

Variation and evolution of sex ratios at the northern range limit of a sexually polymorphic plant

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Abstract

Gender strategies involve three fundamental sex phenotypes – female, male and hermaphrodite. Their frequencies in populations typically define plant sexual systems. Patterns of sex-ratio variation in a geographical context can provide insight into transitions among sexual systems, because environmental gradients differentially influence sex phenotype fitness. Here, we investigate sex-ratio variation in 116 populations of *Sagittaria latifolia* at the northern range limit in eastern N. America and evaluate mechanisms responsible for the patterns observed. We detected continuous variation in sex phenotype frequencies from monoecy through subdioecy to dioecy. There was a decline in the frequency and flower production of females in northerly populations, whereas hermaphrodite frequencies increased at the range limit, and in small populations. Tests of a model of sex-ratio evolution, using empirical estimates of fitness components, indicated that the relative female and male contribution of males and hermaphrodites to fitness is closer to equilibrium expectations than female frequencies. Plasticity in sex expression and clonality likely contribute to deviations from equilibrium expectations.

Introduction

Sex ratios most often concern the relative frequencies of female and male individuals in populations of dioecious organisms. As a result of negative frequency-dependent selection, equilibrium sex ratios are expected to approach 1:1 in populations (Fisher, 1930), although deviations from unity often occur (Hardy, 2002). Plant sex ratios present a more complex situation than in most animal groups because of their diverse sexual systems, facility for sexual and asexual reproduction, and plasticity in gender expression (Geber *et al.*, 1999). In many sexually polymorphic species, sex ratios commonly involve hermaphroditic individuals in addition to females and/or males (Delph, 1990; Barrett, 1992; Fleming *et al.*, 1994; Caruso & Case, 2007), and therefore, the costs and benefits of unisexuality vs. hermaphroditism play an important role in governing

patterns of sex-ratio variation. The frequent occurrence of clonal propagation in sexually polymorphic species also commonly leads to disparities between phenotypic and genotypic sex ratios and deviations from equilibrium expectations (Delph, 1999; Barrett *et al.*, 2010). Finally, environmental and size-dependent gender modification can present difficulties in the assignment of individuals to a particular sexual morph (Lloyd & Bawa, 1984; Sarkissian *et al.*, 2001). These biological realities of plant gender complicate studies of sex-ratio evolution in comparison with many animal groups.

The occurrence of hermaphrodites in sexually polymorphic plant populations is often associated with evolutionary transitions in sexual systems. Although cosexuality represents the fundamental sexual condition of most flowering plants, dioecy (female and male plants) has evolved from hermaphroditism at least ~100 times (Charlesworth, 2002), despite its low frequency in the angiosperms (6–7%; Renner & Ricklefs, 1995). The transition to dioecy commonly involves the invasion of cosexual populations by females resulting in gynodioecious (females and hermaphrodites) and subdioecious (females, males and hermaphrodites) populations. Determining the relative fitness of sexual

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morphs in these populations is necessary for understanding their evolutionary dynamics (Lloyd, 1976; Charlesworth & Charlesworth, 1978; Ashman, 2006; Spigler & Ashman, 2011). Whereas gynodioecy is not always a transitional state and indeed gynodioecious species are relatively common (Webb, 1999), the conditions required for the stable maintenance of subdioecy are less obvious (Maurice & Fleming, 1995; Wolf & Takebayashi, 2004; Ehlers & Bataillon, 2007). It is also now recognized that dioecy may not always be an end point of sexual system evolution (Delph, 2009; Barrett, 2013) and, for example, the coexistence of males and hermaphrodites (androdioecy) may sometimes be derived from dioecy (Pannell, 2002). Environmental gradients causing geographical variation in sex ratios can provide insights into the mechanisms driving these sexual system transitions.

Although sex ratios vary widely in plant populations (Barrett *et al.*, 2010; Field *et al.*, 2013a,b), allocation to female and male function may be adjusted in a predictable manner depending on mating opportunities and frequency-dependent selection. For example, in gynodioecious populations, hermaphrodites evolve more male-biased allocation in female-biased populations (Ashman, 1999), whereas selection can favour female-biased allocation in hermaphrodites that co-occur with males (Dorken & Pannell, 2009). Sex allocation in subdioecious populations represents a more complex situation because of the coexistence of three sex phenotypes. Most cases of subdioecy have evolved from gynodioecy, with females producing no pollen and males with variable gender allocations, ranging from genetically determined 'pure males' to 'inconstant males' that produce both pollen and ovules (hermaphrodites), and commonly exhibit plastic sex expression (Lloyd, 1976; Delph & Lloyd, 1991; Barrett *et al.*, 1999; Delph & Wolf, 2005; Ashman, 2006; Ehlers & Bataillon, 2007). If males in subdioecious populations gain fitness primarily through investment in male function, pure males will be selected and dioecy should evolve. In contrast, if males also gain a significant amount of fitness by investing in ovule production, inconstant males functioning as hermaphrodites may be maintained in populations with pure males. This dynamic will be affected by the sex ratio because if female frequency is low, there are opportunities for inconstant males to gain more fitness through ovule investment. By considering the transmission of genes via pollen and ovules, Lloyd (1976) developed theory that makes it possible to predict sex ratios in subdioecious populations and determine whether males have adjusted their sex allocation according to equilibrium predictions. The models can provide evidence that populations containing mixtures of combined vs. separate-sexed plants are evolutionary stable rather than a transient condition.

Sexual system transitions necessarily occur in a geographical context, and adaptive responses to variation in ecological and demographic conditions are com-

monly invoked to explain shifts in reproductive traits (Barrett *et al.*, 2001; Herrera *et al.*, 2006; Pannell, 2006). There is considerable evidence that environmental gradients at both local and geographical scales influence the frequency and performance of sexual morphs owing to differences in their costs of reproduction (Sakai & Weller, 1999; Case & Barrett, 2004; Ashman, 2006). Populations at geographical range limits frequently confront environments that differ from those in the centre of their range, and this can have important ecological and genetic consequences (Hoffmann & Blows, 1994; Gaston, 2003; Eckert *et al.*, 2008). Under the 'abundant centre' model, where a species is less common at range margins (Sagarin & Gaines, 2002), or has high rates of metapopulation turnover (Holt & Keitt, 2000), more peripheral populations might be expected to favour hermaphroditism over unisexuality, because of the ability of single cosexual individuals to found colonies under low-density conditions (Baker, 1955; Lloyd, 1980; Pannell & Barrett, 1998). In sexually polymorphic taxa in which hermaphroditic individuals occur, we might predict an increased incidence of this condition towards range margins, and in small recently colonized populations, potentially resulting in sexual system transitions (Alonso & Herrera, 2011).

In the Northern Hemisphere, northern range margins are generally associated with a shorter growing season and more stressful environmental conditions (Chuine, 2010), potentially influencing the performance of sexual morphs. If range limits are characterized by deteriorating conditions, we might predict lower frequencies of females and/or reduced flowering in comparison with males, due to the higher cost of reproduction for females (Delph, 1999). This could decrease overall seed output and limit population growth. Alternatively, as discussed earlier, demographic conditions at range margins could favour hermaphroditism leading to the replacement of females by hermaphrodites without preventing sexual recruitment in populations. To our knowledge, there have been no large-scale studies of geographical variation in plant sex ratios encompassing northern range limits to test these predictions.

Here, we investigate geographical patterns of sex-ratio variation in *Sagittaria latifolia* (Alismataceae), a wetland herb native to North America. This species is well suited for this type of study because it is widely distributed, particularly in eastern N. America, and possesses three sexual morphs – females, males and self-compatible hermaphrodites – which vary in frequency within and among populations. These three sex phenotypes are genetically determined, with hermaphrodites also exhibiting size-dependent gender modification (Sarkissian *et al.*, 2001). Sampling throughout most of the species range, including much of eastern N. America (Dorken & Barrett, 2004a) and central California to southern British Columbia, where disjunct populations occur (S.B. Yakimowski & S.C.H. Barrett, unpublished),

has established that most populations are either monoecious or dioecious, with some dioecious populations containing a low frequency (< 10%) of hermaphrodites (Sarkissian *et al.*, 2001; Dorken *et al.*, 2002; Dorken & Barrett, 2004a). However, our recent observations at the northern edge of the eastern N. American range have revealed a more complex pattern involving a high incidence of subdioecious populations. This finding motivated this study, which documents variation in sex ratios along a latitudinal transect encompassing the range limit of sexually polymorphic populations and investigates whether subdioecious populations are likely to be evolutionarily stable.

Our study comprises an extensive geographical survey of sex ratios in 116 populations and a glasshouse experiment involving a comparison of fitness components of progeny from females and hermaphrodites from subdioecious populations. The specific questions we address are as follows: (i) What are the patterns of sex-ratio variation at the northern range limit, and is there evidence of changes in the frequencies of sexual morphs with latitude? We predicted a decrease in frequency of females and an increase in hermaphrodites with latitude; (ii) Does flower production decline with increasing latitude owing to a shorter growing season? We predicted that females would be most sensitive to such an effect because of their higher cost of seed production; (iii) In subdioecious populations, are observed female frequencies and the sex allocation of males and inconstant males (hermaphrodites) predicted by models of equilibrium sex allocation? We combined observations of sex ratios and sex allocation in the field with data from our glasshouse experiment, to predict female frequencies and equilibrium sex allocation for gender morphs with male function, using Lloyd's (1976) model of the transmission of genes via pollen and ovules in subdioecious populations.

Materials and methods

Study species

Sagittaria latifolia (Alismataceae) is a clonal aquatic that inhabits wetland habitats including lakes, rivers, wetlands, marshes, ephemeral ponds and ditches. In common with many sexually dimorphic species, dioecious populations often contain a low frequency of males producing female flowers. Following Lloyd (1976), we refer to this condition as male sex inconstancy. In dioecious populations, we distinguish 'inconstant males' from 'pure males' because the latter produce only male flowers. In contrast, in monoecious populations of *S. latifolia*, small plants often produce only male flowers, before switching when they are larger to the hermaphroditic condition (Sarkissian *et al.*, 2001). We distinguish this sex phenotype from the

male and hermaphrodite sex phenotypes of gender dimorphic populations.

Clones of *S. latifolia* are composed of ramets, which can be either vegetative or reproductive, and at the end of the growing season, they senesce producing perennating corms at the end of axillary stolons. In common with most studies of sex ratios in clonal plants, all sampling involved flowering ramets, sampled at least 2 m apart to reduce the probability of resampling genets. We used this approach because of the difficulty in distinguishing individual genets without genetic markers, which would not have been feasible given the scope of our study. Using microsatellite markers, we have recently demonstrated a close correspondence between ramet and genet sex ratios among 11 dioecious populations of *S. latifolia* (S.B. Yakimowski & S.C.H. Barrett, unpublished), so the sex ratios we report are unlikely to be greatly influenced by differential clonal growth.

In north-eastern N. America, monoecious populations mostly occupy ephemeral habitats, whereas dioecious populations are characteristic of stable wetlands and the edges of lakes and rivers (Dorken & Barrett, 2003). Common garden studies of populations from this region indicate that this ecological segregation is associated with life-history differentiation: ramets in monoecious populations are smaller and flower earlier (peak flowering July) than dioecious ramets, which are larger and flower in mid-August to September (Dorken & Barrett, 2003). Ecological and life-history differentiation contribute towards reproductive isolation of the sexual systems, as indicated by genetic differentiation between monoecious and dioecious populations (Dorken *et al.*, 2002; Dorken & Barrett, 2004a), despite interfertility between hermaphroditic and unisexual individuals (Dorken & Barrett, 2004b).

Sex determination in *S. latifolia* is consistent with a nuclear model involving two linked diallelic loci with dominance (Dorken & Barrett, 2004b). Female function is suppressed by a dominant female-sterility allele, whereas male function is expressed by a dominant male-sterility allele. Females are homozygous recessive at both loci, males are heterozygous at both loci, and inconstant males are homozygous for the recessive female-sterility allele and heterozygous at the male-sterility locus. In monoecious populations, plants are homozygous for the allele dominant to male sterility and the allele recessive to female sterility.

Geographical sampling of sex-ratio variation

To investigate sex-ratio variation in *S. latifolia*, we surveyed 116 populations (Fig. 1, Appendix S1) from NB, ON, QU in Canada and MI, NJ, NY in the USA. Sampling spanned 39.6–48.5°N and 66.2–86.2°W and encompassed the northern range limit for dioecious populations, which occurs at ~46°N. Monoecious

populations are distributed well beyond the northern limit of dioecious populations (Dorken & Barrett, 2004b; S.C.H. Barrett, unpublished).

We sampled a mean of 65 (range = 4–213) flowering ramets per population at peak flowering along transects, with sampling effort determined by variation in population size. We estimated population size by multiplying counts of the number of ramets in 5–20 m² plots distributed throughout the population, by the area occupied by the population. For each ramet, we counted the total number of female and/or male flowers per inflorescence, which is straightforward as the gender of withered flowers and buds is easily determined. Each flowering ramet was classified as female, male or hermaphrodite. Populations were classified as dioecious (females and males, and in some populations, a low frequency of hermaphrodites ranging from 1–10%), 'mixed' (unisexuals and hermaphrodites from 11–79%) and monoecious (hermaphrodites from 80–100%). As discussed below, dioecious and mixed populations are also distinguished from monoecious populations by their cpDNA haplotypes and phenotypic characteristics (Dorken & Barrett, 2003; S.B. Yakimowski & S.C.H. Barrett, unpublished).

Sex ratios and inflorescence size

We used a generalized linear model for categorical data (Proc Catmod, SAS 9.1 TS Level 1M3, SAS Institute Inc. Cary, NC, USA) with a multinomial distribution to assess the relation between the frequencies of the three

sex phenotypes and latitude (fixed effect) for 5520 individuals sampled from 73 dioecious and mixed populations (listed in Appendix S1). This subset of populations was distinguished from monoecious populations by cpDNA and SSR markers (Dorken & Barrett, 2004a; Barrett *et al.*, 2010; S.B. Yakimowski & S.C.H. Barrett, unpublished); however, we also included 13 populations exhibiting genetic admixture of the monoecious and dioecious haplotypes, and three androdioecious populations with the monoecious haplotype. We included these functionally androdioecious populations in our survey because, unlike monoecious populations, the males in these populations were of equivalent size to hermaphrodites, rather than the small size typical of the male phase of hermaphrodite development in monoecious populations.

We investigated the relation between the frequency of each sex phenotype and latitude and population size [\log_{10} (number of ramets)] using a generalized linear model with a binomial distribution (R version 2.8.1; R Development Core Team, 2008). The occurrence of each sex phenotype was tested against the summed occurrence of the other two sex phenotypes and a Bonferroni correction applied to each to obtain *P*-values. We compared models including quadratic terms for latitude and population size, but model fit did not improve and thus simpler models are presented. Logistic regression lines of best fit were calculated by estimating the intercept and slope for latitude and population size separately for each sex phenotype. We also investigated variation in the ratio of total female to

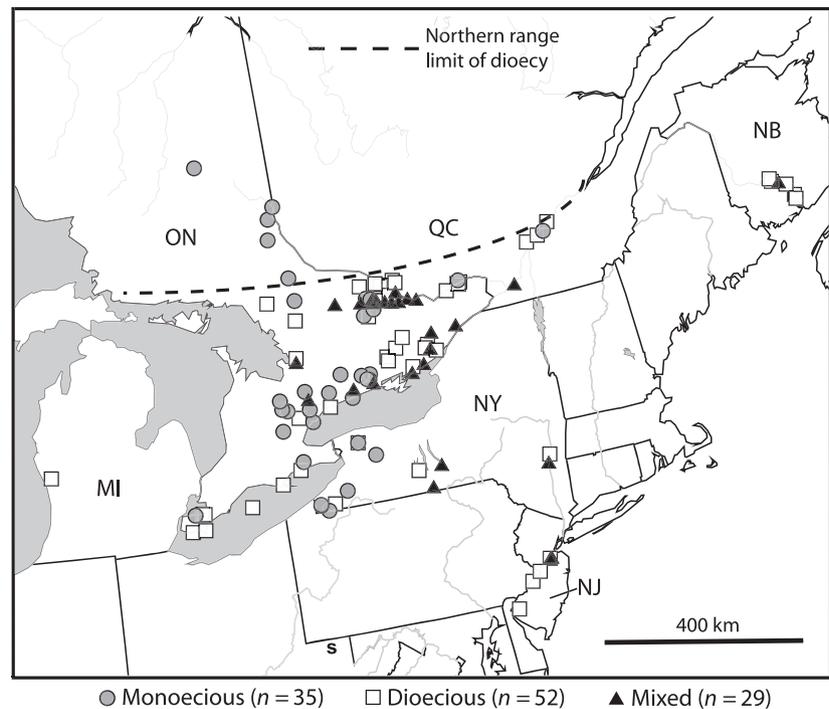


Fig. 1 The geographical distribution in north-eastern N. America of monoecious, dioecious and mixed populations of *Sagittaria latifolia* sampled in this study. See text for definitions of these three classes of populations.

male flowers in populations using a generalized linear model with a binomial distribution. We performed this analysis on the same subset of 73 dioecious and mixed populations discussed earlier, and separately for 33 monoecious populations in our survey.

We examined the relation between variation in inflorescence size (total number of flowers per ramet) and latitude for 5540 ramets sampled from the same set of dioecious and mixed populations as above with latitude, sex, and latitude \times sex as fixed effects, and population as a random effect (Proc Mixed, Satterthwaite df method, SAS 9.1 TS Level 1M3). We performed planned contrasts for the relation between inflorescence size and latitude between sex phenotypes and a likelihood ratio test to determine whether population was a significant effect.

Comparison of fitness components between female and hermaphrodite seed families

We conducted a common glasshouse comparison of fitness of progeny from female and hermaphrodite seed families. These fitness estimates were used to compare predicted and observed frequency of females and patterns of sex allocation. In October 2005, we collected 160 open-pollinated seed families from females and hermaphrodites in eight populations. To capture observed variation in the frequency of hermaphrodites, the experiment included four dioecious populations with low frequencies of hermaphrodites (HMR-ON = 0.02, LNG-ON = 0.08, RRM-ON = 0.08, SPT-ON = 0.07) and four subdioecious populations with high frequencies of hermaphrodites (FHR-ON = 0.37, PTC-QC = 0.28, RNW-ON = 0.29, TR2-ON = 0.49); for further information, see Appendix S1. We predicted a larger fitness difference between females and hermaphrodites in subdioecious populations owing to potentially higher selfing in these populations, as previously reported in a subdioecious population (see Barrett, 2003; Fig. 5b).

We dried seed at room temperature for 2 weeks, and it was stored at 4 °C. Three weeks before germination, we added ~200 seeds per family to individual microtubes of distilled water, and these were returned to 4 °C. We germinated seed from 128 female and 80 hermaphrodite families in May 2006 on soil in 10.2-cm-diameter pots, placed in water-filled trays to which we added No-Damp fungicide (Plant Products Co., Ancaster, ON, Canada). High rates of germination were obtained (> 90%), and seedlings were used in a 2-year glasshouse study. In a separate trial, we also measured germination rate (202 families), using 10 seeds per family and 5.7-cm-diameter pots, over 18 days at which time germination had stopped.

Two weeks after germination, seedlings were transplanted into plug trays, and following establishment, seedlings were fertilized weekly using a 1% 20 : 20 : 20 N : P : K fertilizer solution. At the begin-

ning of August, up to three randomly chosen plants per family, a total of 622 plants, were transplanted individually into 5.7-cm pots and randomized into three blocks of water-filled trays. In September, we transplanted plants to 7.6-cm pots and placed them in individual plastic buckets (25 cm diameter) with water levels maintained ~2 cm above the soil. Day length was extended to 14 h to simulate summer conditions. After senescence, in late December, we collected corms from each pot and counted and weighed them to the nearest hundredth of a gram using an electronic balance. In April 2007, three randomly chosen corms per plant were repotted into 10.2-cm pots and placed back in the glasshouse and growth resumed. Plants were harvested in September 2007, and corms were counted and weighed. Flower number was counted for each inflorescence produced during both growing seasons.

From the germination trial and 2-year glasshouse experiment, we analysed the following fitness components: proportion seed germinated per maternal family, probability of survival and flowering, number of flowers produced, corm production and total corm mass (g). We analysed data on germination, survival, flowering and corm production using logistic regression models (SAS, PROC GENMOD) with a binary distribution and a logit link function. Model factors included family type (female or hermaphrodite), frequency of hermaphrodites in sampled population (low or high frequency), family type \times frequency of hermaphrodite interaction and population treated as a repeated variable nested within frequency of hermaphrodites. We determined significance for each of these factors by a likelihood ratio test, and Chi-squared and *P*-values for each of these factors are reported in Table 1.

We square-root-transformed number of flowers and total corm mass to improve normality and analysed the data with mixed models (Proc Mixed, Satterthwaite df method, SAS 9.1 TS Level 1M3). Similar to the above analysis, family type, frequency of hermaphrodites and family type \times frequency of hermaphrodites were fixed effects, and we report *F*- and *P*-values for each (Table 1). Population was treated as a random factor and nested within frequency of hermaphrodites. We compared models that structured population variance in three ways (using 'repeated' and 'group' statements): one variance parameter for all populations, different variance parameters for low- vs. high-frequency hermaphrodite populations and different variance parameters for each of the eight populations. The fit of these models did not differ substantially based on AICc values (Akaike Information Criterion corrected for sample size); therefore, we used the latter structure of population variance because it should best describe population variance. We removed the effect of population and compared models with a likelihood ratio test to determine significance of this random effect; Chi-squared and *P*-values are reported in Table 1.

Table 1 Analysis of fitness components from a 2-year glasshouse study under uniform conditions of female and hermaphrodite seed families of *Sagittaria latifolia*. We modelled binomial fitness components (marked * = probability of survival, probability of flowering, probability of corm production) with logistic regression, and χ^2 values are presented. We analysed continuous variables (number of flowers, total corm mass) with mixed-effect models, and F values are presented. Abbreviated codes for fitness components are used in eqn 4 in Materials and Methods. Boldface P -values significant following Bonferroni correction (see text for further details).

Fitness component	Model factors							
	Family type		Frequency of hermaphrodites		Family type \times frequency of hermaphrodites		Population	
	χ^2 or F	P	χ^2 or F	P	χ^2 or F	P	χ^2	P
Probability of survival 2006* (Sv06)	$\chi^2 = 1.57$	0.21	$\chi^2 = 4.16$	0.04	$\chi^2 = 0.00$	1.00	28.45	<0.0001
Probability of flowering 2006* (Fw06)	$\chi^2 = 0.96$	0.33	$\chi^2 = 15.73$	<0.0001	$\chi^2 = 0.06$	0.79	36.81	<0.0001
$\sqrt{\text{Number of flowers 2006 (Nf06)}}$	$F_{1,56.9} = 0.00$	0.98	$F_{1,4.15} = 5.29$	0.08	$F_{1,56.9} = 0.50$	0.48	12.3	0.14
Probability of corm production 2006* (Cm06)	$\chi^2 = 1.01$	0.31	$\chi^2 = 0.48$	0.49	$\chi^2 = 0.03$	0.86	10.35	0.11
$\sqrt{\text{Total corm mass 2006 (Mc06)}}$	$F_{1,372} = 0.55$	0.46	$F_{1,6} = 4.32$	0.08	$F_{1,372} = 2.42$	0.12	45.40	<0.0001
Probability of survival 2007* (Sv07)	$\chi^2 = 0.73$	0.39	$\chi^2 = 3.08$	0.08	$\chi^2 = 1.55$	0.21	27.06	0.001
Probability of flowering 2007* (Fw07)	$\chi^2 = 0.01$	0.90	$\chi^2 = 9.60$	0.002	$\chi^2 = 1.20$	0.27	8.43	0.49
$\sqrt{\text{Number of flowers 2007 (Nf07)}}$	$F_{1,234} = 1.82$	0.18	$F_{1,5.97} = 2.62$	0.16	$F_{1,234} = 3.69$	0.06	16.8	0.05
Probability of corm production 2007* (Cm07)	$\chi^2 = 0.84$	0.36	$\chi^2 = 0.49$	0.49	$\chi^2 = 0.17$	0.68	7.15	0.62
$\sqrt{\text{Total corm mass 2007 (Mc07)}}$	$F_{1,301} = 22.60$	<0.0001	$F_{1,5.65} = 1.88$	0.22	$F_{1,301} = 0.04$	0.83	0	1

Due to the number of statistical tests, we used a Bonferroni-corrected alpha value of 0.0005 to determine the significance of model factors, and because of the *a priori* prediction that fitness components of progeny from hermaphrodite families would be lower than progeny from female families, a one-sided P -value was applied ($\alpha = 0.00025$).

Predicting sex phenotype frequencies and male sex allocation

We used Lloyd's (1976) model for predicting the frequencies of sexual morphs in gender polymorphic populations, and to understand how males gain fitness, based on empirical estimates of fitness components from female and inconstant male (hermaphrodite) seed families. The model was developed for gynodioecious-subdioecious populations, thus females are constant in sex expression (only female function), whereas males can be constant (only male function) or inconstant (i.e. hermaphrodite). The predicted relations between sex phenotype frequencies and relative fitness of females and hermaphrodites can be applied to any system where sex is determined by nuclear genes, as in *S. latifolia* (Dorken & Barrett, 2004b). In the model, the frequency of females depends on the relative seed production (N) and subsequent seed fitness of hermaphrodite and female offspring (Q). The product of N and Q is C , the relative hermaphrodite-female ovule contribution (see Fig. S2 for detailed explanation). Assuming males and females survive at the same rate, the frequency of females (p) can be calculated as:

$$p = (1 - 2C) / [2(1 - C)] \quad (1)$$

(Lloyd, 1976).

If $C < 0.5$, then $0 < p \leq 0.5$; if $C > 0.5$, then $p < 0$ because it is assumed that female seed fitness is at least $2 \times$ hermaphrodite seed fitness, as required for females to invade cosexual populations (Lloyd, 1975; Charlesworth & Charlesworth, 1978).

In gynodioecious/subdioecious populations, the maintenance of hermaphrodites and the evolution of pure males depend on male constancy, defined as the proportion of genes contributed via pollen to adults of the next generation (Lloyd, 1975). Male constancy quantifies the pollen contribution in relation to the total (pollen + ovule) contribution that males make to the following generation (Lloyd, 1976). The contribution of pollen to fitness will depend on female frequency, because if p is low, there is little opportunity for pollen donation. In contrast, if female frequency is high, hermaphrodites gain less fitness through ovules. Male constancy (mc) is calculated on a scale from 0.5 to 1.0 because hermaphrodites contribute at least 0.5 of the genes to the next generation via pollen, and possibly more if they gain additional fitness through ovules:

$$mc = [C(1 - p) + p] / [2C(1 - p) + p] \quad (2)$$

(Lloyd, 1976).

This equation incorporates variation in C and can be used to predict male constancy. As populations approach equilibrium, this equation simplifies to:

$$mc = 1/2(1 - p). \quad (3)$$

We estimated the two components of C for populations as follows: N is the mean number of female flowers produced by hermaphrodites divided by the mean number produced by females for 51 sexually polymorphic populations containing both females and hermaphrodites in our field survey. We used our estimates of relative seed fitness for female and hermaphrodite seed families from the glasshouse experiment to estimate Q averaged separately for populations with a low vs. a high frequency of hermaphrodites, and for each population individually. We calculated mean fitness for female and hermaphrodite families separately using the following equation, with least-square means from the statistical models described above for germination (G_m) and each of the fitness components in Table 1. This equation estimates multiplicative fitness gain through sexual reproduction (total number of flowers – top line) and asexual reproduction (total corm mass – bottom line) in 2006 and 2007.

$$\text{Fitness} = G_m \left(\frac{SV_{06}(FW_{06} \times Nf_{06}) + (SV_{07} \times FW_{07} \times Nf_{07})}{+(Cm_{06} \times Mc_{06}) + (SV_{07} \times Cm_{07} \times Mc_{07})} \right) \quad (4)$$

Finally, we calculated Q by dividing the mean hermaphrodite family fitness by mean female family fitness. For subdioecious populations in our field survey, C was calculated using separate average Q s for high- and low-frequency hermaphrodite populations; the former was applied to populations with > 0.1 hermaphrodite frequency, and the latter was applied to populations with ≤ 0.1 hermaphrodite frequency. For each population in the glasshouse experiment, a population-specific Q was used to calculate C . Estimates of Q were highly correlated ($r = 0.92$) with estimates of Q using corm number rather than corm mass (Mc) in eqn 4. We performed Pearson correlations and linear regression

between predicted and observed values of female frequency and male constancy for both average and population-specific Q methods.

Results

Variation in sex ratios

The frequency of hermaphrodites, females and males within populations of *S. latifolia* varied near continuously from monoecy through mixed populations to dioecy (Fig. 2). Our survey included two unisexual populations, 50 dioecious populations, of which 18 contained no hermaphrodites, and 36 monoecious populations, of which 14 were exclusively composed of hermaphrodites, and 22 contained mostly hermaphrodites with occasional male and very rarely female phenotypes. The remaining 28 populations, which we identify as 'mixed', included those that can be classified functionally as subdioecious ($n = 17$), androdioecious ($n = 10$) and gynodioecious ($n = 1$). Among all dioecious and subdioecious populations, mean female, male and hermaphrodite frequencies were 0.35 (range 0.02–0.79), 0.50 (range 0.02–0.91) and 0.15 (range 0.0–0.80), respectively. Among monoecious populations, mean hermaphrodite, male and female frequencies were 0.95 (range 0.82–1.0), 0.04 (range 0.0–0.18) and 0.01 (range 0.0–0.13), respectively.

Sex-ratio variation, latitudinal gradients and population size

Among the 73 sexually polymorphic populations, there was evidence of geographical variation in sex phenotype frequencies (Fig. 3a–c), with an overall effect of latitude on sex phenotype (CATMOD: $\chi^2_2 = 131.84$, $P \leq 0.0001$). As predicted, female frequencies decreased in more northerly populations (GLM: $r_{72} = -0.46$, $\beta = -0.19$,

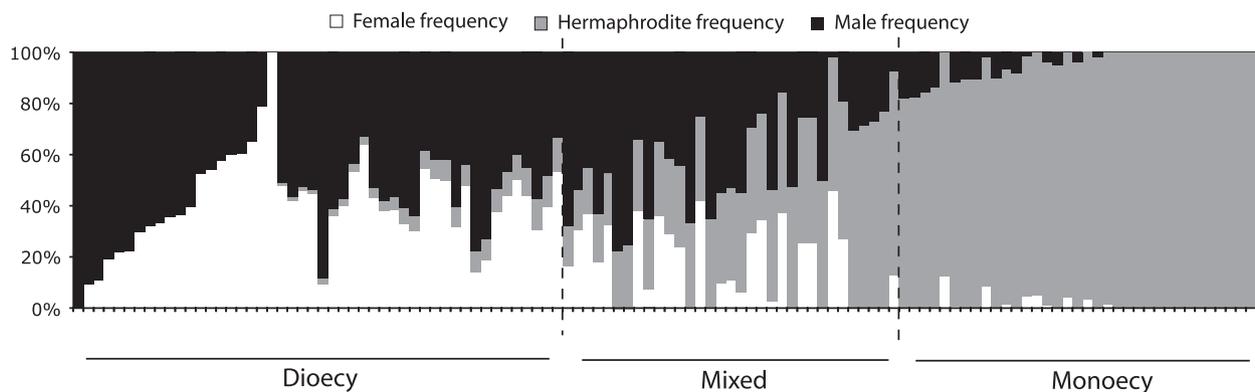


Fig. 2 Variation in sex phenotype distribution for 116 *Sagittaria latifolia* populations ordered from left to right by increasing hermaphrodite frequency. Each vertical bar represents a single population. The proportion white is female frequency, grey is hermaphrodite frequency, and black is male frequency. The vertical dotted lines identify boundaries between dioecy, mixed and monoecious populations across the observed near continuous variation.

$P \leq 0.0001$), whereas male (GLM: $r_{72} = +0.35$, $\beta = +0.14$, $P < 0.0001$) and hermaphrodite (GLM: $r_{72} = +0.11$, $\beta = +0.07$, $P = 0.005$) frequencies increased. There was no significant association between population size and latitude for dioecious and mixed populations ($r_{71} = +0.005$, $P = 0.55$). However, there was a strong negative relation between population size and hermaphrodite frequency (GLM: $r_{72} = -0.22$, $\beta = -0.37$, $P < 0.0001$) and weaker positive relations with female frequency (GLM: $r_{72} = +0.22$, $\beta = +0.12$, $P = 0.0008$) and male frequency (GLM: $r_{72} = +0.02$, $\beta = +0.08$, $P = 0.03$; Fig. 3d–f). All but the last relation remained significant after Bonferroni correction ($\alpha = 0.02$).

Floral sex ratios (proportion of summed total female to male flowers per population) in the 73 sexually polymorphic populations also varied significantly with latitude, with the proportion of female flowers decreasing and male flowers increasing (GLM: $\beta_{\text{female},72} = -0.21$, $P < 0.0001$). There was no relation between population size and floral sex ratios (GLM: $\beta_{\text{female},72} = +0.01$, $P = 0.26$). In monoecious populations, there was no relation between the proportion of female flowers and

latitude (GLM: $\beta_{\text{female},33} = +0.002$, $P = 0.86$) or population size (GLM: $\beta_{\text{female},33} = +0.04$, $P = 0.18$).

Flower production of the sex phenotypes with latitude

Among the 73 sexually polymorphic populations, total flower production per ramet varied significantly among populations (LRT: $\chi^2_1 = 852.73$, $P < 0.0001$) and decreased with latitude ($F_{1,75,3} = 10.51$, $P = 0.002$), but as predicted there were significant differences among the sex phenotypes (Fig. 4; latitude \times sex interaction $F_{2,5467} = 19.56$, $P < 0.0001$). Flower production of females exhibited the steepest decline with latitude ($F_{1,5501} = 144.34$, $P < 0.0001$), and this relation differed significantly from hermaphrodites ($F_{1,5445} = 15.36$, $P = 0.0001$) and males ($F_{1,5482} = 34.19$, $P < 0.0001$). Flower production of males exhibited a moderate decline compared with females ($F_{1,5501} = 27.58$, $P < 0.0001$), and flower production of hermaphrodites did not decline significantly with latitude ($F_{1,5501} = 3.42$, $P = 0.06$); however, the slopes of these

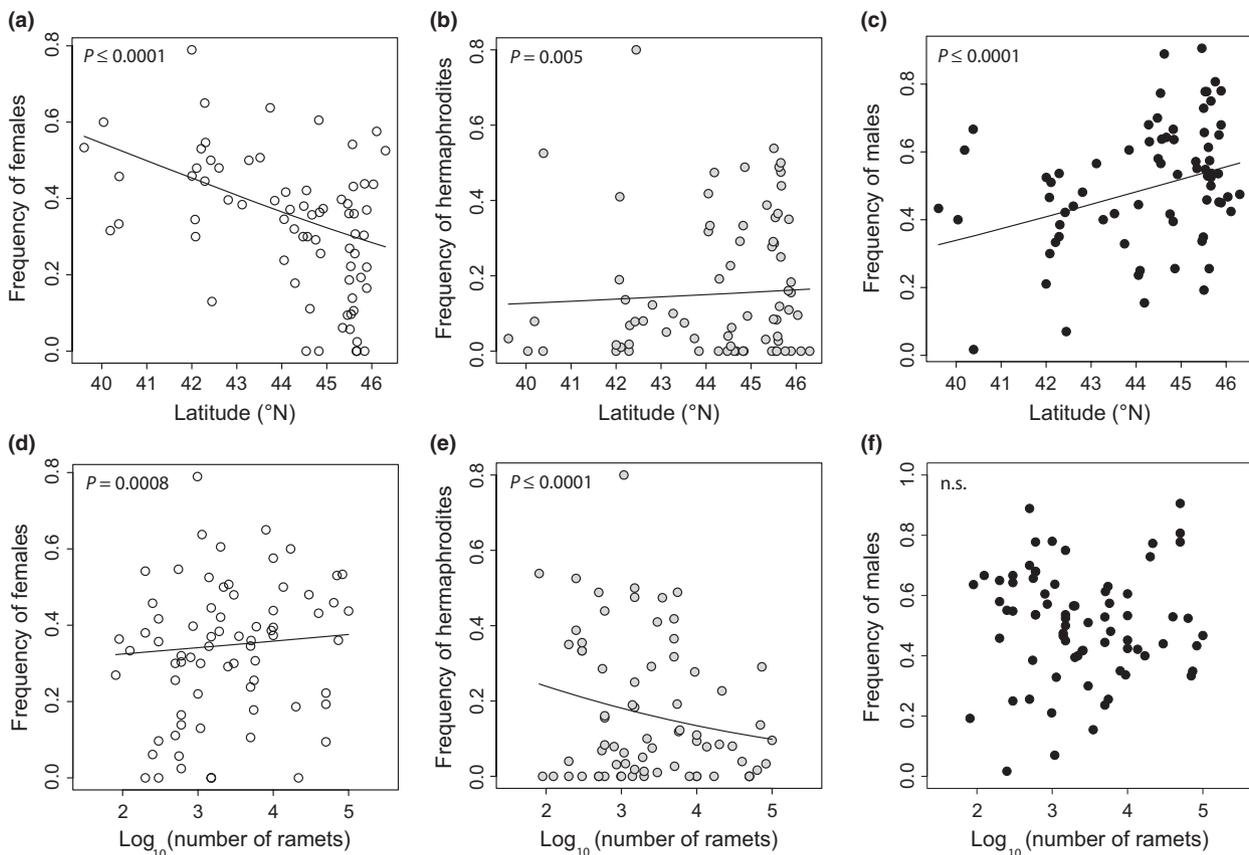


Fig. 3 Relations between female (white), hermaphrodite (grey) and male (black) frequencies and latitude (a–c) and population size (d–f) among 73 polymorphic populations of *Sagittaria latifolia*. The lines of best fit were calculated from separate models for latitude (a–c) and population size (d–f).

relations did not differ from one another ($F = 0.07_{1,5417}$, $P = 0.79$).

Comparison of fitness components between female and hermaphrodite seed families

Populations varied significantly in germination ($\chi^2_1 = 31.96$, $P < 0.0001$) with the proportion of seeds germinating from female families significantly higher (LS mean = 0.87) than from hermaphrodite families (LS mean = 0.79; $\chi^2_1 = 11.75$, $P = 0.0006$). This result was predicted based on the assumption that hermaphrodites in this self-compatible species produce some selfed offspring whereas females cannot. Germination was also significantly higher in low- (LS mean = 0.79) than high-frequency hermaphrodite populations (LS mean = 0.38) ($\chi^2_1 = 58.17$, $P < 0.0001$), as predicted. There was no significant interaction between family type and frequency of hermaphrodites ($\chi^2_1 = 0.00$, $P = 0.98$).

Over two seasons of growth, differences between female and hermaphrodite seed families were only evident for total corm mass (Table 1). Significantly, plants from female families produced 27% more corm mass (LS mean = 34.0 g) than plants from hermaphrodite families (LS mean = 24.9 g). No significant difference in total corm mass was detected at the end of the 2006 growing season, although the trend was in the same direction as in 2007. Populations with high hermaphrodite frequencies may experience lower recruitment of juveniles from both seed and corms.

The only fitness component exhibiting a significant difference between low- vs. high-frequency hermaphro-

dite populations was the probability of flowering. In 2006, plants from low-frequency hermaphrodite populations were more likely to flower (LS mean = 0.73) than plants from high-frequency hermaphrodite populations (LS mean = 0.33; Table 1). The same trend was observed in 2007, although the difference was not significant following Bonferroni correction. However, we note that high-frequency hermaphrodite populations were sampled from more northern sites. Future studies involving paired comparisons from across the range would be important for understanding the extent to which latitude might contribute to the observed differences in flowering propensity. There was no interaction between family type and the frequency of hermaphrodites in populations for any fitness component in either year.

The average N value (relative seed production of females and hermaphrodites) based on variation in female flower number across the 51 field-sampled populations was 0.37 (range 0.09–0.81). These estimates of N reflect the finding that female plants produce more female flowers than hermaphrodite plants in populations where the two sex phenotypes co-occur (Fig. S4; $F_{1,172} = 265.42$, $P < 0.0001$). The average Q value based on the fitness components across four low- and four high-frequency hermaphrodite populations was 0.88 and 0.67, respectively. Applying the low- and high-frequency Q values as described in the Materials and Methods, the mean C value for the 51 populations with three sex phenotypes was 0.27 (range 0.08–0.62). We also calculated individual Q values for the eight populations, and these ranged from 0.14 to 1.21 (mean = 0.83) yielding C values ranging from 0.08 to 0.56 (mean = 0.27). All Q values are tabulated in Table S3.

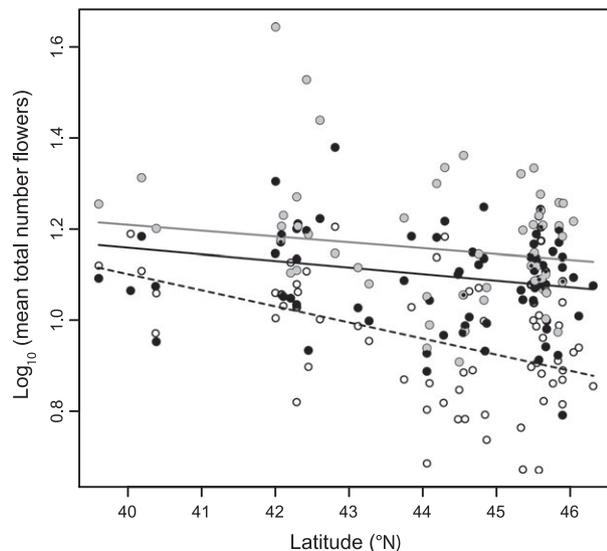


Fig. 4 Relations between population mean flower production and latitude for female (open circles, dotted line), hermaphrodite (grey points and line) and male (black points and line) phenotypes in 73 polymorphic populations. Lines of best fit were calculated from mixed-model estimates.

Predicting the frequency of sex phenotypes

We detected a moderate positive relation between predicted and observed equilibrium female frequency among 51 populations of *S. latifolia* using the average values of Q (Fig. 5a circles; $r_{49} = +0.31$, $P = 0.03$). This relation was not significant (Fig. 5a triangles; $r_6 = +0.16$, $P = 0.56$) using individual Q values for the eight populations. We detected a strong positive correlation between predicted and equilibrium estimates of male constancy ($r_{49} = +0.84$, $P < 0.0001$) using the average values of Q , with most populations close to the 1 : 1 relation of the models intended range of 0.5–1.0 (Fig. 5b circles). A similar positive relation using population-specific estimates of Q (Fig. 5b triangles; $r_6 = +0.76$, $P = 0.02$) was also found. Note that values > 1 occur for ‘equilibrium male constancy’ when observed female frequency is > 0.5 .

Discussion

Our study of the geography of sex ratios at the northern range limit of *S. latifolia* in eastern N. America

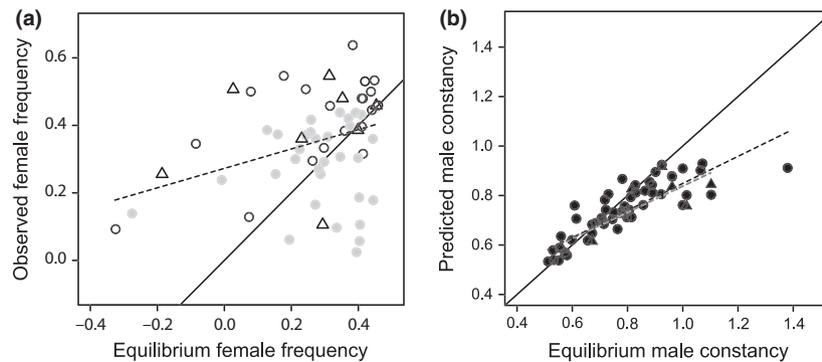


Fig. 5 Relations between observed and predicted equilibrium (a) female frequency and (b) male constancy (see Lloyd, 1976) using average Q values (circles; $n = 51$) and population-specific Q values (triangles; $n = 8$). Black and grey dotted lines represent the linear regression between predicted and observed values for circles and triangles, respectively. In (a), open circles represent southern populations (39.6–43.7°N), and grey circles represent northern populations (44.1–46.0°N). Solid line identifies the 1 : 1 relation.

revealed striking variation in the distribution of the three sex phenotypes. The 116 populations surveyed could be classified into five sexual systems traditionally recognized by botanists – monoecy, dioecy, subdioecy, androdioecy and gynodioecy – based on the sex ratio of flowering ramets. Our preference here is not to give undue importance to these categories, but instead to focus on the quantitative nature of plant gender (Fig. 2), how this variation is maintained and its implications for understanding evolutionary transitions in plant sexual systems.

Geographical variation in sex ratios

Our survey confirmed that most populations of *S. latifolia* are readily classified as either monoecious or dioecious, with 76% of populations composed of either predominantly hermaphroditic or unisexual plants. However, the remaining 24% were largely comprised of three sex phenotypes at varying frequencies. This variation was geographically structured, with increased frequencies of mixed populations at the northern range limit of dioecious populations, as a result of an increase in the frequency of hermaphrodites (Figs 1 and 3). Both monoecious and dioecious populations occur throughout eastern N. America, but monoecious populations are distributed hundreds of kilometres beyond the northern range limit of dioecious populations, outside the area we sampled intensively. This suggests that hermaphrodites possess advantages over unisexuals in northern environments, and similar advantages may explain their increased frequency in subdioecious populations at dioecious range limits.

Two hypotheses may explain the apparent advantage of hermaphroditism at northern range limits in *S. latifolia*. First, the ability of single, self-compatible, hermaphrodite plants to found colonies following dispersal may provide an advantage under low-density conditions (Baker, 1955; Pannell & Barrett, 1998). Increased hermaphrodite fre-

quencies in mixed populations that were smaller in size support this hypothesis (Fig. 3e). However, two issues need to be considered: (i) among mixed and dioecious populations, we found no relation between latitude and population size indicating that low-density conditions do not necessarily characterize populations at range limits. (ii) Because they are monoecious, hermaphrodites of *S. latifolia* possess unisexual flowers and are therefore incapable of autonomous self-pollination, requiring pollinators to ensure seed set. Thus, any colonization advantage must be due to the ability of hermaphrodites to establish sexually reproducing populations from a single plant rather than from reproductive assurance due to autonomous selfing (see Cheptou, 2011). In this regard, it is worth noting that unlike many dioecious clonal aquatics (Barrett *et al.*, 1993), populations of *S. latifolia* composed of only a single gender rarely occur (Fig. 2). This suggests that sexual recruitment is necessary for the persistence of populations even at range limits, regardless of their sexual system.

A second hypothesis, that may explain the increased frequency of hermaphrodite plants in subdioecious populations, concerns the relative costs and timing of seed maturation for females vs. hermaphrodites, under the shorter growing season of northern environments. We found that an increase in hermaphrodite (and male) frequency with latitude was accompanied by a decrease in the frequency of females (Fig. 3a–c). Moreover, there was also a decline in female frequency (GLM: $r_{38} = -0.39$, $\beta = -0.13$, $P \leq 0.0001$) and an increase in male frequency (GLM: $r_{38} = -0.41$, $\beta = +0.12$, $P \leq 0.0001$) towards the northern range limit among pure dioecious populations, and those with a low frequency of hermaphrodites (< 10%), of very similar magnitude to the 73 polymorphic populations. This suggests that although hermaphrodites may increase in association with female decline in some range limit populations, the decline in female frequency can occur independently of this process.

The decline in female frequency in northern range limit populations was also associated with a steep decline in the flower production of females compared with males and hermaphrodites (Fig. 4). These patterns may result from time constraints on seed maturation in northern environments, where the growing season is shorter relative to southern regions (Montague *et al.*, 2008; Chuine, 2010). Female flower number is a good predictor of seed production as female flower number is well correlated with both total seed mass ($r_{19} = 0.88$, $P < 0.0001$) and mean number of seeds per plant ($r_{19} = 0.94$, $P < 0.0001$; calculated from Table 2 in Dorken & Mitchard, 2008). Because female ramets produce many more flowers on which to mature fruit than hermaphrodites (Fig. S4), they require substantially more resources and time for complete seed maturation. At the northern range limit, resources for maternal function must be acquired rapidly before the growing season ends in late September and early October. Thus, it is possible that the sex allocation of hermaphrodites is more suited to northern conditions, as suggested by the more northerly distribution of monoecious populations. We do not know whether the decreased frequency of female flowering in northern populations and changes in their display size result from adaptive evolutionary responses and/or phenotypic plasticity. Regardless, these responses with latitude cause changes to the operational sex ratio of populations and thus determine mating opportunities and the sex ratios of subsequent generations.

Predicting sex ratios from fitness components

In comparison to organisms with separate sexes, the occurrence of three sex phenotypes in plant populations complicates predictions of sex ratios. We used Lloyd's (1976) model of evolution in sexually polymorphic populations to evaluate the extent to which female frequencies and male allocation fit equilibrium predictions. In this model, the relative fitness (C) of the two seed bearing phenotypes – females and hermaphrodites – is fundamental to predicting sex ratios, and we obtained estimates of this parameter from a 2-year glasshouse study. Our estimate of 0.27 indicated that, as predicted, hermaphrodite seed families experienced significantly lower fitness in terms of the number and quality of seed produced than progeny from female families.

Our estimate of C is similar to values reported by Dorken & Mitchard (2008) for *S. latifolia* grown under high and low fertilizer treatments ($C = 0.54$, 0.29, respectively), based on seed number (N) only. However, the deficit experienced by hermaphrodite seed families in our study was mostly driven by their lower production of female flowers (average $N = 0.37$), rather than consistently lower seed quality (average low-frequency $Q = 0.87$; average high-frequency $Q = 0.63$), compared with females. Population estimates of Q ranged from 0.12 to 1.21, suggesting that the relative fitness of hermaphro-

dite families may vary considerably depending on population context. Considerable variation in selfing rates is evident among monoecious populations (see Fig. 2 in Dorken *et al.*, 2002), and hermaphrodites of subdioecious populations may show similar variation.

We also detected a significant positive correlation between observed and predicted female frequency using the average Q values, although the line of best fit differs substantially from the 1 : 1 line, and the analysis using population estimates although positive was not significant (Fig. 5a, triangles). The populations with higher female frequencies than predicted by the model tended to be southern populations, whereas populations that exhibited lower female frequencies tended to be northern populations (Fig. 5a). This suggests that even after accounting for the lower number of flowers produced by females in northern populations, female frequency is lower than expected. In contrast, we detected a strong positive correlation between observed and predicted equilibrium male constancy using the average Q values, and a weaker but still significant positive relation using data from individual populations. For the intended range of male constancy values (0.5–1.0), the model provided a good fit between observed and expected equilibrium values, with most data close to the 1 : 1 line (Fig. 5b). Overall, this suggests that male constancy may adjust more easily to the sex-ratio context of a population, whereas female frequency may be more influenced by geographical location (i.e. latitude, season length and onset of winter conditions) and local ecological conditions (e.g. nutrient availability, level of disturbance), thus preventing females from reaching expected equilibrium frequencies. Therefore, sex ratios in subdioecious populations may be governed by a balance between the difficulties experienced by females in performing well at northern latitudes and the ability of hermaphrodites to replace female function.

Several additional factors may also account for the deviation of parameter values from equilibrium expectations. Lloyd's (1976) model is purposely simple to facilitate empirical tests using data from natural populations. However, it does not account for two ecological features of *S. latifolia* populations likely to influence sex ratios and equilibrium expectations. First, plasticity in ovule production of inconstant males (Delph & Wolf, 2005; Ehlers & Bataillon, 2007) may cause variation in estimates of C (Dorken & Mitchard, 2008; Spigler & Ashman, 2011) if environmental conditions vary. Studies using resource manipulation in *S. latifolia* have demonstrated this type of plasticity (Dorken & Barrett, 2004b), and this may result in a weaker fit to the equilibrium predictions of female frequency and male constancy. Second, *S. latifolia* propagates by sexual and clonal reproduction, and progress to sex-ratio equilibrium may be protracted and sensitive to founding genotypes and the balance between these reproductive modes. A recent model based on genetic and life-history

features of *S. latifolia* demonstrated that subdioecy could be maintained over very long periods of time in a non-equilibrium state with sex-ratio dynamics affected by the composition of the sex-determining genotypes of founders and the degree of clonal propagation (Barrett *et al.*, 2010). Indeed, given this finding, it is somewhat surprising that the predictions from Lloyd's (1976) model were as good as they were and suggests that severe founder events may be limited by extensive waterborne seed dispersal, and frequent sexual recruitment may occur despite populations occurring at range limits.

General conclusions and implications for sexual system evolution

Sagittaria latifolia maintains enormous variation in sex ratios, especially towards the northern range limit. This finding should caution against characterization of the sexual system of plant species based on a limited geographical sampling of populations, particularly in wide-ranging taxa. Populations of *S. latifolia* can be functionally classified into any one of five recognized sexual systems, but with monoecy and dioecy predominating. Subdioecious populations become more common towards the northern range limit of dioecy, thus demonstrating the importance of geographical context for the origin and maintenance of sexual system diversity. Subdioecy is most often viewed as a transient sexual system on the evolutionary pathway to dioecy (Lloyd, 1976; Ehlers & Bataillon, 2007). However, our study of *S. latifolia* provides a novel perspective on this complex sexual polymorphism as subdioecy evolves from dioecious populations containing standing genetic variation for male inconstancy. The increased incidence of hermaphroditism at northern range limits may allow sexually polymorphic populations to persist in the shorter growing seasons that characterize populations in this region.

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References

Alonso, C. & Herrera, C.M. 2011. Back-and-forth hermaphroditism: phylogenetic context of reproductive system evolution in subdioecious *Daphne laureola*. *Evolution* **65**: 1680–1692.

- Ashman, T.-L. 1999. Determinants of sex allocation in a gynodioecious wild strawberry: implications for the evolution of dioecy and sexual dimorphism. *J. Evol. Biol.* **12**: 648–661.
- Ashman, T.-L. 2006. The evolution of separate sexes: a focus on the ecological context. In: *Ecology and Evolution of Flowers* (L.D. Harder & S.C.H. Barrett, eds), pp. 204–219. Oxford University Press, Oxford, UK.
- Baker, H.G. 1955. Self-compatibility and establishment after "long-distance" dispersal. *Evolution* **9**: 347–349.
- Barrett, S.C.H. 1992. Gender variation and the evolution of dioecy in *Wurmbea dioica* (Liliaceae). *J. Evol. Biol.* **5**: 423–444.
- Barrett, S.C.H. 2003. Mating strategies in flowering plants: the outcrossing–selfing paradigm and beyond. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **358**: 991–1004.
- Barrett, S.C.H. 2013. The evolution of plant reproductive systems: how often are transitions irreversible? *Proc. R. Soc. B Biol. Sci.* **280**: 20130913.
- Barrett, S.C.H., Eckert, C.G. & Husband, B.C. 1993. Evolutionary processes in aquatic plant populations. *Aquat. Bot.* **44**: 105–145.
- Barrett, S.C.H., Case, A.L. & Peters, G.B. 1999. Gender modification and resource allocation in subdioecious *Wurmbea dioica* (Colchicaceae). *J. Ecol.* **87**: 123–137.
- Barrett, S.C.H., Dorken, M.E. & Case, A.L. 2001. A geographical context for the evolution of plant reproductive systems. In: *Integrating Ecological and Evolutionary Processes in a Spatial Context* (J. Silvertown & J. Antonovics, eds), pp. 341–364. Blackwell, Oxford, UK.
- Barrett, S.C.H., Yakimowski, S.B., Field, D.L. & Pickup, M. 2010. Ecological genetics of sex ratios in plant populations. *Philos. Trans. R. Soc. B Biol. Sci.* **365**: 2549–2557.
- Caruso, C.M. & Case, A.L. 2007. Sex ratio variation in gynodioecious *Lobelia siphilitica*: effects of population size and geographic location. *J. Evol. Biol.* **20**: 1396–1405.
- Case, A.L. & Barrett, S.C.H. 2004. Environmental stress and the evolution of dioecy: *Wurmbea dioica* (Colchicaceae) in Western Australia. *Evol. Ecol.* **18**: 145–164.
- Charlesworth, D. 2002. Plant sex determination and sex chromosomes. *Heredity* **88**: 94–101.
- Charlesworth, B. & Charlesworth, D. 1978. A model for the evolution of dioecy and gynodioecy. *Am. Nat.* **112**: 975–997.
- Cheptou, P.-O. 2011. Clarifying Baker's Law. *Ann. Bot.* **109**: 633–641.
- Chuine, I. 2010. Why does phenology drive species distribution? *Philos. Trans. R. Soc. B Biol. Sci.* **365**: 3149–3160.
- Delph, L.F. 1990. Sex-ratio variation in the gynodioecious shrub *Hebe strictissima* (Scrophulariaceae). *Evolution* **44**: 134–142.
- Delph, L.F. 1999. Sexual dimorphism in life history. In: *Gender and Sexual Dimorphism in Flowering Plants* (M.A. Geber, T.E. Dawson & L.F. Delph, eds), Springer-Verlag, Berlin, Germany.
- Delph, L.F. 2009. Sex allocation: evolution to and from dioecy. *Curr. Biol.* **19**: R249–R251.
- Delph, L.F. & Lloyd, D.G. 1991. Environmental and genetic control of gender in the dimorphic shrub *Hebe subalpina*. *Evolution* **45**: 1957–1964.
- Delph, L.F. & Wolf, D.E. 2005. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. *New Phytol.* **166**: 119–128.
- Dorken, M.E. & Barrett, S.C.H. 2003. Life-history differentiation and the maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **57**: 1973–1988.
- Dorken, M.E. & Barrett, S.C.H. 2004a. Chloroplast haplotype variation among monoecious and dioecious populations of

- Sagittaria latifolia* (Alismataceae) in eastern North America. *Mol. Ecol.* **13**: 2699–2707.
- Dorken, M.E. & Barrett, S.C.H. 2004b. Sex determination and the evolution of dioecy from monoecy in *Sagittaria latifolia* (Alismataceae). *Proc. R. Soc. B Biol. Sci.* **271**: 213–219.
- Dorken, M.E. & Mitchard, E.T.A. 2008. Phenotypic plasticity of hermaphrodite sex allocation promotes the evolution of separate sexes: an experimental test of the sex-differential plasticity hypothesis using *Sagittaria latifolia* (Alismataceae). *Evolution* **62**: 971–978.
- Dorken, M.E. & Pannell, J.R. 2009. Hermaphroditic sex allocation evolves when mating opportunities change. *Curr. Biol.* **19**: 514–517.
- Dorken, M.E., Friedman, J. & Barrett, S.C.H. 2002. The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **56**: 31–41.
- Eckert, C.G., Samis, K.E. & Loughheed, S.C. 2008. Genetic variation across species' geographical ranges: the central-marginal hypothesis and beyond. *Mol. Ecol.* **17**: 1170–1188.
- Ehlers, B.K. & Bataillon, T. 2007. "Inconstant males" and the maintenance of labile sex expression in subdioecious plants. *New Phytol.* **174**: 194–211.
- Field, D.L., Pickup, M. & Barrett, S.C.H. 2013a. Comparative analyses of sex-ratio variation in dioecious flowering plants. *Evolution* **67**: 661–672.
- Field, D.L., Pickup, M. & Barrett, S.C.H. 2013b. Ecological context and metapopulation dynamics affect sex-ratio variation among dioecious plant populations. *Ann. Bot.* **111**: 917–923.
- Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford University Press, Oxford, UK.
- Fleming, T.H., Maurice, S., Buchmann, S.L. & Tuttle, M.D. 1994. Reproductive biology and relative male and female fitness in a trioecious cactus, *Pachycereus pringlei* (Cactaceae). *Am. J. Bot.* **81**: 858–867.
- Gaston, K.J. 2003. *The Structure and Dynamics of Geographic Ranges*. Oxford University Press, Oxford, UK.
- Geber, M.A., Dawson, T.E. & Delph, L.F. 1999. *Gender and Sexual Dimorphism in Flowering Plants*. Springer-Verlag, Berlin, Germany.
- Hardy, I.C.W. 2002. *Sex Ratios: Concepts and Research Methods*. Cambridge University Press, Cambridge, UK.
- Herrera, C.M., Castellanos, M.C. & Medrano, M. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. In: *Ecology and Evolution of Flowers* (L.D. Harder & S.C.H. Barrett, eds), pp. 278–294. Oxford University Press, Oxford, UK.
- Hoffmann, A.A. & Blows, M.W. 1994. Species borders: ecological and evolutionary perspectives. *Trends Ecol. Evol.* **9**: 223–227.
- Holt, R.D. & Keitt, T.H. 2000. Alternative causes for range limits: a metapopulation perspective. *Ecol. Lett.* **3**: 41–47.
- Lloyd, D.G. 1975. The maintenance of gynodioecy and androdioecy in angiosperms. *Genetica* **45**: 325–339.
- Lloyd, D.G. 1976. Transmission of genes via pollen and ovules in gynodioecious angiosperms. *Theor. Popul. Biol.* **9**: 299–316.
- Lloyd, D.G. 1980. Demographic factors and mating patterns in angiosperms. In: *Demography and Evolution in Plant Populations* (O.T. Solbrig, ed.), pp. 67–88. Oxford University Press, Oxford, UK.
- Lloyd, D.G. & Bawa, K.S. 1984. Modification of the gender of seed plants in varying conditions. *Evol. Biol.* **17**: 255–338.
- Maurice, S. & Fleming, T. 1995. The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphisms. *Oikos* **74**: 55–60.
- Montague, J.L., Barrett, S.C.H. & Eckert, C.G. 2008. Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *J. Evol. Biol.* **21**: 234–245.
- Pannell, J.R. 2002. The evolution and maintenance of androdioecy. *Annu. Rev. Ecol. Syst.* **33**: 397–425.
- Pannell, J.R. 2006. Effects of colonization and metapopulation dynamics on the evolution of plant sexual systems. In: *Ecology and Evolution of Flowers* (L.D. Harder & S.C.H. Barrett, eds), pp. 223–238. Oxford University Press, Oxford, UK.
- Pannell, J.R. & Barrett, S.C.H. 1998. Baker's Law revisited: reproductive assurance in a metapopulation. *Evolution* **52**: 657–668.
- R Development Core Team. 2008. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org>.
- Renner, S.S. & Ricklefs, R. 1995. Dioecy and its correlates in the flowering plants. *Am. J. Bot.* **82**: 596–606.
- Sagarin, R.D. & Gaines, S.D. 2002. The "abundant centre" distribution: to what extent is it a biogeographical rule? *Ecol. Lett.* **5**: 137–147.
- Sakai, A.K. & Weller, S.G. 1999. Gender and sexual dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. In: *Sexual and Gender Dimorphism in Flowering Plants* (M.A. Geber, T.E. Dawson & L.F. Delph, eds), pp. 1–31. Springer-Verlag, New York, NY, USA.
- Sarkissian, T.S., Barrett, S.C.H. & Harder, L.D. 2001. Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters? *Ecology* **82**: 360–373.
- Spigler, R.B. & Ashman, T.-L. 2011. Sex ratio and subdioecy in *Fragaria virginiana*: the roles of plasticity and gene flow examined. *New Phytol.* **190**: 1058–1068.
- Webb, C.J. 1999. Empirical studies: evolution and maintenance of dimorphic breeding systems. In: *Sexual and Gender Dimorphism in Flowering Plants* (M.A. Geber, T.E. Dawson & L.F. Delph, eds), pp. 31–90. Springer Verlag, New York, NY, USA.
- Wolf, D.E. & Takebayashi, N. 2004. Pollen limitation and the evolution of androdioecy from dioecy. *Am. Nat.* **163**: 122–137.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Geographical location, the number of flowering ramets surveyed (N), frequencies of sex phenotypes and population size for 116 populations of *Sagittaria latifolia* sampled in this study.

Figure S2 Schematic illustrating the relations among Lloyd's (1976) model parameters C , N and Q .

Table S3 Mean estimates of life-history components of fitness derived from the logistic regression and mixed-effect models presented in Table 1.

Figure S4 Mean number of female flowers (\pm SE) produced on female (white) and hermaphrodite (black) ramets in dioecious, mixed and monoecious populations of *Sagittaria latifolia*.

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