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Functional characterization of gynodioecy in *Fragaria vesca* ssp. *bracteata* (Rosaceae)

Junmin Li[2], Matthew H. Koski[1] and Tia-Lynn Ashman[1,*]

[1]*Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260-3929, USA and* [2]*Institute of Ecology, Taizhou University, Linhai 317000, China*
** For correspondence. E-mail tia1@pitt.edu*

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• *Background and Aims* Gynodioecy is a phylogenetically widespread and important sexual system where females coexist with hermaphrodites. Because dioecy can arise from gynodioecy, characterization of gynodioecy in close relatives of dioecious and sub-dioecious species can provide insight into this transition. Thus, we sought to determine whether *Fragaria vesca* ssp. *bracteata*, a close relative to *F. chiloensis* and *F. virginiana*, exhibits the functional and population genetic hallmarks of a gynodioecious species.
• *Methods* We compared reproductive allocation of females and hermaphrodites grown in the greenhouse and estimated genetic diversity (allelic diversity, heterozygosity) and inbreeding coefficients for field-collected adults of both sexes using simple sequence repeat (SSR) markers. We estimated mating system and early seed fitness from open-pollinated families of both sex morphs.
• *Key Results* Under greenhouse conditions, females and hermaphrodites allocated similarly to all reproductive traits except flower number, and, as a consequence, females produced 30 % fewer seeds per plant than hermaphrodites. Under natural conditions, hermaphrodites produce seeds by self-fertilization approx. 75 % of the time, and females produced outcrossed seeds with very little biparental inbreeding. Consistent with inbreeding depression, seeds from open-pollinated hermaphrodites were less likely to germinate than those from females, and family-level estimates of hermaphrodite selfing rates were negatively correlated with germination success and speed. Furthermore, estimates of inbreeding depression based on genetic markers and population genetic theory indicate that inbreeding depression in the field could be high.
• *Conclusions* The joint consideration of allocation and mating system suggests that compensation may be sufficient to maintain females given the current understanding of sex determination. *Fragaria vesca* ssp. *bracteata* exhibited similar sex morph-dependent patterns of mating system and genetic diversity, but less reproductive trait dimorphism, than its sub-dioecious and dioecious congeners.

Key words: Dioecy, *Fragaria chiloensis*, *Fragaria vesca* ssp. *bracteata*, *Fragaria virginiana*, gynodioecy, inbreeding depression, selfing rate, SSR, strawberry.

INTRODUCTION

Gynodioecy is a sexual system in flowering plants where females (i.e. male-sterile plants) coexist with hermaphrodites. It is common (approx. 7 % of species; Richards, 1997), phylogenetically widespread (50 families, Jacobs and Wade, 2003) and of importance both as an evolutionary stable sexual system and as the first step in the evolutionary pathway from hermaphroditism to dioecy (Charlesworth and Charlesworth, 1978; Jacobs and Wade, 2003; Bailey and Delph, 2007). Although cytoplasmic–nuclear sex determination is common (Dufay and Billard, 2011), nuclear sex determination has been found in several species and these can have closely related taxa that are dioecious (e.g. S*chiedea*, Weller and Sakai 1991; *Fuschia*, Berry *et al.*, 2004; *Fragaria*, W. Njuguna *et al.*, unpubl. res.; *Carica*, Wu *et al.*, 2010). Thus, these gynodioecious species are key to gaining insight into how gynodioecy gives rise to dioecy (Charlesworth and Charlesworth, 1978; but see Maurice *et al.*, 1994).

For gynodioecy to exist, females must compensate for their loss of male function, and the degree of compensation required is dictated by the genetic determination of male sterility. When sex determination is nuclear, females need a 2-fold advantage to be maintained (Lewis, 1941; Lloyd, 1975). This advantage is often achieved by females producing a greater quantity of seeds and/or better quality seeds than hermaphrodites (Darwin, 1877). Females are expected to be able to produce more seeds by reallocating resources not spent on pollen (e.g. Ashman, 1994), and higher seed production by females is often observed (reviewed in Shykoff *et al.*, 2003; Dufay and Billard, 2011). One common avenue by which females produce higher quality seeds than hermaphrodites is through the avoidance of inbreeding depression. Since females must receive pollen from other individuals, they are obligate outcrossers, whereas self-compatible hermaphrodites are capable of self-pollination. These differences can lead to the progeny of females being more outcrossed than those of hermaphrodites (reviewed in Collin and Shykoff, 2003). Since inbreeding unmasks deleterious recessive alleles and reduces heterozygosity, progeny fitness is expected to decrease with increased inbreeding (reviewed in Charlesworth and Willis, 2009). Indeed, seed quality decreases with increased inbreeding

(e.g. Richards, 2000), and differences in quality (i.e. germination timing and rate, seedling size and survival) of seeds produced by females and hermaphrodites have been observed in many gynodioecious species (Shykoff *et al.*, 2003; Dufay and Billard, 2011). The frequency of females in a population is thus predicted to be a function of selfing rate, relative seed production and inbreeding depression (Darwin, 1877; Lloyd, 1974; Charlesworth and Charlesworth, 1978).

Features of the sexual system not only influence the sex ratio but also influence the distribution of genetic diversity within gynodioecious populations (Tarayre and Thompson, 1997). First, because hermaphrodites are capable of mixed mating whereas females are obligately outcrossed, genetic diversity will depend on sex morph. Specifically, when male sterility is dominant (e.g. females are heterozygous 'Fh' and hermaphrodites are homozygous 'hh'), females will always be the product of outcrossing, whereas hermaphrodites may be the product of female outcrossing, hermaphrodite outcrossing or hermaphrodite selfing. When females derive exclusively from female lineages, they are expected to have lower inbreeding coefficients than hermaphrodites (Ashman, 1992; Ashley *et al.*, 2003; Collin *et al.*, 2009). Secondly, if there is inbreeding depression, then individuals produced by inbreeding may be less likely to survive to adulthood. For these reasons, females are expected to be more heterozygous than hermaphrodites, and so the overall population heterozygosity will increase as female frequency increases (Gouyon and Couvet, 1987; Tarayre and Thompson, 1997; Cuevas *et al.*, 2006). While there have been numerous studies of selfing rates of the sex morphs (reviewed in Shykoff *et al*., 2003), much less attention has been paid to the effects of gender dimorphism on the distribution of genetic diversity within populations (but see Tarayre and Thompson, 1997; Akimoto *et al.*, 1999; Medrano *et al.*, 2005; Cuevas *et al.*, 2006).

Fragaria vesca ssp. *bracteata* is reported to be gynodioecious, and is the only diploid *Fragaria* species that is not hermaphroditic (Staudt, 1999). Recent phylogenetic analyses based on whole chloroplast genome sequencing (Njuguna, 2010) implicate it as the maternal donor to the octoploid *Fragaria* (*F. virginiana* and *F. chiloensis*) which subsequently evolved sub-dioecy and dioecy, respectively (W. Njuguna *et al.*, unpubl. res.). Thus, *Fragaria vesca* ssp. *bracteata* is in a key position to provide insight into the evolution of dioecy from gynodioecy (Goldberg *et al.*, 2010; Spigler *et al.*, 2010). However, characterization of gynodioecy in *F. vesca* ssp. *bracteata* has been based solely on floral morphology and segregation of progeny from controlled crosses (Ahmadi and Bringhurst, 1991; Staudt, 1999). In this work we sought to determine whether *F. vesca* ssp. *bracteata* exhibits the functional and population genetic hallmarks of a gynodioecious species. Specifically, we predicted that females would produce more seeds than hermaphrodites. We also predicted that hermaphrodites would have higher selfing rates than females and that this would contribute to the higher quality of female's seeds relative to those of hermaphrodites. Lastly, given that male sterility is reported to be determined by a dominant nuclear allele (Ahmadi and Bringhurst, 1991), we predicted that adult females would be more heterozygous and have lower inbreeding coefficients than hermaphrodites.

MATERIALS AND METHODS

Study system

Fragaria vesca ssp. *bracteata* is a herbaceous perennial plant that inhabits shady damp localities such as forest edges, meadows, rivers and roadsides in mountainous regions from British Columbia to Mexico (Staudt, 1999). It blooms in the spring and is insect pollinated. It reproduces clonally by plantlets on runners and sexually by seeds. Hermaphrodites are self-compatible (Staudt, 1999). Flowers of females are smaller (petal area: $70{\cdot}0 \pm 4{\cdot}0$ vs. $85{\cdot}1 \pm 5{\cdot}4$ mm^2; $t = -2{\cdot}6$; $P < 0{\cdot}01$) and have fewer ($19{\cdot}8 \pm 0{\cdot}3$ vs. $21{\cdot}1 \pm 0{\cdot}4$; $t = -2{\cdot}5$; $P < 0{\cdot}01$) shorter stamens ($2{\cdot}5 \pm 0{\cdot}1$ vs. $3{\cdot}8 \pm 0{\cdot}2$ mm; $t = -5{\cdot}9$; $P < 0{\cdot}01$) than hermaphrodites (M. Koski, R. Dalton and T.-L. Ashman, unpubl. res.).

Plant material and sampling

For use in the following investigations we collected 87 adult plants of *F. vesca* ssp. *bracteata* from a roadside population on Mary's Peak, Oregon, USA (N44°29′18·4″, W123°32′14·7″) in the spring of 2010. We collected plants at least 1 m apart along three transects on two occasions during the flowering season (12 May 2010 and 7 July 2010). These were transplanted into 266 mL pots with a 2:1 mixture of Fafard #4 to sand. Plants received fertilizer and water as needed. Sex was determined by visual inspection of 2–3 flowers per plant. Females were identified on the basis of flower and anther size and the absence of pollen in their anther sacs. This population is 30 % female (T.-L. Ashman, unpubl. res.).

Reproductive allocation and seed production

In the greenhouse, we produced one clone from each of 43 of the field-collected plants (22 females and 21 hermaphrodites). These were grown in 266 mL pots in a 2:1 mix of Fafard #4 soil and sand for 4 months and then were subjected to a month-long winterization period (total dark and 4 °C) before returning to the greenhouse where they experienced 10 h daylength and 16–18 °C daytime temperatures for the approx. 3 month duration of flowering and fruiting. To ensure full potential seed set, we hand-pollinated all plants three times per week with outcross pollen. Once flowering ceased, we counted flower and fruit number, and collected the second fruit on each plant. On these we scored the number of fertilized ovules (achenes) and unfertilized ovules per fruit. Seed set was determined as the number of fertilized ovules divided by the number of total ovules. We calculated the proportion of fruit set as the number of fruits divided by the total number of flowers, and estimated seed production per plant as the number of fertilized ovules/fruit multiplied by fruit number for each plant. We also scored plant size as the product of leaf number and the diameter of the central leaflet (measured in mm), and vegetative reproduction as the number of plantlets and runners produced by the end of flowering. For two hermaphrodites, fruit set was missed and thus total plant seed fertility was not estimated for these plants.

Mating system and open-pollinated seed quality

For 28 (16 female and 12 hermaphrodite) of the field-collected adults we harvested a single open-pollinated fruit per plant. From each of these, up to 16 seeds were planted individually in 96-well trays filled with Sunshine #3 germination mix. These were placed in a growth chamber under 14/10 h light and 20/15·5 °C day/night conditions. We recorded germination daily for 43 d. For each maternal family we calculated the germination rate as the number of germinated seeds divided by the number of seeds planted, and mean time to germination for germinated seedlings.

DNA extraction and simple sequence repeat (SSR) genotyping

Genomic DNA was extracted from young leaf tissue of the 87 field-collected adults and 393 progeny from the 28 fruits following a procedure modified by Penet *et al.* (2008). DNA was quantified using a Nanodrop 2000 spectrophotometer (ThermoScientific) and was diluted to 3 ng μL^{-1} with sterilized ddH_2O.

We screened 11 SSR primer pairs on the 47 females and 40 hermaphrodite adult plants to estimate population genetic parameters. We selected primers from published studies of congeners [CX661264, CX661603, CX662180, CO816938 (Spigler *et al.*, 2008, 2010); EMFv160AD (Hadonou *et al.*, 2003); UDF002 (Sargent *et al.*, 2004); EMFn153 (Sargent *et al.*, 2006); ARSFL7, ARSFL27 (Lewers *et al.*, 2005); Fvi11 (Ashley *et al.*, 2003); and BFACT10 (Rousseau-Gueutin *et al.*, 2008)]. For all of these, we followed the 'poor man's PCR' protocol (Schuelke, 2000) and the reaction conditions described in Spigler *et al.* (2008). Prior to capillary electrophoresis on an ABI 3730 DNA Analyzer (Applied Biosystems), 1 μL of each reaction was mixed with 0·5 μL of LIZ500 standard and 8·5 μL of Hi-Di formamide. Electrophoretic products were visualized and product sizes assessed using GeneMapper (Applied Biosystems). In addition, we scored four SSRs (ARSFL7, EMFv160AD, UDF002 and CX661264) that were polymorphic and in linkage equilibrium (see the Results) in the progeny to estimate the family-level mating system for 16 female and 12 hermaphrodites.

Data analyses

Reproductive allocation. We determined whether reproductive or vegetative traits differed between the sex morphs using two-tailed *t*-tests (PROC TTEST, SAS Institute, 2008).

Genetic diversity and mating system. We calculated several population genetic parameters for females and hermaphrodites separately. We determined the observed (n_a) and effective number of alleles (n_e) and Shannon information index (I) as a measure of allelic diversity for each SSR locus using POPGEN32 software ver. 1·31. In addition, for each locus and across all loci, we used Fisher's exact test to determine whether females and hermaphrodites differed in genotypic diversity using GENEPOP ver. 4·0 (Raymond and Rousset, 1995). We calculated PIC (polymorphic information content) values and observed heterozygosity (H_o) using Microsatellite Toolkit ver. 3·1·1 (Park, 2001). We tested for differences between the sexes in H_o with *t*-tests. We also calculated the inbreeding coefficient F_{IS} (Wright, 1951) for the whole population and for the sex morphs separately using GENEPOP ver. 4·0.

We used ARLEQUIN ver. 3·1 (Excoffier *et al.*, 2005) to test for linkage disequilibrium between each pair of polymorphic SSR loci and determined significance of deviations from equilibrium via randomization. Significance values were Bonferroni adjusted to account for multiple tests (Rice, 1989).

We used MLTR ver. 3·2 (Ritland and Jain, 2002) to estimate family-level multilocus (t_m) and single locus (t_s) outcrossing rates and converted these to selfing rates using $s = 1 - t$. We used the 'method of moments' procedure for small sample sizes, and estimated standard errors by bootstrapping. We compared mean family-level selfing rates between the sexes with a *t*-test. We estimated biparental inbreeding as $t_m - t_s$ for each sex and compared it with 0 with a *t*-test.

Progeny seed quality. We tested the effect of maternal sex on progeny germination rate using a Generalized Linear Model with a binomial distribution (PROC GENMOD, SAS Institute, 2008) and on germination time using an analysis of variance (ANOVA, PROC GLM, SAS Institute, 2008). In the ANOVA, maternal family was defined as a random variable and was nested within maternal sex. In addition, for hermaphrodite families only, we used linear regression (PROC REG, SAS Institute, 2008) to test the prediction that a higher family-level selfing rate leads to a lower family-mean germination rate and longer time to germination. For the regression analyses we used a one-tailed probability for significance testing.

RESULTS

Reproductive allocation

Females and hermaphrodites did not differ significantly in any of the reproductive or vegetative traits measured, except for the estimated number of seeds per plant (Table 1). Hermaphrodites produced approx. 30 % more flowers than females.

TABLE 1. *Mean ± s.e. and* t*-tests for reproductive traits in female and hermaphrodite* Fragaria vesca *ssp.* bracteata *grown in the greenhouse*

Trait	Female	Hermaphrodite	*t*
Plant size (mm)	207·3 ± 31·4	238·4 ± 30·4	−0·7
Flower number/plant	4·2 ± 0·3	5·6 ± 0·7	−1·7
Proportion of fruit set	0·95 ± 0·02	0·87 ± 0·05	1·4
Ovule number/flower	71·7 ± 3·7	75·5 ± 5·9	−0·6
Seed set/fruit	0·73 ± 0·04	0·76 ± 0·06	0·7
Seed number/plant	202·6 ± 19·8	313·4 ± 47·4	−2·2*
Plantlet number/plant	5·4 ± 1·1	5·6 ± 1·1	−0·2
Runner number/plant	2·1 ± 0·23	2·1 ± 0·36	−0·01

*$P < 0·05$.

Genetic diversity

The observed numbers of alleles, effective numbers of alleles, polymorphic information content and Shannon information index were all generally lower in females than in hermaphrodites (Table 2).

Considering all loci, the sex morphs were genotypically differentiated, and this was most pronounced at EMFv160AD and BFACT010 (Table 2). Observed heterozygosities were lower in hermaphrodites than in females, but not significantly so (Table 2; across all loci: paired *t*-test, $t = 0{\cdot}75$, d.f. = 10, $P = 0{\cdot}47$). Across all loci, the inbreeding coefficient, F_{IS}, for the population was 0·213, and was 50 % higher in hermaphrodites than in females (Table 2).

After Bonferroni correction for multiple tests, a few loci showed significant linkage disequilibrium, specifically between CX661264 and CX661603 ($\chi^2 = 53{\cdot}84$, d.f. = 21, $P = 0{\cdot}0001$), EMFn153 and EMFv160AD ($\chi^2 = 323{\cdot}37$, d.f. = 120, $P < 0{\cdot}00001$), CX661603 and EMFv160AD ($\chi^2 = 136{\cdot}37$, d.f. = 84, $P < 0{\cdot}0003$), and CX661603 and Fvi 11 ($\chi^2 = 191{\cdot}05$, d.f. = 112, $P < 0{\cdot}00001$).

Selfing rate

The selfing rate of *F. vesca* ssp. *bracteata* females was low and not different from zero (mean $s = -0{\cdot}140$; range 0·120 to −0·347), whereas that of hermaphrodites was high and variable ($s = 0{\cdot}764$; range 1·108–0·434) (Fig. 1), and these were significantly different ($t = 11{\cdot}37$, $n = 28$, d.f. = 1, $P < 0{\cdot}0001$). Estimated biparental inbreeding ($t_m - t_s$) was significantly different from zero for hermaphrodites ($0{\cdot}093 \pm 0{\cdot}117$; $t = 2{\cdot}74$, d.f. = 1, $P = 0{\cdot}019$), but not females ($-0{\cdot}0003 \pm 0{\cdot}172$; $t = -0{\cdot}01$, d.f. = 1, $P = 0{\cdot}99$).

Seed quality

Seeds from female mothers were more likely to germinate than those from hermaphrodites (mean ± s.e.: $0{\cdot}95 \pm 0{\cdot}02$ vs. $0{\cdot}89 \pm 0{\cdot}04$; Wald $\chi^2 = 5{\cdot}23$, $P = 0{\cdot}022$) and germinated slightly but non-significantly faster ($14{\cdot}5 \pm 0{\cdot}36$ vs. $15{\cdot}9 \pm 0{\cdot}44$ d; $F_{1,26} = 0{\cdot}91$, $P = 0{\cdot}35$). Among hermaphrodite families, those with higher selfing rates had lower germination success ($b = -0{\cdot}35$; $t_{1,10} = -2{\cdot}59$, $P_{1\text{-tail}} = 0{\cdot}014$, Fig. 2A) and germinated more slowly ($b = 9{\cdot}8$; $t_{1,10} = 1{\cdot}83$, $P_{1\text{-tail}} = 0{\cdot}049$, Fig. 2B).

DISCUSSION

We provide the first functional and population genetic evidence of gynodioecy in *F. vesca* ssp. *bracteata*, a key diploid congener of the sexually polymorphic octoploid *Fragaria* (*F. virginiana* and *F. chiloensis*). In the following paragraphs, we evaluate whether reproductive allocation and mating system jointly reflect sufficient compensation to maintain females given the current understanding of sex determination. We also compare features of *F. vesca* ssp. *bracteata* with those of other gynodioecious species and against other sexually polymorphic members of the genus *Fragaria*.

TABLE 2. *Indices of genetic diversity in female (F) and hermaphrodite (H) individuals of* F. vesca *ssp.* bracteata

SSR locus	Sex morph	n_a	n_e	PIC	I	Genotypic differentiation (P-value)	H_o	F_{IS}
CX661264	F	2	1·77	0·3402	0·6262	*	0·3830	0·2039
	H	3	2·10	0·4221	0·8185		0·5676	−0·1800
EMFn153	F	7	3·26	0·6604	1·3272	NS	0·7708	−0·0460
	H	8	3·18	0·7208	1·5405		0·6410	0·1300
ARSFL 7	F	3	1·64	0·3219	0·6172	NS	0·4792	−0·2330
	H	2	1·62	0·3086	0·5693		0·4615	−0·1880
CX661603	F	7	4·69	0·7579	1·7078	NS	0·3750	0·5313
	H	8	4·79	0·7609	1·7217		0·1538	0·8078
EMFv160AD	F	10	5·80	0·8048	1·9052	***	0·7917	0·0872
	H	13	7·86	0·8600	2·2469		0·8205	0·0540
ARSFL 27	F	2	1·02	0·0204	0·0579	NS	0·0208	−0·8100
	H	1	1·00	0·0000	0·0000		0·0000	–
CX662180	F	1	1·00	0·0000	0·0000	–	0·0000	–
	H	1	1·00	0·0000	0·0000		0·0000	–
UDF002	F	1	1·00	0·0000	0·0000	NS	0·0000	–
	H	2	1·08	0·0712	0·1630		0·0769	−1·2560
BFACT 010	F	7	3·15	0·6268	1·3167	*	0·5417	0·2632
	H	10	4·45	0·7490	1·7974		0·5897	0·1979
CO816938	F	3	1·04	0·0406	0·1157	NS	0·0417	0·0838
	H	2	1·05	0·0487	0·1192		0·0513	−0·1270
Fvi 11	F	12	5·65	0·8026	1·9946	NS	0·6458	0·2241
	H	12	4·99	0·7789	1·9486		0·3421	0·5890
All loci (s.d.)	F	5·00 (3·79)	2·73 (1·90)	0·3978 (0·3430)	0·8790 (0·7945)	**	0·3682 (0·3098)	0·1789
	H	5·63 (4·63)	3·07 (2·27)	0·4291 (0·3551)	0·9932 (0·8717)		0·3368 (0·2947)	0·2489

Number of observed alleles (n_a), effective number of alleles (n_e), polymorphic information content (PIC), Shannon information index (I), observed heterozygosity (H_o) and the inbreeding coefficient (F_{IS}) are given for each locus and for all loci combined for each sex morph.

*$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0{\cdot}001$; NS, not significant.

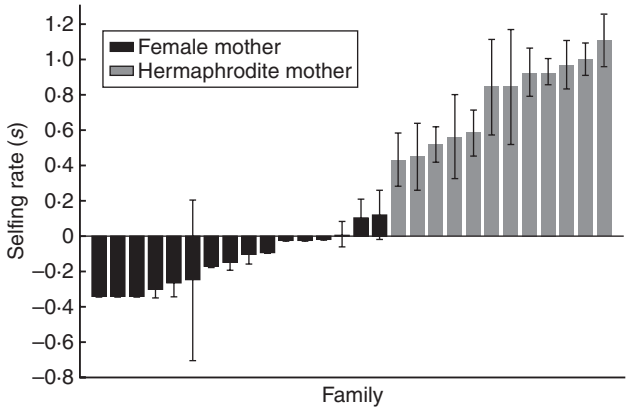

FIG. 1. Selfing rate (mean $s \pm$ s.e.) for open-pollinated seeds of female and hermaphrodite mothers.

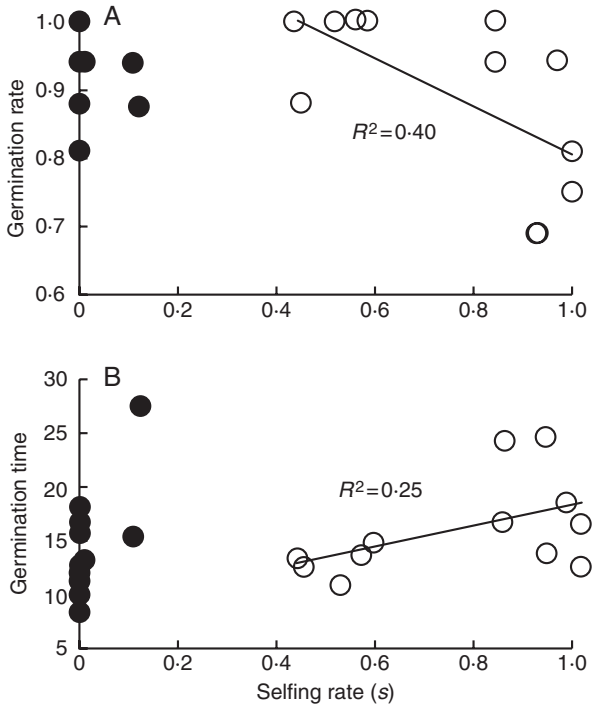

FIG. 2. Relationship of hermaphrodite selfing rate (s) with (A) seed germination rate (proportion of seeds germinating) and (B) mean germination time (days from planting). Data for female families (filled circles) were not included in regression analyses but are shown for comparative purposes.

Do females of F. vesca *ssp.* bracteata *show compensation?*

When male sterility is determined by nuclear genes, females must have a 2-fold advantage to compensate for their loss of pollen fertility and be maintained (Lewis, 1941; Lloyd, 1974; Charlesworth and Charlesworth, 1978). We found that females of *F. vesca* ssp. *bracteata*, which allocate less per flower (in terms of stamens and petals), did not produce more seeds than hermaphrodites under greenhouse conditions with abundant pollination. Rather, they produced 30 % fewer seeds per plant which was due to lower flower production, as other components of seed production (ovule number, seed set and fruit set) showed only minor differences between the sex morphs (Table 1). We have recorded a similar sex differential for flower production per plant (female vs. hermaphrodite: $3{\cdot}73 \pm 0{\cdot}36$ vs. $5{\cdot}77 \pm 0{\cdot}51$; $n = 45$; d.f. $= 1$, $t = 2{\cdot}89$, $P = 0{\cdot}006$), and absence of a differential for proportion of fruit set (female vs. hermaphrodite: $0{\cdot}86 \pm 0{\cdot}04$ vs. $0{\cdot}81 \pm 0{\cdot}05$; $n = 45$; d.f. $= 1$, $t = 1{\cdot}59$, $P = 0{\cdot}25$) for plants under their native pollinator and resource conditions, suggesting that the differences observed in the greenhouse are likely to be reliable.

In seven out of 24 species reviewed by Shykoff *et al.* (2003) females produced fewer flowers than hermaphrodites, but in the vast majority of these species females had higher fruit and/or seed set than conspecific hermaphrodites (Shykoff *et al.*, 2003). Females with lower seed production than hermaphrodites have been found in a handful of other species [e.g. *Daphne laureaola* (Alonso and Herrera, 2001); *Kallstroemia grandiflora* (Garcia *et al.*, 2005); *Trifolium hirtum* (Molina-Freaner and Jain, 1992)] and, in these, alternative mechanisms of female maintenance were sought, such as seed quality differences.

Seed quality could differ between females and hermaphrodites either because of environmental or genetic maternal effects that differ between the sex morphs (e.g. Ashman, 1992; Delph and Mutikainen, 2003) or because seeds of hermaphrodites are more inbred than those of females and the former suffer inbreeding depression (reviewed in Shykoff and Collin, 2003). Our mating system analysis indicates that under natural conditions, hermaphrodites self on average 76 % of the time and that females are highly outcrossed with very little biparental inbreeding (Fig. 1). Relative to the species reviewed by Shykoff *et al.* (2003), *F. vesca* ssp. *bracteata* hermaphrodites are on the extreme end of selfing; only two other species are reported to have higher hermaphrodite selfing rates [*Trifolium hirtum* (Molina-Freaner and Jain, 1992) and *Chionographis japonica* (Maki, 1993)].

Charlesworth and Charlesworth (1978) showed that, for nuclear male sterility to spread, the inequality $sd > (1 - k)/2$ must be met, where k represents relative seed fertility of females over hermaphrodites [(mean seed production by females – mean seed production by hermaphrodites)/mean seed production of hermaphrodites], s is the selfing rate of hermaphrodites and d is inbreeding depression. Our estimates for seed fertility differential ($k = -0{\cdot}35$) and selfing rate ($s = 0{\cdot}764$) in *F. vesca* ssp. *bracteata* predict that inbreeding depression must be approx. 0·87 or greater in the field for females to spread. Inbreeding depression was detected at the germination stage here (Fig. 2), but this value could be an underestimate as it only reflects the early life stage and was assessed under benign conditions (see recent reviews by Fox and Reed, 2011; Cheptou and Donohue, 2011). We can also estimate inbreeding depression from the inbreeding coefficient of the *in situ* adult plants (F_{IS}; Table 2) and the frequency of selfing (s), i.e. $d = 1 - 2\,[F(1 - s)/s(1 - F)]$ (Ritland, 1990; see also Kohn and Biardi, 1995). This estimator has the advantage of integration of inbreeding across the life cycle in the natural environment, but also relies on several assumptions, such as inbreeding equilibrium and selfing as the only form of inbreeding (see discussion in Goodwillie *et al.*, 2005), and the underlying model may not be valid when the population involves two morphs (Medrano *et al.*, 2005), so the estimate of inbreeding depression

needs to be viewed with caution. Using this approach, we estimate inbreeding depression as 0·75, near average for hermaphroditic plant species with mixed mating systems ($d = 0{\cdot}81 \pm 0{\cdot}71$; Goodwillie *et al.*, 2005), and nearly high enough to account for maintenance of females. Alternatively, we can assume the population is at equilibrium and use the observed frequency of females (0·30) and Charlesworth and Charlesworth's (1978) formula for the predicted equilibrium frequency of females [$Z = (k + 2sd - 1)/2(k + sd)$] to solve for inbreeding depression (d). This approach gives an estimate of inbreeding depression of 0·67, fairly close to that obtained above. It is thus unclear whether female's seed quality advantage is sufficient to account for the maintenance of females under the assumption of nuclear inheritance of male sterility. Experimental estimates of the magnitude of inbreeding depression should be obtained. In addition, the genetic determination of male sterility in *F. vesca* ssp. *bracteata* (dominant nuclear male sterility; Ahmadi and Bringhurst, 1991) should be confirmed via larger scale studies that include crosses between, as well as, within populations (Bailey and Delph, 2007). These would reveal whether cytoplasmic components are involved in male sterility. If they were, then models other than those used above (that assume nuclear inheritance) would be appropriate (e.g. Lewis, 1941; Lloyd, 1975; reviewed in Bailey and Delph, 2007; Delph *et al.*, 2007).

How does gynodioecy in F. vesca *ssp.* bracteata *compare with sub-dioecious and dioecious congeners?*

The hermaphrodite selfing rate found in this diploid gynodioecious strawberry is similar to the estimate for hermaphrodites of octoploid sub-dioecious *F. virginiana* ($s = 0{\cdot}72 \pm 0{\cdot}004$; population 'PR'; Rohde and Ashman, 2010). In addition, females were less inbred than hermaphrodites ($F_{IS} = 0{\cdot}043$ vs. $0{\cdot}120$) in this population of *F. virginiana* (Ashley *et al.*, 2003), much as observed here in *F. vesca* ssp. *bracteata*, although in the former mean inbreeding was lower. The seed fertility differential, on the other hand, was much higher in *F. virginiana* (females produce 4–8 times more seeds than hermaphrodites; Ashman, 1999*a*; Spigler and Ashman, 2011). This difference could indicate that female sterility factors evolve only after polyploidization in the octoploid (Goldberg *et al.*, 2010, W. Njuguna *et al.*, unpubl. res.) or the two species may differ totally in their genetic determination of sex. The infrequency with which hermaphrodites contribute seeds to the adult population of the octoploid could also account for the lower inbreeding coefficients in hermaphrodite adults of *F. virginiana* compared with *F. vesca* ssp. *bracteata*, as it would appear that their progeny do not experience very high inbreeding depression (Rohde and Ashman, 2010), as might be expected in a polyploid (e.g. Soltis and Soltis, 2000; Galloway and Etterson, 2007).

Sexual dimorphism is expected to evolve in species with separate sexes when optimal values of a trait differ for male and female fertility (Lande, 1980). One might predict greater dimorphism in species with complete separation of the sexes (e.g. dioecious *F. chiloensis*) than in those with less complete separation of the sexes, i.e. populations still contain an hermaphrodite morph – as in sub-dioecious *F. virginiana* – or do not have males at all – as is the case in gynodioecious *F. vesca* ssp. *bracteata* (reviewed in Eckhart, 1999). In accordance with this prediction, sexual dimorphism was less pronounced in *F. vesca* ssp. *bracteata* than in *F. virginiana*, especially with respect to floral characteristics. Hermaphrodites of *F. vesca* ssp. *bracteata* had 18 % larger petals and 34 % larger stamens than females, but these differentials were 28 and 65 %, respectively, in *F. virginiana* (Ashman and Hitchens, 2000). In addition, there is increasing dimorphism with increasing separation of the sexes for ovule and anther number across all three species (T.-L. Ashman *et al.*, unpubl. res.). Also, while there was more sexual dimorphism in fruit set in *F. virginiana* than in *F. vesca* ssp. *bracteata* (see above) there was less dimorphism in flower number (*F. virginiana* females produce 17 % vs. 25 % fewer flowers than hermaphrodites; Ashman, 1999*a*, *b*). These comparisons indicate that for most traits, gynodioeicous *F. vesca* ssp. *bracteata* exhibits less dimorphism than its dioecious and sub-dioecious congeners, a pattern that is predicted by theory, but has not often been shown for closely related taxa (e.g. Yakimowski *et al.*, 2011).

Population genetic insights into gynodioecy in F. vesca *ssp.* bracteata

Given the proposed genetic mechanism and the potential avenues for mating, we expected females to be more heterozygous and less inbred than hermaphrodites. Adult females did have lower inbreeding coefficients than adult hermaphrodites but they were not significantly more heterozygous than hermaphrodites. This may reflect severe inbreeding depression in the field, i.e. the absence of highly inbred hermaphrodites surviving to adulthood (see above), and/or that adult hermaphrodites mostly have female mothers. It is also possible that historical colonization dynamics affected the sex morphs differently, i.e. perhaps females have undergone a bottleneck (Charlesworth, 2003). The fact that females had fewer alleles/locus on average might lend support to this latter possibility. One must also consider that null alleles can create false homozygotes and thus bias estimates of inbreeding; however, these generally have only small effects (Carlsson, 2008) and to explain the results here null alleles would have to be associated with sex type which seems unlikely.

Although there are far fewer studies for comparison, some have shown lower inbreeding in the unisexual morph relative to the cosexual one. For instance, Molina-Freaner and Jain (1992) found that females had higher levels of heterozygosity than hermaphrodites in one of two populations of *Trifolium hirtum* which has cytonuclear inheritance of sex phenotype. Likewise, in an androdioecious *Schizopepon bryoniaefolius*, males had lower inbreeding coefficients than hermaphrodites (Akimoto *et al.*, 1999). However, no significant difference in inbreeding was found between females and hermaphrodites in *Thymus vulgaris* (Tarayre and Thompson, 1997). Additional insight into the effects of the sexual system on genetic diversity can be achieved through among-population comparisons, as inbreeding is expected to decrease (and heterozygosity to increase) with increasing frequencies of unisexuals, and this has been shown in several species (e.g. *Schizopepon bryoniaefolius*, Akimoto *et al.*, 1999; *Daphne laureola*, Medrano *et al.*, 2005; *Kallstroemia gradiflora*,

Cuevas *et al.* 2006; but see *T. vulgaris*, Tarayre and Thompson, 1997). When such work is combined with information on sex ratios and historical gene flow, additional insight into sexual system evolution can be gained. Such work is underway in *F. vesca* ssp. *braceata.*

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