

A one-locus model of androdioecy with two homomorphic self-incompatibility groups: expected vs. observed male frequencies

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Abstract

Androdioecy, the occurrence of males and hermaphrodites in a single population, is a rare breeding system because the conditions for maintenance of males are restrictive. In the androdioecious shrub *Phillyrea angustifolia*, high male frequencies are observed in some populations. The species has a sporophytic self-incompatibility (SI) system with two self-incompatibility groups, which ensures that two groups of hermaphrodites can each mate only with the other group, whereas males can fertilize hermaphrodites of both groups. Here, we analyse a population genetic model to investigate the dynamics of such an androdioecious species, assuming that self-incompatibility and sex phenotypes are determined by a single locus. Our model confirms a previous prediction that a slight reproductive advantage of males relative to hermaphrodites allows the maintenance of males at high equilibrium frequencies. The model predicts different equilibria between hermaphrodites of the two SI groups and males, depending on the male advantage, the initial composition of the population and the population size, whose effect is studied through stochastic simulations. Although the model can generate high male frequencies, observed frequencies are considerably higher than the model predicts. We finally discuss how this model may help explain the large male frequency variation observed in other androdioecious species of Oleaceae: some species show only androdioecious populations, as *P. angustifolia*, whereas others show populations either completely hermaphrodite or androdioecious.

Introduction

Androdioecy is a mating system, where males and hermaphrodites coexist in a population. Androdioecy is known from a small number of species and its rarity is assumed to be caused by restrictive conditions for the maintenance of males along with hermaphrodites. Initial models (Lewis, 1941; Lloyd, 1975; Charlesworth & Charlesworth, 1978) suggested that (i) because

males can transmit genes only via pollen, males are maintained in a population if the fitness through male reproduction is at least twice as high in males as in hermaphrodites; (ii) as a consequence of this necessity for a large male advantage, male frequencies in populations are expected to be low, reaching 0.5 only when male fertility is infinite relative to hermaphrodites (i.e. when the species is effectively dioecious). In the latter case, the male function of hermaphrodites is either poor (which is called cryptic or leaky dioecy, Charlesworth, 1984) or zero and the species is dioecious. Only four animal genera and eight plant species have been confirmed as stably androdioecious (Pannell, 2002; Weeks *et al.*, 2006), most of them clearly derived from dioecious ancestors by the evolution of

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male function in females, providing reproductive assurance.

Until recently, the mating system of *P. angustifolia*, a wind-pollinated member of the Oleaceae, remained puzzling because female-sterile individuals are observed at frequencies as high as 0.5 in natural populations, and sometimes even higher (Lepart & Dommée, 1992). Saumitou-Laprade *et al.* (2010) found that *P. angustifolia* hermaphrodites have two homomorphic self-incompatibility (SI) groups. Hermaphrodites of a given SI group can only sire seeds of hermaphrodites from the other SI group, whereas males are compatible with both SI hermaphrodite groups. Thus, *P. angustifolia* males can sire twice as many progeny as hermaphrodites of either SI group, potentially offsetting their reproductive disadvantage due to the loss of the female function.

The existence of two self-incompatible groups in hermaphrodites suggests sporophytic diallelic determination of self-incompatibility (Saumitou-Laprade *et al.*, 2010). Pannell & Korbecka (2010) used Lloyd's (1975) phenotypic model, assuming that the relative siring success of males is doubled compared with hermaphrodites to show that, if the hermaphrodites are incapable of selfing, the equilibrium frequency of males is higher than the value $(K - 2)/(2K - 2)$ when there are no SI groups (Lloyd, 1975), where K is their male fertility relative to that of hermaphrodites. With two SI hermaphrodite groups, the equilibrium frequency is increased to $(K - 1)/(2K - 1)$, and males are maintained in the population if the male advantage relative to hermaphrodites is higher than 1, rather than 2 without SI groups. For $K = 1.8$, the expected frequency of males is 0.33 (Pannell & Korbecka, 2010), closer to the observations in Spanish populations than without SI groups.

The equilibrium frequencies of hermaphrodites of the two SI groups remain to be investigated. A model explicitly taking into account the genetic determination of the sex and self-incompatibility phenotypes is not expected to give different expected equilibrium phenotypic frequencies (Lloyd, 1977). However, a genetically explicit model is necessary to analyse the allele frequency dynamics of the SI alleles (i.e. of the two hermaphrodite SI groups). In a two-allele sporophytic system, the alleles' frequencies depend on their dominance: the equilibrium frequencies of the recessive and dominant alleles are, respectively, 0.75 and 0.25, in deterministic or stochastic models (Bateman, 1952; Billiard & Tran, 2012). Dominance also affects alleles' invasion probability and their residence time in finite populations (e.g. Billiard *et al.*, 2007). In metapopulation models of androdioecious species, the frequency of males depends on the genetic determination of sex phenotype: when the female sterility mutation is dominant, males are expected to be more frequent than when it is recessive (Pannell, 2002).

Recessive alleles' frequencies are more sensitive to random variation than dominant ones because recessive mutations are only expressed in homozygotes and blind for selection in heterozygotes (Charlesworth & Charlesworth, 2010). The importance of this effect has for instance been shown when comparing SI expected vs. observed alleles' frequencies in a sporophytic self-incompatible species (Llaurens *et al.*, 2008). Clearly, therefore, the effect of genetic drift should be taken into account when attempting to assess whether the hypothesis outlined above can explain observed male frequencies in *P. angustifolia* populations across the species range.

The genetic determination we assumed in our model is that a single locus determines both self-incompatibility and sex phenotypes. This is motivated by the results of Saumitou-Laprade *et al.* (2010) in which maleness is transmitted only by males (showing that female sterility is nuclear and dominant) and is also completely linked with pollen compatibility. We investigate the conditions for the transition from hermaphroditism to androdioecy, confirm the predicted expected frequencies of males and hermaphrodites in natural populations for varying male selective advantages (following Pannell & Korbecka, 2010) and, in addition, we determine the equilibrium frequencies of the SI alleles and the stability of the equilibria. We also performed simulations in finite populations to investigate the effect of drift on the sex and self-incompatibility polymorphism, as well as on the distribution of male frequencies, which we compared with published data from natural populations of *P. angustifolia*.

Model

We assumed an infinite unstructured population of diploid individuals, with two SI hermaphrodite groups, denoted by H_a and H_b , and a male phenotype, M . An ovule produced by a H_a hermaphrodite can be fertilized only by pollen produced by H_b or M individuals, and an H_b ovule only by a pollen produced by H_a or M individuals. We assumed that these phenotypes are determined by a single locus with three alleles S_1 , S_2 and S_3 . S_1 is recessive to S_2 and S_3 , and S_2 to S_3 . Allele S_3 is assumed to be dominant and to determine female sterility. Hence, the S_1S_3 and S_2S_3 genotypes are males, and can be produced only by crosses between hermaphrodites and males. The S_1S_1 genotypes have the H_a phenotype, and S_1S_2 and S_2S_2 have both the H_b phenotype. The S_3S_3 genotype cannot be produced under our assumptions. The notation and the compatibility of crosses are summarized in Fig. 1.

We assumed that H_a and H_b hermaphrodites produce the same large numbers of ovules and pollen grains, and that males may produce more pollen than hermaphrodites, denoting by K the ratio of the numbers of pollen

Phenotypes			Pollen donor										
			Genotypes		Herma. H_a		Herma. H_b		Male M				
			Alleles		S_1S_1		S_1S_2		S_2S_2	S_2S_3		S_1S_3	
			S_1	S_1	S_2	S_2	S_2	S_2	S_3	S_1	S_3		
Stigma recipient	Herma. H_a	S_1S_1	S_1	Incompatible	S_1S_1	S_1S_2	S_1S_2	S_1S_2	S_1S_3	S_1S_1	S_1S_3		
					H_a	H_b	H_b	H_b	[M]	H_a	[M]		
					1/2	1/2	1	1/2	1/2	1/2	1/2		
	Herma. H_b	S_1S_2	S_1	Incompatible	S_1S_1	Incompatible		S_1S_2	S_1S_3	S_1S_1	S_1S_3		
					H_a			H_b	[M]	H_a	[M]		
								1/2			1/4	1/4	1/4
S_1S_2		H_b	S_2S_2	S_2S_3	S_1S_2			S_2S_3					
				1/2			H_b	[M]	H_b	[M]			
							1/4	1/4	1/4	1/4			
	S_2S_2	S_2		S_1S_2			S_2S_2	S_2S_3	S_1S_2	S_2S_3			
				H_b			H_b	[M]	H_b	[M]			
				1			1/2	1/2	1/2	1/2			

Fig. 1 Genetic control of breeding system in *Phillyrea angustifolia* under a one-locus model with two sporophytic self-incompatibility groups, as suggested in Saumitou-Laprade *et al.* (2010).

grains produced by males vs. hermaphrodites. If males produce more pollen than hermaphrodites, $K > 1$. We denote the frequency of the S_iS_j genotype in the population by x_{ij} . We denote by $X = (x_{11}, x_{12}, x_{22}, x_{13}, x_{23})$, the vector of genotype frequencies, and by $P = (p_1, p_2, p_M)$, the vector of phenotype frequencies, with $p_1 = x_{11}$, $p_2 = x_{12} + x_{22}$, $p_M = x_{13} + x_{23}$ for the respective frequencies of the H_a, H_b and M phenotypes. Finally, $A = (a_1, a_2, a_3)$ is the vector of the frequency of the S_1, S_2 and S_3 alleles, with $a_1 = x_{11} + 1/2x_{12} + 1/2x_{13}$, $a_2 = x_{22} + 1/2x_{12} + 1/2x_{23}$ and $a_3 = 1/2x_{13} + 1/2x_{23}$.

We computed the genotype frequency changes over a generation assuming frequency-dependent selection on the SI types occurring either only through male reproduction (Wright's model, in which all ovules are assumed to be fertilized) or both male and female reproduction, which can include pollen limitation (fecundity selection, where frequency-dependent selection is stronger, because some ovules receive no compatible pollen and are not fertilized), which can alter the dynamics and equilibrium values of genotype and allele frequencies in self-incompatible systems (Vekemans *et al.*, 1998; Billiard *et al.*, 2007). We analysed the stability of all possible equilibria, especially those without males, which allow us to determine the male advantage K under which the male

allele S_3 can invade the population when rare, thus making a transition from hermaphroditism to androdioecy possible.

Selection through male reproduction only

The genotype frequencies in the next generation, denoted by x'_{ij} are given by

$$\begin{cases} x'_{11} = \frac{1}{\sigma} \left(\frac{x_{11} x_{12} + Kx_{13}}{2\theta_1} + \frac{x_{12} 2x_{11} + Kx_{13}}{4\theta_2} \right) \\ x'_{12} = \frac{1}{\sigma} \left(\frac{x_{11} x_{12} + 2x_{22} + Kx_{23}}{2\theta_1} + \frac{x_{12} 2x_{11} + Kx_{13} + Kx_{23}}{4\theta_2} + \frac{x_{22} 2x_{11} + Kx_{13}}{2\theta_3} \right) \\ x'_{22} = \frac{1}{\sigma} \left(\frac{x_{22} Kx_{23}}{2\theta_3} + \frac{x_{12} Kx_{23}}{4\theta_2} \right) \\ x'_{13} = \frac{1}{\sigma} \left(\frac{x_{11} Kx_{13} + Kx_{23}}{2\theta_1} + \frac{x_{12} Kx_{13} + Kx_{23}}{4\theta_2} \right) \\ x'_{23} = \frac{1}{\sigma} \left(\frac{x_{22} Kx_{13} + Kx_{23}}{2\theta_3} + \frac{x_{12} Kx_{13} + Kx_{23}}{4\theta_2} \right), \end{cases} \tag{1}$$

where $\sigma \equiv x_{11} + x_{12} + x_{22}$ is the total quantity of ovules produced by the population, and $\theta_1 \equiv x_{12} + x_{22} + Kx_{13} + Kx_{23}$, $\theta_2 = \theta_3 \equiv x_{11} + Kx_{13} + Kx_{23}$ are, respectively, the total amounts of compatible pollen available for the x_{11}, x_{12} and x_{22} genotypes. Note that $\theta_2 = \theta_3$ because the S_1S_2 and S_2S_2 genotypes both have the same H_b phenotype.

Selection through both male and female reproduction: pollen limitation

We also considered the case with pollen limitation, meaning that there is selection both through male and female reproductive traits. We assumed that, for a given genotype, among all the ovules it produced, only a proportion is fertilized, which we assumed to be equal to the frequency of compatible pollen in the pollen cloud. For instance, the proportion of fertilized ovules produced by hermaphrodites S_2S_2 is equal to $x_{11} + Kx_{13} + Kx_{23}$. The genotype's frequency changes can thus be computed with selection through both male and female reproduction using the same equations as previously, but with $\theta_1 = \theta_2 = \theta_3 = 1$ and with

$$\sigma = 2x_{11}(x_{12} + x_{22}) + K(x_{13} + x_{23})(x_{11} + x_{12} + x_{22}) \quad (2)$$

which is the total quantity of ovules effectively fertilized.

The equilibria and their stability

For both models, we found the equilibria that are possible. We define the vectors \hat{X} , \hat{A} and \hat{P} whose elements are the equilibrium genotype, allele and phenotype frequencies respectively. We focus on biologically realistic equilibria, $x_{ij} > 0$ and $x_{ij} < 1$. We then performed a classical stability analysis for each equilibrium of interest, using the sign of leading eigenvalues of the Jacobian matrix: if positive, the equilibrium is unstable (Otto & Day, 2007). When the leading eigenvalue was zero, we performed deterministic numerical analyses by introducing a rare genotype and finding the conditions for invasion. To determine the conditions under which one allele can invade a population, we computed the eigenvalue at the equilibrium where this allele is not present in the population, and we analysed the range of the parameters where the eigenvalue is negative or positive: when positive, the allele can invade.

Simulations of genetic drift in finite populations

We performed computer simulations to investigate the effect of drift on the dynamics of an androdioecious species with the genetic determination model hypothesized in *P. angustifolia* according to Saumitou-Laprade *et al.* (2010). We especially looked at the maintenance of males and both SI hermaphrodite groups in populations. For that, we performed 10 000 independent replicates of 10 000 generations of a single population, with initial genotype frequency all equal to their deterministic expected value from equations 1. The population size N was fixed. We recorded the genotype frequency at the end of the replicate. The frequency change in a generation was computed in two steps. Step 1: the deterministic frequency change due to reproduction was computed using eqns 1 and 2. Step 2:

drift was simulated by randomly drawing N times in a multinomial distribution with the deterministic frequencies computed during step 1 as parameters. We then computed the proportion of simulations where: males were lost, one of both SI hermaphrodite groups was lost, hermaphrodites H_b were lost, and the proportion of simulations which yielded male frequencies higher than 40%. Finally, we investigated the probability of invasion of alleles S_1 or S_2 when introduced in a single copy into a population at equilibrium, by performing 10 000 independent replicates of 1000 generations and recording if these alleles were lost or not.

Analysis of published sex ratios in natural populations

We compiled estimates of the male frequency observed in 45 natural populations of *P. angustifolia* from different sources (See Tab. 3). Thirty-two populations were in the south of France (Strasberg, 1988; Lepart & Dommée, 1992; Vassiliadis, 1999) and 13 from the Iberian Peninsula (Pannell & Ojeda, 2000). Pannell & Ojeda (2000) hypothesized that the observed high frequencies of males in natural populations could be biased due to a higher annual average flowering probability of males compared with hermaphrodites, which predicts a decrease in male frequencies when the proportion of flowering individuals increases. We used logistic regression to test whether there is such a relationship, following the general framework for the selection of statistical models in generalized linear models by Zuur *et al.* (2009), based on the analysis of deviance and minimizing the AIC. We first searched for the best model (explaining the most deviance with the fewest parameters) through a backward selection model protocol, starting with the full model (with all factors and their interactions) and removing factors and their interactions one by one. We estimated the parameters from the best-fitting model.

Results

The equilibria and their stability

We found four equilibria, whether selection acts through male reproduction or through both male and female reproduction (with exceptions regarding the stability of two equilibria, detailed later). These equilibria are i) populations with only hermaphrodites, at isoplethy $\hat{X}_1 = [1/2, 1/2, 0, 0, 0]$. We call these 'hermaphrodite populations'; the equilibria ii) $\hat{X}_2 = [1/2, 0, 0, 1/2, 0]$ and iii) $\hat{X}_3 = [0, 0, 1/2, 0, 1/2]$ correspond to cases where populations have males plus a single hermaphrodite SI group, in equal frequencies. In the following, we call these 'cryptically dioecious populations'; iv) we will call 'androdioecious populations' populations with all genotypes present in the population $\hat{X}_4 = [\hat{x}_{11}, \hat{x}_{12}, \hat{x}_{22}, \hat{x}_{13}, \hat{x}_{23}]$, the equilibrium genotype frequencies for $K > 1$ are given by

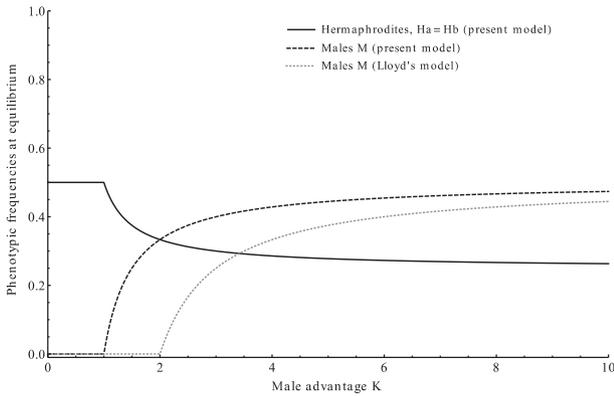


Fig. 2 Male and Hermaphrodite frequencies at equilibrium as a function of the male advantage K according to Lloyd (1975)'s model (dotted curve) and the present model (continuous and dashed curves). The equilibrium values are obtained under the hypothesis of selection through male way only (eqn 4). Both hermaphrodites H_a and H_b have the same expected frequency at equilibrium, hence a single curve is shown.

$$\begin{cases} \hat{x}_{11} = \frac{K}{4K-2} \\ \hat{x}_{12} = \frac{-2K^3 - K^2 + K + (2K-1)\sqrt{2}\sqrt{K^3(1+K)}}{(1-2K)^2(-1+K)} \\ \hat{x}_{22} = \frac{6K^3 - K^2 - K - 2(2K-1)\sqrt{2}\sqrt{K^3(1+K)}}{2(1-2K)^2(-1+K)} \\ \hat{x}_{13} = \frac{-4K^2 + 2K + (2K-1)\sqrt{2}\sqrt{K^3(1+K)}}{2(1-2K)^2K} \\ \hat{x}_{23} = 1 - \hat{x}_{11} - \hat{x}_{12} - \hat{x}_{22} - \hat{x}_{13}. \end{cases} \quad (3)$$

The phenotype and allele frequencies at equilibrium \hat{X}_4 are given by

$$\hat{P} = \left[\frac{1}{2}, \frac{K}{2K-1}, \frac{1}{2}, \frac{K}{2K-1}, \frac{K-1}{2K-1} \right], \quad (4)$$

$$\hat{A} = \left[\frac{(3K-1)(\sqrt{2K(K+1)}-2)}{4(K-1)(2K-1)}, \frac{(3K-1)(2K-\sqrt{2K(K+1)})}{4(K-1)(2K-1)}, \frac{K-1}{4K-2} \right]. \quad (5)$$

The equilibrium frequencies of males are the same as in Pannell & Korbecka (2010). Fig. 2 shows the equilibrium frequencies of H_a , H_b and M phenotypes as functions of the male advantage K . The frequency of the S_1 and S_2 alleles (eqn 5) are very different as S_2 is dominant. When there is no male advantage ($K = 1$), the S_1 and S_2 equilibrium frequencies are 0.75 and 0.25 respectively. Equation 5 shows that the equilibrium frequencies of both alleles decrease when K increases. The importance of the fact that the expected frequency of allele S_2 is lower than S_1 will be discussed when investigating the effect of drift on the maintenance of polymorphism.

Stability of the equilibria with selection through male reproduction only

We analysed the stability of the four equilibria. The leading eigenvalue of the equilibrium \hat{X}_1 (hermaphrodite population), is $K - 1$, hence it is unstable when $K > 1$, that is, males invade the population as soon as they have any advantage through male reproduction relatively to hermaphrodites. The second and third equilibria have males plus a single hermaphrodite genotype (i.e. cryptically dioecious populations). The leading eigenvalue corresponding to the equilibrium \hat{X}_2 is

$$\frac{2 - 3K^2 + \sqrt{4 + K(16 + K(20 + K(16 + 9K)))}}{4K(1 + K)} \quad (6)$$

which is always positive for all $K > 0$, hence this equilibrium is always unstable. In other words, when a hermaphrodite of the missing SI group or a male bearing the missing SI allele S_2 is introduced in the population, it invades. The largest eigenvalues for the third equilibrium \hat{X}_3 is zero, and a numerical analysis of this equilibrium shows that it is unstable for all $K > 0$. The interpretation is the same as in the case of the \hat{X}_2 equilibrium. The leading eigenvalue for equilibrium \hat{X}_3 is zero because allele S_1 is recessive and is thus not favoured until the frequency of the S_1 allele is high enough in the population, allowing its selection through the homozygotes S_1S_1 . This result is important as it means that the invasion of hermaphrodites H_a in populations composed only of hermaphrodites H_b is slow, as hermaphrodites H_a are only slightly favoured when rare. Consequently, such populations would be protected against the invasion of hermaphrodites H_a , especially when drift occurs.

Concerning the fourth equilibrium (i.e. androdioecious populations), we found no simple explicit expression for the eigenvalues. We numerically computed the eigenvalues and concluded that for $0 < K < 1$, there is always a positive eigenvalue, whereas for $K > 1$, all eigenvalues are negative. Hence, for $0 < K < 1$, the equilibrium is unstable, that is, males are disfavoured and eliminated, whereas for $K > 1$, the equilibrium \hat{X}_4 is stable, that is, males and hermaphrodites are maintained in the population, meaning that both sexual and SI polymorphisms are maintained.

Stability of the equilibria with selection through both male and female reproduction: pollen limitation

The stability of the equilibria are qualitatively identical to when there is no pollen limitation, that is, equilibria with two phenotypes \hat{X}_1 , \hat{X}_2 and \hat{X}_3 are unstable, whereas the equilibrium with three coexisting phenotypes \hat{X}_4 is stable. There are, however, quantitative differences for the stability of two of these equilibria: \hat{X}_2 and \hat{X}_4 . First, the leading eigenvalue for equilibrium \hat{X}_2 is equal to

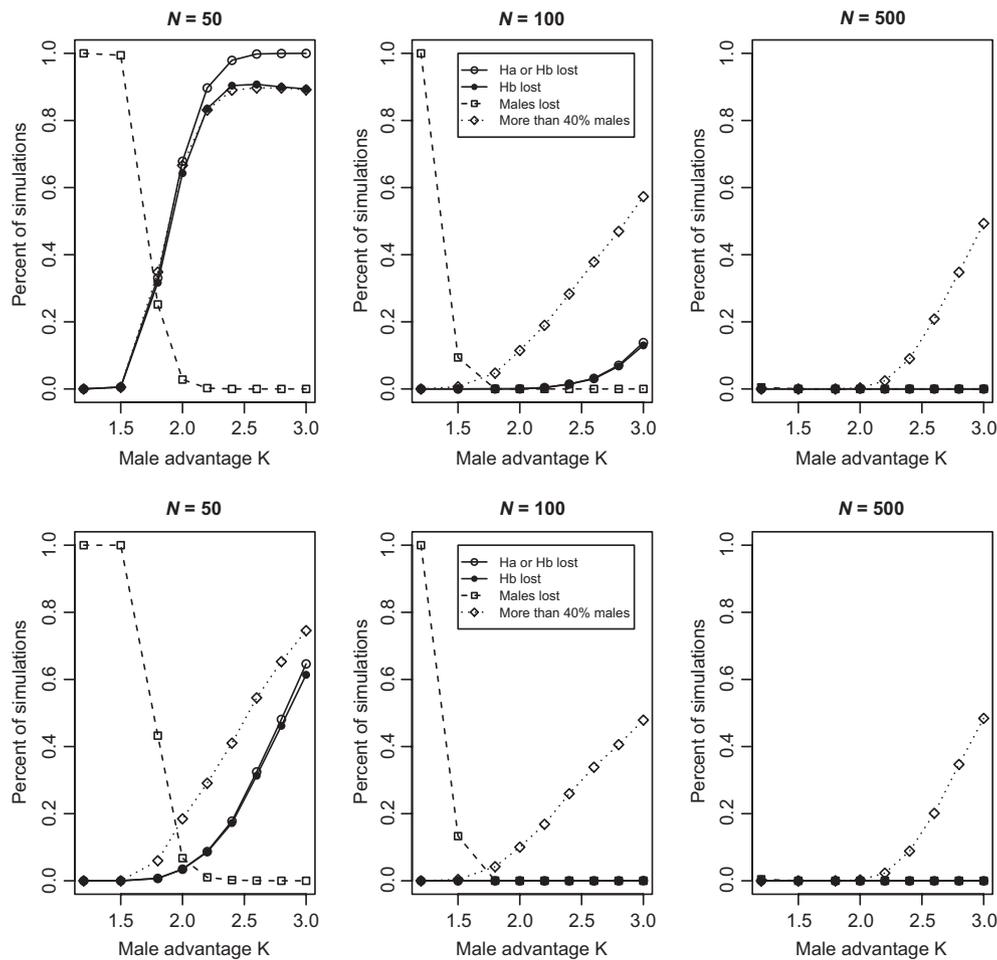


Fig. 3 Maintenance of sexual and self-incompatibility polymorphisms in finite-size populations for varying male advantage K . Top row: results under the model with selection through male reproduction only (no pollen limitation). Bottom row: selection through both male and female reproduction traits (with pollen limitation). In columns are graphics for different population size: $N = 50, 100$ and 500 .

$$\frac{4 - 3K + \sqrt{16 + K(8 + 9K)}}{4K} \quad (7)$$

This eigenvalue is higher under the model with selection through both male and female reproduction than under the model with selection only through male reproduction (compare eqn 6 and 7). As this equilibrium is unstable, this means that when the population composition is near equilibrium, \hat{X}_2 , then the population moves away faster when there is pollen limitation than when there is not. In short, the polymorphism with two hermaphrodites is more protected when there is pollen limitation. For the equilibrium \hat{X}_4 , as in the case with no pollen limitation, we could not compute an explicit expression and thus performed numerical computations for different given values of K . We found that there is a threshold value of K around 1.5. When $K > 1.5$, the leading eigenvalue is lower when there is pollen limitation than when there is not, whereas the

leading eigenvalues are identical for $K < 1.5$. As this equilibrium is stable when $K > 1$, this suggests that when there is pollen limitation, the equilibrium with the three phenotypes is more stable than when there is not. In short, the population goes back faster to the equilibrium after a perturbation when there is pollen limitation. These two results about the eigenvalues have important consequences to interpret results about the maintenance of polymorphisms in finite populations, which we discuss in the sequel.

The effect of drift on maintenance of the polymorphism and male frequencies

Figure 3 shows for different population sizes the proportions of replicates where males or hermaphrodites were lost, as well as the proportions with frequencies of males higher than 40%. A first result is that males are maintained in small populations only if their advantage is

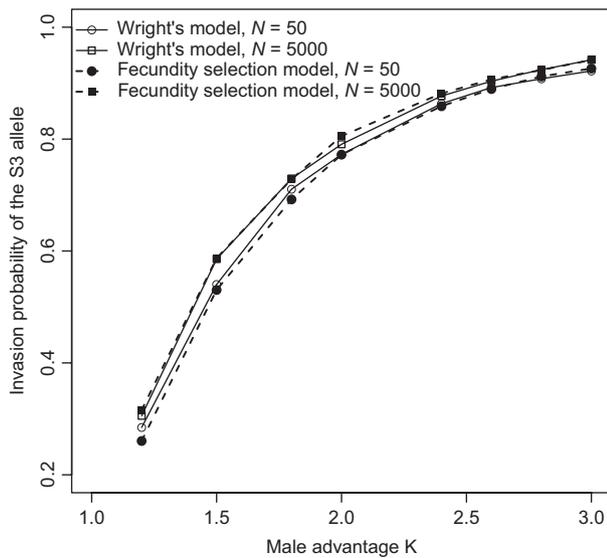


Fig. 4 Invasion probability of the S_3 allele in a hermaphrodite population when introduced in a single copy, for two population sizes and two selection regimes. The invasion of the S_3 allele is considered successful when the male frequency in the population has reached a frequency higher than the expected value under deterministic model (eqn 4).

above a threshold value of the male advantage, K . When K is low, the expected frequency of males is low, and they are therefore likely to be lost by chance. If the male advantage is high enough, however, they are not lost, even with $N = 50$, the smallest population size studied. Second, in small populations, when K exceeds the threshold, one of the hermaphrodite SI groups is frequently lost, especially under the model without pollen limitation. When $N = 50$, $K > 2.5$ and without pollen limitation, one of the hermaphrodite types is always lost, and this is almost invariably hermaphrodite H_b which is lost, because the expected frequency of allele S_2 is much lower than that of allele S_1 (see above). Third, our results show that hermaphrodites are more easily maintained when pollen limitation occurs. This is due to the fact that pollen limitation increases the strength of negative frequency-dependent selection (see above), and thus the eigenvalue for the equilibrium without hermaphrodite H_b is highest with pollen limitation (eqn 6 and 7), so that when a population drifts close to this equilibrium, it rapidly moves away.

We also performed simulations in which we introduced a single copy of either allele S_1 or S_2 in a population at equilibrium. As expected for an outcrossing population, the invasion probability of the dominant S_2 allele is much higher than for S_1 , that is, S_1 increases in frequency slowly when it is rare. For instance, with male advantage $K = 2$, in a population with size $N = 100$, and without pollen limitation, the probability of the allele S_2 invading is 0.59 vs. only 0.07 for allele S_1 (the results for other parameters values are not shown, but are qualita-

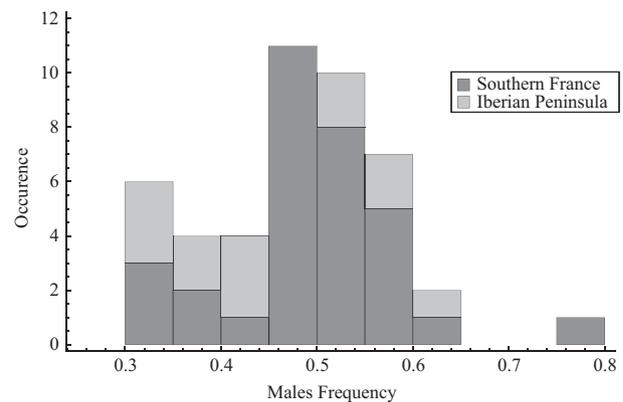


Fig. 5 Distribution of male frequencies among *P. angustifolia* natural populations (data from references in Table 2).

tively similar). Fig. 3 shows that three factors increase the probability of obtaining a male equilibrium frequency above 40%: a high male advantage, a small population size or the loss of a hermaphrodite SI group (which occurs in the smallest population studied $N = 50$). Hence, our results show that populations are unlikely to have both hermaphrodite SI groups and a frequency of males higher than 0.4.

We also investigated the invasion probability of the S_3 allele into a hermaphrodite population. Fig. 4 shows that the two selection regimes behave identically (as expected, as the leading values for the equilibrium without males are identical for both selection regimes). Population size has only a limited effect on the S_3 invasion probability, suggesting that males easily spread when rare.

Sex ratios in natural populations

The male frequencies in natural populations shown in Fig. 5 and Table 2 range from 0.295 to 0.775, with a mean of 0.468. Among 45 populations, 18 have a frequency of males significantly higher than 0.4 (χ^2 test, P -value = 0.05). Comparing the Southern France and the Iberian populations (see Table 2), the mean frequency of males is higher (mean = 0.49 vs. 0.44), the range of frequencies is wider (coefficient of variation = 5.37 vs. 4.43). There is a lower proportion of populations with a frequency of males significantly lower than 0.5 in the Southern France populations than in the Iberian Peninsula populations (4/32 vs. 7/13 populations), whereas the proportion of populations where the frequency of males is not significantly different from 0.5 is higher in the Southern France populations (24/32 vs. 5/13 populations). The frequency of males is marginally significantly lower in the Iberian Peninsula populations than in those from the Southern France (Wilcoxon Rank Test, P -value = 0.086).

The best logistic regression model to explain the data includes two significant factors, the location (Iberian

Table 1 Results of the logistic regression testing the existence of a relationship between male frequency estimation and the frequency of flowering in natural populations.

Models (All populations)	Df	AIC	Deviance (<i>P</i> -value of the LRT vs. the best model)	
Null model (Intercept only)	44	401.7	176.3 (<0.001)	
Flowering frequency	43	403.4	176 (<0.001)	
Location	43	388.2	160.7 (0.006)	
Location + Flowering frequency	42	383.7	154.2 (0.054)	
Location + Flowering frequency + location:Flowering frequency	41	382	150.5 (–)	
Models (Single population)	Df	AIC	Deviance (<i>P</i> -value of the LRT vs. the null model)	GLM estimates
Iberian Peninsula				
Null model (Intercept only)	12	115.5	48.9	
Flowering frequency	11	107.8	39.1 (0.002)	Intercept = 0.46, Flowering frequency = –1.03
Southern France				
Null model (Intercept only)	31	272.6	111.8	Intercept = –0.036
Flowering frequency	30	274.2	111.4 (0.52)	

Df, degrees of freedom; AIC, Akaike Information Criterion; LRT, Likelihood Ratio Test; Deviance, two times the LRT of the model under consideration compared with the saturated model

Peninsula vs. Southern France) and the proportion of flowering individuals, as well as their interaction (which is marginally significant). The flowering frequency is not significant on its own, but only when the location is taken into account. In logistic regressions using single populations separately, the flowering frequency has a significant effect on male frequencies only in the Iberian Peninsula, where it explains 20% of the variation, and male frequencies decrease significantly with an increased proportion of flowering individuals (Table 1, Fig. 6). This is consistent with male frequencies being overestimated in the Iberian Peninsula populations. However, overestimation cannot explain the large number of populations in Southern France with 50%, and more, of males as, in these populations, between 90% and 100% of individuals were flowering and their sex phenotype could be reliably determined (see Fig. 6).

From the GLM (Table 1), we estimated the expected male frequencies if all individuals had flowered and been phenotyped. This correction for overestimation yields a much lower male frequency in the Iberian Peninsula populations, of 0.362 (with 95% confidence interval 0.314–0.409), vs. 0.341–0.628 for the uncorrected frequencies, with a mean of 0.44. For the populations from Southern France, the corrected frequency is 0.491 (0.474–0.508), vs. 0.295 up to one population with a frequency of 0.775 for the uncorrected frequencies, with a mean of 0.49. Hence, when using the predicted values from the logistic regression taking into account overestimation of male frequencies, the expected male frequen-

cies in the Southern France populations are significantly higher than in the Iberian Peninsula populations (the Wilcoxon Rank Test on the uncorrected frequencies showed a marginally significant difference between both locations, see above).

Discussion

Male advantage and androdioecy

In agreement with the results of Pannell & Korbecka (2010), we showed that even a small male advantage can maintain androdioecy in the system believed to exist in *P. angustifolia*, in contrast with the result without diallelic self-incompatibility groups, when the advantage of males relative to hermaphrodite must be at least *two-fold* (Fig. 2). The situation with diallelic SI groups in the hermaphrodites is similar to the condition required for cytoplasmic male sterility mutation to be favoured in gynodioecious species where cytoplasmic genes are only transmitted through female (Lloyd, 1975; Dufaÿ et al., 2007), where even a slight female or cytoplasmic advantage suffices for maintenance of a cytoplasmic male sterility mutation in populations (Lewis, 1941). Gynodioecy is known in about 7% of species (Renner & Ricklefs, 1975), which may be due to the ease with which cytoplasmic male sterility mutations can be maintained. Therefore, when two SI groups exist, we might expect androdioecy to be frequent. The occurrence of diallelic SI is unknown. It

Table 2 Estimates of male frequency in natural populations from the literature.

Region	Locality	Number of males	Number of hermaphrodites	% flowering	Male sex ratio
South France, in 1988 (Strasberg, 1988)					
North Camargue	North BV	44	37	77.14	54.32†
	Cleaning BV	50	52	74.45	49.02
	Saltern BV	60	49	87.90	55.05†
	East BV	75	70	75.92	51.72†
	South BV	44	52	84.21	45.83
	Relongues	32	61	91.18	34.41*
	Islet BB	36	27	46.67	57.14†
	South BB	9	14	52.27	39.13
	Cerisieres	40	78	90.08	33.90*
	Levadon 1	49	60	98.20	44.95
	Levadon 2	32	35	100	47.46
	North CF	51	50	99.02	50.50†
	'CF Grands'	88	63	100	58.28*†
	Centre CF	50	49	91.67	50.51†
	Rushes	72	50	70.52	59.02*†
	Ferigoulet	71	46	94.4	60.7*†
	Cabane Rouge	47	52	97.1	47.5
	Clos de la ville	76	22	97.03	77.55*†
	South Camargue	Islet dune	70	82	59.84
High dune		59	109	97.11	35.12*
Hollow dune		46	49	86.36	48.42
Low dune		48	52	84.75	48
Flat dune		54	50	75.36	51.92†
South France, in 1985–1986–1987 (Lepart & Dommée, 1992)					
Montpellier	La Gardiole	53	38	91.9	58.2†
North Camargue	Tour du Valat 1	63	74	91.9	46.0
Port-la-Nouvelle	Sainte Lucie	61	80	92.2	43.3
South France, 1997 (Vassiliadis, 1999)					
North Camargue	Tour du Valat 2	59	57	91.3	50.9†
South Camargue	Saltern A	9	9	90.0	50.0
South Camargue	Saltern B	12	13	100	48.0
Montpellier	'Grands Travers'	49	56	99.1	46.7
Montpellier	La Boissere	90	83	90.1	52.0†
Montpellier	Montmeze	28	67	89.6	29.5*
South-western Iberian peninsula (Pannell & Ojeda, 2000)					
Portugal	Castalejo	67	128	84.8	34.4*
	Castalejo	52	94	64.0	35.6*
	Castalejo	11	15	76.5	42.3
	Castalejo	92	65	52.3	58.6*†
	Castalejo	30	58	51.8	34.1
	Castalejo	27	16	44.8	62.8†
	St. Vincent	53	80	97.1	39.8*
	St. Vincent	30	51	89.0	37.0*
	St. Vincent	37	36	74.5	50.7
	Spain	Hinojos	86	116	89.0
	Hinojos	46	38	71.8	54.8†
	Alcornocales	44	43	51.5	50.6†
	Cadiz	41	80	66.5	33.9*

The asterisks and the daggers †, respectively, show populations where the frequency of males is significantly different from 0.5 and higher than 0.4 (χ^2 Test, P -value = 0.05).

could be present in other species, including other species in the family Oleaceae, where androdioecious species are known, including *Osmanthus fragrans*, (Hao

et al., 2011) and *Fraxinus ornus*, (Dommée et al., 1999). Interestingly, those two species have populations with a high frequency of males in natural populations.

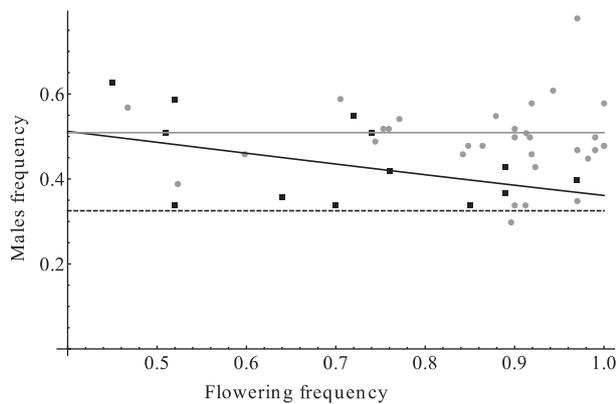


Fig. 6 Observed male frequencies among *Phillyrea angustifolia* natural populations (data from references in Table 2) as a function of the ratio of flowering individuals (Black squares: Iberian peninsula; Gray circles: Southern France). The dashed line is the expected frequency of males for a male advantage $K = 1.93$ as estimated in Vassiliadis et al. (2002) from eqn 4. Black and gray lines are the results of the logistic regression for populations in Iberian Peninsula and Southern France respectively (see Table 1).

The male advantage values measured in previous studies of *P. angustifolia* can, however, only partially explain high male frequencies in natural populations. Pannell & Korbecka (2010) estimated the male advantage $K = 1.8$ from the observed higher flowering frequency of males in Spanish populations (Pannell & Ojeda, 2000, albeit without good estimates of relative flowering frequency), and Vassiliadis et al. (2000) estimated $K = 1.94$, using populations from Southern France, based on the observed numbers of fruits per inflorescence produced by hermaphrodite recipients in a set of controlled pollinations with either male or hermaphrodite pollen. In the model with two SI groups, and $K = 2$, the expected male frequency is 0.33, which is in agreement with observations in some natural populations.

Sex and self-incompatibility polymorphism

Our model shows that, without drift, all three phenotypes should coexist in a population, for any male advantage ($K > 1$). When the population is small, drift often leads to the loss of the sex or self-incompatibility polymorphism, although both polymorphisms are easily maintained when the population is large enough ($n = 500$) and the advantage of males is high enough ($K > 2.0$). Consequently, small and isolated populations are expected to be either hermaphroditic, that is, with both SI hermaphrodite groups and no males, or cryptically dioecious, that is, with a single hermaphrodite SI group and males. If cryptically dioecious natural populations exist, the H_b group is most likely to be lost, because H_a is controlled by the recessive allele S_1 , which is commoner at deterministic equilibrium.

In *P. angustifolia*, the 45 populations observed so far are all androdioecious, suggesting that *P. angustifolia* populations are large enough, and sufficiently connected, to prevent the loss of males. Gene flow is indeed high in *P. angustifolia* populations. A paternity analysis in a small geographically isolated population in Camargue in Southern France, mainly surrounded by seawater, estimated that more than 47% of the seeds were sired by pollen from other populations (Vassiliadis et al., 2002). So far, the number of SI groups has been investigated in five *P. angustifolia* populations, and both SI groups have always been detected (Saumitou-Laprade et al., 2010). Therefore, cryptically dioecious or hermaphrodite populations are unlikely to exist in this species. This can be tested in more populations particularly if genetic markers linked to incompatibility and sex phenotypes become available. In another androdioecious Oleaceae species, *O. fragrans*, hermaphrodite populations are observed (Hao et al., 2011). Some populations of this species may be small or isolated enough to lose a phenotype. *O. fragrans* is entomophilous, whereas *P. angustifolia* is anemophilous, and spatial structure tends to be higher in animal-dispersed pollen plants than in wind-pollinated plants (Vekemans & Hardy, 2004). In another species, *F. ornus*, Verdú et al. (2004) concluded that it is cryptically dioecious, based on populations at the margins of the species range. It is possible that the populations in this study have lost one of the two SI groups, and that both SI groups exist elsewhere in the species.

Observed and expected male frequencies in *P. angustifolia* populations

Among 45 populations, 18 have male frequencies significantly higher than 40%, and male frequencies significantly higher than 50% are recorded in five populations, especially in the populations from Southern France (Table 2), and the excess of males in these populations remains unexplained. We have already excluded the possibility that male frequencies were overestimated in the French populations; although our analysis for the Iberian Peninsula populations supports the overestimation idea, these populations do not have notably high male frequencies before correction for overestimation, compared with the French ones. Furthermore, although our model predicts that populations with 50% males may be cryptically dioecious and include only a single hermaphrodite SI group, this seems unlikely as the *P. angustifolia* populations with 50% males studied so far contain both incompatibility groups, as explained above (Saumitou-Laprade et al., 2010). Perhaps male frequencies are high because of drift, as has been proposed to explain sex ratio variation among populations of a dioecious forest plant (Vandepitte et al., 2010), morph ratio variation in tristylous species (Barrett et al., 1989) or variation in self-incompatible phenotype and genotype frequencies (e.g. in

sporophytic self-incompatibility systems in *Arabidopsis halleri*, Llaurens et al., 2008). This possibility can be tested by using our model to compare the distribution of male frequencies predicted in large vs. small populations, assuming realistic male advantage value, that is, K around 2. Comparing Fig. 3 and Table 2, and given that all three phenotypes coexist in all known populations of *P. angustifolia*, drift also seems unlikely to explain the observation that 18/45 natural populations have male frequencies significantly higher than 0.4. In our simulations, this is predicted only in very small populations when one of the hermaphrodite groups is lost and the male advantage is high. Drift or the loss of a single hermaphrodite SI group might nevertheless explain high male frequencies in other Oleaceae species, such as in *O. fragrans* populations (Hao et al., 2011) and *F. ornus* (Verdú et al., 2004).

The high male frequencies in populations from Southern France must presumably be caused by ecological and/or genetical processes, which remain to be deciphered. One possibility might be spatial heterogeneity and spatial structure. In the androdioecious tropical species *Laguncularia racemosa* (Combretaceae), the large variation of male frequencies among populations (from 0% to 68%) may be due to environmental conditions being more favourable for males in some populations (Landry et al., 2009), perhaps from differences in pollinators' foraging behaviour: males in androdioecious populations had significantly higher visitation rates than hermaphrodites (Landry & Rathcke, 2012). Among genetic possibilities, distorted segregation might possibly occur. In plants, non-1 : 1 sex ratios are known for instance in *Rumex nivalis* (Stehlik et al., 2007) and in *Silene latifolia* (reviewed in Taylor & Ingvarsson, 2003).

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