

'Inconstant males' and the maintenance of labile sex expression in subdioecious plants

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Summary

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- Here, we evaluate the role of pollen limitation and selfing in the maintenance of labile sex expression in subdioecious plant species.
- We used a literature survey to explore which factors correlated with a significant occurrence of hermaphrodites in dioecious species. We developed models to explore the selective maintenance of labile sex expression. The models had similar ecological assumptions but differed in the genetic basis of sex lability.
- We found that a significant frequency of hermaphrodites was associated with animal pollination, and that hermaphrodites were 'inconstant' males with perfect flowers, suggesting evolution through the gynodioecious pathway. Models showed that a modifier converting pure males into inconstant males could be maintained under a wide range of reduction in both male and female fitness. Pollen limitation and self-fertilization facilitated invasion of the modifier. Depending on the genetics of sex determination, we found pure dioecy, stable subdioecy (trioecy), and situations where inconstant males coexisted with either pure females or pure males. Under selfing and pollen limitation, certain conditions selected for inconstant males which will drive populations to extinction.
- We discuss our results in relation to the evolution towards, and the breakdown of, dioecy, and the ecological and evolutionary implications of labile sex expression.

Key words: evolution of dioecy, labile sex, pollen limitation, pollination mode, selfing, sex determination, trioecy.

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Introduction

In dioecious plant species the sexes are separated into male and female individuals. This is a rare condition found in approx. 6% of flowering plants (Renner & Ricklefs, 1995). Dioecy is believed to have evolved from hermaphroditic ancestors mainly through two pathways; either via gynodioecy (the coexistence of two sex morphs, a hermaphrodite and a pure female morph, where the hermaphrodites either produce perfect flowers or are monoecious) or via paradioecy (the coexistence of two sex morphs, both of which evolved from monoecious individuals, one having become specialized to be more male and the other more female). In a few dioecious species, distinct sex chromosomes have evolved. This is

documented in six different families and involves a strict sex determination mechanism with little or no sex lability (see e.g. Dellaporté & Calderon-Urrea, 1993; Ainsworth, 2000).

In many dioecious species, the occurrence of rare hermaphroditic individuals has been reported, reflecting a situation in which three sex morphs may coexist in the same population. This condition is termed 'subdioecy', although other qualifiers are sometimes used in the literature. While subdioecy generally refers to the evolutionary transition from either gynodioecy or paradioecy towards dioecy, the initial breakdown of an already established dioecious mating system could likewise create a situation of three sex morphs. Throughout this paper we refer to subdioecy in a broad sense as the situation in which males and females coexist with a rarer hermaphroditic morph, but

we do not make assumptions as to whether this situation is a transition towards, or a breakdown of, dioecy. The rare hermaphroditic individuals in these populations can either be monoecious or bear perfect flowers.

The occurrence of rare hermaphroditic morphs in subdioecious populations has received various interpretations. Lloyd & Bawa (1984) viewed their presence as mere 'developmental noise'. Others have reported on their possible adaptive value in relation to the abiotic environment (e.g. Charnov & Bull, 1977; Freeman *et al.*, 1980; Webb, 1999; Case & Barrett, 2001, 2004; Delph, 2003).

The adaptive significance of labile sex expression in dioecious species in the context of variation in the resource environment was recently reviewed by Delph & Wolf (2005). They found that most often it was the male morph that showed labile sex expression, being more prone to produce fruits in moist, resource-rich environments than in drier, less resource-rich ones. This indicated resource-dependent gender plasticity, and labile sex expression in males was interpreted to be advantageous as it lowered the cost of producing seeds in resource-poor habitats, hence reducing the possible trade-off between male and female functions.

In our study we also focus on the adaptive significance of labile sex expression, but we ask instead whether the mating environment (pollen limitation and selfing) can favour labile sex expression and thereby affect the maintenance of subdioecy. We emphasize that this view does not exclude the importance of the resource environment, but it may be of value to consider both the resource and the effects of pollen limitation and selfing when trying to understand patterns of sex lability found in subdioecious species.

The idea that the mating environment may be of importance in mating system evolution is not new. Baker (1955) pointed out that a species is much more likely to establish itself after a 'long-distance' dispersal event if individuals are self-compatible plants or are able to evolve self-compatibility, unless the species is able to reproduce vegetatively. Indeed, the situation in which pollen transfer among plants is low (because of a lack of pollinators or compatible mates) has been proposed to be a major selective force in the evolution of selfing as a mode of reproductive assurance (e.g. Schoen, 1982; Wyatt, 1986; Byers & Meagher, 1992; Schoen *et al.*, 1996). The mating environment is also believed to be the major selective force governing sex allocation patterns in hermaphrodites in gynodioecious populations. Hermaphrodites (the pollen-producing morph in gynodioecious populations) are expected to increase their allocation to male function with an increasing frequency of females (Lloyd, 1976). Evidence for such frequency-dependent selection has been found in gynodioecious species on both a spatial (e.g. McCauley & Brock, 1998) and a temporal (Ehlers & Thompson, 2004) scale. The evolution of dioecy may be a final outcome of such frequency-dependent selection where hermaphrodites lose their female function (a female-sterile mutant is favoured) and

become pure males (see e.g. Charlesworth, 1999 for a review). The importance of the mating environment for the maintenance of gynodioecy and androdioecy has also been studied theoretically. Using a metapopulation model, Pannell (1997) demonstrated that hermaphroditic individuals could be maintained because of their greater ability to establish new populations in species whose population dynamics are dominated by recurrent extinction and (re)colonization of local populations.

Here we develop further the idea that the mating environment may be of importance for maintaining labile sex expression in the case of subdioecy. First we carried out a literature survey to investigate which ecological factors are associated with the occurrence of hermaphroditic individuals in significant numbers in subdioecious species. Then we modelled the effects that pollen limitation and selfing may have on the invasion and selective maintenance of a labile sex genotype in a dioecious population. We used an explicit demographic model to explore the invasion of labile sex under different assumptions regarding the genetics of sex determination. We discuss our results and their implications for both the evolution towards and the breakdown of pure dioecy.

Literature survey

We performed a literature survey on Web of Science (WOS; <http://portal.isiknowledge.com/>) and Google scholar (<http://scholar.google.com/>) using the keywords 'sub dioecy', 'near dioecy' and 'trioecy', and traced all relevant references cited. We kept in our survey only studies that included information on the actual frequency of hermaphroditic individuals within at least one natural population. In addition, we searched for information on: (a) the type of flowers on the hermaphroditic individuals (monoecious or perfect flowers); (b) whether the labile sex was the male or the female morph; (c) the major pollination mode (i.e. wind or animal); (d) the importance of vegetative reproduction and (e) where the information was available, pollen limitation in the female morph and self-compatibility in the hermaphrodite morph.

Many studies have reported the frequency of hermaphrodites in dioecious populations. However, here we only present those studies in which hermaphroditic individuals made up at least 5% of individuals in at least one study population. The result of the literature survey is shown in Table 1. We found 32 subdioecious species, representing 21 different families satisfying these criteria (Table 1).

Labile sex expression is a result of inconstant males and is genotype dependent

In 18 of the families in our survey, the inconstant sex was reported. In the majority of these (78%), individuals with labile sex were inconstant (Table 1). Many of the studies in Table 1 also show that the ability of the male morph to

Table 1 Summary of literature survey

Family and species	Frequency of hermaphrodite morph	Habitat	Type of hermaphrodite and inconstant sex	Pollination	Vegetative reproduction	Pollen limitation in females	Hermaphrodite self-compatible	Life form	Reference
Alismataceae <i>Sagittaria latifolia</i>	Dioecious and monoecious pops (12 pops)	Shallow water	Monoecious; IM	Bees, beetles, flies and wasps	Yes	NR	Yes	Aquatic herb	Sarkissian <i>et al.</i> (2001)
Asteraceae <i>Cirsium arvense</i>	< 1–20% (3 pops)	Disturbed 'grassland' habitats	Perfect flowers; IM	Insects	Yes	Yes (when distance to male > 20 m)	Yes	Per. herb colonizer	Kay (1985)
Cactaceae <i>Pachycereus pringlei</i>	0–83% (20 pops)	Desert	Perfect flowers; Triocious	Bats, bees and birds	No	Yes	Yes	Cactus	Fleming <i>et al.</i> (1994, 1998)
Caryophyllaceae <i>Schiedea globosa</i>	0–5%, mean 3% (3 pops)	Dry cliffs	Mainly monoecious; IM	Mainly wind	Yes	NR	NR	Shrub	Sakai & Weller (1991)
<i>Silene acaulis</i> ssp. <i>excapa</i>	2–7%, mean 4.9% (5 pops)	Alpine mountain	Perfect flowers; IM	Insects	No	NR	NR	Per. herb	Maurice <i>et al.</i> (1998)
<i>Silene acaulis</i> ssp. <i>cenisia</i>	18–68%, mean 30% (9 pops)	Alpine mountain	Perfect flowers; IM	Insects	No	NR	NR	Per. herb	Maurice <i>et al.</i> (1998)
Chenopodiaceae <i>Atriplex corrugata</i>	> 10% (1 pop), varies among years	Semiarid shrubland	Monoecious; both sexes labile	Wind	Some	NR	NR	Evergreen shrub	Freeman & McArthur (1984); Freeman <i>et al.</i> (1984)
<i>Atriplex canescens</i>	> 10% (1 pop), varies among years	Semiarid shrubland	Monoecious; both sexes labile	Wind	Some	NR	NR	Evergreen shrub	Freeman & McArthur (1984); Freeman <i>et al.</i> (1984)
<i>Atriplex cuneata</i>	> 10% (1 pop), varies among years	Semiarid shrubland	Monoecious; both sexes labile	Wind	Some	NR	NR	Evergreen shrub	Freeman & McArthur (1984); Freeman <i>et al.</i> (1984)
<i>Atriplex tridentata</i>	> 10% (1 pop), varies among years	Semiarid/saline shrubland	Monoecious; both sexes labile	Wind	Some	NR	NR	Evergreen shrub	Freeman & McArthur (1984); Freeman <i>et al.</i> (1984)
Cluciaceae <i>Clusia nemorosa</i>	Gyno- and dioecious populations	Forest	Perfect flowers; IM	Large bees (e.g. euglossin)	–	Yes	Yes	Tree	Lopes & Machado (1998)
Colchicaceae <i>Wurmbea dioica</i>	0–35%, mean 11% (35 pops)	Open woodland	Perfect flowers; IM	Bees and butterflies	No	Yes but varies	Yes	Per. herb	Barrett (1992)
Cucurbitaceae <i>Ecballium elaterium</i>	Dioecious and monoecious populations	Disturbed sites, e.g. roadside, corrals	Monoecious; IM	Honey and solitary bees	No	NR	Yes	Per. herb colonizer	Costich & Meagher (1992); Costich (1995)
Graminae <i>Buchloe dactyloides</i>	0–70%, mean 15% (8 pops)	Prairie	Monoecious; both sexes labile	Wind	Yes	NR	NR	Per. grass	Huff & Wu (1992)
Loranthaceae <i>Ileostylus micranthus</i>	28% (2 sites)	Forest	Perfect flowers	Insects, flies and bees	No	NR	Yes	Hemiparasitic mistletoe	Ladley <i>et al.</i> (1997)
Oleaceae <i>Fraxinus excelsior</i>	68% (1 pop)	Deciduous forest	Perfect flowers	Wind	–	NR	Yes, at least partly	Colonizing tree	Binggeli & Power (1999); Morand-Prieur <i>et al.</i> (2003)

Table 1 Continued

Family and species	Frequency of hermaphrodite morph	Habitat	Type of hermaphrodite and inconstant sex	Pollination	Vegetative reproduction	Pollen limitation in females	Hermaphrodite self-compatible	Life form	Reference
Onagraceae									
<i>Fuchsia thymifolia</i>	c. 8% (1 pop)	Pine-oak forest	Perfect flowers; IM	Insects (tachinid flies)	No	NR	Yes	Shrub	Arroyo & Raven (1975)
<i>Fuchsia microphylla</i>	c. 8% (1 pop)	Pine-oak forest	Perfect flowers; IM	Hummingbirds and bumble bees	No	NR	Yes	Shrub	Arroyo & Raven (1975)
<i>Fuchsia lycioides</i>	c. 10–50% (5 pops)	Bluff	Perfect flowers; IM	Hummingbirds	No	NR	NR	Shrub	Atsatt & Rundel (1982)
Orchidaceae									
<i>Catasetum viridiflorum</i>	7–19% para; 2% bisexuals	Epiphytic canopy sp.	Perfect flowers; monoecious; mainly IM	Insects (male euglossin)	NR	Yes	NR	Per. herb	Zimmerman (1991)
Resedaceae									
<i>Ochradenus baccatus</i>	0–30%, mean 10% (14 pops)	Desert	Perfect flowers; IM	Bees, flies, beetles and wasps	No	Yes	Yes	Shrub	Wolfe & Shmida (1997)
Roseaceae									
<i>Fragaria chiloensis</i>	4–40%, mean 14% (12 pops)	Sandy beach/ woodland	Perfect flowers; both sex labile	Insects (bees)	Yes	NR	Yes	Per. herb	Hancock (1979)
Rubiaceae									
<i>Nesohedyotis arborea</i>	> 5%	Tree-fern thicket	Perfect flowers; IM	Insects (syrphid flies)	–	Yes	Yes	Endemic tree	Percy & Cronk (1997)
Saxifragaceae									
<i>Astilbe biternata</i>	0–25% (22 pops)	Forest and roadsides	Perfect flowers; IM	Insects	No	Yes	Yes	Shrub	Olson (2001)
Schrophulariaceae									
<i>Hebe subalpina</i>	0–50% (8 pops), varies among years	Subalpine sites	Perfect flowers; IM	Tachinid flies and beetles	No	NR	Yes	Evergreen shrub	Delph & Lloyd (1991)
Sterculiaceae									
<i>Dombeya ciliata</i>	0–50% (3 pops)	Forest	Perfect flowers; IM	Birds and insects (bees and butterflies)	–	NR	NR	Tree	Humeau <i>et al.</i> (2000)
<i>Dombeya delislei</i>	61–77% (among-year variation in 1 pop)	Forest	Perfect flowers; IM	Birds and insects (bees and butterflies)	–	NR	No	Tree	Humeau <i>et al.</i> (1999)
Thymelaeaceae									
<i>Thymelaea hirsuta</i>	17–40%, mean 33% (9 pops)	Desert	Monoecious; both sex labile	Mainly wind	NR	NR	NR	Evergreen shrub	El-Keblawy & Freeman (1999)
Umbelliferae									
<i>Scandia rosaefolia</i>	> 5% (3 pops)	NR	Perfect flowers; IM	Insects	NR	NR	Yes	Per. herb	Webb (1979)
<i>Gingidia decipiens</i>	> 5% (5 pops)	NR	Perfect flowers; IM	Insects	NR	NR	Yes	Per. herb	Webb (1979)
<i>Gingidia montana</i>	> 5% (3 pops)	NR	Perfect flowers; IM	Insects	NR	NR	Yes	Per. herb	Webb (1979)
<i>Gingidia enysii</i>	> 5% (4 pops)	NR	Perfect flowers; IM	Insects	NR	NR	Yes	Per. herb	Webb (1979)

Only studies reporting a frequency of > 5% hermaphroditic individuals in natural populations of subdioecious species are included. Type of hermaphrodite refers to either monoecious individuals or individuals with perfect flowers.

IM, inconstant male; NR, not recorded; Per., perennial; pops, populations; –, irrelevant.

produce fruits varied among genotypes. Empirical studies on the genetic basis for sex lability in the male morph remain rare, but some evidence exists. Delph & Lloyd (1991) studied fruit production in clones of different male genotypes of the subdioecious shrub *Hebe subalpina*. They demonstrated that fruit production varied with environmental conditions. However, the magnitude of the difference among male genotypes stayed the same (no genotype by environment interaction), suggesting that fruit production in hermaphrodites had a strong genetic component. In *Wurmbia dioica* (Ramsey & Vaughton, 2001), *Schiedea globosa* (Sakai & Weller, 1991) and *Astilbe biternata* (Olson & Antonovics, 2000), different male genotypes were examined under controlled favourable glasshouse conditions, and variation among genotypes for a functional hermaphroditic phenotype was found, again indicating a genetic component to the production of fruits. This variation suggests that some male genotypes have developed into a fixed sexual male morph (i.e. show nonlabile sex expression) whereas other male genotypes can still produce fruits.

Subdioecious species with inconstant males evolved preferentially via the gynodioecious pathway

Our literature survey suggests that most of the subdioecious families we report have evolved dioecy via the gynodioecious pathway (Table 1). This is supported by two findings.

First, among the families exhibiting individuals with inconstant sex, significantly more of these were perfect flowered (74%) rather than monoecious (exact binomial test under the null hypothesis of equal occurrence of inconstant sex among both types, $P = 0.02$). The fact that labile sex was mainly of the perfect flowered type directly suggests a gynodioecious pathway.

Secondly, constant females but inconstant males are expected in a species evolving towards dioecy via the gynodioecious pathway (Lloyd, 1976; Charlesworth & Charlesworth, 1978). This asymmetry is not expected if the species evolves towards dioecy from monoecy. This is because the monoecious pathway involves a transient state with sexual specialization through gradual reduction in the proportions of male and female flowers on female and male individuals, respectively. Thus, if subdioecy evolves from monoecy, the labile sex should be equally likely to be male or female. However, among the 18 families in which the labile sex morph was recorded, 14 of these were inconstant males, which differs significantly from an equal probability of labile sex in male and female morphs (exact binomial test, $P = 0.015$). This confirms previous findings (e.g. Westergaard, 1958; Charlesworth & Charlesworth, 1978; Delph & Wolf, 2005) that labile sex in subdioecious species is mostly demonstrated by the male morph.

Renner & Ricklefs (1995) showed that most dioecious species probably evolved from the monoecious pathway. Our finding, however, suggests that evolutionary transitions from monoecy to dioecy may in fact not be more frequent than transitions from gynodioecy to dioecy. The observation that

most extant dioecious species probably evolved from monoecious ancestors does not necessarily imply that most changes from cosexual to dioecious were from monoecy to dioecy. This observation could also arise if dioecious species evolved from monoecious ancestors have subsequently speciated more often, or have become extinct less often, than those that evolved from perfect flowered ancestors

High frequency of labile sex and biotic pollination

We found that 79% of the subdioecious families in our survey (Table 1) had biotic pollination. Renner & Ricklefs (1995) reported on the pollination biology of 157 dioecious families and found that 66% of these were biotically pollinated. While this figure is lower than the 79% we found for the subdioecious families in the present study, the values are not significantly different (exact binomial test, $P = 0.17$). However, a major result of the survey by Renner & Ricklefs (1995) was that dioecy was strongly associated with both monoecy and an abiotic pollination mode (see also Vamosi *et al.*, 2003), and that monoecy itself was associated with abiotic pollination. Of the subdioecious families in Table 1 with perfect flowered hermaphrodites, 93% of families (14 of 15) were biotically pollinated, which is significantly higher than the 66% of biotically pollinated dioecious families reported by Renner & Ricklefs (1995) (binomial exact test, $P = 0.01$). Of 157 dioecious families reported by Renner & Ricklefs (1995) whose major pollination mode was determined, 54 had no record of monoecy, and hence may represent families that evolved dioecy via a nonmonoecious pathway. Of these 54 families, 61% had biotic pollination, which is also significantly lower (binomial exact test, $P = 0.01$) than the proportion of biotically pollinated families we found in subdioecious species with perfect flowered labile sex. Hence, subdioecy in our survey is strongly associated with animal pollination, and the pollination biology of these species differs from that of the majority of dioecious species.

The fact that species with a significant occurrence of hermaphroditic individuals also showed little or no vegetative reproduction indicates that these species strongly rely on sexual reproduction for population growth. Because of their dependence on animal pollen vectors, these species may periodically experience seasons with low pollinator services where seed set in females is reduced. Reduced seed set in females can likewise occur at low population density where the pollen-producing (male) morph is scarce (see e.g. Ashman *et al.*, 2004, and Knight *et al.*, 2005 for a recent review on pollen limitation). Indeed, evidence of pollen limitation in females was found in all subdioecious species where this was studied (Table 1). In addition, the hermaphrodites are mostly self-compatible and in many instances known to produce at least some seeds through self-pollination.

Evidence for an advantage of the hermaphrodite morph through reproductive assurance is mentioned in several of

the studies represented in Table 1. For example, *Ocbradenus baccatus* (Resedaceae) flowers throughout the year but peak flowering is in February–March. Outside peak flowering, the number of flowering plants varies widely. The authors suggest that during these periods, in which numbers of flowering plants are low, populations may suffer reduced pollinator service, giving hermaphrodites a selective advantage through autogamous selfing (Wolfe & Shmida, 1997). In the bee-pollinated *Wurmbea dioica* (Colchicaceae), hermaphrodites have larger flowers and are more often visited than female flowers, suggesting an advantage under pollinator-limited situations (Vaughton & Ramsey, 1998; Ramsey & Vaughton, 2001). In the aquatic herb *Sagittaria latifolia* (Alismataceae) populations vary from monoecy to dioecy (Sarkissian *et al.*, 2001; Dorken & Barrett, 2004). Monoecious populations are mainly found in ephemeral, newly colonized habitats, whereas dioecious populations are found in stable permanent habitats. This suggests that hermaphroditic individuals are better colonizers than females. Although autogamous selfing cannot be present in monoecious individuals, the ability to self and to found a population is nevertheless expected to be higher for a monoecious individual because only a single individual is needed to found a sexually reproducing population, whereas with dioecy at least two individuals of opposite sexes are needed. Different species of *Dombeya* were studied on La Reunion Island by Humeau *et al.* (1999, 2000). Variation from strict dioecy to subdioecy was found both within and among species. Strict dioecy occurs in nondisturbed forests at high latitude whereas subdioecy occurs in the lowlands in small fragmented populations. The authors suggest that the higher frequency of hermaphrodites in the lowlands may be caused by selection for reproductive assurance if fragmented populations experience more frequent extinction and colonization events.

Models

Charlesworth & Charlesworth (1978) modelled the evolution of dioecy from a hermaphroditic ancestral condition via the gynodioecious pathway. This pathway involves the sequential spread of two gene mutations, the first one affecting pollen production (male sterility) and the second affecting ovule production (female sterility). They demonstrated that the most likely pathway to dioecy involves first a recessive male sterility gene entering a hermaphroditic population, causing the population to become gynodioecious. Subsequently, invasion of a dominant female sterility gene will result in the evolution of dioecy. The likelihood of this sequence of events depends on the linkage between the two sterility genes. Complete dioecy only arises under tight linkage, and looser linkage between the two modifiers results in a population containing males, females and hermaphrodites, hence a subdioecious population. However, tighter linkage between the male and female sterility loci is expected to evolve as this would prevent the production of neuter individuals.

Dioecy evolving via this pathway will result in a genetic sex determination mechanism involving homogametic females and heterogametic males.

Our literature survey suggests that most subdioecious species evolved from the gynodioecious pathway. We therefore assume a sex determination model where a single locus segregates for two alleles A and a . Females are homogametic AA and males are the heterogametic Aa sex in accordance with the theoretical predictions of Charlesworth & Charlesworth (1978), supported by empirical studies cited therein. Our literature survey also confirmed previous studies showing that it is the male morph that most often is the labile sex. We therefore model two types of male genotype: one male genotype has fixed male sex expression (i.e. a pure male) and the other male genotypes, so called inconstant males, carry a dominant modifier which gives them a probability h of producing a hermaphrodite phenotype and a probability $1 - h$ of developing into a pure male phenotype. In this respect, our models differ from previous models on the evolution of dioecy, in that we specifically consider a labile-sex male genotype. We ask whether a dominant modifier turning the male morph into an inconstant male can be maintained in a population that has evolved dioecy as described above. In particular, we study the effect of pollen limitation in females and selfing in inconstant males (when they are functionally hermaphrodites) on the maintenance of this labile sex modifier.

When a population consists of AA females and Aa males, the aa genotype cannot be produced through outcrossing alone. However, if a modified Aa male genotype results in a hermaphroditic phenotype, aa genotypes may be produced. Such aa genotypes carry two copies of the female sterility allele (a) and we therefore expect the phenotype of aa genotypes to be male. In a population that very recently evolved dioecy, such aa genotypes will probably result in a viable male. Conversely, if a species has undergone strict dioecy for many generations, this could allow the gradual accumulation of recessive deleterious mutations tightly linked to the a allele which are always masked in normal Aa males. This situation – very similar in essence to a system of sex chromosomes with XX females and XY males – is likely to result in aa genotypes being either sterile or nonviable (just as YY) because of linked deleterious homozygous mutations. Thus, under a scenario of ancient dioecy, we will assume that aa genotypes are in fact nonviable.

In the next two sections, we develop two related models that make identical ecological assumptions but differ in their assumptions regarding the genetic basis of sex lability. For each model we contrast scenarios of ancient vs recent dioecy by considering both nonviable and viable aa genotypes.

Model I: a single tri-allelic sex-determining locus carrying the inconstant male modifier

Under this model we assume that sex is determined by a single biallelic nuclear sex-determining locus (A/a) with AA females

Table 2 Genotypes and their phenotypes under models I and II

Sex phenotype	<i>N</i>	Genotype	Gametic frequencies	Progeny produced through selfing
Model I				
♀	n_1	AA	1A	
♂	n_2	Aa	$1/2A$ $1/2a$	
♂ 1 - <i>h</i>	n_3	Aa*	$1/2A$ $1/2a^*$	$1/4AA$ $1/2Aa^*$ $1/4a^*a^*$
♀ ♂ <i>h</i>				
♂	n_4	aa		
♂ 1 - <i>h</i>	n_5	aa*	$1/2a$ $1/2a^*$	$1/4aa$ $1/2aa^*$ $1/4a^*a^*$
♀ ♂ <i>h</i>				
♂ 1 - <i>h</i>	n_6	a*a*	1a*	a*a*
♀ ♂ <i>h</i>				
Model II				
♀	n_1	AA MM	1AM	
♀	n_2	AA Mm	$1/2AM$ $1/2Am$	
♀	n_3	AA mm	1Am	
♂ 1 - <i>h</i>	n_4	Aa MM	$1/2AM$ $1/2aM$	$1/4aa$ MM $1/2Aa$ MM $1/4AA$ MM
♀ ♂ <i>h</i>				
♂ 1 - <i>h</i>	n_5	Aa Mm	$1/4AM$ $1/4aM$	$1/12AA$ MM $1/6AA$ Mm $1/12AA$ mm
♀ ♂ <i>h</i>			$1/4Am$ $1/4am$	$1/6Aa$ MM $1/3Aa$ Mm $1/6Aa$ mm
♂	n_6	Aa mm	$1/2Am$ $1/2am$	
♂ 1 - <i>h</i>	n_7	aa MM	1aM	aa MM
♀ ♂ <i>h</i>				
♂ 1 - <i>h</i>	n_8	aa Mm	$1/2am$ $1/2aM$	$1/4aa$ mm $1/2aa$ Mm $1/4aa$ MM
♀ ♂ <i>h</i>				
♂	n_9	aa mm	1am	

Sex: ♀, a female; ♂, a male; ♀ ♂, an individual functionally hermaphrodite. Under model I, genotypes *aa*, *a*a* and *a*a** are nonviable when 'ancient dioecy' is assumed. Under model II, genotypes *aa MM*, *aa Mm* and *aa mm* are nonviable when 'ancient dioecy' is assumed (see section 'Models' for details).

and *Aa* males. We then introduce a modifier at the same locus, *a**. Genotypes carrying the *a** modifier allele are inconstant males that have a probability *h* of developing into a hermaphrodite phenotype and a probability $1 - h$ of developing into a male (see Table 2 for a summary). We assume that, if dioecy is ancient, the *a** allele, a modified version of the *a* allele, also carries a cohort of tightly linked recessive mutations inherited from *a*. Accordingly, under the hypothesis of ancient dioecy, we assume that *aa*, *a*a* and *a*a** genotypes are nonviable and the population potentially contains three distinct diploid genotypes that correspond to three sex morphs: *AA* (females), *Aa* (males) and *Aa** (inconstant males that develop either as males or as hermaphrodites). When assuming recent dioecy, *aa*, *a*a* and *a*a** genotypes are considered perfectly viable. The population may contain up to six distinct diploid genotypes (Table 2).

Model II: a two-locus model with a single sex-determining locus and an unlinked locus carrying a (inconstant) male modifier

Under this model we assume (as in model I) that sex is determined by a single biallelic locus (*A/a*) with *AA* females and *Aa* males. We then consider a second locus unlinked to the sex-determining locus. This second locus carries a dominant

modifier *M* which turns males into inconstant male genotypes with a probability *h* of producing a functional hermaphrodite phenotype and a probability $1 - h$ of retaining a pure male phenotype. We assume the effect of *M* to be dominant, such that genotypes *a_Mm* and *a_MM* have a probability *h* of producing a hermaphrodite phenotype, whereas *a_mm* genotypes are pure males (see Table 2). We assume that the modifier has no effect in females. Depending on the fate of the *aa* genotypes, the population potentially consists of six (ancient dioecy, *aa* genotypes nonviable) or nine (recent dioecy, *aa* genotypes viable) distinct diploid genotypes (see Table 2 for details).

Ecological assumptions common to both models

The notations of our model are summarized in Table 3. We assume that the seed set of females is potentially pollen limited and increases with the number of pollen donors in the population. To incorporate pollen limitation in females, the number of offspring produced by a female, Φ , was modelled as an increasing function of the number of pollen donors *P* with initial slope α and asymptotic optimum α/β :

$$\Phi(P) = \frac{\alpha P}{1 + \beta P} \quad \text{Eqn 1}$$

Table 3 Notations common to both models I and II

Symbol	Definition
Φ	Number of offspring produced by pure females as a function of the number of pollen donors (P) in the population; $\Phi = \alpha P / (1 + \beta P)$.
α, β	Parameters modulating the intensity of pollen limitation in females. Under optimal pollen conditions, females have a fertility α/β .
ω	Parameter modulating the number of (selfed) offspring produced by a hermaphrodite relative to a female under optimal pollination. <i>A priori</i> $\omega > 1$ and the number of selfed offspring is fixed to $\alpha/\omega\beta$.
π	Parameter modulating the amount of pollen contributing to the outcrossed pollen pool produced by a hermaphrodite relative to a male. <i>A priori</i> $\pi > 1$ and the contribution of a hermaphrodite is $1/\pi$ relative to a male contributing 1.
K	Carrying capacity of the population.

P is defined as the effective number of pollen donors, that is the number of individuals (males and hermaphrodites) that can contribute pollen weighted by their pollen export (hermaphrodites weight only $1/\pi$ relative to pure males). The female fitness of hermaphrodites, measured in number of offspring, is decreased by a factor ω relative to pure females. Hermaphrodites, depending on the scenario we study, either produce $\alpha/(\omega\beta)$ offspring via complete self-pollination or, when reproducing through outcrossing, are also potentially pollen limited and produce $\Phi(P)/\omega$ offspring. In addition, hermaphrodites, regardless of whether they self or not, always contribute pollen to the outcross pollen pool, albeit less than pure males. The male fitness of hermaphrodites, pollen production, is $1/\pi$ relative to pure males, which have a pollen production of 1. Population growth is density dependent and generations are discrete. The population grows logistically until carrying capacity K .

Given the assumptions already stated, and under each genetic model, a set of recursion equations (given in the Appendix) tracking the numbers of each genotype was derived. These were iterated numerically starting at either high or low density. For each model, high-density iterations began with 100 females, 100 males and two inconstant males (to check whether inconstant males could invade). We considered that equilibrium was reached when the frequencies of genotypes no longer changed through time. We then iterated the model under identical parameter values but starting with 100 females and 100 inconstant males and then adding two pure males (i.e. to check whether males could invade a subdioecious population with inconstant males and females). If the outcome stayed the same, we considered the equilibrium we found to be stable, and if initial conditions (numbers of sexual morphs) changed the outcome we reported the equilibrium as unstable. Low-density iterations were performed in a similar way but here we began iterations with 25 females, 25 males (or inconstant males) and two inconstant males (or males). We typically ran iterations for 1000–5000 generations, although equilibrium was often reached much more quickly. An exception occurs when h is set to very small values as selection becomes very weak (data not shown). The results of our models shown below are all based on a pollen limitation function, Φ , where α is set to 0.05 and β to 0.01. The shape of the

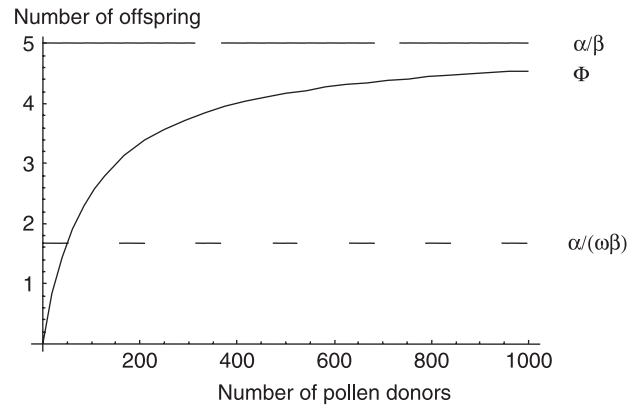


Fig. 1 Number of offspring per female, Φ , as a function of the number of pollen donors in the population. The upper horizontal line (long dashes) represents the asymptotic number of offspring per female, α/β . The lower horizontal dashed line represents the number of offspring produced by hermaphrodites under complete selfing, $\alpha/(\omega\beta)$. Parameter values are $\alpha = 0.05$, $\beta = 0.01$ and $\omega = 3$. α, β, ω , see Table 3 for definitions.

Φ function under these parameter values is shown in Fig. 1. We also iterated scenarios with both more and less severe pollen limitation (slope α) to examine whether this changed the outcome of the models. Unless specifically stated, the outcomes of our models did not vary with α . For all iterations we assumed a carrying capacity of $K = 1000$.

Under both model I and model II we considered four different scenarios.

- We examined conditions for the invasion of a modifier/inconstant males when there is no pollen limitation (i.e. female offspring production is set to a constant α/β and hermaphrodite offspring production to $\alpha/\omega\beta$) and when both females and hermaphrodites reproduce through outcrossing.
- We examined the effect of pollen limitation. Females are pollen limited, that is Φ is a function of P , the number of pollen donors in the population as in Eqn 1, and hermaphrodites are outcrossing and also suffer pollen limitation, their offspring production being $\Phi(P)/\omega$.
- We examined the effect of selfing in hermaphrodites when females are not pollen limited (i.e. Φ is constant and set to its optimum α/β) and hermaphrodites produce offspring through complete selfing.

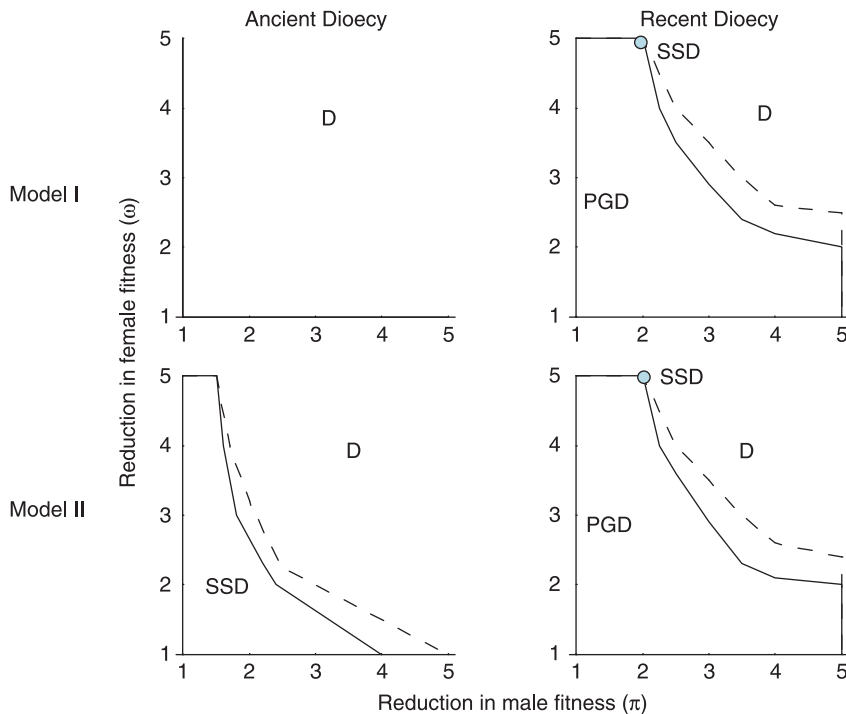


Fig. 2 Outcomes of models I and II as a function of the reduction in female (ω) and male (π) fitness of hermaphrodites. Hermaphrodites are incapable of self-fertilization and reproduce through outcrossing, and the probability of producing a hermaphrodite phenotype, h , is 0.5. Solid line, no pollen limitation; dotted line, pollen limitation. SSD, stable subdioecy; PGD, pseudo gynodioecy; D, dioecy. Note that, under model I with ancient dioecy, both the dotted line and the solid line overlap with the y-axis.

(d) We examined the combined effect of pollen limitation in females and selfing in hermaphrodites.

All of these scenarios were studied under what we term 'ancient' and 'recent' dioecy by assuming that aa and, when applicable, a^*a^* and aa^* genotypes were, respectively, non-viable and perfectly viable. Comparison of (a) and (b) allows us to examine how pollen limitation alone affects the invasion of inconstant males, and comparison of (a) and (c) allows us to examine how selfing alone affects the invasion of inconstant males. The last scenario (d) allows us to examine the joint effect of both pollen limitation and selfing on conditions for the invasion of inconstant males.

Model results

We begin by reviewing the variety of evolutionary outcomes we obtained in our models. We then describe the effects of pollen limitation and selfing separately and in combination. We examine how the different genetic assumptions we made (one vs two loci, and ancient vs recent dioecy) affect the conditions for invasion and maintenance of inconstant males. We concentrate on results based on iterating our models by starting at a relatively high population density, but we also briefly discuss scenarios starting at a low density.

Outcomes of models

Under both sex determination models (I and II), we find that inconstant males can invade a population of pure females and males under a range of conditions (Figs 2, 3). We find five

different stable outcomes that vary with respect to the number and type of sexual morphs maintained in the population at equilibrium. The three most common outcomes are as follows. (1) 'Pseudogynodioecy' (PGD), where only two types of genotypes, pure females and inconstant males, are maintained, and pure males are eliminated. However, this population would appear subdioecious as the inconstant male genotype displays a hermaphrodite phenotype with probability h . The population therefore comprises three different sex phenotypes: females, males and hermaphrodites. (2) 'Stable subdioecy' (SSD), where all three sexual genotypes are stably maintained. (3) 'Dioecy' (D), where inconstant males cannot invade and the equilibrium population consists of only pure males and pure females (in a 1 : 1 sex ratio). In addition to these three outcomes (found in both models I and II and assuming both ancient and recent dioecy), we observe two other stable outcomes, but only when recent dioecy is assumed. These are: (4) 'inconstant males' (IC), where the population at equilibrium consists only of inconstant males and the pure sexes are no longer present, and (5) 'pseudo-androdioecy' (PAD), where only pure males and inconstant males are maintained and pure females are eliminated. In addition, we observe under some scenarios instances in which the population initially grows but pollen limitation becomes so severe that the whole population eventually goes extinct (population extinction (PE)).

The effect of pollen limitation

With one exception – model I assuming ancient dioecy – pollen limitation increases the range of conditions allowing

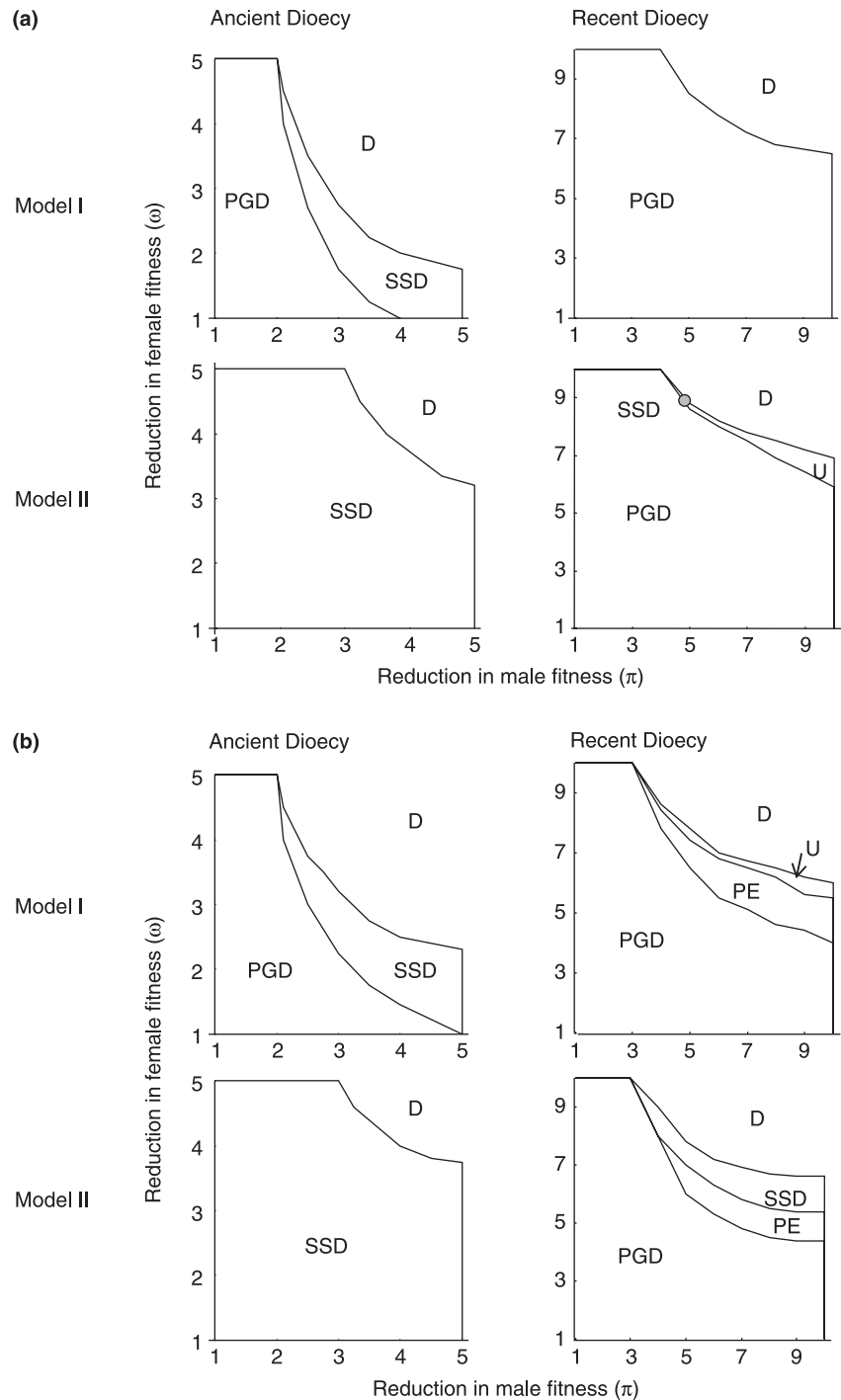


Fig. 3 Outcomes of models I and II as a function of the reduction in female (ω) and male (π) fitness of hermaphrodites. Hermaphrodites reproduce by complete selfing, and the probability of producing a hermaphrodite phenotype, h , is 0.5. (a) No pollen limitation in females. (b) Females suffer pollen limitation. SSD, stable subdioecy; PGD, pseudo gynodioecy; D, dioecy; PE, population goes extinct; U, unstable zone where outcome (coexistence of all three sex morphs or population extinction) depends on the initial conditions. The circle in (a) of recent dioecy represents the limited zone of SSD.

inconstant males to invade (Fig. 2; dotted vs solid line). This is because pollen limitation reduces the difference in female fitness (offspring production) between pure females and inconstant males compared with a situation in which pure females are not pollen limited. Pollen limitation thus allows inconstant males with a lower female fitness (higher values of ω) to invade compared with a situation with no pollen limitation.

Under model I and assuming ancient dioecy, pollen limitation does not facilitate the invasion of inconstant males (Fig. 2). The main reason for this difference is that, when aa genotypes are nonviable, severe selection against the modifier a^* occurs as it is selected out in the a^*a^* and a^*a genotypes. The modifier a^* can still be transmitted through pollen when it fertilizes the ovules of a pure female. However, when an inconstant male acts as a female, the a^* allele is selected against

whenever a or a^* pollen lands on its stigma. Effectively, therefore, a maximum of only a quarter of the offspring of an inconstant male will carry the a^* allele (when an a^* ovule fuses with an A pollen). Although pollen limitation reduces the difference in female fitness between inconstant males and pure females, only male fitness (pollen production) is important for invasion of the a^* allele in this case. Because of pollen competition with the a allele from pure males, the pollen production of an inconstant male must be at least as good as that of pure males in order for the modifier to invade. An inconstant male with a pollen production similar to that of pure males will invade, but it will not increase in frequency unless its pollen production is higher than that of pure males ($\pi < 1$).

Effect of selfing in hermaphrodites

When inconstant males are allowed to reproduce through selfing, the range of (π , ω) values allowing their invasion is greatly increased (compare Figs 2 and 3). This is generally expected as a result of the segregating advantage conferred by selfing. Even in the ancient dioecy scenarios in which up to a quarter of the progeny produced by selfing of Aa^* (model I) are nonviable, selfing still confers a significant fitness advantage compared with scenarios without selfing (compare Fig. 2, no selfing, with Fig. 3a, selfing). Assuming recent dioecy (aa genotypes are viable), the advantage of selfing in both models I and II almost doubles the values of π and ω under which inconstant males may invade compared with ancient dioecy (note the scale difference in Fig. 3 for selfing under ancient vs recent dioecy). In all cases, but for model II assuming ancient dioecy, outcomes of PGD are observed. Stable SSD was found in both models I and II under ancient dioecy. Under recent dioecy, no stable coexistence of all three sexual morphs is found in model I and only under a very limited range of (π , ω) values in model II (Fig. 3).

Joint effect of selfing in hermaphrodites and pollen limitation in females

When the effects of pollen limitation and selfing are combined, outcomes vary greatly depending on whether ancient or recent dioecy is assumed. Under ancient dioecy, selfing in combination with pollen limitation further facilitates the invasion of inconstant males compared with the scenarios of pollen limitation and selfing alone (compare Fig. 3b (selfing and pollen limitation) with Fig. 2, dotted line (pollen limitation and no selfing) and Fig. 3(a) (selfing and no pollen limitation)). However, the possible outcomes of the models (PGD, SSD and D) do not differ from those found under selfing alone. In contrast, when recent dioecy is assumed, the combination of selfing and pollen limitation results in new outcomes. These include unstable zones where the equilibrium of the models depends on the initial conditions, and population extinction (Fig. 3b). In model I, a range of π , ω values results

in unstable SSD whereas the SSD equilibrium is stable in model II.

When inconstant males can self and when females are pollen limited, we observe scenarios in which the invasion of inconstant males under some conditions causes population extinction. Invasions of inconstant males can cause populations to go extinct although the population would grow to carrying capacity if inconstant males were not present. The selfing advantage allows the spread of inconstant males that are very poor in both pollen and offspring production. The invasion and subsequent spread of inconstant males is accompanied by a reduction in pure males, thereby increasing further pollen limitation in females (Fig. 5a). Inconstant males hence increase their own female fitness relative to that of females as they increase in frequency. However, as inconstant males increase in frequency, they may deteriorate the pollen environment to a point where offspring production in pure females is so low that the population eventually crashes, as the inconstant males alone cannot maintain the population (Fig. 5b). This, however, only happens in scenarios in which pure females are severely pollen limited (i.e. we find this for $\alpha = 0.05$ and $\beta = 0.01$, whereas under less severe pollen limitation ($\alpha = 0.15$ and $\beta = 0.03$) the zone of population extinction changes to an unstable zone where the population develops into PGD, SSD or D depending on the starting conditions).

A population starting at low density, consisting only of pure males and females, goes extinct when the number of pollen donors is too low for females to produce enough offspring to maintain the population. In these cases, adding one functional hermaphrodite with the ability to self autonomously can be enough to prevent populations from going extinct. When populations do not go extinct, the equilibrium frequencies of the genotypes do not differ from those found when starting to iterate the recurrence equations at higher densities. Even when inconstant males are not maintained at equilibrium, a situation we term 'transient inconstant males' can arise. Here, females are severely pollen limited and the inconstant males are kept in the population for a considerable number of generations (typically 40–400 generations depending on the scenario) before they are lost and the population eventually returns to pure dioecy. Inconstant male genotypes transiently reach high frequencies, probably because selfing is advantageous at low densities where females are severely pollen limited ('transient inconstant males' are not observed when hermaphrodites are strictly outcrossing). Under such conditions, a population subject to frequent fluctuations in size or mate availability (e.g. a female-biased sex ratio, or low pollinator availability) may never reach the equilibrium point where inconstant male genotypes are lost.

Effect of sex determination

We here examine how the genetic assumptions regarding sex determination (i.e. model I vs model II, and ancient vs recent

dioecy) affect the invasion of inconstant males. One important result is that, under model II (for both recent and ancient dioecy) and under model I assuming recent dioecy, inconstant males can invade with a reduced fitness of both their male and female functions compared with pure sexes. This invasion is possible in the absence of both selfing and pollen limitation (Fig. 2, solid line). While selfing and pollen limitation to a great extent further increase the probability of invasion of inconstant males, it is noteworthy that the genetic assumptions behind sex determination alone play an important role in the invasion of labile sex.

Assuming that dioecy has persisted for many generations (ancient dioecy), and hence that *aa* genotypes are nonviable, significant differences in outcomes between models I and II are found. When the locus carrying the modifier is unlinked from the sex determination locus (model II), inconstant males can invade under a greater range of reductions in both male and female fitness (π , ω values) than when the modifier is introduced at the sex determination loci (model I) (compare model I with model II in Figs 2 and 3). This is a result of the selection against the modifier a^* in homozygotes, as discussed in the previous section. However, under model II the inconstant males cannot outcompete pure males, resulting in SSD instead of PGD. In contrast, in model I, the modifier can only invade when inconstant males are selfing, but when inconstant males invade they can outcompete pure males under a large range of π , ω values and the range of conditions yielding SSD is much smaller than in model II.

When selection against *aa* genotypes is relaxed (recent dioecy), the range of conditions under which inconstant males can invade is greatly increased in all scenarios and for both model I and model II. Moreover, under model II, inconstant males can actually outcompete pure males, which was never the case under ancient dioecy. Relaxing the selection against *aa* genotypes and allowing inconstant males to reproduce through selfing creates a situation that is not unlike that of the spread of a sexually transmitted virus. The modifier allele can invade even when both the male fitness and the female fitness of a hermaphrodite are less than 1/6 of those of the pure sexes (e.g. Fig. 3, recent dioecy). The invasion of inconstant males with very poor pollen and offspring production creates situations in which the equilibrium outcomes depend on initial conditions and in which the invasion of the modifier eventually may drive the population to extinction.

Under ancient dioecy, the value of h does not change the qualitative outcome of the models; however, it does affect both the speed at which equilibrium is reached and the equilibrium frequency of inconstant males (not shown). In contrast, under recent dioecy, varying the value of h affects the qualitative outcome of the models. In the absence of selfing (both with and without pollen limitation), the range of (π , ω) values yielding PGD at $h = 0.5$ (Fig. 2) yields PAD when higher values of h are assumed ($h = 0.9$; data not shown). Under selfing and no pollen limitation, the range of (π , ω)

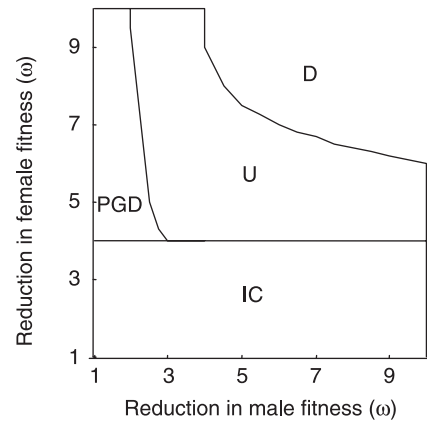


Fig. 4 Outcomes of model I assuming recent dioecy, as a function of the reduction in female (ω) and male (π) fitness of hermaphrodites. Females are pollen limited and hermaphrodites reproduce by complete selfing, and the probability of producing a hermaphrodite phenotype, h , is 0.9. PGD, pseudo gynodioecy; D, dioecy; U, unstable zone where the equilibrium (coexistence of all three sex morphs or population extinction) depends on the initial conditions. IC, population consists entirely of inconstant males.

values yielding PGD (Fig. 3a) still yields PGD for higher h values. However, when selfing and pollen limitation are combined, PGD is only conserved over a narrower range at high values of h (Fig. 4) and new outcomes are found (compare Fig. 3b with Fig. 4). New outcomes include a broad unstable zone leading to either SSD or PE depending on initial conditions and a zone in which inconstant males eliminate both pure sexes (IC; Fig. 4).

Discussion

Empirical studies on subdioecious species report a wide range of reductions in female and male fitness of hermaphrodites relative to the pure sexes. For example, pure females produce on average three (*Hebe subalpina*; Delph & Lloyd, 1991), four (*Astilbe biternata*; Olson, 2001) and up to five (*Schieda globosa*; Sakai & Weller, 1991) times as many seeds as hermaphrodites. In *Ochradenus baccatus*, pure females may even produce 9–20 times more seeds (Wolfe & Shmida, 1997), but in *Cirsium arvense* (Kay, 1985) the offspring production of hermaphrodites relative to that of pure females potentially varies from 0.13 to as high as 1.29. Pollen production in pure males has been found to be equal to (*Schieda globosa*) or 1.3 times (*Astilbe biternata*) or twice (*Ochradenus baccatus*) as high as the pollen production in hermaphrodites, but large variations are found among individuals (e.g. pollen production in hermaphrodites of *Cirsium arvense* varies from 0.25 to 0.85 relative to pure males). These studies confirm that the π , ω parameter space we investigate in our models lies in the range of conditions that occur in natural populations. Our models show that, under a variety of genetic assumptions regarding sex determination, inconstant males can invade a dioecious

population despite reductions in both their male and their female fitness relative to that of pure sexes. Pollen limitation and selfing facilitate the invasion and maintenance of a labile sex genotype under a broader range of (π , ω) values.

When dioecy has persisted for a long time, the probability of invasion of a labile sex modifier is reduced compared with a situation in which dioecy has evolved more recently. However, under both ancient and recent dioecy, a labile sex modifier can invade a population under conditions that may be found in natural populations. This strongly suggests that evolution towards and breakdown of dioecy is a very dynamic process, and that dioecy is not a 'deterministic endpoint' from which no further evolution is possible (see also Maurice & Fleming, 1995; Wolf & Takebayashi, 2004).

In natural populations, it is rarely obvious whether a subdioecious species represents a transition towards or a breakdown of dioecy. The observation of both gynodioecious and subdioecious populations within one species, or in closely related ones (e.g. *Clusia nemorosa*, *Hebe subalpina*, *Fuchsia lycioides* and *Schiedea globosa*; Table 1), suggests that the breeding system here may be evolving towards dioecy. In other species, observations of both strictly dioecious and subdioecious populations (e.g. *Buchloe dactyloides* and *Fragaria chiloensis*; Table 1) are less suggestive as to whether the subdioecious populations are on their way to evolving pure dioecy or whether they in fact represent situations in which dioecy is 'breaking down'.

Biotic pollination and labile sex

The literature survey showed that a significant frequency of hermaphrodites in subdioecious populations is associated with animal pollination and perfect flowered hermaphrodites. Reviews by Aide (1986) and Vogler & Kalisz (2001) both found a striking difference in the outcrossing rates between animal- and wind-pollinated species. While wind-pollinated species show a strongly bimodal distribution of outcrossing rates (most having outcrossing rates above 80%), animal-pollinated species show a much wider range, with about 50% having intermediate outcrossing rates. Hence there seems to be empirical support for animal-pollinated species on average experiencing higher levels of selfing than wind-pollinated ones. Even if inconstant males do not self autonomously, the fact that they are insect pollinated may increase the amount of selfing through either within-flower or among-flower (geitonogamous) selfing and thereby promote the maintenance of inconstant males. Charlesworth & Charlesworth (1978) argued that a reduction in pollen production in hermaphrodites with perfect flowers is unlikely to reduce the proportion of selfed ovules, whereas a reduction in pollen production in male flowers of monoecious individuals is more likely to reduce the chance of self-fertilization of its female flowers.

Our models show that selfing in hermaphrodites greatly facilitates the invasion and maintenance of inconstant males.

If both insect pollination and perfect flowers increase self-fertilization rates, this may, at least partly, explain why high frequencies of hermaphrodites in subdioecious populations are mainly found in such species.

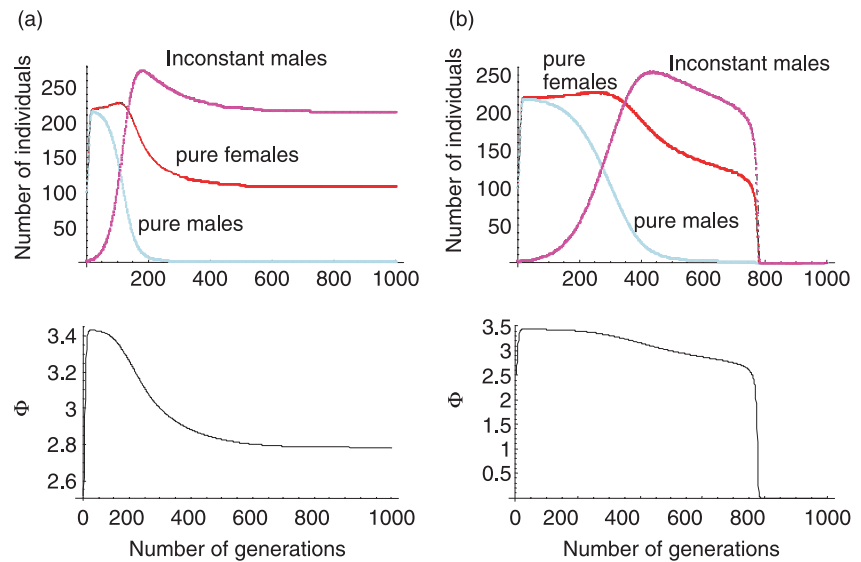
Subdioecy and pollen limitation in females

Pollen limitation in flowering plants is a common phenomenon (e.g. reviews by Burd, 1994; Larson & Barrett, 2000; Knight *et al.*, 2005). However, the best evidence for pollen limitation in plants is based on reviews of animal-pollinated plant species. Knight *et al.* (2005) compared pollen limitation among biotically and abiotically pollinated species and found no significant difference. However, the authors stressed that this comparison was based on a very low sample size of abiotically pollinated species, limiting their ability to detect differences in pollen limitation among species with different pollination modes. There is at present no empirical evidence for insect-pollinated species being more pollen limited than wind-pollinated ones. Nevertheless, in our literature survey, pollen limitation in females was found in all cases where this was studied.

In our models, pollen limitation facilitates invasion of inconstant males by reducing the difference in female fitness between inconstant males and pure females, thereby allowing inconstant males with a poorer female fitness to invade compared with a situation with no pollen limitation. The spread of inconstant males in a population is accompanied by a reduction in the frequency of pure males and stronger pollen limitation in females. Inconstant males thereby increase their own female fitness relative to that of females as they increase in frequency. This effect is largest at low to medium population densities. At high population densities, the number of pollen donors, even if these are mainly inconstant males, may still be large enough to prevent pollen limitation in females. However, depending on the genetic assumption of sex determination and on the ability of hermaphrodites to self or not, the spread of inconstant males with very low pollen production may create a scenario in which the population goes extinct because of severe pollen limitation in females (Fig. 5).

The effect of pollen limitation in females for the maintenance of trioecy was investigated theoretically by Maurice & Fleming (1995). Using a different modelling assumption (they modelled pollen limitation as a function of pollen donor frequency, whereas we modelled it as a function of numbers of pollen donors), they found that pollen limitation in females could give hermaphrodites a selective advantage which, under some scenarios, would result in evolution to trioecy, and under a restricted range of conditions would lead to androdioecy. Wolf & Takebayashi (2004), using essentially the same model as Maurice & Fleming (1995) but regarding pollen limitation as a function of pollen donor availability (as we do), showed that androdioecy could evolve from dioecy under a much broader range of (π , ω) conditions (with $\pi = 1/ph$ and

Fig. 5 Effect of inconstant males on pollen availability and population viability. Recent dioecy (*aa* genotypes are viable) is assumed, hermaphrodites reproduce by self-fertilization, and pure females are pollen limited ($\alpha = 0.05$ and $\beta = 0.01$). Upper graphs show the change over time in numbers of pure females, pure males and inconstant males. Lower graphs show the concomitant increase in pollen limitation illustrated by the decrease in the number of offspring per female, Φ . (a) Increased pollen limitation with the spread of inconstant males ($\pi = 6$ and $\omega = 5$). (b) Population extinction ($\pi = 6$ and $\omega = 6$). α , β , π , ω , see Table 3 for definitions.



$\omega = 1/\text{oh}$ in their parametrization). Interestingly, unlike Maurice & Fleming (1995), they did not find conditions leading to stable trioecy. This illustrates how different assumptions regarding pollen limitation can lead to qualitative differences in model outcome.

Our models, which incorporate a form of pollen limitation similar to the 'decelerating function' used by Wolf & Takebayashi (2004; see their fig. 4, curve D), yield a rich series of evolutionary outcomes comprising all outcomes reported earlier. Apart from the fact that we incorporate explicit demographics and density-dependent growth, we (1) incorporate sex lability instead of a set of genotypes with fixed phenotypes and (2) explore a broad range of genetic assumptions for sex determination. This may explain why we find qualitatively different conditions for invasion of a hermaphroditic morph.

If we consider model I (under recent dioecy) and fairly high values of h (0.9–1), we obtain a situation in which inconstant males are closer and closer to fixed hermaphrodites. This situation is then comparable to the case examined by Wolf & Takebayashi (2004), who modelled a single sex-determining locus (albeit with a different dominance hierarchy between sex-determining alleles). Under these assumptions, the PGD zone previously obtained at lower h values when hermaphrodites cannot self (Fig. 2) is now replaced by an androdioecy zone where inconstant males and hermaphrodites coexist. The addition of selfing and pollen limitation yields a sort of androdioecy whereby only inconstant males are maintained in the population and the population will phenotypically be androdioecious with rare males (Fig. 4).

An important difference between our model and previous ones concerns inbreeding depression. Inbreeding depression is considered one of the main selective factors in mating system evolution. Inbreeding depression is mostly modelled as the reduction in survival of offspring produced by self-fertilization relative to that produced by outcrossing. In our

models we do not explicitly incorporate inbreeding depression as a separate parameter. However, we incorporate, through ω , the reduction in number of offspring produced by an inconstant male relative to an outcrossed female. In scenarios in which selfing occurs, ω values higher than 1 may be viewed as the combined effects that resource allocation and inbreeding depression have on reducing the female fitness of a hermaphrodite. It remains to be explored whether a separation of the effects of resource allocation and inbreeding depression in selfing hermaphrodites may change the predictions of our models.

The evolutionary significance of labile sex expression

We predict that genotypes exhibiting labile sex expression may be selectively maintained under various scenarios. It is therefore relevant to consider whether labile sex has any ecological and evolutionary consequences for a population. We briefly discuss some aspects of this in the following paragraphs.

First, labile sex expression can affect the maintenance of a local population. Our models predict that the presence of a selfing hermaphrodite in populations at low density may save a population from extinction when pollen limitation in females is too severe to ensure population growth. This finding is somewhat similar to that described by Pannell (1997), who showed how hermaphrodites may be stably maintained in gynodioecious and androdioecious populations subject to recurrent situations of low density by frequent extinction/colonization events. He showed that a high population turnover in a metapopulation could maintain hermaphrodites because of their selfing advantage under pollen-limited situations. Our models also predict instances where invasion of inconstant males may cause population extinction. We know of no empirical studies that can support this 'extinction scenario'.

Secondly, labile sex expression may also have important consequences for the distribution of the species among different

types of habitat. The importance of labile sex expression in relation to the abiotic environment has been addressed by several authors (recently reviewed by Delph & Wolf, 2005). Many studies show that inconstant males are more prone to produce fruit in moist and resource-rich environments but stay male in more resource-poor ones. A trade-off in the hermaphrodite morph between male fitness and female fitness has been documented in several species (e.g. *Hebe subalpina* (Delph & Lloyd, 1991), *Wurmbea dioica* (Barrett *et al.*, 1999) and *Astilbe biternata* (Olson & Antonovics, 2000). Such a trade-off is expected to be a major selective factor driving the evolution of gender specialization. In a gynodioecious species evolving towards dioecy, a labile sex genotype may be at a selective advantage in poor growing conditions as it lowers the cost of producing fruits (Delph & Wolf, 2005). Moreover, if inconstant males only produce fruits under favourable conditions the trade-off may have no, or minimal, effect on pollen production (i.e. no trade-off) and this plasticity may thus hinder the evolution of dioecy (Barrett *et al.*, 1999). A labile sex and the ability to lessen the trade-off between male fitness and female fitness may thus also be important in terms of the species habitat range as it should allow the species to grow under a wider range of abiotic conditions.

We find that a stable coexistence of males, females and inconstant males may occur without invoking adaptive significance of labile sex expression in response to variation in resource among environments. Nevertheless, our way of modelling sex lability through the probability, h , that an inconstant male produces a hermaphroditic phenotype can be extended to vary among environments. It would be interesting to examine how a trade-off between male fitness and female fitness in inconstant males may vary between resource-rich and resource-poor habitats, allowing h to evolve. Such a modelling approach is potentially one way of synthesizing the importance of mating and resource environment in further elucidating the dynamics behind labile sex in plants.

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Appendix

Model I: recursion equations for the genotypic composition of the population

Genotypes are listed Table 2. Assuming that at time t the population is composed of $n_1, n_2 \dots n_6$ individuals and that hermaphroditic individuals only have a contribution π to the pollen pool relative to pure males, the male gametic frequencies $^M\gamma_A, ^M\gamma_a$ and $^M\gamma_{a^*}$ for, respectively, the A, a and a^* alleles in the outcrossed pollen pool are (dropping time superscript on n s and γ s)

$$^M\gamma_A = \left\{ \frac{1}{2}n_2 + \frac{1}{2} \left[(1-h) + \frac{h}{\pi} n_3 \right] \right\} / P$$

$$^M\gamma_a = \left\{ \frac{1}{2}n_2 + n_4 + \frac{1}{2} \left[(1-h) + \frac{h}{\pi} n_5 \right] \right\} / P \quad \text{Eqn A1}$$

$$^M\gamma_{a^*} = \left\{ \frac{1}{2}n_2 + \left[(1-h) + \frac{h}{\pi} \left(\frac{1}{2}n_3 + \frac{1}{2}n_5 + n_6 \right) \right] \right\} / P$$

$$P = n_2 + n_4 + [(1-h) + h\pi](n_3 + n_5 + n_6).$$

Note that under model I females are homogametic AA and thus trivially produce only one gametic type A . Then, assuming random union of gametes for seeds produced by females and assuming self-fertilization for the seeds set by

hermaphroditic individuals, one can obtain the set of recursion for the composition of the population at time $t + 1$ as a function of the composition of the population at time t and the male gametic frequencies in Eqn A1 above:

$$\begin{aligned} n_1^{t+1} &= \exp[\log(\Phi)\Delta]^M \gamma_A n_1^t + \frac{h}{4} \exp\left\{\log\left(\frac{\alpha}{\omega\beta}\right)\Delta\right\} n_3^t \\ n_2^{t+1} &= \exp[\log(\Phi)\Delta]^M \gamma_a n_1^t \\ n_3^{t+1} &= \exp[\log(\Phi)\Delta]^M \gamma_a n_1^t + \frac{h}{2} \exp\left\{\log\left(\frac{\alpha}{\omega\beta}\right)\Delta\right\} n_3^t \\ n_4^{t+1} &= \frac{h}{4} \exp\left\{\log\left(\frac{\alpha}{\omega\beta}\right)\Delta\right\} n_5^t \\ n_5^{t+1} &= \frac{h}{2} \exp\left\{\log\left(\frac{\alpha}{\omega\beta}\right)\Delta\right\} n_5^t \\ n_6^{t+1} &= \left(\frac{h}{4} n_3^t + \frac{h}{4} n_5^t + h n_6^t\right) \exp\left\{\log\left(\frac{\alpha}{\omega\beta}\right)\Delta\right\} \end{aligned} \quad \text{Eqn A2}$$

($\Delta = 1 - (n_1 + n_2 + n_3 + n_4 + n_5 + n_6)/K$; $\Phi = \alpha P/(1 + \beta P)$ (note again that the time superscript t is being dropped from n_1, n_2, \dots etc. to simplify notations).)

The set of recursion equations above (Eqns A1 and A2) assumes recent dioecy, that hermaphrodites self and that females are pollen limited. Under the ancient dioecy assumption, terms involving n_4, n_5 and n_6 are dropped from the recursions. Accordingly, Eqn A1 can be slightly modified to explore scenarios involving no pollen limitation in females. When assuming no pollen limitation in females the Φ function in Eqn A1 is just set to its asymptotic value $\Phi = \alpha/\beta$.

When hermaphrodites are assumed not to self, the male gametic frequencies described in Eqn A1 are still valid but the set of recursion equations for the composition of the population at time $t + 1$ as a function of the composition of the population at time t is:

$$\begin{aligned} n_1^{t+1} &= \exp[\log(\Phi)\Delta]^M \gamma_A n_1^t + \exp[\log(\Phi')\Delta]^M \gamma_A n_3^t \\ n_2^{t+1} &= \exp[\log(\Phi)\Delta]^M \gamma_a n_1^t \\ &\quad + \exp[\log(\Phi')\Delta]^M \left(\gamma_A \frac{h}{2} n_5^t + \gamma_a \frac{h}{2} n_3^t \right) \\ n_3^{t+1} &= \exp[\log(\Phi)\Delta]^M \gamma_a n_1^t + \exp[\log(\Phi')\Delta]^M \\ &\quad \left[\gamma_A \left(\frac{h}{2} n_3^t + \frac{h}{2} n_5^t + h n_6^t \right) + \gamma_a \frac{h}{2} n_3^t \right] \\ n_4^{t+1} &= \exp[\log(\Phi')\Delta]^M \gamma_a \frac{h}{2} n_5^t \\ n_5^{t+1} &= \exp[\log(\Phi')\Delta]^M \left[\gamma_a \left(\frac{h}{2} n_3^t + \frac{h}{2} n_5^t + h n_6^t \right) + \gamma_a \frac{h}{2} n_5^t \right] \\ n_6^{t+1} &= \exp[\log(\Phi')\Delta]^M \gamma_a \left(\frac{h}{2} n_3^t + \frac{h}{2} n_5^t + h n_6^t \right) \end{aligned} \quad \text{Eqn A3}$$

(Φ' , the modified Φ function describing pollen limitation in hermaphrodites with a maximum at $\alpha/(\beta\omega)$.)

Last, if hermaphrodites outcross but neither females nor hermaphrodites are pollen limited, Eqn A3 can be slightly modified by setting Φ and Φ' to their maxima.

Model II: recursion equations for the genotypic composition of the population

Genotypes are listed Table 2. Assuming that at time t the population is composed of n_1, n_2, \dots, n_9 individuals of each corresponding genotype, $^F\gamma_{AM}$ (respectively $^M\gamma_{AM}$) denotes the frequency of the 'AM' gametic genotypes in ovules (respectively pollen) in the whole population.

One obtains the following expression for the set of female and male gametic frequencies as a function of the composition of the population at time t :

$$\begin{aligned} ^F\gamma_{AM} &= \left(n_1 + \frac{1}{2} n_2 + 0 \right) / C_F \\ ^F\gamma_{Am} &= \left(0 + \frac{1}{2} n_2 + n_3 \right) / C_F \\ ^M\gamma_{AM} &= \left[\frac{1}{2} \left(1 - h + \frac{2h}{\pi} \right) n_4 + \frac{1}{4} n_5 + 0 \right] / C_M \\ ^M\gamma_{Am} &= \left[0 + \frac{1}{4} \left(1 - h + \frac{h}{\pi} \right) n_5 + \frac{1}{2} n_6 \right] / C_M \\ ^M\gamma_{AM} &= \left[\frac{1}{2} \left(1 - h + \frac{2h}{\pi} \right) n_4 + \frac{1}{4} \left(1 - h + \frac{h}{\pi} \right) n_5 + 0 \right] / C_M \\ ^M\gamma_{am} &= \left[0 + \frac{1}{4} \left(1 - h + \frac{h}{\pi} \right) n_5 + \frac{1}{2} n_6 \right] / C_M \end{aligned} \quad \text{Eqn A4}$$

$C_F = n_1 + n_2 + n_3$ and $C_M = [(1 - h) + h/\pi](n_4 + n_5 + n_7 + n_8) + n_6 + n_9$.

Note that the gametic frequencies and the vector of composition of the population are time dependent (see Eqn A5 below), although the time subscripts have been dropped from the equation above to simplify notations. The frequency of the two-locus diploid genotypes is obtained under random union of gametes for females and under self-fertilization for hermaphroditic individuals (see Table 3)

$$\begin{aligned} n_1^{t+1} &= \exp[\ln(\Phi)\Delta]^M \gamma_{AM}^t \gamma_{AM}^t + \frac{h}{4} \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] n_4^t \\ &\quad + \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \frac{h}{12} n_5^t \\ n_2^{t+1} &= \exp[\ln(\Phi)\Delta]^M \left(\gamma_{Am}^t \gamma_{AM}^t + \gamma_{AM}^t \gamma_{Am}^t \right) \\ &\quad + \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \frac{h}{6} n_5^t \end{aligned}$$

$$\begin{aligned}
 n_3^{t+1} &= \exp[\ln(\Phi)\Delta]^M \gamma_{AM}^t \gamma_{AM}^t + \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \frac{h}{12} n_5^t \\
 n_4^{t+1} &= \exp[\ln(\Phi)\Delta]^M \gamma_{am}^t \gamma_{AM}^t + \frac{h}{2} \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] n_4^t \\
 &\quad + \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \frac{h}{6} n_5^t \\
 n_5^{t+1} &= \exp[\ln(\Phi)\Delta]^M \left(\gamma_{am}^t \gamma_{AM}^t + M \gamma_{am}^t \gamma_{AM}^t\right) \\
 &\quad + \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \frac{h}{3} n_5^t \quad \text{Eqn A5} \\
 n_6^{t+1} &= \exp[\ln(\Phi)\Delta]^M \gamma_{am}^t \gamma_{AM}^t + \frac{h}{6} \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] n_5^t \\
 n_7^{t+1} &= \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \left(\frac{h}{4} n_4^t + h n_7^t + \frac{h}{4} n_8^t\right) \\
 n_8^{t+1} &= \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \frac{h}{2} n_8^t \\
 n_9^{t+1} &= \exp\left[\ln\left(\frac{\alpha}{\omega\beta}\right)\Delta\right] \frac{h}{4} n_8^t
 \end{aligned}$$

($\Delta = 1 - (n_1 + n_2 + n_3 + n_4 + n_5 + n_6 + n_7 + n_8 + n_9)/K$;
 $\Phi = \alpha P / (1 + \beta P)$; $P = [1 - h + (h/\pi)](n_4 + n_5 + n_7 + n_8)$
 $+ n_6 + n_9$.)

The set of recursion equations in Eqns A4 and A5 assumes recent dioecy, that hermaphrodites self and that females are pollen limited. Under the ancient dioecy assumption, terms involving n_7 , n_8 and n_9 are dropped from the recursions. Accordingly, Eqn A5 can be slightly modified to explore scenarios involving no pollen limitation in females. When no pollen limitation in females is assumed, the Φ function in Eqn A5 is just set to its asymptotic value $\Phi = \alpha/\beta$.

When it is assumed that hermaphrodites do not self, the male gametic frequencies described in Eqn A4 are still valid but the set of recursion for the composition of the population at time $t + 1$ as a function of the composition of the population at time t is:

$$\begin{aligned}
 n_2^{t+1} &= \exp[\ln(\Phi)\Delta]^M \left(\gamma_{AM}^t \gamma_{AM}^t + M \gamma_{AM}^t \gamma_{AM}^t\right) \\
 &\quad + \exp[\ln(\Phi')\Delta] \left[M \gamma_{AM}^t \frac{h}{4} n_5^t + M \gamma_{AM}^t \left(\frac{h}{2} n_4^t + \frac{h}{4} n_5^t\right)\right]
 \end{aligned}$$

$$\begin{aligned}
 n_2^{t+1} &= \exp[\ln(\Phi)\Delta]^M \left(\gamma_{AM}^t \gamma_{AM}^t + M \gamma_{AM}^t \gamma_{AM}^t\right) \\
 &\quad + \exp[\ln(\Phi')\Delta] \left[M \gamma_{AM}^t \frac{h}{4} n_5^t + M \gamma_{AM}^t \left(\frac{h}{2} n_4^t + \frac{h}{4} n_5^t\right)\right] \\
 n_3^{t+1} &= \exp[\ln(\Phi)\Delta]^M \gamma_{am}^t \gamma_{AM}^t + \exp[\ln(\Phi')\Delta] \frac{h}{4} n_5^t \\
 n_4^{t+1} &= \exp[\ln(\Phi)\Delta]^M \gamma_{am}^t \gamma_{AM}^t \\
 &\quad + \exp[\ln(\Phi')\Delta] \left[M \gamma_{am}^t \left(\frac{h}{2} n_4^t + \frac{h}{4} n_5^t\right) \right. \\
 &\quad \left. + M \gamma_{AM}^t \left(\frac{h}{2} n_4^t + \frac{h}{4} n_5^t + h n_7^t + \frac{h}{2} n_8^t\right)\right] \\
 n_5^{t+1} &= \exp[\ln(\Phi)\Delta]^M \left(\gamma_{am}^t \gamma_{AM}^t + M \gamma_{am}^t \gamma_{AM}^t\right) \\
 &\quad + \exp[\ln(\Phi')\Delta] \left[M \gamma_{am}^t \left(\frac{h}{2} n_4^t + \frac{h}{4} n_5^t\right) \right. \\
 &\quad \left. + M \gamma_{AM}^t \frac{h}{4} n_5^t + M \gamma_{AM}^t \left(\frac{h}{2} n_4^t + \frac{h}{4} n_5^t + h n_7^t + \frac{h}{2} n_8^t\right) \right. \\
 &\quad \left. + M \gamma_{AM}^t \left(\frac{h}{4} n_5^t + \frac{h}{2} n_8^t\right)\right] \\
 n_6^{t+1} &= \exp[\ln(\Phi)\Delta]^M \gamma_{am}^t \gamma_{AM}^t + \exp[\ln(\Phi')\Delta] \\
 &\quad \left[M \gamma_{am}^t \frac{h}{4} n_5^t + M \gamma_{AM}^t \left(\frac{h}{4} n_5^t + \frac{h}{2} n_8^t\right)\right] \quad \text{Eqn A6} \\
 n_7^{t+1} &= \exp[\ln(\Phi')\Delta]^M \gamma_{am}^t \left(\frac{h}{2} n_4^t + \frac{h}{2} n_5^t + h n_7^t + \frac{h}{2} n_8^t\right) \\
 n_8^{t+1} &= \exp[\ln(\Phi')\Delta] \left[M \gamma_{am}^t \left(\frac{h}{2} n_4^t + \frac{h}{4} n_5^t + h n_7^t + \frac{h}{2} n_8^t\right) \right. \\
 &\quad \left. + M \gamma_{AM}^t \left(\frac{h}{4} n_5^t + \frac{h}{2} n_8^t\right)\right] \\
 n_9^{t+1} &= \exp[\ln(\Phi')\Delta]^M \gamma_{am}^t \left(\frac{h}{4} n_5^t + \frac{h}{2} n_8^t\right)
 \end{aligned}$$

(Φ' , the modified Φ function describing pollen limitation in hermaphrodites with a maximum at $\alpha/(\beta\omega)$.)

Last, if hermaphrodites outcross but neither females nor hermaphrodites are pollen limited, Eqn A6 can be slightly modified by setting Φ and Φ' to their maxima.