doi:10.1111/evo.12613

Selfish male-determining element favors the transition from hermaphroditism to androdioecy

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Received April 1, 2014
Accepted January 9, 2015

According to the current, widely accepted paradigm, the evolutionary transition from hermaphroditism toward separate sexes occurs in two successive steps: an initial, intermediate step in which unisexual individuals, male or female, sterility mutants coexist with hermaphrodites and a final step that definitively establishes dioecy. Two nonexclusive processes can drive this transition: inbreeding avoidance and reallocation of resources from one sexual function to the other. Here, we report results of controlled crosses between males and hermaphrodites in *Phillyrea angustifolia*, an androdioecious species with two mutually intercompatible, but intraincompatible groups of hermaphrodites. We observed different segregation patterns that can be explained by: (1) epistatic interactions between two unlinked diallelic loci, determining sex and mating compatibility, and (2) a mutation with pleiotropic effects: female sterility, full compatibility of males with both hermaphrodite incompatibility groups, and complete male-biased sex-ratio distortion in one of the two groups. Modeling shows that these mechanisms can explain the high frequency of males in populations of *P. angustifolia* and can promote the maintenance of androdioecy without requiring inbreeding depression or resource reallocation. We thus argue that segregation distortion establishes the right conditions for the evolution of cryptic dioecy and potentially initiates the evolution toward separate sexes.

KEY WORDS: **Male advantage, oleaceae, plant mating systems, sex ratio distortion, sporophytic diallelic self-incompatibility system.**

The evolution from hermaphroditism toward separate sexes (dioecy) is one of the most frequent mating system transitions in plants (Charlesworth and Charlesworth 1978; Pannell 2002) and animals (Weeks et al. 2006). Two basic evolutionary pathways can account for the emergence of dioecy: through a monoecious (unisexual reproductive organs on the same individual) stage, which probably occurred in the Asteraceae for instance (Torices et al. 2011), or through unisexual intermediates (Charlesworth and Charlesworth 1978; Ross 1978; Barrett 1992; Ehlers and Bataillon 2007). This latter pathway enjoys widespread acceptance although there is no clear evidence for it in plant (for a review see Spigler and Ashman 2012) or animal families (Weeks 2012).

According to theoretical studies, the transition from hermaphroditism to dioecy through unisexual intermediates occurs in two successive steps: first, some individuals give up one of their reproductive functions (either male or female) and second, the remaining hermaphrodites specialize in the other function. Theory, built on the nuclear control of sex, predicts that unisexual individuals can be maintained in hermaphrodite populations only under stringent conditions. Namely, unisexual individuals compensate for the loss of one sexual function via a fitness

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

advantage, either through efficient resource reallocation to the remaining reproductive function or, if inbreeding depression is high, through the avoidance of self-fertilization, rendered impossible due to the separation of sexual function in different individuals (Charlesworth and Charlesworth 1978). The effect is however asymmetrical for males and females, and theory predicts that the conditions under which male-sterile mutants can evolve are much less stringent than the conditions under which female-sterile mutants can be maintained in a hermaphroditic population (Lewis 1941; Lloyd 1975; Charlesworth and Charlesworth 1978). Data are in agreement with these predictions: androdioecy (populations in which males and hermaphrodites coexist) is exceedingly rare in nature (Pannell 2002), much rarer than gynodioecy (Webb 1999; populations in which females and hermaphrodites coexist). In gynodioecy, male sterility under nuclear determination is rare and the vast majority of gynodioecious species are associated with a cytoplasmic male-sterility mutation for which the constraints on female resource reallocation are far less stringent (Dufaÿ and Billard 2012). For female sterility, the selective constraint appears so strong that the existence of functional androdioecy was long challenged (Charlesworth 1984) and the few confirmed cases of androdioecy were thought to have evolved from dioecy (Pannell 2002; Delph and Wolf 2005; Delph 2009).

Several observations suggest that separate sexes and self-incompatibility (SI) systems evolve conjointly (Rosas and Dominguez 2009; Barrett 2010; Liu et al. 2012). Recent theoretical and empirical advances on an androdioecious Oleaceae species, *Phillyrea angustifolia*, shed new light on how an SI system can help overcome the resource reallocation constraint and favor the evolution of androdioecy (Saumitou-Laprade et al. 2010; Husse et al. 2013). A newly described sporophytic SI system separates hermaphrodites into only two homomorphic SI groups: individuals of a given SI group can only sire seeds of hermaphrodites from the other group, whereas males are compatible with both SI groups (Saumitou-Laprade et al. 2010). The existence of two SI groups facilitates the evolution and maintenance of compatible males because they can sire twice as many progeny as hermaphrodites of either SI group. This "siring advantage" of pollen from unisexual males potentially offsets the reproductive disadvantage of males due to the loss of the female function (Pannell and Korbecka 2010).

Androdioecy under nuclear sex determination detected in *P. angustifolia* appears to evolve as easily as gynodioecy under nuclear–cytoplasmic genetic control (Husse et al. 2013). In particular, even the slightest resource reallocation to the remaining sexual function is enough for unisexual individuals to evolve in both cases. This weak constraint may explain the extraordinarily high frequencies of androdioecious populations, and males within those populations, observed in Oleaceae species (Dommée et al. 1999; Hao et al. 2011; Husse et al. 2013). Furthermore, 11% of the 330 species in the tribe Oleeae are androdioecious (Wallander 2001), a figure proportionally much higher than that reported in plants and animals (Pannell 2002).

However, as recently highlighted (Husse et al. 2013), there are many features of androdioecy in the Oleaceae that are still unknown and misunderstood. First, the genetic architecture of the sex-determination system and its interaction with the SI system remain unknown. Second, although the SI system effectively explains the maintenance of androdioecy, it fails to explain how males reach the very high frequencies (50% and higher) typically observed in some natural populations (Husse et al. 2013). These high male frequencies are striking in the face of the low male fitness advantage (in terms of resource reallocation), which is either very weak (Vassiliadis et al. 2000; Pannell and Korbecka 2010) or not even detectable (Vassiliadis et al. 2002). Hence, males must enjoy an additional cryptic advantage, not associated with siring or reproductive output, whose nature remains to be determined.

To identify the nature of this cryptic male advantage, we performed controlled crosses between *P. angustifolia* hermaphrodites and males to better describe the genetic architecture of sex and SI traits, and their interaction. Examination of the crossing results revealed an unexpected and strong segregation distortion toward males, which may further contribute to the male advantage. Based on these results, we constructed a genetic determination model that accounts for the segregation of both sex and SI, integrating the observed segregation distortion toward males. Finally, based on this genetic model, we developed and analyzed a population genetics model, and demonstrated that incorporating these new features substantially improves the fit between the predicted and observed proportions of males in natural populations.

Materials and Methods

CONTROLLED CROSSES

Between spring 2008 and 2010, 16 males and 16 hermaphrodites were selected for controlled crosses from a population located in Fabrègues (referred as population 1 in Fig. S1 in Saumitou-Laprade et al. 2010) and maintained at the CEFE-CNRS experimental garden in Montpellier, France. In this slow-growing shrub, a total of 26 crosses between males and hermaphrodites belonging to the two SI groups produced a significant number of seeds allowing genetic analysis. After germination, seedling paternity was verified with polymorphic markers (Saumitou-Laprade et al. 2000) and sex and SI group were determined for the 623 confirmed progenies during their first flowering season, two years later. The hermaphrodite individuals were assigned to an SI group using the stigma test as previously described in Saumitou-Laprade et al. (2010). Progeny phenotypes were confirmed during their second

flowering season, demonstrating that the sexual phenotypes are perfectly stable.

Because we aimed at determining the genotypes present in the male and hermaphrodite phenotypes, the crossing design included crossing single hermaphrodites with different males and single males with hermaphrodites belonging to the two SI groups identified in Saumitou-Laprade et al. (2010).

EVOLUTIONARY MODELS

To explore how the genetic determination model of sex and SI (Appendix), constructed on the basis of our crossing experiment results, affects the maintenance and evolution of androdioecy and the frequency of males, we implemented a deterministic population genetic model. We investigated the expected frequency of all genotypes and phenotypes at equilibrium as a function of the male advantage K (heuristically, the amount of pollen contributed by males relative to that contributed by hermaphrodites). We built a genetic model, and not a phenotypic model, due to the assumed sex-ratio distortion that implies a selection at the haploid stage. A genetic model is thus necessary to analyze the evolutionary outcomes of the model (Lloyd 1977).

We assumed an infinite, unstructured population of diploid individuals with two SI hermaphrodite groups, denoted H_a and H_b, and a male phenotype, M. An ovule produced by an H_a hermaphrodite can be sired only by pollen produced by H_b or M individuals and an ovule produced by an H_b hermaphrodite can be sired only by a pollen produced by H_a or M individuals. We also assumed that the allele M encoding the male phenotype has an epistatic effect on the locus S such that males do not express any SI phenotype. Finally, we assumed that the allele M is fully distorted in crosses between males and hermaphrodites H_b, that is, these crosses only produce male offspring.

We assumed that H_a and H_b hermaphrodites produce the same (high) numbers of ovules and pollen grains, and that males have a higher chance of siring offspring than hermaphrodites: K denotes the male fitness advantage relative to hermaphrodites. The frequency of the H_a (*mmS1S1*), H_b (*mmS1S2*), and males (*mMS1S1*, *mMS1S2*, *mMS2S2*) genotypes in the population is designated, respectively, by x_1, x_2, x_3, x_4, x_5. p_a, p_b, and p_M denote the frequency of H_a and H_b hermaphrodites and males, respectively, in the population, with $p_a = x_1$, $p_b = x_2$, and $p_M = x_3 + x_4 + x_5$.

We computed the genotype frequency changes over one 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 (Wright 1939)) 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 SI systems (Vekemans et al. 1998). For both models, we found all the possible and biologically realistic equilibria, that is, where $0 < x_i < 1$ for all i, by solving the system of equation (1) given below. Then, we analyzed the stability of all possible equilibria, especially those without males, to determine the male advantage K under which allele M can invade the population when rare, and enable the transition from hermaphroditism to androdioecy. To do so, we performed a stability analysis of the systems described hereafter (Otto and Day 2007; Billiard et al. 2011; Husse et al. 2013). The method relies on the calculation of the leading eigenvalue of the Jacobian matrix associated with the system of equations for the equilibrium of interest, using Mathematica 9.0 (Wolfram Research 2012). Typically, the leading eigenvalue is a function of the parameters of the model, and here we had one single parameter: the male advantage K. The final step consists in determining for which value of K the leading eigenvalue is positive, meaning that the equilibrium of interest is unstable. In particular, to examine the conditions under which males can invade a hermaphroditic population, we searched for the value of K for which the leading eigenvalue associated with the equilibrium without males is positive.

Selection through male reproduction only

The genotype frequencies in the next generation, denoted by x', are given by

$$\begin{aligned}
x_1' &= \frac{1}{\sigma}\left(\frac{x_1}{2}\frac{x_2 + Kx_3 + Kx_4/2}{\theta_1} + \frac{x_2}{2}\frac{x_1}{\theta_2}\right)\\
x_2' &= \frac{1}{\sigma}\left(\frac{x_1}{2}\frac{x_2 + Kx_4/2 + Kx_5}{\theta_1} + \frac{x_2}{2}\frac{x_1}{\theta_2}\right)\\
x_3' &= \frac{1}{\sigma}\left(\frac{x_1}{2}\frac{Kx_3 + Kx_4/2}{\theta_1} + \frac{x_2}{2}\frac{Kx_3 + Kx_4/2}{\theta_2}\right)\\
x_4' &= \frac{1}{\sigma}\left(\frac{x_1}{2}\frac{Kx_4/2 + Kx_5}{\theta_1} + \frac{x_2}{2}\frac{Kx_3 + Kx_4 + Kx_5}{\theta_2}\right)\\
x_5' &= \frac{1}{\sigma}\left(\frac{x_2}{2}\frac{Kx_4/2 + Kx_5}{\theta_2}\right),
\end{aligned} \tag{1}$$

where $\sigma = x_1 + x_2$ is the total quantity of ovules produced by the population, and $\theta_1 = x_2 + Kx_3 + Kx_4 + Kx_5$ and $\theta_2 = x_1 + Kx_3 + Kx_4 + Kx_5$ are, respectively, the total amounts of compatible pollen available for H_a and H_b hermaphrodites, respectively.

These equations are obtained from genetic determination model (Appendix) by considering (1) only the possible crosses between individuals, and (2) only the possible progenies for a given cross. For instance, the frequency change x_1' for genotype *mmS1S1* is computed as follows. The only compatible crosses that can produce *mmS1S1* progenies are (*mmS1S1* × *mmS1S2*), (*mmS1S1* × *mMS1S1*), and (*mmS1S1* × *mMS1S2*). The probability that a *mmS1S1* hermaphrodite is sired by a *mmS1S2* hermaphrodite is

Table 1. The three segregation patterns observed for sex and incompatibility groups (males/H_a hermaphrodites/H_b hermaphrodites) in 26 successful controlled crosses performed between hermaphrodites (referenced by numbers) and males (referenced by capital letters) in *Phillyrea angustifolia*.

			Male Pollen donor															
		M Type	M_a								M_b						M_c	
	H Type (1)	Ind. Label	B	D	G	K	N	S	T	U	E	F	H	L	M	O	A	C
Hermaphrodite Recipient	H_a	01					14/12/0 **0.84 NS**											1/0/6 **0.12 NS**
		04	18/14/0 **0.597 NS**										9/6/5 **0.96 NS**				5/0/5 **0.76 NS**	
		06			22/27/0 **0.57 NS**											20/12/15 **0.5 NS**		
		07		16/16/0 **1 NS**							13/13/10 **0.21 NS**							
		10				5/7/0 **0.7 NS**									17/14/15 **0.22 NS**			
		12										9/3/5 **0.82 NS**						
		13															12/0/9 **0.66 NS**	
		20							7/12/0 **0.36 NS**									
		21							13/11/0 **0.84 NS**									
		22							5/18/0 **0.01***									
		23						3/9/0 **0.029***		7/9/0 **0.23 NS**								
	H_b	03																27/0/0 **<0.001***
		05									23/0/0 **<0.001***					21/0/0 **<0.001***		
		08		37/0/0 **<0.001***	37/0/0 **<0.001***													
		09				8/0/0 **<0.01***												
		11										18/0/0 **<0.001***		7/0/0 **0.02***				

Ha and Hb = hermaphrodite types. Ma, Mb, Mc = male types defined a posteriori by the kind of progeny they produce on Ha hermaphrodites; Ma: produce males and Ha hermaphrodites only; Mb: produce males and Ha and Hb hermaphrodites; Mc: produce males and Hb hermaphrodites only.

(1) H_a and H_b hermaphrodites respectively named group1 (G_1) and group2 (G_2) in (Saumitou-Laprade et al. 2010)

(*) Significantly different from the expectation under Mendelian segregation (multinomial exact test with a likelihood-ratio test comparing the probability of the observation under a Mendelian segregation hypothesis or under the best-fitting multinomial distribution, the exact*p*-value is given in bold).

x_2/θ_1, while the probability that a *mmS1S2* hermaphrodite is sired by *mmS1S1* hermaphrodite is x_1/θ_2. One-half of the progeny for these two crosses has the *mmS1S1* genotype. The probability that a *mmS1S1* hermaphrodite is sired by a *mMS1S1* male is Kx_3/θ_1 and by a *mMS1S2* male is Kx_4/θ_1. Respectively, one-half and one-quarter of the progeny of these two crosses has the *mmS1S1* genotype. Note that no hermaphrodites can be produced when males sire ovules produced by the *mmS1S2* hermaphrodite because we assume full sex-ratio distortion toward males in this type of cross, as demonstrated by empirical results (Table 1).

Selection through both male and female reproduction: pollen limitation

We also considered the case with pollen limitation, meaning that there is selection through both male and female reproductive traits. We assumed that, for a given genotype, among all the ovules produced, only some are fertilized, and this proportion is equal to the frequency of compatible pollen in the pollen cloud. The proportion of fertilized ovules produced by H_a and H_b hermaphrodites is equal to $x_2 + Kx_3 + Kx_4 + Kx_5$ and $x_1 + Kx_3 + Kx_4 + Kx_5$, respectively. The genotype frequency changes can thus be computed with selection through both male and female reproduction using the same equations as above, but with $\theta_1 = \theta_2 = 1$ and with

$$\sigma = 2x_1x_2 + Kx_1(x_3 + x_4 + x_5) + Kx_2(x_3 + x_4 + x_5),$$

which is the total quantity of ovules effectively fertilized.

Results

TOWARD A GENETIC DETERMINATION MODEL

The results of the 26 controlled crosses are summarized in Table 1. Seven different males were crossed with and

sired progeny on both types of hermaphrodites, and nine hermaphrodites were crossed with and produced progeny sired by more than one male. Table 1 gives the number of males, H_a hermaphrodites, and H_b hermaphrodites produced in each cross. We tested whether the observed proportion of phenotypes was significantly different from the proportions expected under Mendelian segregation (multinomial exact test, see Table 1). All crosses performed between males and either type of hermaphrodites produced seeds and flowering progeny, confirming the previously observed compatibility of males with both SI types (Saumitou-Laprade et al. 2010). We analyzed the proportions of the different phenotypes in progenies, which yielded new information.

Three types of segregation patterns observed on H_a hermaphrodites reveal three distinct male genotypes

Table 1 shows that males and hermaphrodites were present in balanced (1:1) proportions in the offspring sired by males crossed with hermaphrodites H_a, but there were differences in the proportions of the incompatibility phenotypes among the hermaphrodites produced. Three kinds of hermaphrodite segregation patterns were detected: (1) only H_a hermaphrodites, (2) both types of hermaphrodites in balanced proportions, and (3) only H_b hermaphrodites. Importantly, two different segregation patterns were scored for the progeny of four distinct H_a hermaphrodite mothers (01, 06, 07, and 10) that were crossed with two different males. Even further, the three kinds of segregation were also observed for a single hermaphrodite (04) crossed with three different males (males A, B, and H respectively). In contrast, progeny of a given male crossed with different H_a hermaphrodites always followed the same segregation pattern (e.g., males A and T). This pure paternal effect strongly suggests that the three segregation patterns correspond to three different genotypes at the incompatibility locus in the male parent. Therefore, we split the male phenotype into three male types M_a, M_b, and M_c according to their ability to produce only H_a, H_a and H_b, or only H_b hermaphrodites, respectively, when crossed with H_a hermaphrodites.

Crosses between males and H_b hermaphrodites produce only male offspring

In contrast to the even segregation of sexual phenotypes in the progeny of H_a hermaphrodites, all eight crosses performed on H_b hermaphrodites produced exclusively male offspring (Table 1). Three H_b hermaphrodites were crossed each with two different male pollinators and produced only male progeny whatever the male pollinator. These same male pollen donors (C, D, E, F, G, K, O) produced hermaphrodites when crossed with H_a hermaphrodites, demonstrating that they produce viable gametes encoding the hermaphrodite phenotype. The absence of hermaphrodites in progeny produced on H_b hermaphrodites assesses a postmeiotic segregation distortion process favoring males during or after fertilization on H_b hermaphrodites.

A genetic determination model based on two diallelic loci plus segregation distortion

Based on crossing data performed in 1987 (see Table S1 in Saumitou-Laprade et al. 2010), we initially proposed two simple genetic determination models (Saumitou-Laprade et al. 2010): either one single locus encoding both sex and SI with three alleles (*S1*, *S2*, and *S3* with *S1* recessive to *S2* and *S3*, and *S2* to *S3*, with allele *S3* assumed to determine dominant female sterility), or two independent diallelic loci encoding sex and SI, respectively. Under the single locus hypothesis, only two male genotypes (*S1S3* and *S2S3*) are possible (Husse et al. 2013). The existence of three distinct groups of males revealed by the present data clearly rules out the possibility that a single gene with three alleles can code for both sex and SI in *P. angustifolia*. Therefore, the most likely genetic determination model involves the existence of two independent diallelic loci, one encoding SI and the other sex (see Appendix for details).

THE EQUILIBRIA AND THEIR STABILITY

The two models (with or without pollen limitation) gave very similar results, both qualitatively and quantitatively, and can be summarized in three main points. First, we found that males can invade as soon as the male advantage K is above a threshold value of 2/3, which means for instance that males can invade a panmictic hermaphroditic population even if males produce one-third less pollen than hermaphrodites. Hence, under these assumptions, and given segregation distortion and full compatibility of males with both types of hermaphrodites, males can invade the population even if their male reproductive output, for example, viable pollen production, is actually lower than that of the hermaphrodites (i.e., when $2/3 < K < 1$). Second, in the case where selection acts through both male and female reproduction, the values of the phenotypic frequencies at equilibrium are:

$$p_a = \frac{K\left(\left(5K^2+12K\right)-(3K-4)\sqrt{K\,(K+16)}\right)}{4\left(K^3+32K-16\right)}$$
$$p_b = \frac{K\left((K+1)\sqrt{K\,(K+16)}-K^2-7K+8\right)}{2\left(K^3+32K-16\right)}$$
$$p_M = 1-p_a-p_b \qquad (2)$$

When selection acts through male reproduction only, the frequencies at equilibrium are too complex to be given here but they are numerically close to the results when selection acts through both male and female reproduction (eq. 2, Fig. 1), except that the frequencies of males are expected to be slightly higher. For instance, the frequency of males is expected to be higher than 1/2

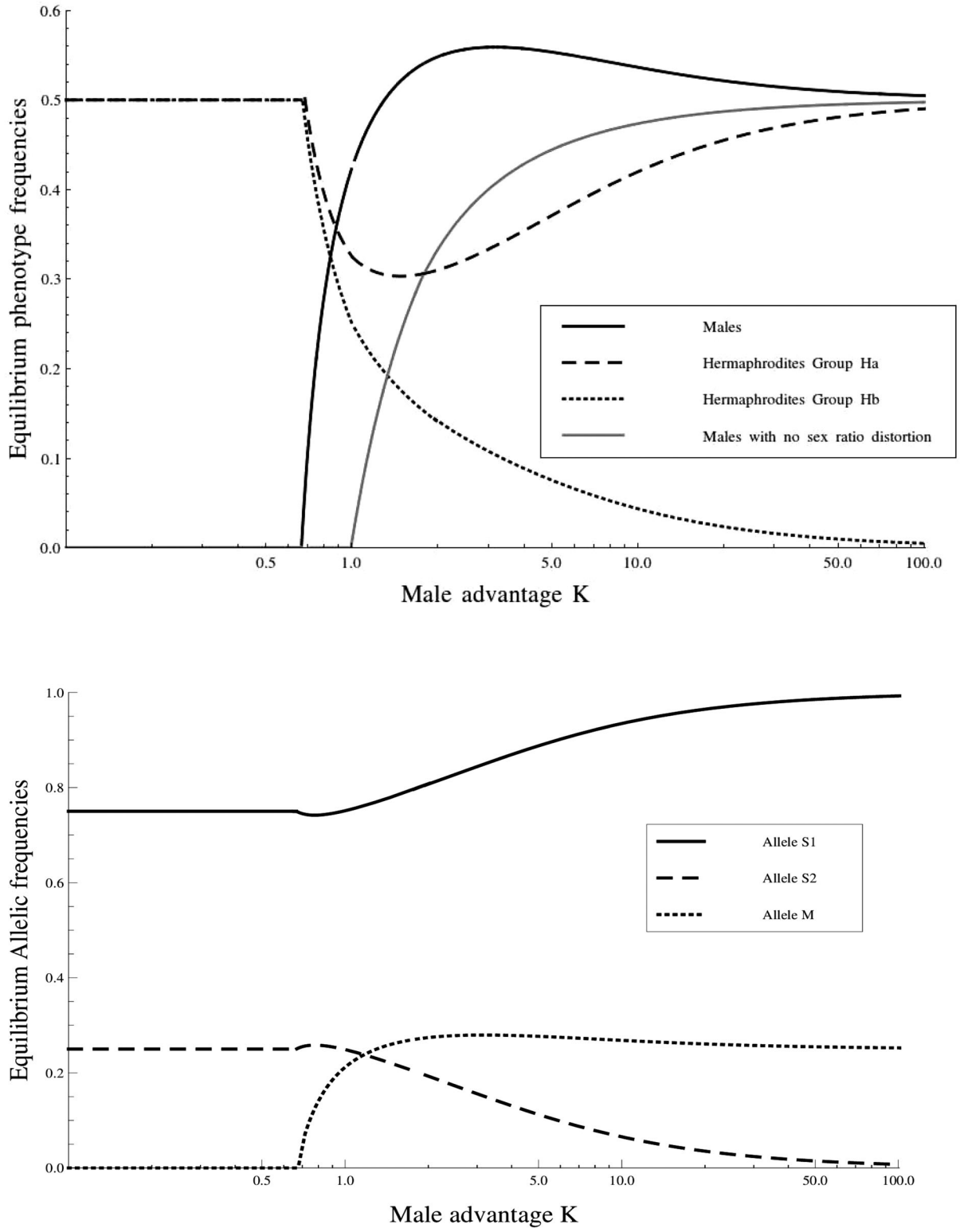

Figure 1. (A) Phenotype and (B) allelie frequencies at equilibrium. The frequency without sex-ratio distortion is from Pannell and Korbecka's (2010) model.

when $K > 2(2-\sqrt{2}) \approx 1.17$ (when there is selection through both male and female reproduction) versus $K > 1.3$ (when there is selection on male reproduction only). Equation 2 and Figure1 show that male frequencies at equilibrium can be high even when males sire fewer ovules than hermaphrodites, on average ($K < 1$), and males can reach frequencies higher than 0.5 with very moderate values of the male advantage ($K > 1.3$), and tend toward 0.5 when K is large. The highest frequency for males at equilibrium is approximately 0.582 and is obtained for $K = \frac{2}{3}\left(3+\sqrt{3}\right) \approx 3.15$.

Third, hermaphrodites of the two groups are expected to have different frequencies: H_b hermaphrodites are expected to have a lower frequency than H_a hermaphrodites due to segregation distortion favoring males over H_b hermaphrodites. The exclusion of H_b hermaphrodites increases as the male advantage K increases, because the frequency of males in the population also increases along with the proportion of ovules that are sired by males. The frequency of H_b hermaphrodites is even expected to go to 0 when K is large, that is, when the population tends to function more and more as a cryptic dioecious population. This asymmetry between the two groups of hermaphrodites explains the nonmonotonous relationship between the equilibrium male frequency and the male advantage, K. This asymmetry also means that H_b hermaphrodites are more likely to be lost in small populations than H_a hermaphrodites. Finally, when $K > 2$ approximately, regarding the allelic frequencies, the frequency of the male allele *M* is almost constant and becomes virtually independent of the value of K (Fig. 1B). Also, the *S2* allele is expected to show a low frequency, especially when K is large (Fig. 1B).

Discussion

THE MALE SEX DETERMINING FACTOR IS EPISTATIC OVER SI AND HAS PLEÏOTROPIC EFFECTS

The locus coding for sex interacts with the S locus, with two different manifestations. First, males, whatever their genotype at the S locus, are compatible with hermaphrodites of both SI groups. We interpret this observation as an epistatic effect of the *M* allele at the sex locus on the S locus, whereby the presence of the dominant *M* allele can suppress the expression of the SI phenotype. This confirms previous observation of male compatibility with hermaphrodites of both SI groups (Saumitou-Laprade et al. 2010). Second, the *M* allele is also associated with segregation distortion, whereby male gametes carrying the haplotype *mS1* or *mS2*, nonetheless viable on H_a hermaphrodites, cannot sire seeds on H_b hermaphrodites. Under strict Mendelian segregation, the crosses [*mmS1S2* × (*MmS1S1* or *MmS1S2* or *MmS2S2*)] should produce males M_a (*MmS1S1*) and/or M_b (*MmS1S2*) and/or M_c (*MmS2S2*), and hermaphrodites H_a (*mmS1S1*) and/or H_b (*mmS1S2*) and/or H_c (*mmS2S2*), but the latter three categories are never observed (Appendix).

Strikingly, the *M* allele at the sex locus has multiple pleiotropic effects. In addition to directly generating female sterility by preventing the formation of a functional stigma, it allows males to be compatible with all hermaphrodites independently of their S genotype (Saumitou-Laprade et al. 2010 and the present study), and it causes full segregation distortion on H_b. We do not know whether the segregation distorter that drives the sex-ratio segregation distortion is a selfish genetic element fully linked to the *M* allele or the *M* allele itself. Although the mechanism of such different pleiotropic effects for a single locus is difficult to explain, we speculate that the sex locus contains several different linked genes, perhaps representing a supergene (Kurian and Richards 1997; Li et al. 2007), as in the case of heterostyly, or a protosexual chromosome (Ming et al. 2011).

MECHANISMS UNDERLYING THE POSTMEIOTIC SEGREGATION DISTORTION PROCESS MAY ACT AT PRE- OR POSTZYGOTIC STAGES

The distortion event we observed in *P. angustifolia* is clearly postmeiotic and the mechanism driving segregation distortion differs from true meiotic drive. The mechanisms underlying segregation distortion may occur at either the pre- or postzygotic stage.

If we assume that segregation distortion toward males is prezygotic, then the *M* allele is necessarily active during the gametophytic phase. Furthermore, because all gametes produced by males, whatever their genotype, are compatible with H_a hermaphrodites, the *M* allele must be active in the sporophytic phase. We propose three associated pleiotropic effects that allow *M* to control its relationship with the S locus. First, the *M* allele prevents the expression of the *S1* allele in the sporophytic phase, otherwise the gametes produced by males with genotype *MmS1S1* would not be compatible on H_a hermaphrodites. Second, the *M* allele expresses incompatibility with *S2* at the sporophytic level, otherwise the *mS1* gamete produced by males *MmS1S1* would be compatible with H_b hermaphrodites, and segregation distortion would be absent in this particular case. Third, the *M* allele prevents incompatibility with *S2* during the gametophytic phase, otherwise the gametes *MS2* would not be compatible with H_b hermaphrodites. A parsimonious explanation of how the M locus works would be to assume that it is a supergene, in which the *M* allele contains (1) a copy of *S2* expressed in the sporophyte during pollen maturation, imprinting all pollen grains produced by males whatever their genotype at the S locus, and (2) an anti-S genetic component closely linked to *S2* and expressed in the gametophyte, suppressing the *S2* signature of pollen with genotypes *MS1* or *MS2*. The way in which the M locus works can be comparable with the toxin–antitoxin system well known in bacteria

(Van Melderen and Saavedra De Bast 2009), involving closely linked genes encoding both a protein "poison" and the corresponding "antidote." Both functions have opposite effects: a negative effect on the whole population (here, incompatibility of all male pollen grains with H_b) and a positive protecting effect on related members that possess the closely linked genes (here, full compatibility of gametes that contain the *M* allele).

In the case of postzygotic segregation distortion, the *M* allele must prevent the expression of SI at the sporophytic phase, otherwise no males would be compatible with both H_a and H_b hermaphrodites. Furthermore, in this case, individuals with the *mm* genotype (hermaphrodites) must either abort as embryos or die as young plants before first flowering (however, we did not observe high rates of plantlet mortality in progeny) when H_b is sired by a male. Both processes imply a transgenerational effect of the *M* allele and a strong ovule discounting effect, incurring a substantial mating cost.

IMPLICATIONS OF SEGREGATION DISTORTION ASSOCIATED WITH HOMOMORPHIC DIALLELIC SI FOR THE EVOLUTION OF MATING SYSTEMS

The existence of two SI groups of hermaphrodites and the full compatibility of males with either group is insufficient to fully explain the male frequencies of 50% or more (up to 77%) observed in natural populations of *P. angustifolia* (Husse et al. 2013). With this simple SI model, expected male frequencies for a realistic male advantage are only around 33%, and for an infinite male advantage, around 50%. Here, the combination of our empirical results and theoretical predictions show that male frequencies of above 50% can be readily explained by two SI groups of hermaphrodites and the full compatibility of males when combined with complete segregation distortion, even for a very slight male advantage (Fig. 1A).

Furthermore, and counterintuitively, we show that female-sterility mutations can be maintained even if they imply a fitness cost (at the zygotic stage) rather than an advantage to males (i.e., male advantage $K < 1$). This prediction contrasts with the current view of unisexuality as resulting exclusively from resource reallocation to the male function (in the case of androdioecy) or the female function (in case of gynodioecy) or the avoidance of inbreeding depression (Charlesworth and Charlesworth 1978; Charnov 1982). In contrast, our model, in which the combination of segregation distortion and universal compatibility are the primary sources of the male advantage, predicts that males can evolve in the absence of resource reallocation even if they have a lower fitness at the zygotic stage. Interestingly, this advantage concerns only the male locus, such that the *M* allele can be interpreted as a selfish genetic element. To our knowledge, this is the first empirical and theoretical result showing that nuclear selfish genetic elements can cause the evolution of unisexuality and males.

Table 2. Number of H_a and H_b hermaphrodites detected using the stigma test among hermaphrodites sampled in the five study sites (according to Table 1 in Saumitou-Laprade et al. 2010).

Studied site	Number of H_a	Number of H_b
1. Fabrègues	16	16
2. Moissac-Vallée-Française	10	09
3. Île Sainte-Lucie	09	10
4. Camargue—La Tour du Valat	16	02
5. Cadix-Pinar de la Algaida	14	05

Finally, because segregation distortion occurs only when H_b hermaphrodites are fertilized by males (Table 1), it introduces an asymmetry between both SI groups of hermaphrodites. The genetic model (Appendix) shows that only two H_a genotypes are eliminated in crosses ($H_b \times M_a$ and $H_b \times M_b$), instead of four H_b genotypes in crosses ($H_b \times M_a$, $H_b \times M_b$, and $H_b \times M_c$). Thus, we predict that, at equilibrium, H_b hermaphrodites are found in lower frequencies than H_a hermaphrodites (Fig. 1A) for any value of the male advantage *K*. Interestingly, in *P. angustifolia*, where the male advantage *K* is close to 1, H_b hermaphrodites were observed at low or moderate frequencies, but never at high frequencies in the five populations sampled in 2010 (Table 2). Another expectation is that H_b hermaphrodites may be lost at extreme values of the male advantage *K*, which could be attained if hermaphrodites reallocate resources to the female function.

Eliminating H_b hermaphrodites by sex-ratio distortion decreases *S2* allele frequencies and increases the probability that it will be lost through stochastic effects. In this case, the population contains only H_a hermaphrodites that cannot mate with each other and must be fertilized by *S1S1* males; the male function of hermaphrodites becomes useless and the population corresponds to a cryptic dioecious population. Such cases of cryptic dioecy have never been observed in *P. angustifolia*, but were reported in Spanish populations of *Fraxinus ornus* (Verdu 2004), another androdioecious species of the Oleaceae family (Dommée et al. 1999). Further, natural *P. angustifolia* populations with very high male frequencies (higher than 75%) reported in North Camargue (Table 2) can be explained by a foundation effect by H_b hermaphrodite(s) only and any male type, whose offspring would be only male, as long as pollen from H_a hermaphrodites is not available in the population.

JOINT EVOLUTION OF SI AND SEX

The evolution of separate sexes and SI are among the most studied mating system transitions in evolutionary biology, but SI and sex have almost exclusively been studied independently.

The strong epistasis we observed between the genes coding for SI and for sex now provides clear evidence that they should be considered jointly. There is an emerging set of observations of such interactions, including the evolution of anisogamy from preexisting mating types in the green algae (Ferris et al. 2010; Billiard et al. 2011; Geng et al. 2014), and the evolution of dioecy from heterostyly in plants (Li et al. 2010; Liu et al. 2012; Zhou et al. 2012). The observation of the joint evolution of SI and unisexuality in *P. angustifolia* confirms androdioecy as a possible route for the evolution of separate sexes from a homomorphic SI.

As demonstrated in studies by Pannell and Korbecka (2010), Husse et al. (2013), and the present article, the existence of two SI groups clearly favors the evolution of androdioecy and its maintenance with high male frequencies. However, the epistatic interaction between sex and SI loci, especially due to the pleiotropic effect of the gene (or locus) encoding sex, opens new theoretical questions on the evolution and maintenance of SI when there are unisexual individuals. In the case of gynodioecy with nucleocytoplasmic sex determination, theoretically the existence of females in a population facilitates the loss of gametophytic SI (Ehlers and Schierup 2008). By analogy, one can wonder whether androdioecy helps or not the evolution and maintenance of SI. Furthermore, the existence of only two SI groups in *P. angustifolia* is striking and unexpected due to the negative frequency-dependent selection acting on the S locus that favors any rare, and especially new, SI group. The existence of only two homomorphic SI groups in plants has been considered as possible, but only transient (Charlesworth and Charlesworth 1979), and the reasons behind a stable homomorphic diallelic SI remain to be explored. In addition to possible molecular constraints (Saumitou-Laprade et al. 2010), androdioecy with fully compatible males may also contribute to the stability of the diallelic system observed in *P. angustifolia*.

SEGREGATION DISTORTION AND THE EVOLUTION OF SEPARATE SEXES

Segregation distortion is frequent in plants and animals. It concerns many traits and is of major importance as an evolutionary force (Taylor and Ingvarsson 2003). Among all known phenomena of segregation distortion, sex-ratio distortion is one of the most ubiquitous and is thought to drive the evolution of genetic determination of sexes in dioecious species (Kozielska et al. 2010). Here, unexpectedly, segregation distortion creates favorable conditions for the evolution of androdioecy and cryptic dioecy, and eventually the evolution of separate sexes. We showed that sex-ratio distortion generates an asymmetry between the two SI groups of hermaphrodites, despite their ecological equivalence, thus favoring the loss of the male function in one of the two SI groups of hermaphrodites. Only one of the two SI groups can be effectively lost, the H_b group. Losing H_a would lead to the extinction of the population because crosses involving H_b hermaphrodites produce only males. Segregation distortion can favor the stochastic loss of the heterozygous hermaphrodites *S1S2* because they are expected to be in lower frequency (Fig. 1). Once the *S2* allele is lost, a population with only *S1S1* hermaphrodites (H_a) and males (M_a) would function as a cryptic dioecious population and, in such an already functionally dioecious population, resources are reallocated to the female function in hermaphrodites and, ultimately, the unused male function is lost, generating a morphologically dioecious population (Mayer and Charlesworth 1991). Therefore, the conjunction of diallelic SI and segregation distortion makes stable androdioecy with high male frequencies observed in Oleaceae, a likely route to dioecy. This is supported by the relatively high proportion (11%) of androdioecious species in the Oleaceae (Wallander 2001), and by phylogeny of *Fraxinus* that shows at least one transition from androdioecy to dioecy in the section *Ornus* (Wallander 2008).

General Conclusion

An open question is thus now how full segregation distortion toward males evolves and how widespread it is in the Oleaceae family. Answering this question requires theoretical work to investigate the conditions under which a female-sterility mutation with segregation distortion can invade a population already fixed for a female-sterility mutation. Empirical studies, for example, screening other androdioecious species (such as *F. ornus*, Dommée et al. 1999, and *O. fragrans*, Hao et al. 2011) for segregation distortion, will lend further insight. Additional questions include the evolution of the diallelic SI system and its distribution in the Oleaceae family and beyond; for example, did it exist in the ancestral heterostylous species and is it still present in dioecious species derived from androdioecious or polygamous species?

ACKNOWLEDGMENTS

The authors thank N. Faure, T. Mathieu, and D. Degueldre for support in caring for the plants at the Lille1 University greenhouse and at the experimental garden at the *Centre d'Ecologie Fonctionnelle et Evolutive* (CEFE-CNRS) in Montpellier. They are very grateful to A.-S. Blervaque for access to microscopy facilities at the *Stress Abiotique des Végétaux* (UMR INRA 1281) laboratory, B. Buatois for biochemical analyses, and M.-P. Dubois for microscopy facilities at the CEFE-CNRS in Montpellier. The authors are very grateful to J. Cuguen for financial support and encouragements. They thank J. Pannel, V. Castric, X. Vekemans, and M. Dufaÿ for scientific discussions and helpful comments on the manuscript. This work was supported by the French National Research Agency (ANR-11-BSV7-013–03). All authors contributed extensively to the work presented in this article. PSL and PV jointly designed and carried out the crossing design and phenotyping strategies with JL. They performed phenotyping and genetic analysis and produced the genetic model together with LH. SB developed and analyzed the population genetic model jointly with LH and PL. AB oversaw the germination and

early growth of the biological material. CG and LH performed genotyping with molecular markers and paternity assessment of the crosses progeny. SB, PV, and PSL wrote the article.

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Associate Editor: L. Fishman
Handling Editor: R. Shaw

Appendix

Genetic determination model for SI and sex

locus, the S locus, encodes the SI system with a dominant allele *S2* and a recessive allele *S1*. Because crosses on H_a hermaphrodites reveal the three groups of males, they can be considered as test crosses that can identify the allelic status of males at the S locus (*S1S1*, *S1S2*, or *S2S2*). We therefore hypothesized that H_a hermaphrodites are homozygous at the S locus for the recessive *S1* allele. H_b hermaphrodites would thus have the heterozygous *S1S2* genotype. Note that in hermaphrodite species with diallelic sporophytic SI (i.e., heterostylous species), dominant alleles cannot form homozygote combinations, such that *S2S2* genotypes cannot be produced in hermaphrodites. However, in the present androdioecious species case, because males do not express their SI phenotype, *S2S2* genotypes can exist.

Some gamete combinations between males and hermaphrodites H_b are eliminated, but all possible combinations are transmitted in crosses between males and H_a. The Mendelian segregations obtained in male progeny when crossedwith H_a are

				Paternal											
	Phenotypes:			H_a	H_b		M_a		M_b				M_c	
		Genotypes:		mm S1S1	mm S1S2		Mm S1S1		Mm S1S2				Mm S2S2	
			Gametes	mS1	mS1	mS2	MS1	mS1	MS1	MS2	mS1	mS2	MS2	mS2
Maternal	H_a	mm S1S1	mS1	Inc	H_a mm S1S1	H_b mm S1S2	M_a Mm S1S1	H_a mm S1S1	M_a Mm S1S1	M_b Mm S1S2	H_a mm S1S1	H_b mm S1S2	M_b Mm S1S2	H_b mm S1S2
	H_b	mm S1S2	mS1	H_a mm S1S1	Inc	Inc	M_a Mm S1S1	*H_a mm S1S1*	M_a Mm S1S1	M_b Mm S1S2	*H_a mm S1S1*	*H_b mm S1S2*	M_b Mm S1S2	*H_b mm S1S2*
			mS2	H_b mm S1S2	Inc	Inc	M_b Mm S1S2	*H_b mm S1S2*	M_b Mm S1S2	M_c Mm S2S2	*H_b mm S1S2*	*H_c mm S2S2*	M_c Mm S2S2	*H_c mm S2S2*

"Inc" indicates missing allelic combinations due to incompatibility reactions observed in progeny from controlled crosses. Barred cells indicate missing allelic combinations (in italics) due to sex-ratio distortion.

We assumed the existence of two diallelic loci. The first locus, which we called the M locus, determines sex with a dominant allele *M* coding for the male phenotype (i.e., the female-sterility mutation) and a recessive allele *m* coding for the hermaphrodite phenotype, such that *mm* individuals are hermaphroditic and *mM* individuals are male (note that males cannot mate with each other and therefore *MM* individuals cannot be produced). The second distorted toward males when crossed with H_b. These segregation distortions, conditional to the type of hermaphrodite recipient, can be qualified as full because progeny are entirely constituted of male individuals in crosses between males and H_b hermaphrodites. Under this hypothesis, three different groups of males with different genotypes (*mMS1S1*, *mMS1S2*, and *mMS2S2*) can be produced in each generation, whereas only two groups of hermaphrodites can be produced (*mmS1S1* and *mmS1S2*) because hermaphrodite H_c (*mmS2S2*) is never produced due to incompatibility in ($H_b \times H_b$) and sex-ratio distortion in ($H_b \times M_b$) or ($H_b \times M_c$) crosses.