

## How much better are females? The occurrence of female advantage, its proximal causes and its variation within and among gynodioecious species

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• **Background** Gynodioecy is a reproductive system of interest for evolutionary biologists, as it poses the question of how females can be maintained while competing with hermaphrodites that possess both male and female functions. One necessary condition for the maintenance of this polymorphism is the occurrence of a female advantage, i.e. a better seed production or quality by females compared with hermaphrodites. Theoretically, its magnitude can be low when sterility mutations are cytoplasmic, while a 2-fold advantage is needed in the case of nuclear sterility. Such a difference is often thought to be due to reduced inbreeding depression in obligatory outcrossed females. Finally, variation in sex ratio and female advantage occur among populations of some gynodioecious species, though the prevalence of such variation is unknown.

• **Scope** By reviewing and analysing the data published on 48 gynodioecious species, we examined three important issues about female advantage. (1) Are reduced selfing and inbreeding depression likely to be the major cause of female advantage? (2) What is the magnitude of female advantage and does it fit theoretical predictions? (3) Does the occurrence or the magnitude of female advantage vary among populations within species and why?

• **Conclusions** It was found that a female advantage occurred in 40 species, with a magnitude comprised between 1 and 2 in the majority of cases. In many species, reduced selfing may not be a necessary cause of this advantage. Finally, female advantage varied among populations in some species, but both positive and negative correlations were found with female frequency. The role of reduced selfing in females for the evolution of gynodioecy, as well as the various processes that affect sex ratios and female advantage in populations are discussed.

**Key words:** Female advantage, female compensation, male sterility, gynodioecy, inbreeding depression, mating system, resource allocation, sex ratio, self-incompatibility.

### INTRODUCTION

Gynodioecy, the co-occurrence of females and hermaphrodites within the same species, is a relatively common sexual system in flowering plants (reviewed in Webb, 1999) and has been a model of interest for evolutionary biologists for several reasons. First, like any other polymorphism, it poses the question of its appearance and its maintenance within populations. Because this particular polymorphism involves the occurrence of female individuals that have lost one of their sexual functions with hermaphrodites that possess both, its maintenance at equilibrium is particularly intriguing. Secondly, because gynodioecy is sometimes viewed as an intermediate step in the evolution of dioecy (co-occurrence of female and male plants), its understanding appears crucial for the study of the evolution of separate sexes, which is a common transition in the evolutionary history of Angiosperms (Charlesworth, 1999; Barrett, 2002). Thirdly, since gynodioecy results in many cases from interactions between cytoplasmic and nuclear genes (see below), it constitutes a choice model for the study of nuclear–cytoplasmic conflicts (Saumitou-Laprade *et al.*, 1994).

Two main sex determination systems in gynodioecious species have been investigated theoretically and documented in

nature: (1) mutations responsible for male sterility are nuclear (Godley, 1955; Kohn, 1989) or (2) mutations of male sterility are cytoplasmic (CMS, for cytoplasmic male sterility) and their effect can be counteracted by nuclear alleles, named restorers, that restore pollen production (Cosmides and Tobby, 1981; reviewed in Chase, 2007). In the latter case, because they are purely maternally inherited, the loss of pollen production does not reduce the transmission of cytoplasmic genes, while it clearly constitutes a cost for nuclear genes that are bi-parentally transmitted, leading to the so-called nuclear–cytoplasmic conflict. For both kinds of sex determination, theory predicts that females should benefit from a better female fitness than hermaphrodites, in order for mutations of male sterility to invade a population. Such a fitness difference has been called female advantage (FA) or female compensation in the literature (Darwin, 1877) and is a central parameter of the evolution of gynodioecy. In a meta-analysis conducted on 29 different gynodioecious species, Shykoff *et al.* (2003) found indeed that females produced more flowers, set more fruits and produced more seeds that were larger and germinated better than those of hermaphrodites from the same populations, thus showing that the occurrence of a FA is a general trend in gynodioecious species.

Practically, FA, when it occurs, can have several proximal causes including (a) reallocation of resources from the male towards the female function; (b) sex differences in interactions with herbivores (hermaphrodite-biased predation); and (c) reduced selfing and associated inbreeding depression (ID) in females. The latter process has been investigated in theoretical models (e.g. Charlesworth and Charlesworth, 1978) and is sometimes considered to be the main process responsible for the fitness advantage of females, and thus for gynodioecy (e.g. Kubota and Ohara, 2009); however, the strength and the generality of this effect remain poorly known.

Besides the occurrence of FA, both the among-population variation and the magnitude of this effect are potentially important for the evolutionary dynamics of gynodioecy. First, although most theoretical models – even those that consider species within a meta-population context (Frank, 1989; Pannell, 1997; Couvet *et al.*, 1998; Dufay and Pannell, 2010) – generally assume the FA to be a constant parameter of the species, it is thought possibly to vary among populations, as a function of a biotic or abiotic component of the environment and/or of the population sex ratio. Such a variation in the FA among populations has been shown to facilitate the maintenance of the sexual polymorphism in some cases (McCauley and Taylor, 1997; Frank and Barr, 2001). Secondly, although a FA is theoretically needed in all gynodioecious species, its magnitude should vary according to the details of genetic determination of male sterility. On the one hand, the invasion and the maintenance of nuclear-transmitted male sterility requires a 2-fold FA, that compensates the loss of pollen production for nuclear alleles of male sterility (Lewis, 1941; Lloyd, 1976). On the other hand, a FA comprised between 1 and 2 (i.e. females are better, but not necessarily twice better, than hermaphrodites) should be sufficient for CMS to invade a population (Gouyon *et al.*, 1991; Bailey *et al.*, 2003), although other conditions (i.e. a cost of restorer alleles and/or recurrent local extinctions and invasions of demes in a metapopulation context) are necessary for the stable maintenance of the sexual polymorphism in that case (Gouyon *et al.*, 1991; Couvet *et al.*, 1998; Bailey *et al.*, 2003). To date, no clear synthesis is available regarding the effective magnitude of FA that has been empirically measured in gynodioecious plants nor about its possible variation among the populations of a given species.

This work reviews experimental studies that have investigated the occurrence of FA in 48 different gynodioecious species. The main purpose of the current study was not to verify the overall occurrence of a FA (already shown by Shykoff *et al.*, 2003), but rather to examine other questions related to this fitness effect. (a) Is reduced selfing and associated ID likely to be the major cause of FA? (b) What is the magnitude of the FA, how does it vary among species and does it fit with theoretical predictions? (c) Does the occurrence or the magnitude of the FA vary among populations within species and why?

## METHODS

### *Literature search and data extraction*

We searched the bibliographic database Web of Science up to July 2009 for all papers containing the words ‘gynodioecy’, ‘male sterility’, ‘female advantage’ or ‘female compensation’

in the title or keywords. We retained all studies that showed a statistical comparison of at least one of the reproductive traits listed below, between females and hermaphrodites, either in natural populations or in controlled conditions (greenhouse or experimental garden). The present work reviews the data published in 82 studies, based on 48 gynodioecious species, belonging to 39 different genera and 27 families (Table 1).

For all investigated species, we collected the results of statistical comparisons between open-pollinated females and open-pollinated hermaphrodites for the following reproductive traits: number of flowers (NFL), fruit set (FS), defined as the fruit/flower ratio, seed set or number of seeds per fruit (SS), seed size or seed mass (SM), total number of fruits per plant (NF), total number of seeds per plant (NS) and seed germination rate (G). Female–hermaphrodite comparisons were also recorded for offspring or adult survival rate (Surv). For each of these traits, it was noted whether the reviewed study found a statistical advantage for females, a statistical advantage for hermaphrodites or a non-significant difference. In a second step, the results obtained for the different reproductive traits for each species were summarized: a FA was considered to occur in a given species as soon as a significantly higher value was found in females for at least one of the traits. When only female disadvantages or non-significant differences were found on the several investigated traits, it was considered that no FA effectively occurred. Species for which different studies found contradictory results were classified in a separate category (see Results).

For traits related to fruit or seed production and quality (FS, SS, NS, NF, G and SM), the results of other types of female–hermaphrodite comparisons (experimentally cross-pollinated females vs. experimentally cross-pollinated hermaphrodites; experimentally cross-pollinated females vs. experimentally self-pollinated hermaphrodites) were also recorded when available, because they could provide some information on the proximal causes of the FA (Table 2). Indeed, a FA that has been found by comparing seed production or quality between open-pollinated female flowers and open-pollinated hermaphroditic flowers reflects an advantage that females should experience in natural conditions, and was subsequently reported in Table 1, but such a FA may be attributable to different factors, including differences in the number of ovules per flower, in the ability to set and mature seeds, in the quantity of resources that can be invested in seeds and in the average quantity of pollen deposited on stigmas. The proportion of self-pollen deposited on stigmas and the overall quality of selfed/outcrossed seeds in hermaphrodites are other important factors that can influence various components of female reproduction. Thus, when only open-pollinated flowers had been compared between females and hermaphrodites, we could assess whether a FA effectively occurred, but it was not possible to assess which of these factors was responsible for it. However, comparisons performed on hand-pollinated (outcrossed) flowers could provide additional information, because one can expect that (a) the quantity of pollen deposited on stigmas was similar between female and hermaphroditic flowers and that (b) only outcrossed pollen had been deposited, consistently leading to an inbreeding level that should be similar between seeds produced by the two sexes. Consequently, if a FA was found in such a case, it

TABLE 1. Main characteristics of the 48 gynodioecious species and occurrence of female advantage

Species	Family	SI/SC	Poll	Life cycle	Genetics	FA?	Fitness traits			References
							Females are better	Females are not better	No differences	
<i>Beta vulgaris</i>	Amaranthaceae	SI	W	P	NC	NO			NFL, SS, NS, G	Boutin-Stadler (1987); Dufay <i>et al.</i> (2009)*
<i>Gingidia flabellata</i>	Apiaceae			P		FA	FS	NFL		Webb (1981)
<i>Cirsium chikushiense</i>	Asteraceae	SI	E	P		NO		NFL		Kawabuko (1994)
<i>Echium vulgare</i>	Boraginaceae	SC	E	Bisa	NC	FA	FS, SS, NS		NFL, SM, G	Klinkhamer <i>et al.</i> (1991*, 1994)
<i>Eritrichum aretioides</i>	Boraginaceae	pSC	E	P		FA	SM, G		NFL, SS	Puterbaugh <i>et al.</i> (1997)
<i>Phacelia linearis</i>	Boraginaceae	SC	E	A	N	FA	NS		SM, SS, G, Surv	Eckhart (1992a, b)
<i>Raphanus sativus</i>	Brassicaceae	SI	E	A	NC	NO		NFL, NS, G	SM	Miyake <i>et al.</i> (2009)
<i>Opuntia quimilo</i>	Cactaceae	SC	E	P		FA	FS		NFL,SS	Diaz and Cocucci, 2003
<i>Lobelia siphilitica</i>	Campanulaceae	SC	E	P	NC	FA	NFL, NS		SS, NFL	Caruso and Yakobowski (2008); Caruso and Case (2007*); Caruso <i>et al.</i> (2003)
<i>Dianthus sylvestris</i>	Caryophyllaceae	SC	E	P		FA	NFL, SM		SS, G	Collin <i>et al.</i> (2002)
<i>Moehringia lateriflora</i>	Caryophyllaceae	pSC	E	P		VAR	FS, SS		FS, SS	Sugawara (1993)
<i>Schiedea adamantis</i>	Caryophyllaceae	SC	W/E	P	N	FA	FS, SM, SS		NFL, G, Surv-Ad,	Sakai <i>et al.</i> (1997)
<i>Schiedea salicaria</i>	Caryophyllaceae	SC	W/E	P	N	VAR	G, SM, SS		NFL, FS, SM, SS, G	Weller and Sakai (2005)
<i>Silene acaulis</i>	Caryophyllaceae	SC	E	P	NC	FA	FS		NFL	Shykoff (1992*); Hermanutz and Innes (1994)
<i>Silene italica</i>	Caryophyllaceae	SC	E	P	NC	FA	FS, SM	NFL	NFL	Maurice (1999); Lafuma and Maurice (2006)
<i>Silene nutans</i>	Caryophyllaceae	SC	E	P	NC	NO			NFL	Dufay <i>et al.</i> (2010)
<i>Silene stockenii</i>	Caryophyllaceae	SC	E	A	NC	NO			FS, SS	Talavera <i>et al.</i> (1996)
<i>Silene vulgaris</i>	Caryophyllaceae	SC	E	P	NC	FA	FS, SM, SS		SS, G	Dulberger and Horovitz (1984); Olson <i>et al.</i> (2006); Andersson <i>et al.</i> (2008*)
<i>Clusia nemorosa</i>	Clusiaceae	SC	E	P		FA	FS			Lopes and Machado (1998)
<i>Wurmbea biglandulosa</i>	Colchicaceae	SC	E	P		VAR	NFL, SS, NS, G	SM, NS	SS, Surv	Ramsey and Vaughton (2002); Vaughton and Ramsey (2004)
<i>Cucurbita foetidissima</i>	Cucurbitaceae	SC	E	P	N	FA	NFL,NS	SS	FS,SM	Kohn (1989)
<i>Erythroxylum havanense</i>	Erythroxylaceae	SI	E	P		FA	FS, Surv-Ad			Avila-Sakar and Dominguez (2000); Rosas <i>et al.</i> (2005)
<i>Geranium maculatum</i>	Geraniaceae	SC	E	P		FA	NFL, FS, SM, SS, NS, G, Surv		NFL, FS, G, Surv	Agren and Willson (1991); Chang (2006)
<i>Geranium sylvaticum</i>	Geraniaceae	SC	E	P		VAR	FS, SS, NS		NFL,SM,G,Surv	Asikainen and Mutikainen (2003, 2005); Ramula and Mutikainen (2003)
<i>Nemophila menziesii</i>	Hydrophyllaceae	SC	E	A	NC	FA	NS		NFL	Barr (2004)

Continued

TABLE 1. *Continued*

Species	Family	SI/SC	Poll	Life cycle	Genetics	FA?	Fitness traits			References
							Females are better	Females are not better	No differences	
<i>Iris douglasiana</i>	Iridaceae	SC	E	P		Amb.	SS		SS	Uno (1982)
<i>Glechoma longituba</i>	Lamiaceae	SC	E	P		VAR	SM, SS	SS		Zhang <i>et al.</i> (2008)
<i>Lycopus maackianus</i>	Lamiaceae	.	E	P		FA	NS			Hong and Moon (2003)
<i>Thymus vulgaris</i>	Lamiaceae	SC	E	P	NC	FA	NS, G			Assouad <i>et al.</i> (1978)
<i>Limnanthes douglasii</i>	Limnanthaceae	SC	E	A	NC	FA	NFL			Kesseli and Jain (1984, 1985)
<i>Nototriche compacta</i>	Malvaceae	SC	E	P		NO		SS	FS, SS	Garcia-Franco and Arroyo (1995)
<i>Sidalcea hendersonii</i>	Malvaceae	SC	E	P	N	VAR	SS		FS, SS	Marshall and Ganders (2001)
<i>Sidalcea malviflora</i>	Malvaceae	SC	E	P	NC	FA	SS			Graff (1999)
<i>Chionographis japonica</i>	Melanthaceae	SC	E	P		FA	NS		NFL	Maki (1993)
<i>Satyrium ciliatum</i>	Orchidaceae	SC	E	P		FA	FS			Huang <i>et al.</i> (2009)
<i>Hebe subalpina</i>	Plantaginaceae	SC	E	P		FA	FS		NFL	Delph (1993)
<i>Plantago coronopus</i>	Plantaginaceae	SC	W	A	NC	Amb.	NFL, SM	SS	SS, Surv	Koelewijn (1996, 1998); Koelewijn and VanDamme (1996*)
<i>Plantago lanceolata</i>	Plantaginaceae	SI	W	P	NC	FA	NFL, NS, SM, Surv-Ad		SM, SS, G, Surv	Poot <i>et al.</i> (1996); Vandamme and Vandelden, (1982*, 1984)
<i>Plantago maritima</i>	Plantaginaceae	Mainly SI	W	P	NC	VAR	NFL, SM, NS		FS, SS, NS	Nilsson and Agren (2006)
<i>Chionochloa bromoides</i>	Poaceae	SC	W			NO			NFL, SS	Connor (1990)
<i>Cortaderia richardii</i>	Poaceae	SC	W	P	N	FA	SM, G			Connor (1965)
<i>Fragaria virginiana</i>	Rosaceae	SC	E	P	N	FA	FS, NFL, NS	NFL		Ashman (1999, 2003); Case and Ashman (2007)
<i>Prunus mahaleb</i>	Rosaceae	pSC	E	P		FA	NFL, SM, NF	FS	G	Jordano (1993)
<i>Bequaertiodendron magalismontanum</i>	Sapotaceae		E	P		FA	FS			Steyn and Robbertse (1990)
<i>Saxifraga granulata</i>	Saxifragaceae	SC	E	P	NC	Amb.	SM, G	SS	NFL	Stevens and Richards (1985*); Stevens (1988)
<i>Daphne laureola</i>	Thymelaeaceae	SC	E	P		NO	NFL	FS	SM, FS	Alonso and Herrera (2001); Alonso (2005); Alonso <i>et al.</i> (2007)
<i>Gnidia wikstroemiana</i>	Thymelaeaceae	SC	E	P		FA	NFL		FS	Beaumont <i>et al.</i> (2006); Smith (2009*)
<i>Kallstroemia grandiflora</i>	Zygophyllaceae	SC	E	A		Amb.	FS	SS	NS, SM	Cuevas <i>et al.</i> (2008)

For each species is listed: whether it is self-compatible (SC), partially self-compatible (pSC) or self-incompatible (SI); its pollination mode (Poll), if it is either wind pollinated (W) or entomophilous (E); whether it is perennial (P), bisannual (Bisa) or annual (A) and, where known, the determination of sex (NC, nuclear–cytoplasmic; N, nuclear). This table reports the traits for which studies have found a female advantage, a female disadvantage or no difference between females and hermaphrodites. When the same trait is found in several categories for a given species, it means that either different studies found contradictory results or that results vary according to the population or year. Abbreviation for the traits are the followings: NFL, number of flowers; FS, fruit set; SS, seed set; NS, number of seeds per plant; NF, number of fruits per plant; SM, seed mass or size; G, germination; Surv, survival of offspring; Surv-Ad, adult survival. Only results obtained on open-pollinated flowers are reported here. The column 'FA' summarizes all reported results, by indicating whether a female advantage has been statistically demonstrated by the original studies for at least one trait (FA), ambiguous cases (Amb.), species for which female advantage varies with populations (VAR) and species for which no female advantage has been found (NO). References tagged with an asterisk are those that were used for reporting data on sex ratios but not on fitness traits.

TABLE 2. How to interpret different types of comparison between females and hermaphrodites for a given species

Comparison of outcrossed flowers	Comparison of open-pollinated flowers		
	F < H	F = H	F > H
F < H	FD	–	–
F = H	FD due to pollen limitation	No FA	FA mainly due to the avoidance of ID
F > H	FD due to pollen limitation. FA may be pollinator dependent*	FA may be pollinator dependent*	FA not (entirely) due to the avoidance of ID

When female (F) vs. hermaphrodite (H) comparisons were performed in both open-pollinated and artificially outcrossed flowers, several combinations of results could be found for a given trait. Whereas comparisons of open-pollinated flowers highlight whether females benefit from a female advantage (FA) or disadvantage (FD) in natural conditions, comparisons based on hand-pollinated (outcross) flowers provide information on whether the avoidance of self-pollination and associated inbreeding depression (ID) may be one proximal explanation of this fitness advantage, as soon as they were performed in the same ecological conditions as open pollinations.

A dash indicates situations that have not been reported in this review. In cases marked by an asterisk, a female advantage could occur (probably due to resource reallocation, rather than avoidance of ID) in non-pollen-limited situations.

could not be attributable to a difference in the level of pollen limitation nor to a reduced selfing and associated ID in females (Table 2). We also extracted from the reviewed articles any additional information that would help to clarify this (e.g. measurements of the selfing rate occurring in populations; measurements of the magnitude of ID on fitness traits).

In order to investigate the possible variation of occurrence of a FA among populations, within a given species, we then focused on studies in which at least one of the fitness traits listed above was measured on plants originating from different populations. Based on these studies, we recorded whether the statistical analyses showed a significant FA in all populations or whether a variation of FA occurrence was found within the same study. Any details that could explain such among-populations variation were also recorded.

Finally, for each species, the following reproductive characteristics were also reported, when information was available: the sex ratio in natural populations (average and/or minimum and maximum female frequencies), mode of pollination (entomophilous vs. anemophilous), sex determination (nuclear vs. nuclear–cytoplasmic), life cycle (annual vs. perennial) and potential for self-pollination in hermaphrodites (self-compatible vs. self-incompatible species). Such information can be useful for the understanding of the dynamics of gynodioecy since (a) sex differences in interactions with pollinators may lead to sex differences in reproductive success in entomophilous species; (b) the magnitude of FA is expected to differ according to the details of sex determination; (c) sex differences in reproductive success may be caused by differences in terms of survival in perennial species; and (d) information on self-(in)compatibility will help us in investigating the possible role of reduced selfing and ID in the expression of FA (see below).

### Quantifying female advantage

It was possible to estimate the magnitude of the FA for 28 gynodioecious species. For this analysis, species for which either the number of fruits or the number of seeds per plant had been measured or estimated for both females and hermaphrodites were retained. This was done in order to (a) build a homogeneous data set and (b) obtain reliable estimates of the female reproductive success. Then, the FA was computed as the ratio of the average value reported in females divided by the average value in hermaphrodites. We recorded either the number of seeds or fruits that was directly measured by the study, or an estimation of this number, by computing data measured on other reproductive traits (number of flowers × fruit set or number of flowers × number of seeds per fruit). We only used data that had been recorded on open-pollinated flowers, in order to obtain values as close as possible to the effective variation in female fitness in natural populations.

In a few species (*Wurmbea biglandulosa*, *Geranium maculatum*, *Geranium sylvaticum* and *Fragaria virginiana*), several values were available, from different studies. In such cases, we retained the study that had been performed using the highest number of populations (i.e. Asikainen and Mutikainen, 2005 for *G. sylvaticum*; and Vaughton and Ramsey, 2004 for *W. biglandulosa*). In *G. maculatum* and *F. virginiana*, for which such criteria could not help in choosing one of the studies, we retained the study that provided an average value for the FA (i.e. Agren and Willson, 1991, instead of Chang, 2006 that provided a range of values for *G. maculatum*; and Ashman, 1999 instead of Case and Ashman, 2007 for *F. virginiana*).

Finally, in order to investigate possible variation in the magnitude of FA, we focused on studies that contained such data measured on plants from different populations, and recorded the different values of the numbers of seeds or fruits per plant, corresponding to each studied population. In comparing populations, we preferred to rely only on data from single studies, rather than combining data from different studies, which may have used different methods.

## RESULTS

### Occurrence of a female advantage

Out of the 48 investigated species, a FA was found with no ambiguity on at least one fitness-related trait in 29 different species. In a few cases among these 29 species (*Cucurbita foetidissima*, *F. virginiana*, *Gingidia flabellata*, *Prunus mahaleb* and *Silene italica*), one other trait, either the number of flowers, fruit set or seed set, was found to favour hermaphrodites; however, the total number of seeds or fruits per plant was found in all cases to be larger in females, showing that the advantage for females was not cancelled out by their disadvantage in other fitness components. For all of these 29 species, we thus considered that a FA occurred (Tables 1 and 3).

In four additional species, although a FA was found on one or several traits, it was difficult to reach a firm conclusion. In *Iris douglasiana*, hermaphrodites experienced stronger pre-dispersal seed predation by caterpillars, but the quantitative

TABLE 3. Summary of the different situations in terms of female advantage: occurrence (FA), variation in female advantage among populations (VAR), ambiguous cases (Amb.) and no female advantage (NO), according to the genetic determination of sex and the mating system

	FA	VAR	Amb.	NO	Total
Self-compatible (SC) species					
Nuclear	5	2	0	0	7
Nuclear–cytoplasmic	9	0	2	2	13
Unknown	10	4	2	3	19
Total	24	6	4	5	39
Self-incompatible (SI) species					
Nuclear	0	0	0	0	0
Nuclear–cytoplasmic	1	1	0	2	4
Unknown	1	0	0	1	2
Total	2	1	0	3	6
No information on SI/SC or on determination of genetics	3	0	0	0	3
Total	29	7	4	8	48

consequence on female seed set relative to hermaphrodites was not detailed (Uno, 1982). In *Plantago coronopus*, one study found a FA (Koelewijn, 1996), while another found the opposite result (Koelewijn, 1998). Finally, in the two last species, a FA was found for one trait, but a hermaphroditic advantage was found for at least one other. In *Saxifraga granulata*, females produced heavier seeds with a higher germination rate, but showed a lower seed set than hermaphrodites, and no quantitative measurement of these contradictory effects was available (Stevens, 1988). In *Kallstroemia grandiflora*, females showed a higher fruit set but a lower seed set than hermaphrodites, apparently leading to a globally similar seed number among females and hermaphrodites (Cuevas *et al.*, 2008). These four species were scored as ‘ambiguous’ cases for the occurrence of a FA (‘Amb.’ in Tables 1 and 3).

In 25 species, at least one study was carried out on the reproductive success of females and hermaphrodites originating from more than one population. Among them, seven species were found to show a variation in the occurrence of FA, a statistical advantage being found for females in some data sets but no significant difference (or a significant disadvantage) being detected in other data sets generated by the same study (‘VAR’ category, Tables 1 and 3; details provided in Table 4). In some of these cases, the occurrence of FA seemed to be related to the sex ratio, either at the patch or at the population level, but both positive and negative correlations with female frequency were reported (Table 4).

Finally, in the eight remaining species, no FA could be found (Tables 1 and 3) and, in some cases, females even seemed to be overall disadvantaged in terms of their female fitness compared with hermaphrodites. In some of these species (*Cirsium chikushiense*, *Silene nutans* and *Chionochloa bromoides*), such a result was not very informative since very few traits were compared between females and hermaphrodites. Some others (*Raphanus sativus*, *Beta vulgaris* and *Daphne laureola*) constitute intriguing cases, in which no benefit could be found in favour of females, when comparing sex types for many different traits, in several populations.

Only six species were defined as self-incompatible (*B. vulgaris* spp. *maritima*, *C. chikushiense*, *R. sativus*,

*Erythroxylum havanense* and *Plantago lanceolata*) or mainly self-incompatible (*Plantago maritima*). For three other species, we could not find any information on self-compatibility/incompatibility. All the other (39) species were defined as self-compatible or at least partially self-compatible (Table 1), meaning that hermaphrodites have the potential for self-pollination, producing some inbred seeds that might suffer from ID. Because of the very low number of self-incompatible species and also because these data are not phylogenetically independent from each other, a statistical comparison of self-incompatible and self-compatible species would be difficult to interpret and would probably lack statistical power. However, as shown in Table 3, it is interesting to note that species for which no FA was found represent half of the self-incompatible species, but only 13% of the self-compatible species.

#### Proximal causes of the female advantage

Among the 40 species in which a FA was detected on at least one fitness trait, in at least one population (categories ‘FA’, ‘Amb’ and ‘VAR’ in Tables 1 and 3), we could hypothesize on the proximal cause of such a fitness difference in 21 cases (Table 5). In 14 of these species, the FA could not be entirely explained by reduced self-pollination and associated ID: (a) when the species was self-incompatible (three cases); (b) when a FA was also clearly found when comparing outcrossed seeds or fruits between females and hermaphrodites (eight cases); and (c) when the selfing rate of hermaphrodites was found to be naturally low in populations or when the magnitude of ID was found to be low, at least for the trait on which a FA had been found (three cases). In contrast, in seven other species, the FA could be mainly attributed to reduced self-pollination and associated ID, either by using direct evidence (no FA when comparing outcrossed seeds or fruits; one case) or by using other information, based on the selfing rate or occurrence of ID (six cases; Table 5).

*Geranium maculatum* and *Schiedea adamantis* were two additional species in which females had a higher female fecundity compared with open-pollinated hermaphrodites but not with outcrossed hermaphrodites. However, in both these species, the result of open pollinations was observed in natural populations (Agren and Wilson, 1991; Sakai *et al.*, 1997; Chang, 2006) whereas outcrossed pollinations had been performed in greenhouses (Sakai *et al.*, 1997; Van Etten *et al.*, 2008). Thus, the difference in the occurrence of FA could be explained not only by a difference in the pollination treatment, but also by a difference in ecological conditions, such as soil fertility, moisture or light availability. It was thus decided not to include these inconclusive results in Table 5.

The fact that FA was, or was not, due to reduced ID seemed not to affect the type of reproductive trait on which the FA was detected. For instance, a FA was found through a higher seed mass or germination regardless of whether reduced ID had apparently played a role (e.g. *S. adamantis*, *Schiedea salicaria* and *Silene vulgaris*) or not (e.g. *Silene acaulis*, *E. havanense*, *Thymus vulgaris*, *P. coronopus*, *P. lanceolata*, *P. maritima*, *Cortaderia richardii*, *P. mahaleb*). Similarly, fruit set was often found to be higher in females that apparently benefited

TABLE 4. Variation of female advantage among populations

Species	No. of populations	Measure	Results	Details	References
Qualitative variation					
<i>Moehringia lateriflora</i>	2	Seed set	1.02; 1.46*	FA in patches with low female frequency	Sugawara (1993)
<i>Wurmbea biglandulosa</i>	3	Nb seeds	0.79; 1.07; 1.63*	Variation in the magnitude of pollen limitation, which affects females more than hermaphrodites	Ramsey and Vaughton (2002)
<i>Geranium sylvaticum</i>	2	Nb seeds	1.2; 3.3 *	FA in populations with high female frequency	Ramula and Mutikainen (2003)
<i>Glechoma longituba</i>	6	Seed set	FA in 3 populations; FD in 3 populations	FA in populations with low female frequencies	Zhang <i>et al.</i> (2008)
<i>Sidalcea hendersonii</i>	6	Seed set	0.4; 0.8; 1; 1.15; 1.16*; 1.21*	FA in populations with high female frequencies	Marshall and Ganders (2001)
<i>Plantago maritima</i>	12	Nb seeds	From 0.4 to 2.2*	FA in populations with high female frequencies	Nilsson and Agren (2006)
<i>Schiedea salicaria</i>	2 × 5 years	Germination	From 1.24 to 2.3*	Strong ID reduces seed germination in hermaphrodites; selfing rate varies among populations and years	Weller and Sakai (2005)
Quantitative variation					
<i>Gingidia flabellata</i>	4	Nb fruits	From 1.23* to 2.07*	Altitude and abiotic conditions could affect fruit set of hermaphrodites, while females always show a very high fruit set	Webb (1981)
<i>Phacelia linearis</i>	6	Nb seeds	From 1.31* to 2.52*	No correlation between FA and female frequency; FA may increase with water availability	Eckhart (1992b)
<i>Silene vulgaris</i>	2	Nb seeds	From 1.7* to 2.7*	Local female frequency and magnitude of FA are negatively correlated	Olson <i>et al.</i> (2006)
<i>Nemophila menziesii</i>	23	Nb seeds	From 1.31* to 2.3*	FA is higher in dry sites compared with sites with higher moisture	Barr (2004)
<i>Thymus vulgaris</i>	7	Nb seeds	From 1.5* to 8.5*	Plant size varies among populations and affects seed production; the highest FA was found in the populations with the highest density	Assouad <i>et al.</i> (1978)
<i>Plantago coronopus</i>	5	Nb seeds	From 1.4* to 2.5*	The highest FA was found in the populations with the highest female frequency	Koelewijn (1996)

The first part of the table includes the seven species for which a significant female advantage was found in some but not all the data sets within a given study ('VAR' category). When available, the different numbers for the magnitude of female advantage are reported, and numbers tagged with an asterisk indicate populations/years for which female advantage was found to be significant. The second part of the table includes species for which a female advantage was always found to be significant but with a varying magnitude (defined as the number of fruits or seeds per plant) among populations. FA, female advantage; FD, female disadvantage (females < hermaphrodites); ID, inbreeding depression.

from reduced ID (e.g. *Opuntia quimilo*, *S. adamantis*, *S. vulgaris*), but also when female advantage had other proximal causes (e.g. *S. acaulis*, *E. havanense*, *Kallstroemia grandiflora*).

Finally, some of the studies reported sex differences in biotic interactions that could possibly affect the occurrence of a FA. In four cases, hermaphrodites were reported to be more affected by herbivory or parasitism than females. In *Sidalcea hendersonii*, sex-biased seed predation led to higher seed production in females, at least in populations that contained many females (Marshall and Ganders, 2001), while in the other three examples [more nectar robbing in hermaphrodites of *Glechoma longituba* (Zhang *et al.*, 2009) and more pre-dispersal predation in hermaphrodites of *Iris douglasiana* (Uno, 1982) and *Dianthus sylvestris* (Collin *et al.*, 2002)]; the consequences on the reproductive success of females relative to hermaphrodites was not quantified. Regarding interactions with pollinators, it was possible to integrate the results obtained in some of the studies on both open- and outcross-pollinated flowers and reach conclusions about differences in the magnitude of pollen limitation between females and hermaphrodites (as explained in Table 2). However,

in all cases (*D. laureola*, *P. mahaleb*, *S. granulata*, *S. hendersonii* and *G. longituba*) pollen limitation affected female plants more strongly than hermaphroditic plants. This means that when a FA occurred in these particular species, this was not because of but rather in spite of these differences in pollinator interactions.

#### Magnitude of the female advantage

The values of FA, estimated as the average ratio between females and hermaphrodites in the number of seeds or fruits produced per plant, varied from 0.64 (*D. laureola*) to 37.7 (*Lycopus maackianus*). These values were mostly comprised between 1 and 2 (17 species), and females producing more than twice the number of seeds or fruits compared with hermaphrodites were found in only six cases (Table 6; Fig. 1). Because the magnitude of the FA is theoretically expected to vary with sex determination, it would have been interesting to compare these values among species with nuclear vs. cytoplasmic male sterility. Unfortunately, (a) the genetic system is only known for a very low number of species and statistical comparisons would be meaningless and (b) for some of the few species with nuclear

TABLE 5 When is female advantage likely to be due to an avoidance of inbreeding depression?

Species	FA due to ID avoidance?	Type of evidence
<i>Cortaderia richardii</i>	No	FA found on outcrossed flowers
<i>Erythroxylum havanense</i>	No	SI species
<i>Fragaria virginiana</i>	No	FA found on outcrossed flowers
<i>Gnidia wikstroemiana</i>	Unlikely	No difference between females and self-pollinated hermaphrodites; FA occurs through a higher number of flowers
<i>Kallstroemia grandiflora</i>	Unlikely	No ID found on fruit set in another study <sup>1</sup>
<i>Phacelia linearis</i>	Unlikely	Selfing rate was found to be low; females have more resources than hermaphrodites
<i>Plantago coronopus</i>	No	FA found on outcrossed flowers
<i>Plantago lanceolata</i>	No	SI species
<i>Plantago maritima</i>	No	Mainly SI species
<i>Prunus mahaleb</i>	No	FA found on outcrossed flowers
<i>Sidalcea hendersonii</i>	No	FA found on outcrossed flowers <sup>2</sup>
<i>Silene acaulis</i>	No	FA found on outcrossed flowers <sup>3</sup>
<i>Thymus vulgaris</i>	No	FA found on outcrossed flowers
<i>Satyrion ciliatum</i>	No	High selfing rate, strong ID measured on hermaphrodites <sup>4</sup>
<i>Cucurbita foetidissima</i>	Likely	Selfing rate of hermaphrodites high in gynodioecious populations; ID reduces number of flowers and survival
<i>Limnanthes douglasii</i>	Likely	Strong ID <sup>5</sup>
<i>Lobelia siphilitica</i>	Likely	FA found in open but not outcrossed flowers
<i>Opuntia quimilo</i>	Yes	Strong ID measured on self-pollinated flowers
<i>Schiedea adamantis</i>	Likely	High selfing rate, strong ID, which reduces – among others – germination rate
<i>Schiedea salicaria</i>	Likely	Strong ID, affects all stages of life cycle <sup>6</sup>

FA, female advantage; ID, inbreeding depression; SI, self-incompatible.

When some evidence was found in published studies other those listed in Table 1, the additional reference is noted: <sup>1</sup>Cuevas *et al.* (2005); <sup>2</sup>Keller and Schwaegerle (2006); <sup>3</sup>Thompson and Tarayre (2000); <sup>4</sup>Kohn and Biardi (1995); <sup>5</sup>Mutikainen and Delph (1998); <sup>6</sup>Gaetli and Goudet (2006).

determination of male sterility, no data on the number of seeds or fruits were available. However, one can note that (a) among the species that could be included in this study, no species with nuclear male sterility were found having a FA <1.5 and (b) the second strongest advantage (6.68) was found for *F. virginiana* in which male sterility has a nuclear determination (see also Fig. 1A). For *L. maackianus*, which had the strongest FA, no information was available on sex determination. Finally, among the three self-incompatible species for which this could have been estimated, the female–hermaphrodite ratio of the number of seeds was <1 in one species (no FA) and <1.5 in all three cases (Fig. 1B).

The magnitude of the FA appeared to increase significantly with the average female frequency reported in natural populations (Fig. 2A;  $P = 0.02$ ;  $R^2 = 0.3$ ;  $n = 17$ ). It must be noted, however, that the significance of the test was explained by the three species having the highest FA in this data set (2.17, 2.5 and 3.44) and being indeed associated with the highest recorded average female frequencies (0.55, 0.60 and 0.5, respectively). When these three values were removed from the data set, thus focusing on species in which the FA was <2, no further statistical correlation could be found ( $P = 0.46$ ;  $R^2 = 0.04$ ;  $n = 14$ ). Finally, the magnitude of FA was found not to be significantly dependent on the maximum female frequency reported in populations (Fig. 2B;  $P = 0.8$ ;  $R^2 = 0.02$ ;  $n = 21$ ).

Finally, in six species, it was found that a FA always occurred but with a magnitude that varied among populations (Table 4). In some cases, this seemed to depend on environment quality or on the size or vigour of the plant, which suggests a sex difference in the ability to produce seeds according to the quantity of available resources. In two other species, the magnitude of FA apparently varied with the sex ratio: females in *S. vulgaris* benefitted from a lower FA when occurring at high frequencies, while in *P. coronopus*, the highest value of FA was found in the population with the highest female frequency. One must note that a quantitative variation in other fitness parameters (such as fruit or seed set) was frequently reported among populations in the reviewed studies. However, for the reasons explained above, this study of FA magnitude only included estimations of fruit or seed number per plant. The current study thus presents only a part of the possible variation of FA within investigated species.

## DISCUSSION

Gynodioecy is a fascinating sexual polymorphism that has triggered numerous theoretical and empirical studies. A number of recently published reviews on the topic illustrate the growing interest in gynodioecy in ecology and evolution, but, to date, they mainly focused on either the genetic determination of gynodioecy (Touzet *et al.*, 2003; Chase *et al.* 2007; Delph *et al.*, 2007; McCauley and Olson, 2008) or the effect of population structure on the reproductive system (e.g. McCauley and Bailey, 2009), while only Shykoff *et al.* (2003) reviewed and analysed the data published on the FA. The current study extends the results highlighted by Shykoff *et al.* (2003) by investigating in more detail the occurrence, magnitude, possible causes and variation of this fitness effect.

*A female advantage was found in most, but not all, gynodioecious species*

Consistently with the meta-analysis by Shykoff *et al.* (2003), this review showed evidence for the occurrence of a FA, at least on one fitness trait, in a majority of the investigated gynodioecious species. This fits theoretical predictions that mutations responsible for male sterility should benefit from a better transmission through female fitness in order to invade hermaphroditic populations (Lewis, 1941; Frank, 1989; Gouyon *et al.*, 1991; Bailey *et al.*, 2003; Dufay *et al.*, 2007).

TABLE 6. Magnitude of the female advantage in the 28 species for which information was available

Species	SI/SC	Genetics	FA	Measurement/calculation	Reference
<i>Beta vulgaris</i>	SI	NC	0.93	NS	Boutin-Stadler (1987)
<i>Gingidia flabellata</i>			1.49	NFL × FS*	Webb (1981)
<i>Echium vulgare</i>	SC	NC	1.42	NS	Klinkhamer <i>et al.</i> (1994)
<i>Phacelia linearis</i>	SC	N	1.68	NS	Eckhart (1992b)
<i>Opuntia quimilo</i>	SC		0.88	NS	Diaz and Cocucci (2003)
<i>Lobelia siphilitica</i>	SC	NC	1.31	NS	Caruso and Yakobowski (2008)
<i>Dianthus sylvestris</i>	SC		1.62	NFL × NSF*	Collin <i>et al.</i> (2002)
<i>Silene italica</i>	SC	NC	1.22	NS	Lafuma and Maurice (2006)
<i>Silene vulgaris</i>	SC	NC	2.17	FS × NSF	Olson <i>et al.</i> (2006)
<i>Wurmbea biglandulosa</i>	SC		1.77	NS	Vaughton and Ramsey (2004)
<i>Cucurbita foetidissima</i>	SC	N	1.50	NS	Kohn (1989)
<i>Geranium maculatum</i>	SC		1.60	NS	Agren and Willson (1991)
<i>Geranium sylvaticum</i>	SC		1.68	NS	Asikainen and Mutikainen (2005)
<i>Nemophila menziesii</i>	SC	NC	1.67	NS	Barr (2004)
<i>Lycopus maackianus</i>			37.73	NS	Hong and Moon (2003)
<i>Thymus vulgaris</i>	SC	NC	2.5	NS	Assouad <i>et al.</i> (1978)
<i>Chionographis japonica</i>	SC		1.4	NS	Maki (1993)
<i>Hebe subalpina</i>	SC		3.44	NFL × FS*	Delph (1993)
<i>Plantago coronopus</i>	SC	NC	1.72	NS <sup>#</sup>	Koelwijn (1996)
<i>Plantago lanceolata</i>	SI	NC	1.38	NS	Vandamme and Vandelden (1984)
<i>Plantago maritima</i>	SI	NC	1.01	NS	Nilsson and Agren (2006)
<i>Chionochloa bromoides</i>	SC		0.91	NFL × NSF*	Connor (1990)
<i>Fragaria virginiana</i>	SC	N	6.68	NFL × FS *	Ashman (1999)
<i>Prunus mahaleb</i>	pSC		1.40	NF	Jordano (1993)
<i>Saxifraga granulata</i>	SC	NC	0.72	NFL × NSF*	Stevens (1988)
<i>Daphne laureola</i>	SC		0.65	NS	Alonso <i>et al.</i> (2007)
<i>Gnidia wikstroemiana</i>	SC		3.58	NFL × FS*	Beaumont <i>et al.</i> (2006)
<i>Kallstroemia grandiflora</i>	SC		1.29	NS	Cuevas <i>et al.</i> (2008)

The table shows the average FA, defined as the female fitness of females relative to hermaphrodites. The fourth column indicates whether this value was obtained by directly measuring the number of seeds or fruits per plant (NS and NF, respectively) or by estimating seed or fruit production through a calculation. NFL, number of flowers; NSF, number of seeds per fruits; FS, fruit set. Lines tagged with an asterisk show cases for which we performed the calculation ourselves, based on values available in the original study, and a hash sign refers to a study in which the number of seeds was estimated through the number of spikes per plant.

For each species is listed: whether it is self-compatible (SC), partially self-compatible (pSC) or self-incompatible (SI) and, where known, the determination of sex (NC, nuclear–cytoplasmic; N, nuclear).

In eight species, however, no FA was found. In half of the cases, because the measurements were carried out on a restricted number of fitness traits or individual plants/populations, future complementary studies involving more genotypes and/or more fitness traits may detect an advantage for females. In other species, no FA could be found even though many different traits were compared between females and hermaphrodites. In such species, it seems unlikely that females are maintained in so many different populations [as in *B. vulgaris* (Dufay *et al.*, 2009), *D. laureola* (Alonso and Herrera, 2001) and *R. sativus*: Murayama *et al.* (2004)] without benefiting from a selective advantage, and thus relying only on mutations that would be maintained by stochastic processes. In these three species, since females were found at high frequencies in some populations, one should keep in mind that a high increase in female frequency may lead to a strong decrease in FA in some cases (see the end of the Discussion and Fig. 3). Such a phase in the evolutionary dynamics of a gynodioecious population may thus lead to a failure to detect a FA. However, while this constitutes an interesting explanation for the absence of FA in some populations of some species, it is less likely to occur in every studied population of *B. vulgaris*, *R. sativus* and *D. laureola*, in particular in those exhibiting a low female frequency.

In species with a nuclear–cytoplasmic determination of sex, such as *B. vulgaris* and *R. sativus*, a FA of a very low

magnitude is theoretically sufficient for the maintenance of females, possibly at high frequencies (Gouyon *et al.*, 1991; Dufay *et al.*, 2007) and thus may be particularly difficult to detect statistically. One other likely hypothesis is that a FA occurs on some fitness traits that have not been investigated yet (e.g. germination and seed quality in the annual *R. sativus*; adult survival in the perennials *B. vulgaris* and *D. laureola*). More generally, many traits, such as adult survival, probability of flowering and age at first flowering, are rarely investigated. In perennial species (which is the case in 38 of the 48 species included in the current study), better performance through one of these traits could still increase seed production by females over their lifetime (see Morris and Doak, 1998 for an example in *S. acaulis*). This constitutes a perspective for the study of gynodioecious species, in particular those in which FA has not yet been found.

#### *The magnitude of female advantage varies greatly among gynodioecious species*

First of all, one needs to consider these results with caution, since the magnitude of FA was estimated through the number of fruits or seeds, neglecting other traits such as differences in seed quality or adult survival. Nonetheless, we found a spectacular variation in the value of FA among species, that varied

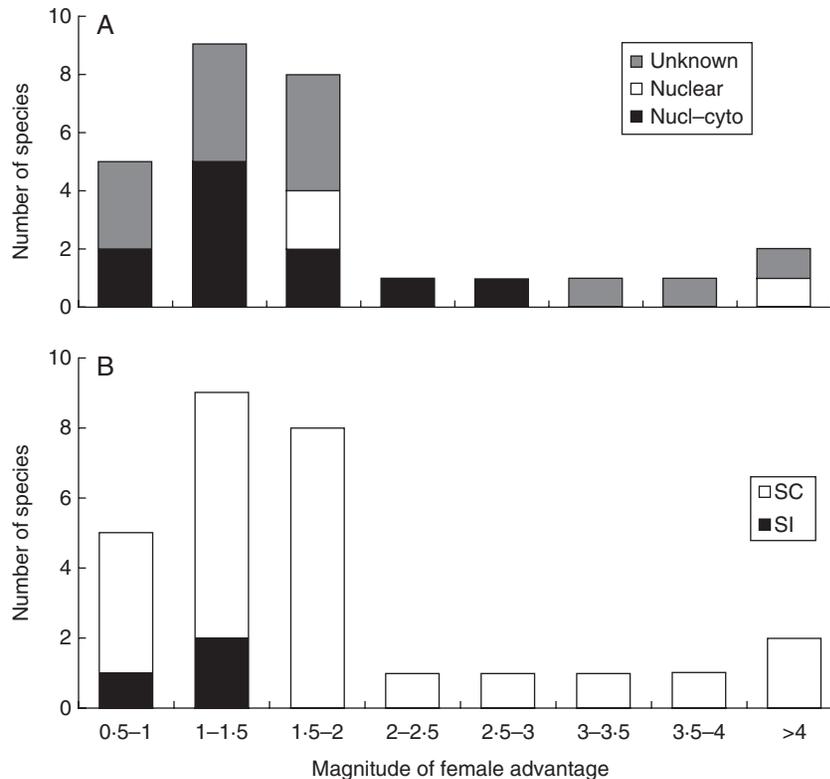


FIG. 1 Distribution of the magnitude of female advantage among 28 gynodioecious species according to (A) their genetic determination of sex (nuclear–cytoplasmic, nuclear, or not known, as indicated) and (B) the ability of hermaphrodites to self-pollinate (self-incompatibility or self-compatibility, as indicated). A female advantage  $<1$  corresponds to species in which either no female advantage or a female disadvantage was reported.

from  $<1$  to  $>30$ . A large majority of species was found to have a FA comprised between 1 and 2, which corresponds to theoretical expectations under nuclear–cytoplasmic determination of sex (Gouyon *et al.*, 1991; Dufay *et al.*, 2007). Regarding the few species that seemed to have a large, or even extremely large, FA, some of them may be sub-dioecious or on the way to evolving towards dioecy (Lewis, 1941).

Although a large FA appeared to be associated with high female frequencies, no correlation was found between FA and sex ratio in the case of species having their FA comprised between 1 and 2 (Fig. 2; see also Couvet *et al.*, 1990). As explained below, processes affecting the sex ratio are numerous and the relationships between female frequency and FA are quite complex, and undoubtedly non-linear. Moreover, in the case of nuclear–cytoplasmic determination, theory predicts that frequency-dependent selection can lead to large oscillations of female frequency within a population (and thus, also among different populations) even when the value of FA remains fixed (Gouyon *et al.*, 1991; Bailey *et al.*, 2003; Dufay *et al.*, 2007). In the light of these different predictions, the absence of correlation between FA and female frequency is thus not very surprising.

#### *Differences in biotic interactions seem not to often lead to a female advantage*

Because hermaphrodites often carry larger flowers than females (this was documented in 26 of the species included

in the study; see also Shykoff *et al.*, 2003), they are likely to attract more insects, which can either be beneficial in the case of pollinators or costly in the case of natural enemies. A higher rate of predation or parasitism was reported in the hermaphrodites of four species, and was found to provide females with a fitness advantage in at least one of these cases. Sex differences in pollination efficiency were also found in some species, with females suffering more from pollen limitation than hermaphrodites (although this may not be a general trend, as suggested by Shykoff *et al.*, 2003; see also Dudle, 1999 for an example of stronger pollen limitation in hermaphrodites). In all of the cases investigated in the current study, this led to a lower seed or fruit set in females compared with hermaphrodites; thus, when a FA occurred, this was not because of, but rather in spite of differences in interactions with pollinators.

#### *Female advantage may be caused by reduced selfing in some cases but not all*

In seven species, it was found that reduced ID could be responsible – at least partly – for the occurrence of FA, while in 14 other species, it appeared that reduced ID was not a necessary cause of the FA. This result was quite surprising, since the reduced self-pollination and associated ID is sometimes cited as the main proximal cause for FA in gynodioecious species (e.g. Kubota and Ohara, 2009). This does not mean that no ID occurs within these species (in some

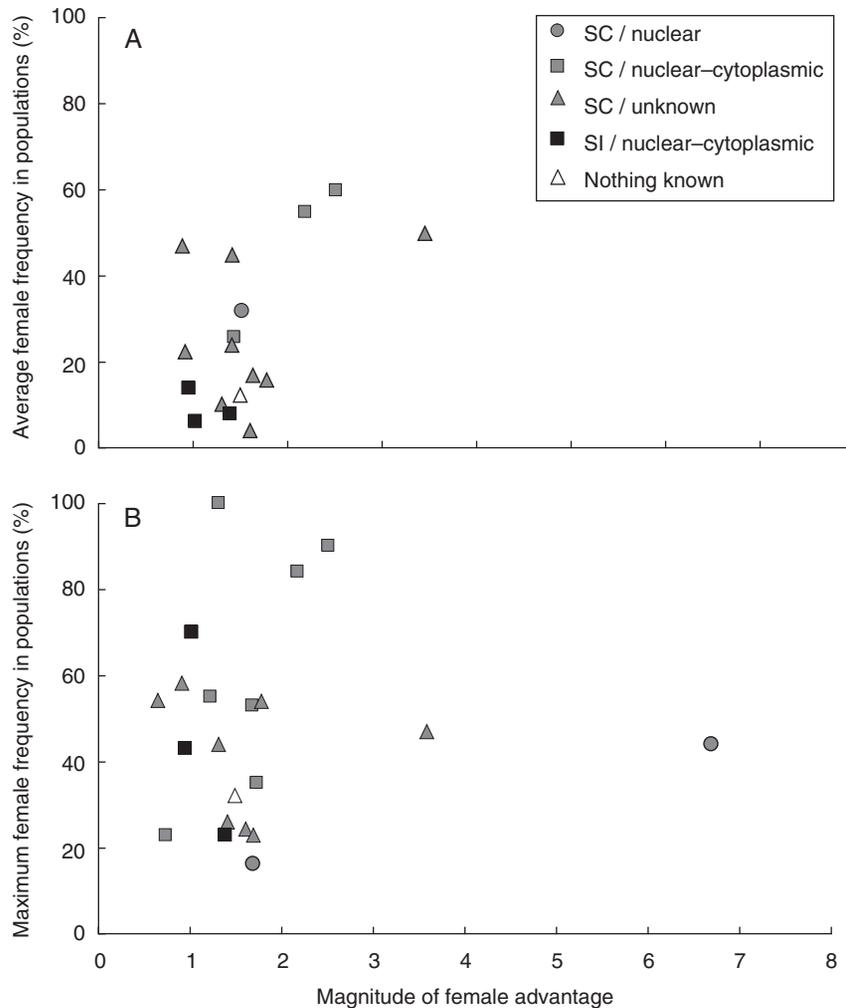


FIG. 2. Sex ratio in natural populations as a function of the magnitude of female advantage based on (A) average female frequencies and (B) the highest female frequencies reported in natural populations. Among the 28 species for which the magnitude of female advantage was estimated, data on the average sex ratio and the maximum sex ratio were available for 17 and 21 species, respectively. The colours and the shapes of symbols provide information about the self-incompatible (SI)/self-compatible (SC) status and the genetics determination of sex, respectively.

cases, e.g. *S. hendersonii* and *P. mahaleb*, self-pollinated flowers were shown to have a low seed set or to produce seeds of low quality), but this could mean that selfing rates are sufficiently low in these species to not disadvantage hermaphrodites, at least under the conditions examined in the reviewed studies. However, one must note that a FA possibly results from a combination of different mechanisms, and that reduced selfing and ID, while not being the only necessary cause of it, may increase the magnitude of FA in some species. This may explain why this magnitude is low in the investigated self-incompatible species, while it reaches very high values in some self-compatible species (Fig. 1B).

Our study contradicts the view that selfing and ID are a necessary process for a FA to occur and persist. Importantly, this means that gynodioecy should not be particularly difficult to evolve in self-incompatible species (in which neither females nor hermaphrodites self-pollinate), as argued sometimes in the literature (e.g. Kubota and Ohara, 2009). It is true, however, that few gynodioecious species are known to

be self-incompatible, even though no phylogenetic analyses on the occurrence of these two traits have been made to confirm their negative association within Angiosperms. In addition, in the two species *Trillium camschatcense* and *P. maritima*, both exhibiting self-compatible and self-incompatible populations on the one hand, and gynodioecious and hermaphroditic populations on the other hand, gynodioecy and self-incompatibility appeared to be negatively correlated at the population level (Nilsson, 2005; Nilsson and Agren, 2006; Kubota and Ohara, 2009). However, even though reduced selfing and ID in females may help in selecting for male sterility in self-compatible species, such an explanation may not be true in all cases, as suggested by the current study. Alternatively, the model of Ehlers and Schierup (2008) has shown that the occurrence of male sterility could enhance the breakdown of self-incompatibility. Consequently, such negative association may sometimes result from a higher probability of self-incompatibly breakdown within gynodioecious lineages, rather than from a stronger selection for male sterility in self-compatible lineages.

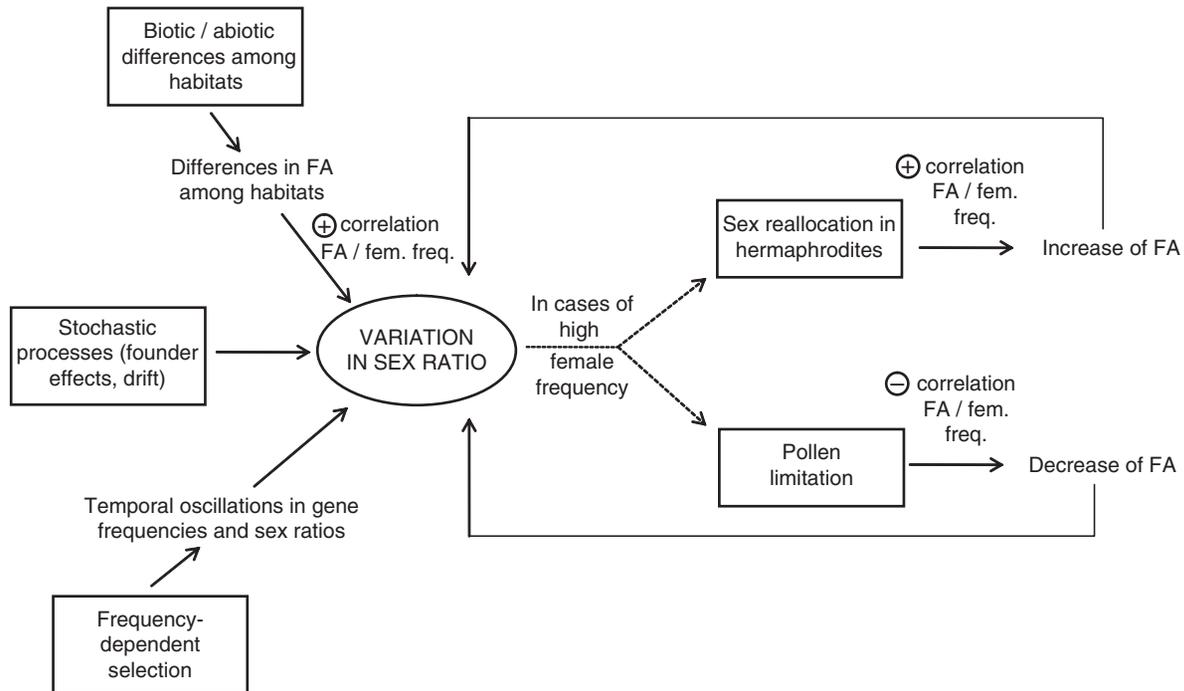


FIG. 3. Schematic representation of the causes and consequences of sex ratio variation among gynodioecious populations, and its interplay with female advantage.

#### Why does the female advantage vary within species, among populations?

Among species for which one study documented the FA in more than one population, at least half of them showed a variation in the occurrence or in the magnitude of FA. In some cases, the population variation in FA may have been due to sex-specific responses to environmental variation. In addition, in many cases, FA was found to vary with the population sex ratio, but both negative and positive correlations were highlighted among the reviewed studies. In order to understand this puzzling result, one needs to consider all processes likely to affect both FA and the sex ratio, and also to understand when female frequency is the cause vs. the consequence of the FA (Fig. 3 and see also the review by McCauley and Bailey, 2009).

First, three main processes can theoretically affect the sex ratio in a gynodioecious population: drift and founder effects that lead to non-equilibrium situations (Nilsson and Agren, 2006); frequency-dependent selection that leads to oscillations in the sex ratio over time in nuclear–cytoplasmic systems (Gouyon *et al.*, 1991; Dufay *et al.*, 2007, 2009); and the magnitude of FA (Dufay *et al.*, 2007). The latter point simply means that a large FA induces a strong positive selection for male sterility, leading to high female frequencies. Thus, for this reason, positive correlations between female frequencies and FA values should be observed, at least in some cases (Fig. 3). If female frequency reaches high values in a population, two very different processes can then occur. On the one hand, as postulated by Ramula and Mutikainen (2003), a high female frequency could lead hermaphrodites to allocate more resources to male function, consequently decreasing their seed production and thus increasing the value of FA.

This is another reason for observing positive correlations between FA and female frequency. Such a mechanism should theoretically have a positive feedback on female frequency and is sometimes considered as a step towards the evolution of dioecy (Charlesworth and Charlesworth, 1978; Maurice *et al.*, 1994). On the other hand, in populations containing a lot of females, the overall availability of pollen should decrease. As already outlined, females seem sometimes to be more affected by pollen limitation than hermaphrodites, for several reasons: (a) in pollen-limited situations, self-compatible hermaphrodites have the potential to self-pollinate; (b) hermaphroditic flowers are often more attractive to pollinators than female flowers; and/or (c) because of the spatial clustering of cytoplasmic haplotypes and thus sex types within populations (e.g. McCauley, 1998; Klaas and Olson, 2006; De Cauwer *et al.*, 2010), females have a higher risk of experiencing local depletion of pollen. In such a case, we expect female frequency to decrease in the next steps of the dynamics. Consequently, one could imagine that both positive and negative correlations between FA and female frequencies could be encountered in the same set of populations, depending on the phase of the dynamics of sex ratio oscillations. For instance, in a first step, populations in which FA is the highest should experience an increase in female frequency, but once female frequency has reached some threshold value, this could lead to a decrease of female seed production, which should ultimately lead females to be counter-selected (Fig. 3).

#### CONCLUSIONS AND PERSPECTIVES

One of the main results suggested by the current study is that reduced selfing may not be the major cause of the occurrence

of FA, and thus of the maintenance of gynodioecy. However, this could be investigated in only a part of the reviewed species: while female–hermaphrodite comparisons of female fitness based on open-pollinated flowers are necessary to know about the possible occurrence of a FA in natural conditions, many studies lack some data based on outcrossed and selfed hand pollination, which are helpful to discriminate among the possible mechanisms responsible for the FA. We propose that future studies should document female–hermaphrodite fitness comparisons using both open-pollinated and hand-pollinated flowers, in order to assess the role of reduced selfing in the evolution of gynodioecy in a larger number of species.

One other very important type of data that were surprisingly missing from many studies was the genetic details of sex determination. It was thus not possible to test the theoretical predictions about how sex determination should be related to the sex ratio in populations and to the magnitude of FA. Indeed, many studies used these theoretical predictions to infer sex determination from data obtained on FA or the sex ratio. Although such an argument may be very useful, as proposed by Bailey and Delph (2007), direct evidence obtained from crossings would considerably enhance our knowledge of gynodioecy. Because both nuclear (Charlesworth and Charlesworth, 1978) and nuclear–cytoplasmic gynodioecy (Maurice *et al.*, 1994) may theoretically lead to dioecy, but involving slightly different genetic and/or ecological mechanisms, this could also help us in understanding one major and common transition in the evolutionary history of plant mating systems.

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