly, an increase in male frequencies in populations was correlated with a decrease in inbreeding coefficient (F_{is}) (Akimoto et al. 1999).

As Charlesworth (1984) noted, some species have been described as androdioecious, but in many cases hermaphrodites were found to be male-sterile (functionally female). In general,

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1 : 1 sex ratios were also described in these species, suggesting functional dioecy (e.g., *Solanum*; see Anderson and Symon 1989).

The co-occurrence of males and hermaphrodites in equal frequencies and the self-fertility of hermaphrodites has been described in two species in the Oleaceae, *Phillyrea angustifolia* and *Fraxinus ornus* (Lepart and Dommée 1992; Dommée et al. 1999). In *F. ornus*, an entomophilous tree, a study based on a small number of crosses showed that although male frequencies are 50%, hermaphrodites produce viable pollen and are self-fertile (Dommée et al. 1999). In *P. angustifolia*, a wind-pollinated shrub, preliminary studies realized in natural conditions and based on a small number of crosses (five in Lepart and Dommée 1992 and seven in Traveset 1994) suggested that morphologically male plants never set seed, while morphologically hermaphroditic plants produce viable pollen as well as seeds and are often self-fertile. In addition, of the few crosses performed, both unsuccessful crosses among hermaphrodites and self-sterility were observed in some populations, suggesting possible incompatibility, but these results also exhibit a strong variability (Lepart and Dommée 1992; Traveset 1994).

The aim of this study was to explore in detail the nature of the breeding system of *P. angustifolia* from a natural population in southern France. Several hundred single-donor crosses and self-pollinations were performed to assess the male fertility of hermaphrodites, to estimate relative male function of hermaphrodites, and to extensively investigate the suspected self-incompatibility in hermaphrodites. We analyzed the germination success of pollen tubes on stigmas and fruit set in order to (1) determine whether hermaphrodite pollen is able to grow through the pistil and sire seeds, (2) compare the fertilization abilities of male and hermaphrodite pollen at both pre- and postzygotic levels, and (3) test the hypothesis that hermaphrodites are self-incompatible.

Material and Methods

Study Species

Phillyrea angustifolia L. (Oleaceae) is a wind-pollinated evergreen shrub that is widespread throughout the western part of the Mediterranean basin and Portugal. It is a common but rarely dominant species in the western Mediterranean garrigue formations (Strasberg 1988).

The flowers are tightly clumped in contracted racemes (or inflorescences), bearing four to nine flowers in the axils of the leaves on the twigs produced during the previous year. In March–April, flowers open asynchronously within an individual, and flowering occurs over 3–4 wk within a given population. Hermaphrodite flowers are protogynous, although anther dehiscence occurs while the stigma is still receptive. Within an inflorescence, the pollen from an early flower can easily reach the stigmas of the latest ones, so protogyny does not prevent potential geitonogamy. Males and hermaphrodites do not differ in inflorescence architecture as observed, for example, in *Mercurialis annua* (Pannell 1997d); therefore, males do not have an advantage over hermaphrodites with respect to pollen dispersal.

The ovary contains four ovules. Fruits are one-seeded drupes, ovoid while young and subglobose at maturity and

are dispersed by birds. This species has a mast flowering process for males and hermaphrodites (Lepart and Dommée 1992; J. Lepart, personal observation).

Plant Material

All pollination experiments were carried out with individuals grown from seeds collected in 1987 from 14 hermaphrodites (labeled families A–N) sampled in a previously studied population in La Gardiole near Montpellier, France, composed of 91 individuals—53 males and 38 hermaphrodites (Lepart and Dommée 1992). The seedlings were planted in 1987 in the experimental field at Montpellier, and sexual phenotypes were recorded over several flowering seasons. No sexual lability was observed on a total of 222 plants (14–22 individuals per family). The mean proportion of males in these families was 0.59 (SE = 0.19, $n = 14$, not significantly different from 0.5, χ^2 df = 1, $P = 0.07$). The 18 hermaphrodite recipient plants in our experimental pollinations (see below) were members of families A–J; the 16 hermaphrodite pollen donors came from families A–F, G, I, J, and O; and the 18 male pollen donors from families A–F, K, L, M, and N. Plants from the same family were derived from open-pollination and are, therefore, at least half-sibs.

Pollen Tube Growth Experiment

One week before the flowering period, in February 1995, hermaphrodites and males were chosen in the experimental field (table 1). On hermaphrodite recipients, twigs were covered with polyester bags (PBS International, Scarborough). Anthers were collected from randomly chosen open flowers on 10 male and eight hermaphrodite pollen donors (plus the self-pollen donors) and placed in small plastic tubes. To induce anther dehiscence, tubes were left in an air dryer with some Silicagel crystals for 1 h. Pollen was deposited on the stigmas of flowers before their own anthers dehisced.

Two to three hermaphrodites and two to four males were selected for crosses with each of the six pollen recipients, on which two to 11 flowers were pollinated with either male or hermaphrodite self- or outcross pollen. A total of 224 flowers were observed: 108 crossed with male donors, 75 crossed with hermaphrodite donors, and 41 selfed (table 1).

In order to detect and evaluate any family effects, at least one hermaphrodite and one male pollen donor came from the same family as the recipient, while the other pollen donors came from different families. We performed preliminary studies that showed that pollen tubes reach the ovary within 3 d. Therefore, 3 d after pollination, flowers were fixed in formalin–acetic acid–alcohol (FAA 1 : 1 : 8) for at least 24 h and then rinsed with water and soaked in Z-colorant (Jahier et al. 1992) for 30 min at 65°C. The gynoecium of each flower was then gently crushed and examined with UV epifluorescence microscopy in a drop of aniline blue solution (Martin 1959; Jahier et al. 1992). Pollen tubes were easily distinguished from maternal tissues (fig. 1) but could not be precisely localized. The presence of at least one pollen tube in the style (fig. 1C) was used as a criterion for successful pollination in comparisons among treatments (see table 2).

Table 1
Number of Flowers with at Least One Pollen Tube in the Style
over the Total Number of Flowers Pollinated in the
Pollen Tube Growth Experiment

Pollen donors	Hermaphrodite recipients					
	A.11	B.8	C.10	D.3	E.2	F.5
Males:						
A.8	7/8	2/4
A.12	6/7	3/7
B.5	6/8	1/4
B.11	4/5	0/1
C.5	5/6	4/6
C.11	4/5	5/5
D.12	6/7	3/6
D.4	3/3	0/3*
E.9	4/7	3/4
F.1	4/6	5/6
Total: 75/108	23/28	6/16	18/21	12/20	8/13	8/10
Hermaphrodites:						
A.5	7/7	0/3*
A.1	3/3	1/4
B.2	0/7*
C.22	3/6
C.8	5/11	3/3
D.5	7/7	0/3*
E.11	0/5*	1/5
F.11	1/5	6/6
Total: 37/75	10/17	1/7	15/24	3/6	1/10	7/11
Self: 1/41	0/7*	1/10*	0/6*	0/9*	0/4*	0/5*

* No pollen tubes observed in any of the flowers' styles (see text).

Fruit Set Experiment

Because seed production can be influenced by many factors that occur after the pollen grows through the stigma, we also measured fruit set. Pollen recipients were randomly chosen among those plants in the experimental field that were flowering in April 1996. They were not the same plants that were used in the 1995 study because most did not flower in 1996 (see table 3). A few days before the beginning of flowering, one to two twigs per treatment were randomly selected on each of the 13 recipients. The twigs were bagged, except those chosen for open-pollination, which were used as a positive control for seed production for each recipient.

Because of their small size, the number of flowers per twig was difficult to count for each pollination. Therefore, only the number of inflorescences per twig was determined (mean = 162, SE = 15, $n = 127$ twigs); we counted a total of 20,919 inflorescences and 19,541 fruits on 127 twigs (7394 inflorescences and 5951 fruits on 48 twigs for pollination by hermaphrodites, 8013 inflorescences and 9778 fruits on 44 twigs for pollination by males, 3194 inflorescences and 494 fruits on 18 twigs for self-pollinations, and 2318 inflorescences and 3318 fruits on 17 twigs for open-pollinations).

In order to avoid any contamination by cross-pollen, self-pollinated twigs were only allowed to self within the bags. Because of the large amount of pollen produced by flower and the high number of flowers open at the same moment in the

bag, self-pollen was not considered as limiting. For the outcross pollinations, eight males and eight hermaphrodites were used as pollen donors (see table 3). To obtain pollen, two flowering twigs per pollen donor were cut, placed in paper bags, and stored in a greenhouse protected from any outside source of pollen for 12–24 h. Pollen was collected on a plastic sheet that was placed inside the bag under the twigs. In order to collect the maximum amount of pollen possible, approximately one volume of neutral talcum powder was mixed with one volume of pollen from each plant (Cuevas and Rallo 1990). The same volume of mixture for each pollination measured in a small pill was then placed in a small pollen-dispersing spray gun (Institut National de la Recherche Agronomique [INRA], Orléans). The absence of any talcum effect on pollen viability was previously verified by *in vitro* germinability tests. The pollen was dispersed into the bag around the recipient twigs through a small hole. Each pollination was repeated at least twice during the flowering time in order to pollinate as many flowers as possible.

In September, total fruit number was counted on each open-, cross-, and self-pollinated twig. Fruit set was assessed by dividing the number of fruits per twig by the number of inflorescences present before bagging (table 3).

To facilitate comparison with other species, the number of flowers per inflorescence was also estimated on nine hermaphrodite individuals (mean over all individuals = 5.08,

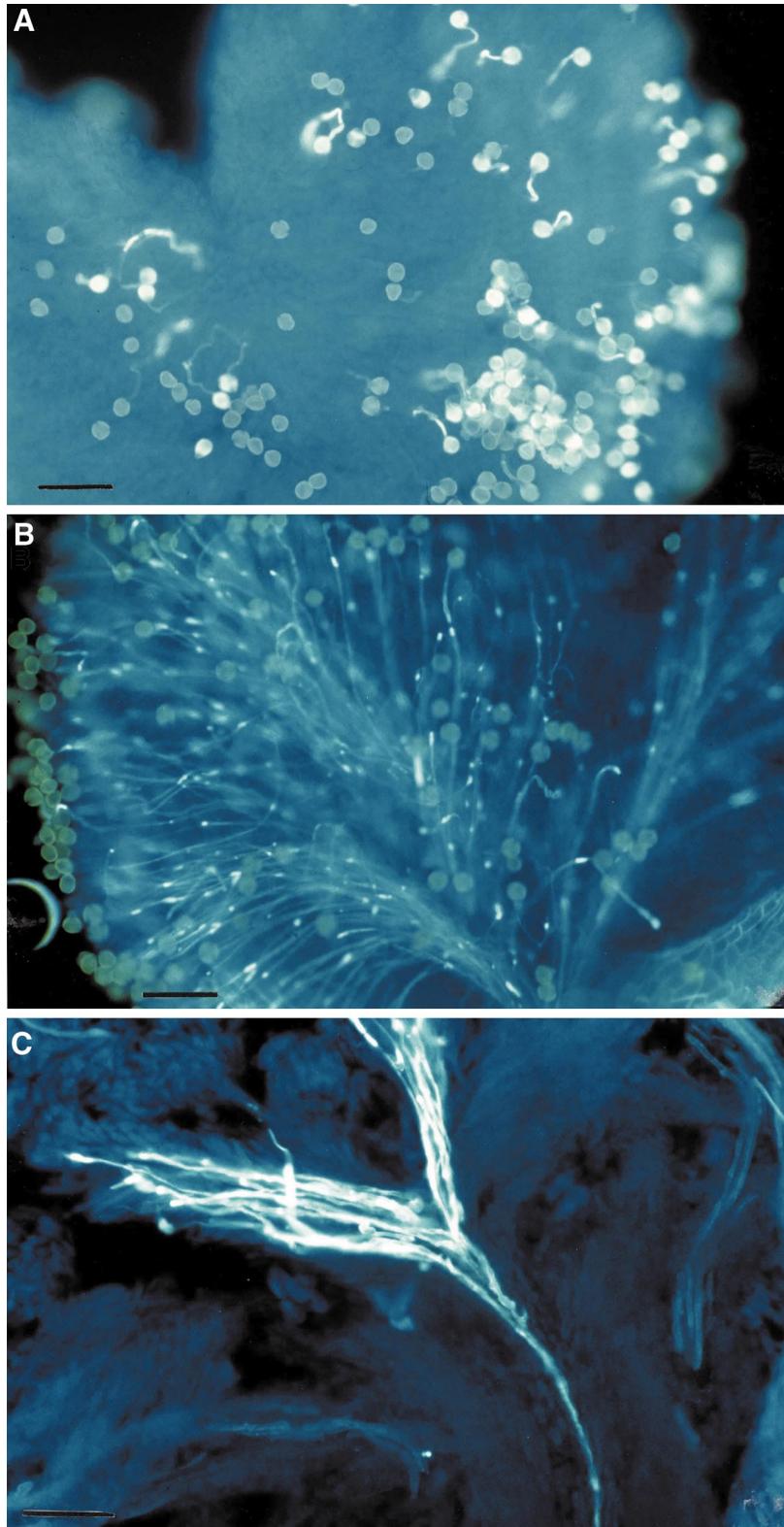


Fig. 1 Pollen tube growth (white) within stigma and style of flowers that were fixed 9 d after hand pollination. Bars: 100 μm *A*, Self-pollinated stigma. Self-pollen tubes have emerged from pollen grains and have just grown on the long stigmatic cells (blue). Some are longer and irregular with forked ends; most are short and curly and can hardly be distinguished from the stigma cells. *B*, Cross-pollinated stigma. Long pollen tubes have grown through the stigma to the style. They are guided by transmitting tissue bundles converging at the upper end of the style. Only one (rarely two, 6.2% of total observed cross-pollinated pistils) pollen tube grows to the ovary, where it reaches one of the four ovules. *C*, Cross-pollinated pistil showing pollen tube bundles from the stigma to the style and only one pollen tube in the style. The pistil was gently crushed in order to remove external fluorescent tissues and to easily detect pollen tubes.

Table 2
Results of the Fisher Exact Test Analysis of Pollen Tube Growth on Six Hermaphrodite Recipients after the Different Pollination Treatments

	Pt/tot			P		
	× M	× H	Self	Self vs. × H	Self vs. × M	Self vs. × H, × M
Hermaphrodite recipients:						
A.11	23/28	10/17	0/7	0.019	<0.001*	<0.001*
B.8	6/16	1/7	1/10	0.669	0.190	0.330
C.10	18/21	15/24	0/6	0.017	0.0003*	0.001*
D.3	12/20	3/6	0/9	0.045	0.003*	0.007*
E.2	8/13	1/10	0/4	0.714	0.082	0.021
E.5	8/10	7/11	0/5	0.033	0.007*	0.031
Total	75/108	37/75	1/41	<0.001*	<0.001*	<0.001*

Note. Pt = number of flowers with at least one pollen tube in the style; tot = total number of flowers observed; × H = flowers crossed with hermaphrodite pollen; × M = with male pollen; self = selfed flowers.

* Still significant after Bonferroni correction performed on each column.

range = 4.5–6 according to the individual, $n = 1516$ inflorescences, $SD = 1.63$). Differences in number of flowers per inflorescence between and within individuals were observed, but this should not bias our conclusions based on fruit set. First, because the differences between individuals were included in the recipient effect (see below), and second, because twigs were randomly chosen, the differences between twigs within individuals were randomly distributed among the different treatments.

Statistical Analyses

Pollen tube growth. Differences in germination success between self- and outcross-pollination treatments were tested using Fisher exact tests (STRUC program in GENEPOP; Raymond and Rousset 1995), and global differences between male and hermaphrodite pollination treatments were tested by a G-test for heterogeneity (Sokal and Rohlf 1995).

The presence of a pollen tube (from a pollen donor) in a given style was modeled using logistic regression with the GENMOD procedure of the SAS Institute (1995). The model examined the effects of pollen donor's sex (two levels: male or hermaphrodite), recipient identity, the sex × recipient interaction, and family type (two levels: within or among families).

Fruit set. The fruit set variable was square root transformed in order to homogenize the variances. The various effects were tested using ANOVA models in the MIXED procedure of the SAS Institute (1995).

To test the relative fertility according to the effect of the pollen donor's sex (two levels: male or hermaphrodite, fixed), the recipient (random), and the pollen donor identity (nested within sex effect, random), we used a mixed partially nested ANOVA model.

We performed a complete two-way ANOVA with a recipient effect (random) and a treatment effect (t , either open- or self-pollination, fixed) to compare fruit set after self- and open-pollination. The Tukey's Studentized range test was used to compare means for different treatments (open-, self-, × M, and × H pollinated).

Results

Pollen Tube Growth

Male fertility of hermaphrodites. Pollen from hermaphrodites germinated on the stigmas of 37 of the 75 pollinated flowers (table 2, col. × H). Therefore, hermaphrodites produce potentially functional pollen.

Relative pollen fertilities of hermaphrodites and males. Male pollen germinated better than hermaphrodite pollen (fig. 2); the difference between male and hermaphrodite donors in the proportion of flowers with a pollen tube in the style was significant (G-test for heterogeneity, $P = 0.009$). Male pollen was 1.49 times more capable of growing into the style than hermaphrodite pollen, and its germination success rate was more homogeneous ($CV = 16.6\%$ vs. 53.6%). The variance in pollen success was significantly lower among male pollen donors than among hermaphrodite pollen donors (F -test, $P = 0.004$). Pollen tube growth was also significantly influenced by the stylar tissue (Fisher's exact test, recipient effect, $P < 0.0001$). The logistic regression results showed significant sex ($P < 0.0001$) and recipient ($P < 0.0001$) effects, but neither the sex × recipient interaction nor the family effect was significant ($P = 0.36$ and $P = 0.47$, respectively).

Self-incompatibility. Both self- and outcross-pollen grains germinated on the stigma surface. However, after self-pollination, pollen tubes penetrated neither the stigma nor the style (fig. 1A): only one of 41 selfed flowers showed pollen tubes within the style or the ovary 3 d after pollination (table 2, self). Conversely, within cross-pollinated flowers (fig. 1B), pollen tubes grew along stigmatic transmitting tissues: the difference in pollen tube growth between self- and cross-pollinated flowers was significant for four of the six recipients (table 2, both self vs. × M, and self vs. × H). Self-incompatibility therefore occurred at the prezygotic level.

Generally, two bundles of pollen tubes reached the upper end of the style. At this level, a single pollen tube (rarely two) was able to grow through the style toward the ovary (fig. 1C). This is an extreme case of pollen tube attrition. In the ovary, a callose-rich segment at the end of the pollen tube can usually be seen in contact with the ovule.

Table 3

Mean Number of Fruit per Inflorescence (Number of Twigs) for Each Pollination in the Fruit Set Experiment

Pollen donors	Hermaphrodite recipients												
	G.2	H.3	E.11	I.1	G.22	G.12	E.1	B.12	G.13	J.10	A.1	A.11	A.13
Males:													
K.2	0.645 (2)	1.278 (2)	0.752 (2)	2.171 (2)
L.1	0.301 (1)	2.251 (2)	0.818 (2)	1.260 (1)
M.1	1.185 (2)	1.682 (2)	0.850 (1)
L.9	1.542 (2)	1.975 (2)	0.579 (2)
N.16	1.805 (2)	1.902 (2)	1.978 (2)
L.21	1.530 (2)	1.539 (2)	0.692 (2)
L.11	1.069 (2)	m	m
N.12	3.342 (2)	1.545 (2)	0.292 (1)
Hermaphrodites:													
E.12	0.109 (2)	2.288 (2)	0.741 (2)	1.545 (2)
G.22	0.714 (2)	0.538 (2)	0.049 (1)*	0.340 (2)
I.1	0.814 (2)	0.724 (2)	1.596 (2)
J.4	0.828 (2)	1.246 (2)	0.882 (1)
D.1	0.987 (2)	0.278 (2)	0.695 (2)
G.1	0.335 (2)**	1.542 (2)	m
O.1	0.447 (2)	0.222 (2)	0.013 (2)*
D.2	2.089 (2)	0.119 (2)	0.032 (2)*
Self-pollination	0.023 (1)	0.343 (1)	0.194 (1)	0.062 (1)	0.265 (2)	0.202 (1)	0.171 (1)	0.192 (2)	m	0.213 (2)	0.088 (3)	0.062 (1)	0.033 (2)
Open pollination	0.851 (1)	0.757 (1)	1.236 (1)	1.530 (1)	1.441 (2)	2.446 (2)	1.187 (1)	2.858 (2)	0.992 (1)	3.186 (1)	1.365 (1)	1.246 (2)	0.732 (1)

Note. Fruit set value after self-pollination is used as a threshold for cross-incompatibility detection. In order to take into account possible recipient effect, fruit set value in cross-fertilization was corrected by dividing by open-pollinated fruit set value; m = missing value.

* Incompatible crosses without correction.

** Incompatible crosses with correction.

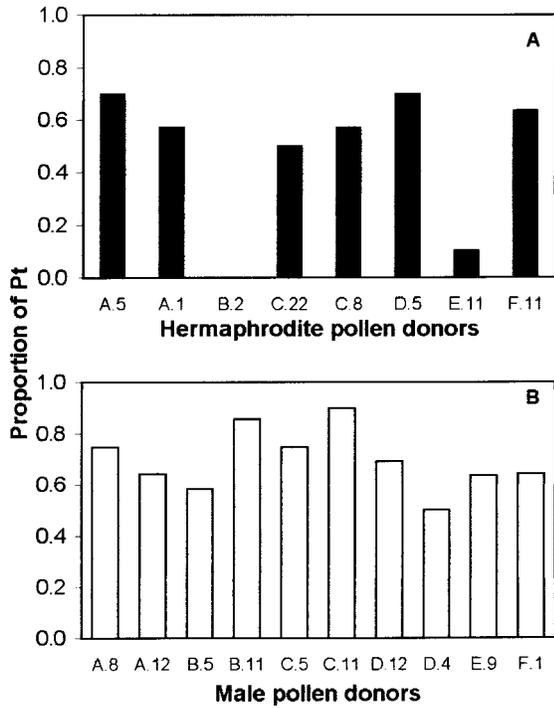


Fig. 2 Proportion of flowers with a pollen tube in the style after cross-pollination with pollen from hermaphrodites (A) and males (B) performed on two hermaphrodite recipients each (total of six recipients; see table 2). The proportion of flowers containing pollen tubes was significantly higher for male pollen donors (0.70, SD = 0.12, $n = 10$) than for hermaphrodite pollen donors (0.47, SD = 0.25, $n = 8$; pollen donor's sex effect, $P = 0.009$). Male donors were potentially 1.49 times more effective at fertilizing ovules than hermaphrodites.

Fruit Set

Male fertility of hermaphrodites. Hermaphrodites sired seeds on all of the 13 hermaphrodite recipients tested and, thus, are potentially male-fertile.

Relative male fertilities of hermaphrodites and males and cross-incompatibility. Male pollen donors sired significantly more fruits (1.93 times on average) than hermaphrodite pollen donors (table 4, effect of pollen donor sex). Again, male pollen donors exhibited more evenly distributed fertilization success than hermaphrodite donors (fig. 3). In addition, a significant difference among hermaphrodite recipients was observed (table 4, recipient effect). Actually, variation in fruits per inflorescence within families after open-pollination was high, and the recipient effect was again significant ($F_{12,11} = 5.84$, $P = 0.0044$). Thus, some recipients were better fruit producers than others in the year of the study. Further, the interaction between hermaphrodite recipient and donor identity nested within sex (table 4, recipient \times donor[sex]) was highly significant, demonstrating differences in receptivity of hermaphrodites according to the donor's identity.

Self-incompatibility. Self-pollinated twigs set very few fruits compared with the other types of crosses (0.14 fruits per inflorescence, SE = 0.04, $n = 18$, i.e., 0.028 fruits/flower with 5.08 flowers/inflorescence), that is to say, 11 times fewer than

open-pollinated twigs (1.58 SE = 0.22, $n = 17$; fig. 4), and this difference was highly significant (treatment effect, $F_{1,12} = 78.54$, $P < 0.0001$). Controlled pollinations using male pollen were as successful as open pollinations (paired t -test, $P = 0.404$). Therefore, the poor performance of self-pollen is not an artifact of the bagging of the twigs. One hermaphrodite (H.3), however, set only 2.2 times less than by open pollination, which suggests that self-incompatibility may not be strict.

Cross-incompatibility. Since we noticed that some crosses had low fruit set and were thus putatively incompatible, we explored in more detail the recipient \times donor(sex) interaction. As there is considerable difference in fruit set after self-pollination, we have used the value of each fruit set after self-pollination as a threshold below which crosses are deemed incompatible. Using this threshold, we found three incompatible crosses out of the 49 performed (single asterisk in table 3); all were H \times H crosses. This was not because of a higher relatedness between pollen donors and recipients in H \times H crosses than in M \times H crosses. Indeed, the three crosses between siblings, G.22 \times G.2, E.12 \times E.11, and G.1 \times G.13, were fully compatible. When the fruit set values were corrected for the previously mentioned recipient effect (by dividing by open-pollinated fruit set values), one additional incompatible cross was revealed (table 3, double asterisk). This, again, involved a hermaphrodite pollen donor. After these incompatible crosses were removed from the ANOVA analysis on fertility, no change was observed in the different effect tested.

Discussion

We have established that (1) hermaphrodites are clearly male-fertile, (2) hermaphrodites are strongly self-incompatible, and (3) male pollen donors were more successful than hermaphrodite pollen donors. These three results have been demonstrated at both pre- and postzygotic levels.

Male Fertility of Hermaphrodites

Most hermaphrodites were potentially male-fertile: 80% of hermaphrodite pollen donors showed pollen tube growth in styles of recipient flowers, and 80% successfully produced seeds, with the best hermaphrodites being as fertile as the males. This result, based on a substantial number of crosses, confirms the male fertility of hermaphrodites and thus differs from the earlier work on the same population (Lepart and

Table 4

Results of Mixed, Partially Nested Analysis of Variance on the Number of Fruits per Inflorescence

Source	df	Mean squares	F	P	df
Pollen donor sex	1	2.434	53.75	0.0002	1
Donor (sex)	11	0.165	1.02	ns	9
Recipient	10	0.053	8.26	0.0011	10
Recipient \times sex	9	0.1669	0.31	ns	7
Recipient \times donor (sex)	15	0.1703	3.49	0.0007	10
Residual	42	0.0487	36

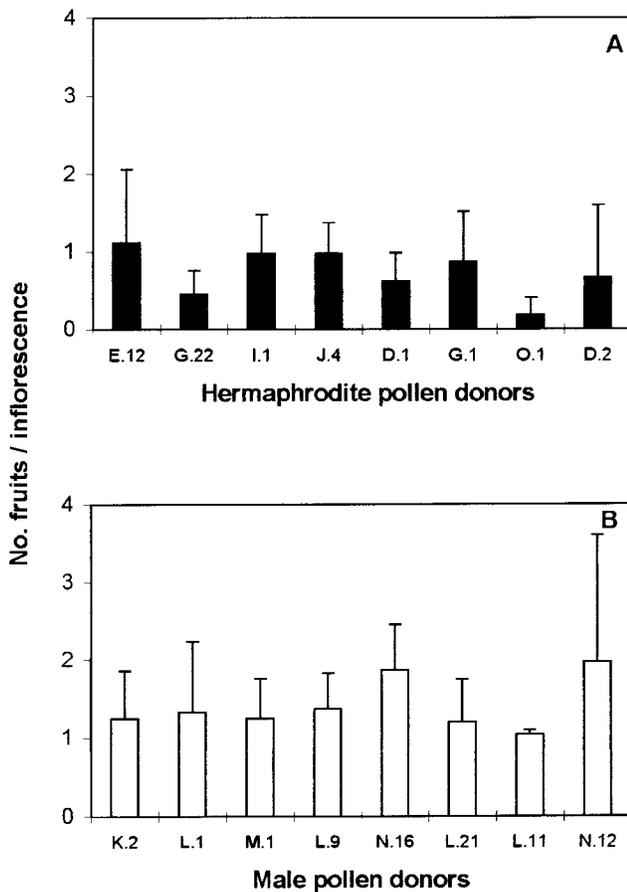


Fig. 3 Mean fruit sets after different pollination treatments performed on three to four hermaphrodite recipients each (total of 13 hermaphrodite recipients; see table 2), one or two twigs per treatment per individual. A, H \times H crosses; B, M \times H crosses.

Dommée 1992) but confirms results on other populations (Lepart and Dommée 1992; Traveset 1994).

Two individuals (B.2 and E.11 in table 1) showed no or very little pollen tube growth in the styles of the two recipients tested. Male sterility is the most likely cause.

Relative Male Fertility

A male fertility advantage was found at both pre- and post-zygotic stages, such that the hermaphrodites were half as fertile as males (table 4, sex effect). The prezygotic male advantage of 1.49 was reinforced at the fruit set level, where the male advantage rose to 1.93. The lower male fertility of hermaphrodites can be attributed to two non-mutually exclusive causes: first, a lower pollen quality of hermaphrodites, and second, unsuccessful crosses more frequent among hermaphrodites, which we will discuss later.

We showed that the hermaphrodites were globally less successful than males in single-donor pollination. This suggests that hermaphrodites may be much less successful at siring seeds in natural conditions. Indeed, different pollen donors are likely to compete on stigmas. In this situation, pollen germination and pollen tube growth are likely to be affected. Because the

hermaphrodites are less efficient pollen donors, and because their male fertility has a high variance, pollen donor competition may decrease the relative male fertility of hermaphrodites (e.g., see Marshall 1991) in *Phillyrea angustifolia*.

Strong pollen attrition (Cruzan 1989; Cruzan and Barrett 1996) was observed, which suggests that pollen competition occurs for access to ovules: after cross-pollination by males or hermaphrodites, pollen tube bundles converged toward the midpart of the style, and thereafter, just one pollen tube grew to reach the ovary (two pollen tubes observed in only 6.2% of pistils). This explains the almost exclusive production of one-seeded drupes (rarely two-seeded; C. Vassiliadis, personal observation) even though flowers contain four ovules. As far as we know, such strong attrition has previously been described only in *Olea europea* (Bradley and Griggs 1963; Cuevas et al. 1994b) and *Pistacia vera* (Martínez-Pallé and Herrero 1998).

In addition, two factors suggest that male relative fitness of hermaphrodites was probably overestimated in our study. First, males seem to flower more frequently and more abundantly than hermaphrodites (J. Lepart and C. Vassiliadis, personal observation), which would lower the relative male fecundity of hermaphrodites. Second, better survival and vegetative growth of males has been observed (Lepart and Dommée 1992), which could strongly reduce the relative hermaphrodite viability.

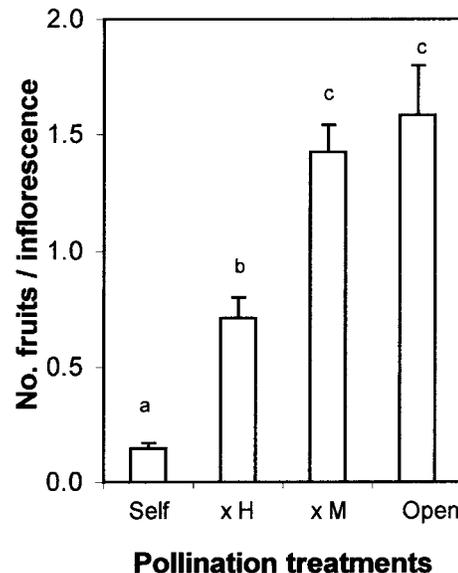


Fig. 4 Mean number of fruit per inflorescence after different pollination treatments on 13 recipients (see table 3). *Self*, self-pollination; *x H*, pollination using pollen from hermaphrodite donors; *x M*, pollination using pollen from male donors; *Open*, open pollination. The difference between the male and hermaphrodite treatments was significant (*t*-test, $P < 0.05$). The mean fruit set for male donors (1.42, SD = 0.78) was not significantly different from open-pollination treatment (1.58, SD = 0.84) but was highly significantly different from hermaphrodite donors (0.71, SD = 0.64). Male donors were twice as effective as hermaphrodite ones at siring seeds.

Self-Incompatibility

We found that hermaphrodite *P. angustifolia* individuals in the population studied were self-incompatible. Almost no self-pollen tubes were found within the style or ovary, effectively preventing fertilization at the prezygotic level. We observed pollen tube growth patterns such as swollen, forked, and coiled tubes and swollen tube ends (fig. 1A) that are indicative of strong SI (Dumas and Knox 1983) and are similar to those reported in self-incompatible varieties of the closely related *O. europea* (Bradley and Griggs 1963; Cuevas and Rallo 1990; Cuevas et al. 1994a). This confirms with a larger number of individuals previous observations in the “La Gardiole” population (Lepart and Dommée 1992), where self-pollinations resulted in very low or no fruit set, suggesting strong SI.

Selfing resulted in very few fruits per flower compared with open-pollination: an 11-fold difference was observed (2.8% vs. 31%). This low relative fertilization success of self-pollen is probably mainly because of SI as observed at the prezygotic level but also because of postzygotic phenomenon occurring during the maturation period, as early inbreeding depression and selective fruit abortion cannot be excluded. The occurrence of self-incompatibility was also inferred from a weak (5%) fruit : flower ratio in one of two *P. angustifolia* populations studied (Traveset 1994). Similar values were obtained in self-incompatible *O. europea* varieties, where the fruit : flower ratio ranged from 0% to 2.8% in selfed twigs, versus 10.5% after cross-pollination (Bradley and Griggs 1963).

Self-incompatibility of hermaphrodites, or very low outcrossing rates, is one of the conditions required by model predictions for a species to be functionally androdioecious (Lloyd 1975). It is interesting to observe that except for the strongly self-sterile *Fraxinus lanuginosa* (Ishida and Hiura 1998) and *P. angustifolia* (this study), most other androdioecious species are self-fertile. However, in the predominantly selfing *Schizopepon bryoniaefolius*, the presence of males reduces the selfing rate (Akimoto et al. 1999), and the other species have high outcrossing rates, e.g., *Datisca glomerata* suffers a high inbreeding depression (Rieseberg et al. 1993). In *Mercurialis annua* and *Fraxinus ornus*, hermaphrodites can still reproduce by self-fertilization in colonizing or low density populations (Pannell 1997d; Dommée et al. 1999).

Unsuccessful Crosses among Hermaphrodites

The substantial number of single-donor cross pollinations allowed us to detect some cases of unsuccessful pollinations among hermaphrodites only. Both a genetic incompatibility system and inbreeding depression can lead to unsuccessful crosses. The occurrence of self-incompatibility at the prezygotic level in this study suggests the existence of a genetic incompatibility system. Nevertheless, the frequency of incompatible crosses among unrelated individuals is limited by the number of *S*-alleles (Vekemans et al. 1998).

Some hermaphrodites had very low siring success (in terms of fruit set) on one recipient plant but high siring success on the other recipient plant on which they were tested (see table

2). This effect is not caused by experimental methods (i.e., bagging effect or age of flowers) since controlled pollinations using males resulted in a fruit set as high as observed in open-pollinations. Nor is this siring success variation caused by a recipient effect or by flower aging, since other crosses performed on the same plant at the same time were successful. In addition, similar contrasting success rates in H × H crosses occurred at the prezygotic level (see table 1). The high variation of pollen tube germination observed in hermaphrodites may be in fact because of cross-incompatibility phenomena.

Maintenance of Male Fertility of Hermaphrodites: Evolutionary Significance of Androdioecy

Even if we suppose that males are better competitors for ovules in populations, the question of the maintenance of the potential male fertility of hermaphrodites still remains. Two elements may allow the resolution of this paradox. First, male fertility of hermaphrodites may ensure their reproduction in a hermaphrodite or newly founded population, where there is no competition with male pollen. This argument would be in agreement with the population dynamics of *P. angustifolia*, which has been shown to be invading Camargue in the past 5 decades (Strasberg 1988). Nevertheless, in *P. angustifolia*, this argument is contradicted by the strong self-incompatibility observed. Second, long-term evolutionary trends of this mixed breeding system can be invoked. Self-incompatibility should suggest breakdown of dioecy rather than an evolution toward dioecy (Charlesworth 1995), but the presence of a relict pistil in male flowers is compatible with an evolution from hermaphroditism to dioecy, as suggested by Lepart and Dommée (1992). Thus, the androdioecy observed in *P. angustifolia* may be transient and recent, i.e., the lower male fertility of hermaphrodites can be interpreted as a female-biased functional gender (as defined in Lloyd 1980), and some of the hermaphrodites may be already male-sterile (as we have observed). Indeed, low male frequencies (from 20% to 38%) and gender inconsistency occur in males and hermaphrodites in Spanish populations (Traveset 1994). Various breeding systems are observed in related genera. Andromonoecy is described in *Olea* species, and both hermaphroditism and dioecy are present in the genera close to *Phillyrea*. Unfortunately, the phylogeny of these genera has not yet been resolved (E. Wallander, personal communication), and thus we cannot speculate on any one evolutionary scenario.

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