

Self- and cross-pollination effects on pollen tube growth and seed set in holm oak *Quercus ilex* L (Fagaceae)

A Yacine^{1*}, F Bouras²

¹ Laboratoire de génétique et évolution des populations végétales, Université des sciences et technologies de Lille-I, 59655 Villeneuve d'Ascq cedex, France;

² Département de foresterie, Institut national agronomique d'El-harrach, Algiers, Algeria

(Received 22 February 1996; accepted 16 September 1996)

Summary – Patterns of the self-incompatibility system have been more often described for hermaphroditic, entomophilous and short-lived plant species. *Quercus ilex* is a long-lived, monoecious, anemophilous and highly self-incompatible species. We used pollination experiments to investigate phenotypic responses of the self-incompatibility system. Flowers from 14 individuals of the same stand were hand-pollinated with self-pollen, cross-pollen from a single donor and a mixture of the two types. We observed a slower pollen tube growth and no or nearly no seed production after self-pollination. The more self-pollen tubes reach the style, the more flowers will stop their ovule development, resulting in a high flower abortion rate. In open pollination, pollen load is not a limiting factor, but incompatible pollen may reach stigma simultaneously or before compatible cross-pollen, which will induce an early abortion of flowers. When pollination is qualitatively and quantitatively effective, the regulation of seed production related to the resources availability acts by the late abortion of fruit.

self-incompatibility / pollen tube growth / seed set / *Quercus ilex* L

Résumé – Effets de l'auto- et de l'allopollen sur la croissance des tubes polliniques et la production de fruits chez le chêne vert (*Quercus ilex* L). Les systèmes d'auto-incompatibilité ont été plus fréquemment étudiés chez des espèces hermaphrodites, entomophiles et à courte génération. *Quercus ilex* est une espèce monoïque anémophile et à longue génération. Des pollinisations contrôlées ont été réalisées pour l'analyse des réponses phénotypiques du système d'auto-incompatibilité. Des fleurs de 14 individus ont été pollinisées avec de l'autopollen, de l'allopollen simple donneur et un mélange d'auto- et d'allopollen. Nous avons observé une plus lente croissance des tubes polliniques et peu ou pas de production de fruits après autopollinisation. Plus le nombre de tubes d'autopollen attei-

* Correspondence and reprints
Tel: (33) 320 43 67 48; fax: (33) 320 43 69 79

gnant le style est élevé, plus la proportion d'avortements précoces des fleurs sera importante. En pollinisation libre, le pollen n'est pas un facteur limitant, néanmoins si du pollen incompatible atteint le stigmate avant ou en même temps que de l'allopollen compatible, il peut diminuer significativement la production de fruits par un avortement précoce des fleurs. Quand la pollinisation est assurée par du pollen compatible, la régulation de la production de fruits, compte tenu de la disponibilité des ressources, s'opère par un avortement tardif des fruits.

auto-incompatibilité / tube pollinique / production de fruits / *Quercus ilex* L

INTRODUCTION

Many plants have adaptations that prevent self-fertilization such as the genetic self-incompatibility system. Such plants present an accumulation of detrimental recessive mutations; as a consequence, inbreeding depression may act as a major selective obstacle to the evolution of self-fertilization mechanisms (Hamilton and Mitchell-Olds, 1994). When genetic self-incompatibility occurs, a plant that produces functional male and female gametophytes is unable to produce selfed offspring. Fertilization occurs only between gametophytes of different genotypes (Heslop-Harrison, 1983).

In incompatible matings, pollen tube growth is inhibited on the stigma, in the style or in the ovary (Dumas and Knox, 1983; Seavey and Bawa, 1986) where callose formation is related to rejection phenomena.

Martin (1959) and Heslop-Harrison et al (1973) have shown that callose formation provides a useful phenotypic bioassay for the rapid diagnosis of pollination by staining callose selectively with water soluble aniline blue, which fluoresces in ultraviolet light.

Two basic forms of genetic self-incompatibility are recognized. In systems referred to as sporophytic, recognition and rejection of self-pollen mostly take place on the stigmatic surface. In most plant families, the reaction is controlled by a single locus S possessing a large number of alleles (de Nettancourt, 1977).

The number of alleles can be very high, in *Brassica oleracea*, for example, more than 50 alleles have been reported, and complex interactions exist between S alleles (Beschoner et al, 1995).

In gametophytic systems, rejection generally occurs within the style by heavy callose deposition (Ebert et al, 1989), and the fate of the pollen is determined by its own haploid gametophytic genotype. As exhibited in most plant species, the control is exerted through the action of a single multi-allelic locus (Richards, 1986). Concurrently with these two principal systems, other variants of incompatibility expression have been described.

A phenomenon that has been defined as pseudo-incompatibility was first reported by Darwin (1876). Seeds are obtained after selfing in species that are normally self-incompatible. The functional incompatibility system is transiently broken down but continues to be inherited in the offspring (Richards, 1986).

Late-acting incompatibility has been reviewed by Seavey and Bawa (1986); rejection can occur in the ovary, before or after fertilization. Theoretical approaches to distinguish between postzygotic self-incompatibility and inbreeding depression are developed by these authors. The postzygotic rejection of selfs is often excluded from definitions of self-incompatibility owing to the difficulty of distinguishing such an effect from inbreeding influences (Barrett, 1988).

Quantitative variation in pollination success after crosses using a single donor has

generally been described as partial incompatibility (de Nettancourt, 1977; Mulcahy and Mulcahy, 1985; Waser, 1992).

Some species exhibit a delayed incompatibility system as in *Asclepias syriaca* (Morse, 1994), in which self-pollination strongly reduces the success of the later cross-pollination. Cryptic self-incompatibility has also been found and described as a slower pollen tube growth and higher attrition rates of self-pollen (Cruzan, 1989) or the preferential success of cross-pollen in achieving fertilization when it competes with self-pollen (Lloyd and Schoen, 1992). Interference between male and female function could reduce female success by clogging stigmas with self-pollen and styles or micropyles with self-pollen tubes (Bertin, 1993).

Incompatibility barriers of self-incompatibility systems act essentially at different prezygotic levels and inbreeding depression, which occurs after fusion of gametes, is a postzygotic effect.

The relative weight of both mechanisms, self-incompatibility and inbreeding depression, has been analyzed for some species such as *Amsinckia grandiflora* by Weller and Ornduff (1989, 1991); *Amsinckia douglasiana* by Casper et al (1988); *Campsis radicans* by Bertin et al (1989) or *Aquilegia caerulea* by Montalvo (1992).

It has been shown that oak species are highly self-incompatible (Ducousso et al, 1993) and as in all predominantly outbreeding species we would expect a high level of inbreeding depression (Whisler and Snow, 1992) when self-fertilization occurs.

As stated by Hagman (1975), self-incompatibility in *Quercus* genus species would be due to a gametophytic control of the pollen tube growth in the style.

Indirect studies of the mating system based on genetic analyses of offspring from open pollination in *Q. ilex* (Yacine and Lumaret, 1988), *Q. robur* and *Q. petraea*

(Bacilieri et al, 1994) have shown that these species are nearly strictly allogamous. Self-incompatibility systems are assumed to be present, although not clearly described in most cases. Moreover, genetic differences between offspring using allozyme markers have been shown in *Q. ilex* (Yacine and Lumaret, 1988). This suggests that crosses between individuals of the same location are non-random. This is a common phenomenon in plant populations (Vaughton, 1995) but the mechanisms responsible for such a pattern vary among species.

Even if outbreeding is prevalent in *Quercus* species, a slight deficit in heterozygotes can occur as shown by genetic structure analyses of *Q. macrocarpa* and *Q. gambelii* populations (Schnabel and Hamrick, 1990). This deficit has been explained by structuration within populations (Sork et al, 1993) which induces a Wahlund effect (Ducousso et al, 1993) and by assortative mating.

In this study we have tried to answer the following questions for the *Q. ilex* species: i) If there is evidence of a self-incompatibility system, what are its phenotypic responses? ii) What are the differences in phenotypic response after pollination with different sources of pollen (self-, cross-/single donor, mixed and open pollination)? iii) If non-random crosses occur, we would suspect variation in compatibility between individuals receiving pollen from the same source.

The results may lead to a better understanding of outbreeding, non-random crosses and their consequences for the genetic structure of populations.

MATERIALS AND METHODS

Quercus ilex is a long-lived, monoecious and wind-pollinated species. Its geographic distribution is in the occidental part of the Mediterranean Basin. Flowering occurs in spring. A substantial lag in the flowering period has been observed among trees of the same stand (Michaud et al, 1992). The duration between pollination and fertilization is about 55 to 60 days

(Corti, 1959). Seed maturation occurs in November or December; it requires only a single growth season in contrast to the *Quercus* species of the subgenus *Erythrobalanus*, which require two seasons (Elena-Rossello et al, 1993).

Male flowers are grouped in catkins. They are produced in the basal portion of the stem of the same year or in the distal part of the stem of the previous year. All catkins carry about 20 flowers. One to three female flowers are initiated from axils of leaves produced the same year. Each flower carries six ovules (this trait is stable among all species of *Quercus*), only one ovule becoming seed (Corti, 1959; Mogensen, 1975). Mogensen (1975) stated that in *Quercus* species, the first fertilized ovule suppresses the growth of the others. While the development of the catkins occurs before that of the female flowers, anthesis and female receptivity overlap on the same individual for all individuals used in this experiment.

In a population of *Q. ilex* in the Chrea park, located in the Tellian Mountains in Algeria at an elevation of 950 m (longitude: 2°52' E and latitude 36°27' N), flowering and acorn production have been studied for 5 years (1989–1993). In this study concerning experiments carried out in 1992, 14 individuals were selected as the female parent for their longer flowering time and their relatively large and stable seed production. Individuals that produced predominantly male flowers or had an important production of both female and male flowers were used as pollen source.

Pollination experiments

For each female parent and each pollination type, female flowers were sampled by taking all flow-

ers produced by four 2-year-old twigs, which were bagged before flowering to prevent pollen contamination. We conducted pollination experiments when the female flowers appeared. Each individual was self-pollinated, cross-pollinated using a single donor and pollinated with a mixture of self- and cross-pollen. The cross-pollen used in mixed source and in single donor pollination was the same. For each individual, four twigs carrying open pollinated flowers were bagged after flowering time. For each female parent and each pollination type, 71 to 133 flowers were hand pollinated. Five different sources of pollen were used and each donor was used for more than one recipient (table I). For all donors, pollination was conducted just after the pollen was collected.

For each individual and each treatment, two female flowers were collected at six successive times: 1, 3, 10, 16, 28 and 35 days after pollination. Open-pollinated flowers of the same age were collected at the same time; all flowers were fixed in formalin acetic acid alcohol (1:1:8) and treated with NaOH 8 N for 1 day. Samples were then washed and stained with aniline blue 0.1%.

Using a fluorescence microscope, pollen tube growth was described simultaneously with flower development and i) the number of pollen grains on the stigma and ii) the number of pollen tubes (PT) at five different levels from the stigma to the ovary were counted (fig 1).

Pollen tube attritions were calculated at these different levels (number of pollen tubes not reaching a particular stage). To reduce the effect of pollen load differences between pollinations, we reported all these attritions to the pollen tube number at the upper level (PT1). Thus, the stigmatic attrition is $(PT1-PT2)/PT1$, stylar attrition in the first part of the style is $(PT1-PT3)/PT1$,

Table I. Cross-pollination (Fe: individuals used as female parents, Ma: individuals used as male parents).

Fe →	5	10	11	13	15	17	22	26	27	28	30	37	40	41
Ma ↓														
6							+				+	+	+	+
11					+			+						
26						+			+	+				
37	+			+										
40		+	+											

The individuals are indicated with a number. Four female parents were used also as pollen donor. The same female parents were pollinated with a mixture of self- and cross-pollen from the same single donor used in cross-pollination.

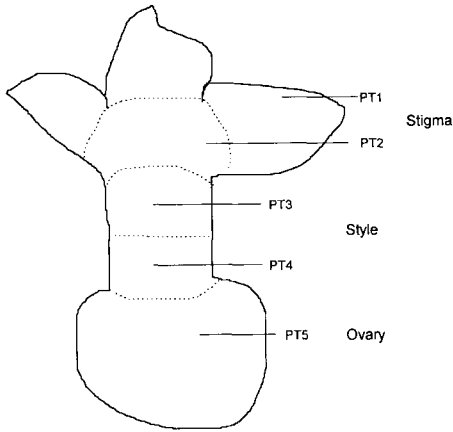


Fig 1. Diagram of holm oak flower showing the five levels at which pollen tubes were numbered: PT1 on stigmatic area, PT2 on the base of the stigma, PT3 on the first part of the style, PT4 on the base of the style and PT5 on the ovary.

stylar attrition in the second part of the style is (PT1-PT4)/PT1 and ovarian attrition is (PT1-PT5)/PT1.

In October, acorns were counted, weighed and tested for their germination rate. Flowers that fell into the bags were also collected and classified into two types: i) aborted flowers or immature flowers that had not yet developed ovules and ii) aborted fruits, characterized by the presence of ovules, one of them always being more developed than the others; fruit abortion has occurred after fertilization. Fruit and aborted flowers were reported to the initial number of flowers carried by the sampled twigs.

Statistic analysis

Comparisons between pollination types

To detect the effect of pollination types and recipients on pollen tube attrition, fruit rate and flower abortion rate, a two-way analysis of variance (ANOVA) was carried out on arcsinus transformed data using the Statgraphics program (STSC, 1991). The Newman-Keuls test was used to compare mean values. For pollen tube attritions, data from open pollinated flowers have not been considered and as many differences

were observed between each harvesting date, all these dates being treated separately.

The relationship between pollen tube attritions on the one hand and fruit and flower abortion rate on the other hand were investigated using a correlation analysis. Each pollination type was treated separately. Correlation analyses were used to investigate the relationship between pollination types for the same variable (pollen tube attritions, fruit and flower abortion rate).

Comparisons between recipients that received pollen from the same single donor

The same procedure was used to detect differences between recipients that received the pollen of the same donor in cross-experiments. A one-way ANOVA was carried out on arcsinus transformed data. A χ^2 test was conducted to investigate the differences between recipients for fruit production.

RESULTS

Flower, fruit development and pollen tube growth

There was a lag between pollen tube growth and the ovule development. Observations of flowers harvested 1 day after pollination showed that most of the pollen tubes were still on the stigmatic surface. Flowers harvested 3 days after pollination showed pollen tubes at the base of the style and those harvested 10 days after pollination showed pollen tubes in the ovary. At this time ovules are not yet developed. Six ovules of the same size were observed in flowers harvested 16 and 28 days after pollination. At 35 days after pollination fertilization has occurred.

Comparison between pollination types

Pollen load, pollen tube growth and pollen tube attrition

The number of pollen grains on the stigma varies from 102 to 353 for the open-poll-

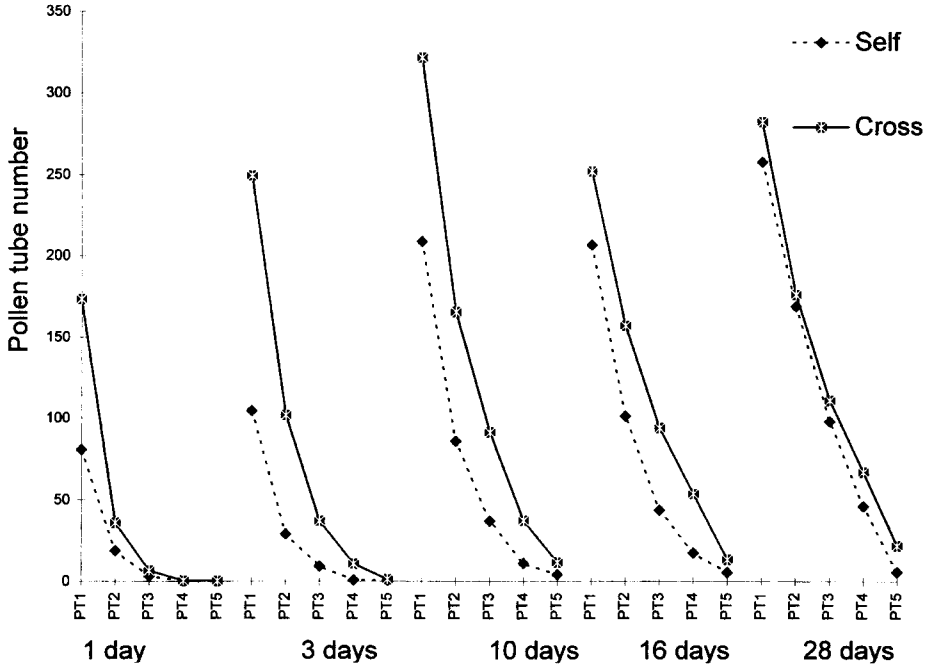


Fig 2. Number of pollen tubes (PT) on the stigma (PT1), on the base of the stigma (PT2), in the first part of the style (PT3), in the second half part of the style (PT4) and in the ovary (PT5) 1, 3, 10, 16 and 28 days after self- and cross-pollination.

nated flowers and from 227 to 422 for hand-pollinated flowers. Differences in the pollen tube number at different levels of the flower is striking between self- and cross-pollinated flowers until 10 days after pollination, with pollen tube growth being slower for selfings (fig 2).

For flowers harvested 1 day after pollination, the ANOVA for pollen tube attritions shows significant differences between recipients but not between pollination types (table II). Interactions between pollination types and recipients is statistically significant for the stigmatic and stylar attrition.

The effect of pollination type on stigmatic attrition and on attrition at the top level of the style is significant for flowers harvested 3 days after pollination. The high-

est attritions are for the selfed and the lowest for the open-pollinated flowers (table III).

For flowers harvested 10 and 16 days after pollination, differences are significant between pollination types for stylar attrition (at the two levels) and between recipients. In all these cases, the highest attrition is for selfed flowers and the lowest for cross-pollinated flowers. For flowers harvested 28 days after pollination, differences between pollination types are for ovarian attrition.

Fruit and flower abortion rate

Variance analysis was conducted for flower abortion rate (early abortion of flowers that have not yet developed ovules), fruit abor-

Table II. The effect of hand-pollination types and recipients on stigmatic, stylar (at the first [1] and at the base [2] level) and ovarian attrition when flowers were harvested 1 day (D1), 3 days (D3), 10 days (D10), 16 days (D16) and 28 days (D28) after pollination.

Source	DF	Stigmatic attrition		Stylar attrition (1)		Stylar attrition (2)		Ovarian attrition	
		F		F		F		F	
D1	Pollination types	2	2.31	0.03	0.44	0.46			
	Recipients	13	8.30***	6.70***	5.08***	5.02***			
	Interactions	39	1.91*	1.98*	1.92*	1.96*			
D3	Pollination types	2	9.20***	5.67**	1.76	0.14			
	Recipient	13	0.89	0.98	1.73	1.30			
	Interactions	39	1.30	1.09	0.92	1.04			
D10	Pollination types	2	2.38	5.99*	10.08***	6.50**			
	Recipient	13	1.74	3.56***	3.73***	5.89***			
	Interactions	39	0.85	1.76	1.25	1.46			
D16	Pollination types	2	2.68	3.75*	3.85*	1.21*			
	Recipient	13	1.78	1.61	1.53	2.49*			
	Interactions	39	0.73	0.97	1.75	1.18			
D28	Pollination types	2	1.30	0.07	0.65	3.75			
	Recipients	13	2.29*	1.86	1.96*	1.57			
	Interactions	39	1.37	1.94*	1.76*	1.07			

DF: degrees of freedom; F: variance ratio; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Table III. Mean of the stigmatic, stylar at the first (1) and at the base (2) level and ovarian attrition when flowers are harvested 1 day (D1), 3 days (D3) 10 days (D10) and 16 days (D16) after pollination.

Pollination types		Stigmatic attrition		Stylar attrition (1)		Stylar attrition (2)		Ovarian attrition	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
D1	Self	76.00	29.73	91.21	26.05	92.85	26.23	92.85	26.23
	Mix	70.12	27.53	91.18	20.24	96.43	18.90	96.43	18.90
	Cross	79.29	21.55	92.61	19.62	96.31	18.88	96.31	18.88
D3	Self	73.09 ^b	23.33	90.17 ^b	20.23	96.11	18.91	96.15	18.9
	Mix	49.13 ^a	17.83	80.43 ^a	13.47	95.53	5.79	99.28	1.50
	Cross	58.03 ^a	22.26	84.70 ^{ab}	11.43	95.28	6.50	99.13	1.83
D10	Self	56.58	27.52	81.74 ^b	16.62	95.51 ^b	8.92	98.57 ^b	3.44
	Mix	46.79	21.76	79.50 ^b	14.45	90.45 ^a	8.82	97.20 ^{ab}	4.99
	Cross	48.57	15.73	72.82 ^a	13.81	88.40 ^a	10.61	96.39 ^a	4.54
D16	Self	44.35	22.81	76.14 ^b	16.42	87.26 ^b	11.79	95.39	5.99
	Mix	36.05	13.85	64.84 ^{ab}	18.65	78.97 ^a	15.53	93.68	9.67
	Cross	37.45	13.73	62.16 ^a	20.53	77.00 ^a	20.99	91.47	19.57
D28	Self	36.18	20.14	62.53	24.55	80.66	21.01	95.08	18.76
	Mix	42.18	13.62	65.66	12.79	79.09	10.91	92.73	5.99
	Cross	41.18	18.70	64.30	13.16	78.80	11.34	92.57	6.17

SD: standard deviation. ^{ab} Values followed by the same letter are not significantly different at the 5% level (Newman-Keul's test).

Table IV. The effect of pollination types (hand [self, mixed, cross] and open pollinations) and recipients on flower abortion rate, fruit abortion rate, fruit rate, seed germination rate and mean weight/fruit.

<i>Source</i>	<i>DF</i>	<i>F</i>
Flower abortion rate		
Poll types	3	5.63**
Recipient	13	3.37***
Fruit abortion rate		
Poll types	3	2.07
Recipient	13	4.41***
Fruit rate		
Poll types	3	9.24***
Recipient	13	2.62*
Seed germination rate		
Poll types	3	11.35***
Recipient	13	1.75
Mean weight/fruit		
Poll types	3	3.56*
Recipient	13	1.34

DF: degrees of freedom; *F*: variance ratio; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

tion rate (late abortion of fruit with fertilized ovules), fruit rate, mean weight/fruit and seed germination rate.

Except for the fruit abortion rate, the effect of pollination type is significant for all variables (table IV). The highest flower abortion rate is observed for the selfed flowers and the lowest rate is for the cross-pollinated flowers (table V). Fruit rate in selfed flowers is also significantly lower than in the other pollination types. Only about 4.23% of the flowers are fertilized when selfed. Seed from selfed flowers have the lowest germination rate and the lowest mean fruit weight.

Female parents do not have the same behaviour with respect to the same pollination type. In table VI, we present results of seed set for each individual (identified with its number) and each pollination type. These results show that individual N°26 did not produce seed from selfed and open-pollinated flowers while fruit rate was 30.00 when flowers were cross-pollinated. For the individuals N°5 and N°37, selfed flowers produced more seeds than cross-pollinated ones. For four individuals (N°37, 5, 13 and 15) fruit rate obtained in open pollination is higher than that obtained in cross-pollination.

Table V. Mean fruit rate, flower and fruit abortion rate, seed germination rate and mean weight/fruit for each pollination type.

	<i>Self</i>		<i>Mix</i>		<i>Cross</i>		<i>Open</i>	
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
Fruit rate	4.24	1.12	15.41	4.20	25.91	4.80	22.24	4.72
Flower abortion rate	34.25	7.05	22.81	4.40	11.61	1.56	19.89	4.40
Fruit abortion rate	7.38	2.43	9.52	2.97	15.66	3.78	10.30	2.92
Seed germination rate	10.93	5.98	40.99	8.27	51.12	6.71	55.12	7.06
Mean weight/fruit	1.87	0.35	2.77	0.26	2.80	0.24	3.17	0.36

SD: standard deviation.

Table VI. Fruit rate for each pollination type (cross single donor, self, mixed and open pollination) and for each female parent indicated by their number and grouped for the same source of cross-pollen and single donor.

No of female parent	Self	Mix	Cross	Open
Donor N°6				
37	3.66	0	0	7.14
22	1.90	14.50	25.14	20.00
41	4.16	10.46	50.00	12.86
40	0	31.42	48.23	7.14
30	0	4.16	24.00	4.28
Donor N°26				
17	4.68	3.57	27.05	15.17
27	8.88	11.56	20.90	28.57
28	4.28	49.23	58.82	47.41
Donor N°37				
5	7.20	5.93	2.29	61.43
13	12.50	5.15	16.66	28.57
Donor N°11				
15	1.29	14.92	6.31	15.71
26	0	1.14	30.00	0
Donor N°40				
10	10.80	44.60	38.66	41.80
11	0	14.92	14.73	21.13

Relationship between pollen, fruit and flower characters

When the different pollination types are treated separately, open and self-pollination show the same results. For these two pollination types, correlation is negative and highly significant between stylar attrition (in the first part of the style) and flower abortion rate (table VII). For open pollination stigmatic attrition is also correlated negatively to flower abortion rate. Fruit abortion rate is negatively correlated with ovarian attrition only when flowers have been out-crossed and fruit rate is positively correlated with attrition at the base of the style only when flowers are pollinated with mixed pollen.

Individuals that have had a higher fruit rate after cross-pollination also have a higher fruit rate after mixed pollination. Correlation is positive and highly significant between the two pollination types ($r = +0.87$, $P = 0.0001$, $N = 14$).

Table VII. Correlations between pollen tube attritions (stigmatic, stylar at the top [1] and at the base [2] level and ovarian) and fruit rate, flower and fruit abortion rate for each pollination type.

		Flower abortion rate	Fruit abortion rate	Fruit rate
Stigmatic attrition	Self	-0.03	-0.38	-0.31
	Mix	-0.05	+0.20	+0.36
	Cross	+0.43	+0.05	-0.21
	Open	-0.72**	-0.01	+0.38
Stylar attrition (1)	Self	-0.60**	+0.24	+0.03
	Mix	-0.18	+0.07	+0.33
	Cross	+0.33	+0.11	-0.28
	Open	-0.68**	+0.38	+0.38
Stylar attrition (2)	Self	-0.25	-0.19	+0.02
	Mix	-0.15	+0.23	+0.56*
	Cross	+0.25	+0.06	-0.17
	Open	-0.28	+0.32	+0.44
Ovarian attrition	Self	-0.03	-0.38	-0.31
	Mix	-0.01	-0.12	+0.09
	Cross	+0.10	-0.55*	-0.40
	Open	+0.06	+0.05	-0.39

*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Comparison between recipients that received pollen from the same single donor

Female parents receiving the same pollen genotype show different levels of compatibility. There are significant differences in pollen tube attritions and in seed set.

Pollen tube attritions

For four donors, the stigmatic attrition shows significant differences between recipients

for flowers harvested 1 day after pollination (table VIII). Significant differences between recipient in stylar (at one or both of the two studied levels) and ovarian attrition is shown depending on the donor and on the harvesting date.

Seed set

The χ^2 test used to compare seed set between recipient and the same single donor shows significant differences for all donors (table IX).

Table VIII. Effect of recipients (in single cross-pollination) on stigmatic, stylar (at the first [1] and at the base [2] level) and ovarian attrition for flowers harvested 1 day (D1), 3 days (D3) 10 days (D10), 16 days (D16) and 28 days (D28) after pollination.

Donor	N°6		N°26		N°11		N°37	N°40
	DF	F	DF	F	DF	F	F	F
D1								
Stigmatic attrition	4	1.81	2	242.07**	1	230.22**	5.46	1031**
Stylar attrition (1)	4	1.44	2	1.00	1	/	0.781	/
Stylar attrition (2)	/	/	2	/	1	/	/	/
Ovarian attrition	/	/	2	/	1	/	/	/
D3								
Stigmatic attrition	4	16.83*	2	0.99	1	60.69*	0.05	1.6
Stylar attrition (1)	4	121.95*	2	321.35**	1	4.75	0.02	0.16
Stylar attrition (2)	4	433**	2	/	1	5.52	0.72	0.60
Ovarian attrition	4	59.66*	2	/	1	18.03*	/	/
D10								
Stigmatic attrition	4	0.24	2	0.35	1	0.00	0.57	1.82
Stylar attrition (1)	4	506.79**	2	0.51	1	1.75	5.76	1.21
Stylar attrition (2)	4	973.49**	2	0.06	1	0.30	0.81	18.81*
Ovarian attrition	4	834**	2	1	1	2.58	2.97	29.14*
D16								
Stigmatic attrition	4	229.13**	2	0.57	1	0.14	10.93	0.00
Stylar attrition (1)	4	112.44**	2	19.62*	1	2.32	20.50*	5.80
Stylar attrition (2)	4	133.25**	2	4.46	1	17.21*	8.42	20.30*
Ovarian attrition	4	/	2	0.68	1	0.12	0.14	3.01
D28								
Stigmatic attrition	4	0.63	2	2.70	1	0.39	2.41	1.60
Stylar attrition (1)	4	39.98*	2	3.18	1	0.01	10.63	2.10
Stylar attrition (2)	4	43.92*	2	0.68	1	0.48	5.79	1.57
Ovarian attrition	4	43.92*	2	0.08	1	1.61	4.12	0.05

DF: degrees of freedom; F: variance ratio; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Table IX. χ^2 values for fruit production for different recipients receiving pollen from the same donor.

Donor	N°6	N°26	N°11	N°37	N°40
DF	4	2	1	1	1
χ^2	63.20**	54.57**	16.52**	18.02**	12.71**

DF: degrees of freedom; ** $P < 0.01$.

DISCUSSION

Phenotypic responses of the self-incompatibility system

The significant differences between pollination types for stigmatic, stylar and ovarian attrition are essentially due to slower and lower pollen germination of the self-pollen. This has both quantitative and qualitative consequences on seed production (no or nearly no production and a low germination rate for seeds from self-pollination). Differences in pollen attrition between pollination types provided evidence of pollen-style interactions. These interactions have been shown in studies of self-incompatibility or when self-pollen is compatible but disadvantaged (Levin, 1975; Casper et al, 1988; Montalvo, 1992) or for some species in which the pollen exhibits a varying performance depending upon the recipient (Gawel and Robacker, 1986; Waser et al, 1987; Quesada et al, 1993).

Differences in pollen performance are probably determined by genetic similarity or dissimilarity between the maternal sporophyte and the microgametophyte.

Biochemical and molecular studies have shown that haploid microgametophytes transcribe and translate a large portion of their genome and that the vast majority of the genes expressed by pollen are also expressed in the sporophytic (diploid) stage of the life cycle (Schlichting et al, 1990).

Even if seed production is very low in selfing, there are some differences in phe-

notypic responses among the 14 individuals. Four of them show no production and have a high flower abortion rate, so they are completely self-incompatible, while six of them have produced fruit that has not germinated. For the others, germination ability varies among individuals and the mean fruit weight is low.

The existence of a self-incompatibility system together with a genetic load gives rise to a low vigour of selfed offspring (Johnston, 1992; Montalvo, 1992). Predominantly outcrossing species commonly exhibit substantial inbreeding depression from the beginning of the life cycle (Barrett and Harder, 1996), so in natural conditions, we expect a low survival probability for self-fruit after germination.

The individual N°26 did not produce fruit either in self- or open pollination (in open pollination, pollen load is not a limiting factor), whereas it has a high fruit rate in cross-pollination.

If self-pollen or incompatible out pollen reaches a stigma before any compatible out pollen arrives, incompatible pollen may have an important effect on flowers, which abort early (ie, before ovule development), leading to a decreased seed production. This hypothesis is supported by the negative and significant correlation between stigmatic and stylar attrition (at the first level) and flower abortion rate both in self- and open pollinations. Thus, a high proportion of self-pollen tubes reaching the style (at the top or the base level) stops early development of flowers. The same situation has been

described for *Asclepias exalta* by Broyles and Wyatt (1993), where self-pollen decreases the probability that ovaries become fruit when mixed with compatible out pollen and for *Asclepias syriaca* by Morse (1994). Early abortion of flowers is a common trait among plant species; it has often been attributed to resource limitations (Stephenson, 1981; Devlin and Stephenson, 1987; Stöcklin and Favre, 1994). Regard-

ing our results, for holm oak, we suggest that incompatible pollen is one of the factors that consistently give rise to early abortion of flowers (fig 3A).

Even if there is a continuous variation among traits in phenotypic responses of self-incompatibility, its expression is predominant at the prezygotic level and suggests a control by pollen-genotype and pollen-pistil interactions. As suggested by Hagman

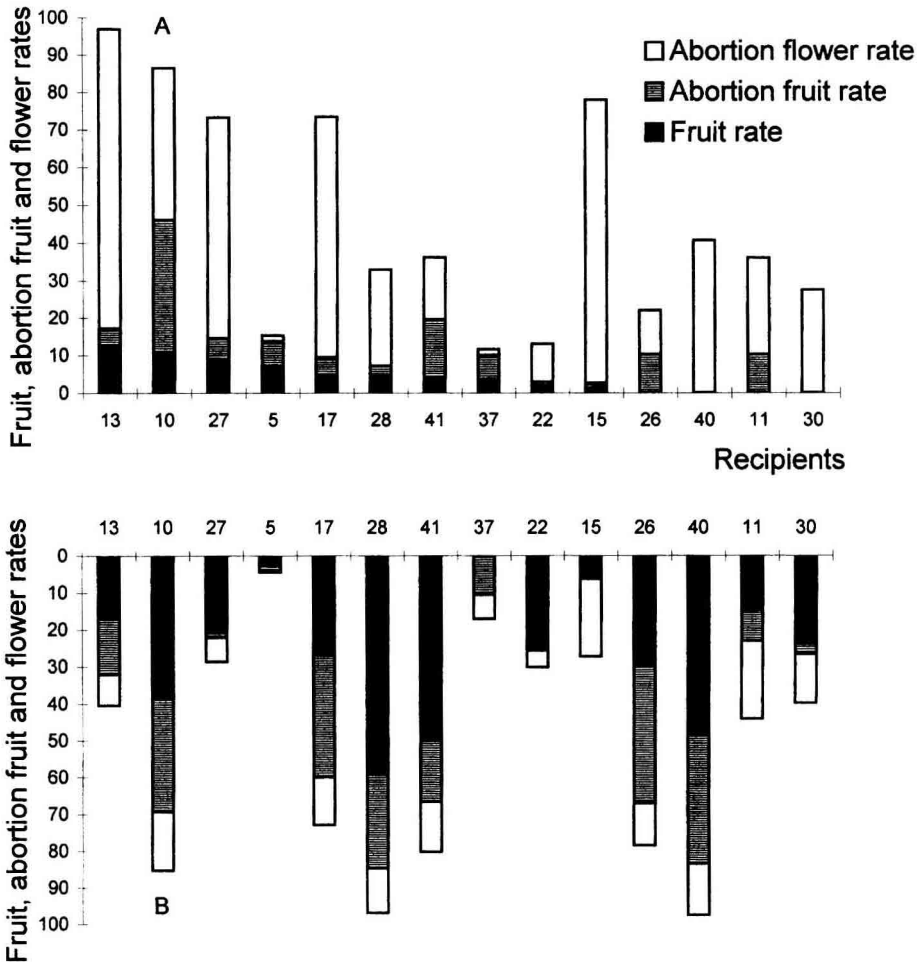


Fig 3. Fruit rate, abortion fruit and flower rate for each recipient: **A**) after self-pollination, **B**) after cross-pollination.

(1975) for oak species, it could be a gametophytic incompatibility system. Self-pollen should be most often incompatible and out pollen should be compatible, partially compatible or even incompatible according to the genotype composition of the pollen tree.

It is difficult to draw conclusions from our experiments about the relative weight of environmental and genetic factors acting on the amount of variability on the response to self-incompatibility. Thus, self-incompatibility could be complete but sometimes broken down by environmental factors as in pseudo self-incompatibility.

Different pollination types and reproductive success

Pollination using a single donor can result in higher seed production than open pollination. When open pollination leads to lower seed set than hand pollination, results have been attributed to pollen limitation, which has been demonstrated in several entomophilous plant studies (Bierzychudek, 1981; Walsh and Charlesworth, 1992; Campbell and Halama, 1993). In our experiments, pollen is not the limiting factor and differences are essentially due to the presence of incompatible pollen on the stigmas, which decreases fruit production. Evidence of inhibition of cross-pollen by self-pollen has been also shown for *Asclepia syriaca* by Morse (1994) and for *Asclepia exalta* by Broyles and Wyatt (1995). In natural conditions low seed set is determined both by pollen efficiency (quantity and quality) and maternal resource availability (Broyles and Wyatt, 1995).

Some individuals (N°37, 5, 15 and 13) have a higher production in self- or open pollination than in cross-pollination using a single donor. These results could be attributed to a self-incompatibility system and/or to outbreeding depression. It appears from these results that even if pollen load

is a limiting factor and self-pollen inhibits the out pollen performance, out pollen quality (its genotype) is a prevalent factor on quality and quantity of offspring.

In self-pollination, pollen–pistil interaction occurs when pollen tubes reach the style, which results in the stopping of ovule development. In mixed pollination, the more pollen tubes stopping their growth before the base of the style, the more seed will be produced. Results obtained in mixed pollination are generally intermediate between those obtained in self- and cross-pollination. As mentioned earlier and reported by Seavey and Carter (1994), the simultaneous presence of self- and cross-pollen on the stigmas will significantly diminish fruit production.

In cross-pollination (using a single donor), the results of the correlation analysis show that if the proportion of pollen tubes reaching the ovary is higher, this results in a higher fruit abortion rate. Therefore, when cross-pollination occurs without the presence of self-pollen, abortion occurs after fertilization (late abortion). Differences among fruit abortion rate could be attributed to the incompatibility system, to inbreeding depression (if the individuals are genetically related) or to maternal resource availability.

In long-lived plant species, when allogamy is the prevalent mating system or when reproduction is partially asexual, we can expect a high level of genetic load. Differences in seed set between cross-pollinated individuals could therefore be attributed only to the genetic load. If this is true we would expect a significant positive correlation for seed set between self- and cross-pollination. This hypothesis has been proposed by Krebs and Hancock (1990) and tested on *Vaccinum corymbosum*. Regarding our results, evidence of a self-incompatibility system could result in observed differences in seed set between cross-pollinated individuals. Nevertheless, it is difficult

to separate at this state incompatibility from inbreeding depression.

Individuals with high seed production in outcross pollination also have a high fruit abortion rate (fig 3B), correlation is positive and highly significant. For these individuals, late abortion could be related to resource limitation.

A self-incompatibility system could result in quantitative variation of compatibility with the cross-pollen; this is shown in pollination experiments using the same donor for several recipients.

Effect of recipients pollinated with the same single donor on pollen tube attritions and seed set

There are significant differences between the recipient for stigmatic, stylar and ovarian attrition and for fruit rate when the same single pollen donor is used. As stated by several authors (Stephenson and Bertin, 1983; Schlichting and Devlin, 1989; Snow, 1990), pollen performance of a particular genotype depends on the recipient plant. These results could be interpreted as a variable level of compatibility between different genotypes attributed to the existence of a self-incompatibility system.

In conclusion, this study clearly shows the role of self-pollen in early abortion of flowers and its inhibitory effect on the cross-pollen and the existence of a self-incompatibility system with various mechanisms acting with variable intensity at different levels.

These mechanisms are prevalent at the prezygotic level (stigmatic and/or stylar level), whereas fruit abortion after cross-pollination occurs later. When pollination is qualitatively and quantitatively effective, the regulation of seed production related to resource availability acts by late abortion of fruit (after fertilization).

Variation in phenotypic responses can be partly attributed to environmental factors and to specific characters of each individual such as the age of the tree, the position of the flowers in the tree, resource allocation and sex ratio.

The genetic differences among offspring of individuals of the same population which are reported for this species (Yacine and Lumaret, 1988) and which are attributed to a wide lag in flowering time and to differences in sex allocation between individuals, could be partly due to the existence of a self-incompatibility system.

ACKNOWLEDGMENTS

We wish to thank Dr J Cuguen, H Van Dijk, R Jean, I Till-Bottraud, P Vernet and the reviewers for their helpful comments on this manuscript. This work has been supported by the Institut national agronomique (Algiers), the Laboratoire d'évolution et de systématiques végétales d'Orsay, université de Paris-Sud and the Laboratoire de génétique et d'évolution des populations végétales de Lille, université des sciences et technologies de Lille-I.

REFERENCES

- Bacilieri R, Ducouso A, Kremer A (1994) Genetic, morphological, ecological and phenological differentiation between *Quercus petraea* (Matt) Liebl and *Quercus robur* L in a mixed stand of France. *Silvae Genet* 44, 1-10
- Barrett SCH (1988) The evolution, maintenance and loss of self-incompatibility systems. In: *Plant Reproductive Ecology* (J Lovett Doust, L Lovett Doust, eds), Oxford University Press, New York, NY, USA, 98-124
- Barrett SCH, Harder LD (1996) Ecology and evolution of plant mating. *Tree* 11, 73-79
- Bertin RI (1993) Incidence of monoecy and dichogamy in relation to self-compatibility in angiosperms. *Am J Bot* 80, 557-560
- Bertin RI, Barne C, Guttman SI (1989) Self sterility and cryptic self fertility in *Campsis radicans* (Boraginaceae). *Bot Gaz* 150, 397-403
- Beschoner M, Plumper B, Odenbach W (1995) Analysis of self-incompatibility interactions in 30 resynthesized *Brassica napus* lines. I. Fluorescence

- microscopic studies. *Theor Appl Genet* 90, 665-670
- Bierzychudek P (1981) Pollinator limitation of plant reproductive effort. *Am Nat* 117, 838-840
- Broyles SB, Wyatt R (1993) The consequences of self pollination in *Asclepias exalta*. A self incompatible milkweed. *Am J Bot* 80, 41-44
- Broyles SB, Wyatt R (1995) A reexamination of the pollen donation hypothesis in an experimental population of *Asclepias exalta*. *Evolution* 49, 89-99
- Campbell DR, Halama KJ (1993) Resource and pollen limitations to lifetime seed and pollen in a natural plant population. *Ecology* 74, 1043-1051
- Casper BB, Sayigh LS, Lee SS (1988) Demonstration of cryptic incompatibility in distylous *Amsinckia douglasiana*. *Evolution* 42, 248-253
- Corti R (1959) Ricerche sul ciclo riproduttivo di specie del genere *Quercus* delle flora italiana. IV. Osservazioni sulla embriologia sul ciclo riproduttivo in *Q ilex* L. *Ann Accad Ital Sci For* 8, 19-42
- Cruzan MB (1989) Pollen tube attrition in *Erythronium grandiflorum*. *Am J Bot* 76, 562-570
- Darwin C (1876) *The Effects of Cross and Self Fertilisation in the Vegetable Kingdom*. Murray, London, UK
- de Nettancourt D (1977) Incompatibility in angiosperms. In: *Monographs on Theoretical and Applied Genetics*. No 3. (R Frankel, GAE Gall, HF Linskens, eds), Springer-Verlag, Berlin, Germany, 223 p
- Devlin B, Stephenson AG (1987) Sexual variations among plant of a perfect flowered species. *Am Nat* 130, 199-218
- Ducouso A, Michaud H, Lumaret R (1993) Reproduction and gene flow in the genus *Quercus* L. *Ann Sci For* 50 (suppl 1), 91s-106s
- Dumas C, RB Knox (1983) Callose and determination of pistil viability and incompatibility. *Theor Appl Genet* 67, 195-219
- Ebert PR, Anderson MA, Bernatzky R, Altschuler M, Clarke AE (1989) Genetic polymorphism of self incompatibility in flowering plants. *Cell* 56, 255-262
- Elena-Rossello JA, de Rio JM, Garcia Valdecantos JL, Santamaria IG (1993) Ecological aspects of the floral phenology of the cork-oak (*Q suber* L): why do annual and biennial biotypes appear? *Ann Sci For* 50 (suppl 1), 114s-121s
- Gawel NJ, Robacker CD (1986) Effects of pollen style interaction on the pollen tube growth of *Gossypium hirsutum*. *Theor Appl Genet* 72, 84-87
- Hagman M (1975) Incompatibility in forest trees. *Proc R Soc Lond [Biol]* 188, 313-326
- Hamilton MB, Mitchell-Olds T (1994) The mating system and relative performance of selfed and outcrossed progeny in *Arabis fecunda* (Brassicaceae). *Am J Bot* 81, 1252-1256
- Heslop-Harrison J (1983) Self incompatibility: phenomenology physiology. *Proc R Soc Lond [Biol]*
- Heslop-Harrison J, Heslop-Harrison Y, Knox RB (1973) The callose rejection reaction: a new bioassay for incompatibility in *Cruciferae* and *Compositae*. *Inc New* 3, 75-76
- Johnston MO (1992) Effects of cross and self fertilization on progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. *Evolution* 46, 688-702
- Krebs SL, Hancock JF (1990) Early acting inbreeding depression and reproductive success in the high-bush blueberry, *Vaccinium corymbosum* L. *Theor Appl Genet* 79, 825-832
- Levin DA (1975) Gametophytic selection in Phlox. In: *Gamete Competition in Plants and Animals*. North Holland, Amsterdam, the Netherlands, 207-217
- Lloyd DG, Schoen DJ (1992) Self and cross-fertilisation in plants. *Int J Plant Sci* 153, 358-369
- Martin FW (1959) Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technol* 34, 125-128
- Michaud H, Lumaret R, Romane F (1992) Variation in the genetic structure and reproductive biology of holm oak populations. *Vegetatio* 99-100, 107-113
- Mogensen HL (1975) Ovule abortion in *Quercus* (Fagaceae). *Am J Bot* 62, 160-165
- Montalvo AM (1992) Relative success of self and outcross pollen comparing mixed and single donor pollinations in *Aquelegia caerulea*. *Evolution* 46, 1181-1198
- Morse DH (1994) The role of self-pollen in the female reproductive success of common Milkweed (*Asclepias syriaca*: *Asclepiadaceae*). *Am J Bot* 8, 22-330
- Mulcahy DL, Mulcahy GB (1985) Gametophytic self incompatibility or the more things change. *Heredity* 54, 139-144
- Quesada M, Winsor JA, Stephenson AG (1993) Effects of pollen competition on progeny performance in a heterozygous cucurbit. *Am Nat* 142, 694-706
- Richards AJ (1986) *Plant Breeding Systems*. Allen and Unwin, London, UK, 259 p
- Schlichting CD, Devlin B (1989) Male versus female reproductive success in the hermaphroditic plant *Phlox drummondii*. *Am Nat* 133, 212-227
- Schlichting CD, Stephenson AG, Small LE (1990) Pollen loads progeny vigor in Cucurbita pepo: the next generation. *Evolution* 44, 1358-1372
- Schnabel A, Hamrick JL (1990) Comparative analysis of population genetics structure in *Quercus macrocarpa* and *Quercus gambelii* (Fagaceae). *Syst Bot* 15, 240-251
- Seavey RS, Bawa SF (1986) Late acting self-incompatibility in angiosperms. *Bot Rev* 52, 195-219
- Seavey SR, Carter SK (1994) Self-sterility in *Epilobium obcordatum* (Onagraceae). *Am J Bot* 81, 331-338

- Snow AA (1990) Effects of pollen load size and number of donors on sporophyte fitness in wild radish (*Raphanus raphanistrum*). *Am Nat* 136, 742-758
- Sork VL, Huang S, Wiener E (1993) Macrogeographic and fine-scale in a North American oak species, *Quercus rubra* L. *Ann Sci For* 50 (suppl 1), 261s-270s
- Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. *Annu Rev Ecol Syst* 12, 253-279
- Stephenson AG, Bertin R (1983) Male competition, female choice and sexual selection in plants. In: *Pollination Biology* (L Real, ed), Academic Press, Orlando, FL, USA, 110-149
- Stöcklin J, Favre P (1994) Effects of plant size and morphological constraints on variation in reproductive components in two related species of *Epilobium*. *J Ecol* 82, 735-746
- Vaughton G (1995) No evidence for selective abortion in the Australian shrub *Grevillea barklyana* (*Proteaceae*). *Int J Plant Sci* 156, 417-424
- Walsh NE, Charlesworth D (1992) Evolutionary interpretations of differences in pollen tube growth rates. *Q Rev Biol* 67, 19-36
- Waser NM (1992) Population structure, optimal outbreeding and assortative mating in angiosperms. In: *The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives* (NW Thornhill, ed), University of Chicago Press, Chicago, IL, USA, 1-13
- Waser NM, Price MV, Montalvo AM, Gray RN (1987) Female choice in a perennial herbaceous wildflower, *Delphinium nelsonii*. *Evol Trends Plants* 1, 29-33
- Weller SG, Ornduff R (1989) Incompatibility in *Amsinckia grandiflora* (*Boraginaceae*): distribution of callose plugs and pollen tubes following inter and intra crosses. *Am J Bot* 76, 27-282
- Weller SG, Ornduff R (1991) Pollen tube growth and inbreeding depression in *Amsinckia grandiflora* (*Boraginaceae*). *Am J Bot* 78, 801-804
- Whisler SL, Snow AA (1992) Potential for the loss of self incompatibility in pollen limited populations of mayapple (*Podophyllum peltatum*). *Am J Bot* 79, 1273-1278
- Yacine A, Lumaret R (1988) Distribution spatiale des génotypes dans une population de chêne vert (*Quercus ilex* L), flux génique et régime de reproduction. *Genet Sel Evol* 20, 181-198