

Ecological context and metapopulation dynamics affect sex-ratio variation among dioecious plant populations

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- **Background and Aims** Populations of dioecious flowering plants commonly exhibit heterogeneity in sex ratios and deviations from the equilibrium expectation of equal numbers of females and males. Yet the role of ecological and demographic factors in contributing towards biased sex ratios is currently not well understood.
- **Methods** Species-level studies from the literature were analysed to investigate ecological correlates of among-population sex-ratio variation and metapopulation models and empirical data were used to explore the influence of demography and non-equilibrium conditions on flowering sex ratios.
- **Key Results** The survey revealed significant among-population heterogeneity in sex ratios and this was related to the degree of sampling effort. For some species, sex-ratio bias was associated with the proportion of non-reproductive individuals, with greater male bias in populations with a lower proportion of individuals that were flowering. Male-biased ratios were also found at higher altitudes and latitudes, and in more xeric sites. Simulations and empirical data indicated that clonal species exhibited greater heterogeneity in sex ratios than non-clonal species as a result of their slower approach to equilibrium. The simulations also indicated the importance of interactions between reproductive mode and founder effects, with greater departures from equilibrium in clonal populations with fewer founding individuals.
- **Conclusions** The results indicate that sex-based differences in costs of reproduction and non-equilibrium conditions can each play important roles in affecting flowering sex ratios in populations of dioecious plants.

Key words: Biased sex ratios, costs of reproduction, dioecy, stress gradients, non-equilibrium conditions, metapopulations.

INTRODUCTION

The classical theoretical arguments of Dusing (1884, see Edwards 2000) and Fisher (1930) predict 1 : 1 primary sex ratios after the period of parental investment when the costs of producing females and males are equal. However, departures from equality are commonly reported in the literature on dioecious angiosperms, although most estimates involve flowering (secondary or operational) sex ratios and only limited information is available on primary (seed) sex ratios (for reviews, see Delph, 1999; de Jong and Klinkhamer, 2002; Barrett *et al.*, 2010; Sinclair *et al.*, 2012; Field *et al.*, 2013). Determining the complex ecological, demographic and genetic factors that cause these departures remains an enduring problem in ecology and evolutionary biology. In a recent comparative analysis of sex-ratio variation in 243 species of flowering plants, Field *et al.* (2013) found that sex-based differences in life-history traits and their influence on the costs of reproduction contribute to among-species variation in sex ratios. But among-population heterogeneity in sex ratios also occurs within species leading to questions regarding the processes that contribute to this variation and if these mirror those underlying cross-species patterns. Given that populations of dioecious species can vary considerably in demographic characteristics and in the environmental conditions in which

they occur, examining the ecological correlates of sex-ratio variation should provide insight into the processes driving deviations from equilibrium expectations.

Differential reproductive expenditure between the sexes is considered one of the primary drivers of biased flowering sex ratios in plant populations (Lloyd and Webb, 1977; Bell, 1980). Greater reproductive investment by females is often associated with male-biased flowering sex ratios because of the earlier onset and more frequent flowering of males, and because of greater female mortality (Lloyd, 1973; Lloyd and Webb, 1977; Meagher, 1981; Allen and Antos, 1993; Delph, 1999; Obeso, 2002; Barrett and Hough, 2013). These processes can drive sex-ratio variation among species and lead to associations between life-history traits related to sex-specific differences in reproductive costs (e.g. fleshy fruits and pollen and seed dispersal mechanisms), as demonstrated in a recent among-species comparative analysis (Field *et al.*, 2013). Differential costs of reproduction between the sexes may also be expected to drive among-population sex-ratio variation for species occupying heterogeneous environments. For example, if females incur greater reproductive costs they may delay flowering or flower less frequently, which could lead to a positive association between male bias and the proportion of non-reproductive individuals (Lloyd, 1973; Meagher, 1981; Allen and Antos, 1993). Similarly, higher

female reproductive expenditure leading to greater susceptibility to stress should also result in more male-biased sex ratios when resources are limited, or with increasing environmental stress along altitudinal, latitudinal or moisture gradients (e.g. Freeman *et al.*, 1976; Dawson and Ehleringer, 1993; Delph, 1999; Pickering and Hill, 2002). Consequently, biased sex ratios among populations of flowering plants are likely to reflect complex interactions between sex-based differences in the costs of reproduction, life history and ecological context.

Stochastic processes are also likely to contribute towards much of the observed variation in sex ratios among populations of dioecious species. Many plants persist in metapopulations in which founder effects interacting with life-history parameters can significantly influence the equilibrium dynamics of sexual polymorphisms (Pannell, 2006). In particular, limited sexual recruitment and high levels of clonal reproduction can preserve non-equilibrium sex ratios for long periods following disturbance and population establishment (Pannell and Barrett, 1998; Barrett *et al.*, 2010). The observed heterogeneity in sex ratios may therefore reflect historical contingencies from which populations of long-lived and clonally reproducing populations have yet to reach equilibrium. In comparison to other plant sexual polymorphisms, in which interactions between stochastic forces and life history on sex phenotype ratios have been explored (e.g. Morgan and Barrett, 1988; Eckert and Barrett, 1995), there has been little consideration of these influences on sex-ratio variation in dioecious populations (but see Barrett *et al.*, 2010).

There are several predictions that can be made when assessing the role of stochastic processes in affecting sex ratios. First, we would expect more variation in sex ratios around equality in small compared with large populations, which would be reflected by a classic funnel-shaped distribution (e.g. Palmer, 1999). Secondly, if clonal reproduction does indeed retard progress to equilibrium, a deeper and more attenuated funnel distribution would be expected in comparison with species in which sexual recruitment dominates. These predictions could be tested by developing models of sex-ratio dynamics in metapopulations and comparing the results with empirical data from surveys of sex ratios in dioecious populations.

Here, we consider within-species variation in sex ratios in relation to ecological correlates, demography and life history. First we examine the frequency of among-population heterogeneity in sex ratios for species with multiple population samples and assess the extent to which sampling effort affects among-population sex-ratio variation. We then consider the influence of demography and environmental conditions on among-population sex-ratio variation in relation to sex-specific differences in reproductive costs. With higher female reproductive costs we predict greater male bias in populations with a higher frequency of non-reproductive plants, and for those in more stressful environments, specifically higher altitudes or latitudes and more xeric sites. Finally, we develop metapopulation models to assess the influence of non-equilibrium conditions, founder effects and clonal reproduction on sex-ratio variation and compare the results with empirical data from the literature on dioecious plants with contrasting reproductive modes.

MATERIALS AND METHODS

Data collection

To investigate the influence of sampling effort, environmental conditions and demographic factors on among-population heterogeneity in sex ratios, we selected species from a large database of sex ratios used in our comparative study of sex-ratio variation in flowering plants (Field *et al.*, 2013). Only articles meeting the following three criteria were included in this database (for more information, see Field *et al.*, 2013): (1) the species included were dioecious only; (2) the study reported the number of individuals sampled; and (3) sex ratio samples were obtained by direct counts and were not based on plant density. For populations of each species, we recorded the number of females and males that were counted, total sample size and, if available, the population size. We also recorded the number of non-reproductive plants, location, latitude and longitude and altitude (metres above sea level). From this database we obtained a sample of $n = 126$ species to examine whether among-population heterogeneity in sex ratios was related to sampling effort (the number of populations sampled). For the altitude and latitude analysis, species were only included if ≥ 3 populations were sampled, there was a continuous distribution of the predictor variable, and there was no correlation between altitude and latitude. For altitude, only species with ranges > 1000 m were considered. This resulted in a sample size (n) of five and 13 species for the altitude and latitude analysis, respectively. For five species we also obtained information on soil moisture gradients (high vs. low soil moisture). From these studies we classified populations categorically, as high or low soil moisture, based on the original soil moisture values presented in each paper. Throughout this article the sex ratio of a population = males / (females + males).

Statistical analysis

Among-population heterogeneity in sex ratios. For species with multiple population samples, we used heterogeneity G -tests (Sokal and Rohlf, 1995) using a script written in R to assess if there was significant among-population heterogeneity in sex ratios. To examine if there was a relationship between heterogeneity in sex ratios and the number of populations sampled for the 126 species with more than one population sample, we used a generalized linear model (GLM) (logistic regression) with a binomial distribution and a logit link function in Genstat for Windows 13th edition. For this analysis, species were scored as 0 (no heterogeneity) or 1 (significant heterogeneity) and the number of populations sampled was added to the fitted model.

Demographic and ecological correlates of sex-ratio variation. We used GLM (logistic regression) analysis with a binomial distribution and logit link function in Genstat for Windows 13th edition to investigate if within-species variation in sex ratio was related to: (a) the number of non-reproductive individuals, (b) altitude, (c) latitude, and (d) soil moisture (high vs. low soil moisture). For each model the population characteristic, species identity and their interaction were added sequentially.

The influence of non-equilibrium conditions and reproductive mode on sex-ratio variation

Metapopulation model. To investigate the influence of colonization–extinction dynamics on population sex ratios within a metapopulation we used an ‘island model’ with a discrete time simulation to track the frequencies of females and males in demes over successive ‘generations’. The metapopulation consisted of a 20×20 lattice of local demes (400 total) each with a non-zero probability of extinction that could be occupied by females and males. Each generation comprised the following steps: (a) within-deme population growth via sexual and clonal propagation with year-to-year survival; (b) probabilistic seed dispersal into demes followed by population size regulation to carrying capacity; and (c) probabilistic extinction of each deme.

For simulations within each deme, we used a genetic model with two gender phenotypes determined by alleles at a sex-determining region consisting of two linked loci *Su* and *S* (Charlesworth and Guttman, 1999). In this model, males are the heterozygous genotype $Su^f S^m / Su^f S^m$ (i.e. XY) and females are homozygous $Su^f S^m / Su^f S^m$ (i.e. XX), where Su^f is a dominant suppressor of female fertility and S^m is a recessive male sterility factor. We simulated the two linked-locus model because the majority of dioecious species are thought to possess this mode of sex determination (Charlesworth, 2002). We let g_{mj} and g_{fj} be the number of male and female individuals in the j th deme at time t . We let g_{mj}' and g_{fj}' be the total number of males and females at time $t + 1$, which is the sum of all components of sexual and asexual reproduction and year-to-year survival for males and females for a given deme. This was obtained using recursion equations to describe each component of reproduction and survival:

$$\begin{aligned} g_{mj}' &= \left[(Og_{fj}) \frac{(g_{mj}/2)}{P_j} \right] + Cg_{mj} + Sg_{mj} \\ g_{fj}' &= \left[(Og_{fj}) \frac{(g_{mj}/2)}{P_j} \right] + Cg_{fj} + Sg_{fj} \end{aligned} \quad (1)$$

where C is the number of ramets produced by each individual as a result of clonal propagation, and S is the proportion of adult individuals surviving from time t to time $t + 1$. We set the parameters C and S to be equal among the sexes in a given simulation. In this model, sexual reproduction occurs via random mating whereby each female produces O seed, and the frequencies of females and males in the seed progeny at time $t + 1$, depends on the proportion of male and female determining pollen grains ($Su^f S^m$ and $Su^f S^m$, respectively) in the total pollen pool. We set the gamete segregation ratios of each male to 1:1 (i.e. $g_{mj}/2$) and the total size of the pollen pool in the j th deme as P_j , which reflects the number of pollen-bearing individuals in the population ($P = g_{mj}$). Thus, 1:1 sex ratios are obtained from sexual reproduction (seed) for time $t + 1$. We assumed that sexual reproduction by females is not pollen limited, unless there are no males in the deme at time t . Although this may not reflect levels of pollen limitation experienced by small populations with skewed sex ratios, we found using more complex pollen limitation functions had little effect on the distribution of sex ratios in the metapopulation. As, for simplicity, this

model also assumes that all seeds germinate, all individuals flower each year, and there is no age-specific mortality, the time (and population size) at which equilibrium sex ratios are obtained may be underestimated.

In the next step of the simulation, seeds can disperse into the demes from a common ‘seed pool’ that is independent of the population size of local demes or whether the deme is currently occupied. We assumed that the number of seeds that immigrated into the j th deme was I_j , which varied according to a Poisson distribution with a mean of λ_j . The number of male and female individuals among the immigrant seed for the j th deme then followed a multinomial distribution with an expected probability of 0.5 for each gender. Thus, locally produced seed, ramets produced by clonal propagation, adults that survived from the previous generation, and immigrant seed all contribute to the next generation of adults. We assumed that demes were density-regulated and could maintain a maximum of K adults. Therefore, any demes with a population size of $t + 1 > K$ were truncated by multiplying the frequency of each genotype by $K/(g_{mj}' + g_{fj}')$. Finally, for each generation the demes had a constant probability E of going locally extinct. For all simulations we used an extinction probability of $E = 0.1$, and demes could only be recolonized in the following generation by seed immigration. The simulation was initialized with 100 males and 100 females in each deme and run for 8000 generations and the sex ratio and population size of all demes in the simulation were recorded every 100 generations.

Simulations were run under a restricted range of parameters to explore the importance of founder effects and modes of reproduction on sex-ratio variation among populations. We initially explored simulations across a range of seed production ($O = 2, 4, 8$ and 10) and survivorship probabilities per generation ($S = 0.2, 0.4, 0.6, 0.7$ and 0.8); however, these parameters had less effect on the distribution of sex ratios compared with the mode of reproduction and founder effects. Therefore, to isolate the effect of clonal reproduction on sex-ratio variation we held these parameters constant using representative values of $O = 2$ and $S = 0.7$. To examine the effect of founder size and mode of reproduction, separate simulations were conducted across a range of mean seed immigration rates ($\lambda_i = 1, 2, 4, 6$ and 8) and rates of clonal propagation ($C = 0, 1, 2, 4$). For each simulation we examined sex-ratio variation in relation to population size. Our choice of parameters is not intended to represent an exhaustive exploration of parameter space, but rather serves to illustrate the role of founder effects and mode of reproduction on sex-ratio variation, and to provide a qualitative comparison to the empirical data for clonal and non-clonal dioecious species.

Comparison of empirical data with model results. To investigate the influence of non-equilibrium conditions on sex-ratio variation in natural populations of clonal versus non-clonal species and compare this with our model results, we assessed the relationship between population size and sex ratio for 14 clonal and nine non-clonal species. Only species with six or more population samples and explicit population counts were included. This resulted in sex ratios for a total of 348 populations, with $n = 217$ for the clonal species (mean = 15.5,

range = 6–44) and $n = 131$ for the non-clonal species (mean = 14.6, range = 6–34).

RESULTS

Is sampling effort associated with among-population heterogeneity in sex ratio?

For the 126 species for which multiple population samples were obtained, 40.2% showed significant among-population heterogeneity in sex ratio (see Supplementary Data Table S1). There was also a significant positive relationship between heterogeneity in sex ratio and the number of populations sampled [GLM $F_{1,125} = 12.98$, $P < 0.001$; $y = e^{-1.125+0.1040x} / (1 + e^{-1.125+0.1040x})$]. The percentage of species with significant heterogeneity increased from 29%, when two populations were sampled, to around half (48%) when $n = 10$, to over 95% when >40 populations were sampled.

Does the magnitude of sex-ratio bias in populations vary with the number of non-reproductive individuals?

Among the seven dioecious species for which data were available, the proportion of non-reproductive individuals in populations had no effect on variation in sex ratio (Table 1A). However, sex ratio varied among species and there was a significant interaction between the proportion of non-reproductive individuals and species (Table 1A), indicating that the effect of non-reproductive frequency on sex ratio varied among species. In three of the seven species (*Populus deltoides*, *Oemleria cerasiformis* and *Smilax herbacea*), there was a significant positive relationship between the proportion of non-reproductive individuals in populations and their sex

TABLE 1. Results of the GLM (logistic regression) analyses to examine the effect of (A) the proportion of non-reproductive plants ($n = 7$ species), (B) altitude ($n = 5$), (C) soil moisture ($n = 5$) and (D) latitude ($n = 13$) on sex-ratio variation among populations of dioecious species

	d.f.	F	P
(A) Proportion of non-reproductive plants			
Non-reproductive proportion	1,102	0.53	0.468
Species	6,102	29.93	< 0.001
Non-reproductive proportion \times species	6,102	11.26	< 0.001
(B) Altitude			
Altitude	1,28	8.12	0.008
Species	4,28	3.32	0.024
Altitude \times species	4,28	2.07	0.112
(C) Moisture stress			
Moisture level (high, low)	1,14	14.40	0.002
Species	4,14	8.92	< 0.001
Moisture level \times species	4,14	4.07	0.022
(D) Latitude			
Latitude	1,176	7.49	0.007
Species	12,176	13.20	< 0.001
Latitude \times species	12,176	1.29	0.229

Significant P values ($P < 0.05$) are highlighted in bold.

ratios ($P < 0.001$ – 0.047); male-biased sex ratios were more common when a greater proportion of the population was non-reproductive.

Is sex-ratio bias associated with gradients of stress?

Altitude had a significant effect on sex ratio (Table 1B) and this was consistent among the five species for which data were available (altitude \times species; $P = 0.112$; Table 1B). Greater male bias was evident at higher altitudes (Fig. 1A). There was also a significant effect of soil moisture on sex ratio for the five species but this varied among species (Table 1C and Fig. 1B). In three of the five species greater male bias occurred at drier sites, but in the remaining two soil moisture was not associated with significant changes in sex ratio. Latitude had a significant effect on sex ratio (Table 1D) and this was consistent among the 13 species included in our study (latitude \times species; $P = 0.229$, Table 1D). Higher latitude populations (in the northern hemisphere) tended to be more male biased and this relationship was strongest in two species *Astilbe biternata* and *Sagittaria latifolia* (data not shown).

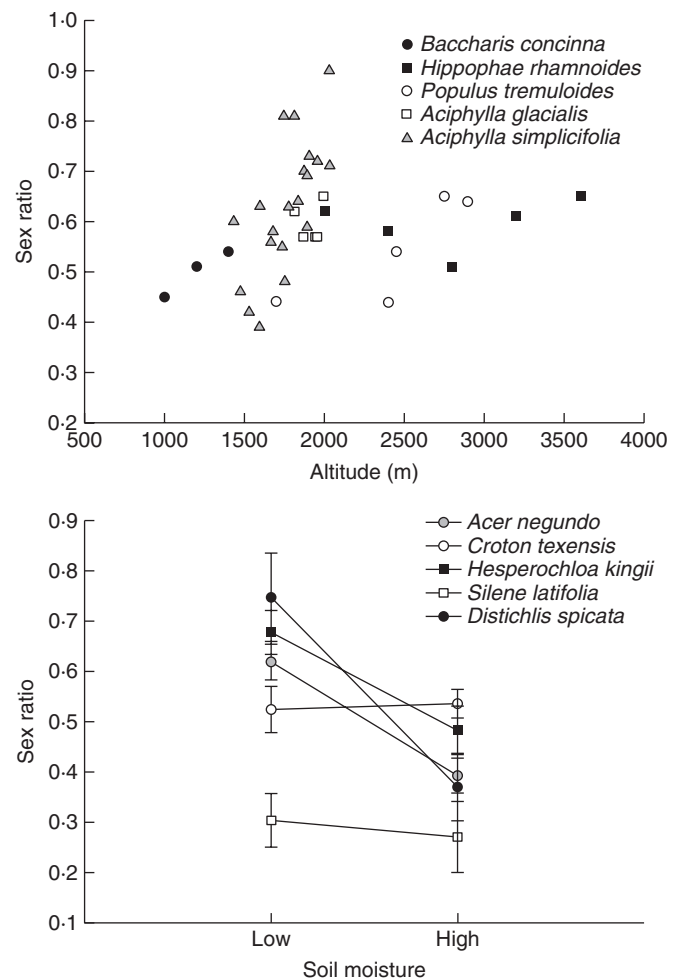


FIG. 1. Sex ratio (proportion male) variation along: (A) altitudinal gradients for five dioecious species, and (B) soil moisture gradients (low vs. high soil moisture) for five dioecious species. Vertical bars are \pm s.e.

Metapopulation dynamics and sex-ratio variation

We used a metapopulation model to examine how the mode of reproduction and number of founders affected variation in sex ratio. For non-clonal species, there was a rapid decline in the variance in sex ratio with population size (Fig. 2A, C), whereas for clonal species this decline was much slower, with highly skewed sex ratios in both large and small populations (Fig. 2B, D). The number of founders also interacted with reproductive mode to influence sex-ratio variation. For

non-clonal species, the number of founders had relatively little effect, with a similar rapid decline in variance with four (Fig. 2A) or two founders (Fig. 2C). In contrast, for clonal species the decline in variance with population size was more rapid if populations were founded by four (Fig. 2B) compared with two (Fig. 2D) individuals.

Empirical data on sex ratios exhibited similar patterns to those predicted from our metapopulation model (Fig. 2E, F). The variance in sex ratio decreased with increase in population

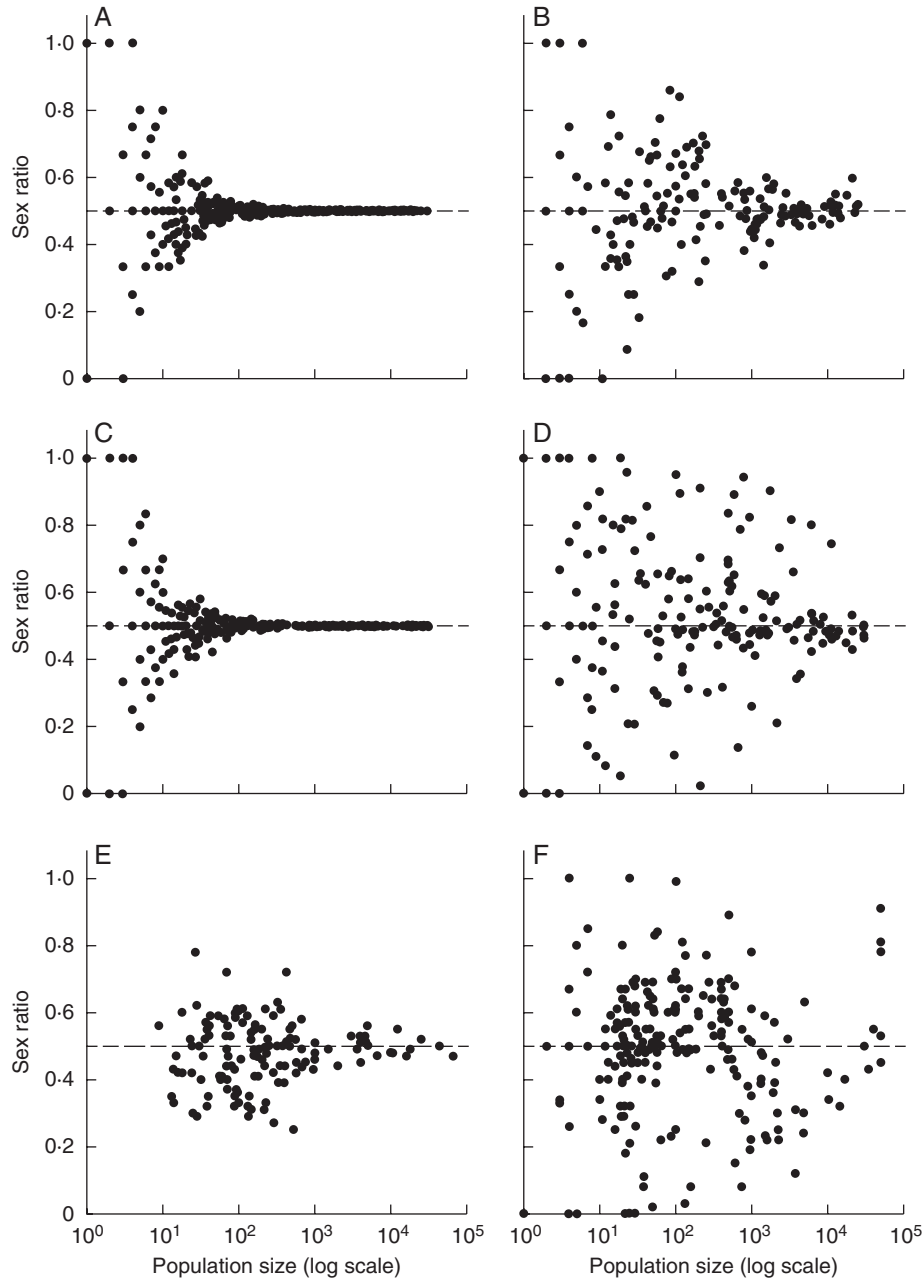


FIG. 2. Predicted and observed relationships between sex ratio (proportion male) and population size (log scale) for non-clonal and clonal populations of dioecious plant species; results of a metapopulation model to examine sex-ratio variation in populations of: (A) non-clonal and (B) clonal species with four founding individuals, (C) non-clonal and (D) clonal species with two founding individuals. The data in (E) and (F) are from natural populations of non-clonal ($n = 9$ species) and clonal species ($n = 14$), respectively. The total number of populations sampled ($n = 348$, mean per species = 14.6 (non-clonal) and 15.5 (clonal)). See Materials and Methods for details of the model and parameter values.

size, and this decline was more rapid in non-clonal species (Fig. 2E) than clonal species (Fig. 2F). For clonal species, highly skewed sex ratios (<0.3 and >0.7) were evident across a wide range of population sizes, whereas for non-clonal species these were only apparent in populations of <1000 individuals.

DISCUSSION

We found considerable heterogeneity in sex ratios among populations of dioecious flowering plants and a significant amount of this variation appears to reflect sampling effort, the demographic characteristics of populations, and differences between the sexes in their response to environmental stress. In addition, we found that founder effects and metapopulation dynamics have the potential to play an important role in shaping the equilibrium dynamics of sex ratios, especially in clonal populations. We begin by discussing patterns of sex-ratio heterogeneity and the importance of sex-specific differences in reproductive costs for among-population variation in sex ratios. We conclude by considering the potential importance of interactions between life-history traits, reproductive mode and stochastic forces for generating biased sex ratios in plant populations.

Among-population heterogeneity in sex ratios is a common feature of dioecious plant species. Of the 126 species included in our survey, approx. 40% with multiple population samples showed significant variation in sex ratios. Predictably, the likelihood of detecting sex-ratio variation among populations varied with sampling effort, with a higher probability of detecting heterogeneity when many populations were sampled. This highlights the need to sample multiple populations to obtain accurate sex ratio estimates for a species and to enable an assessment of the factors causing biased sex ratios. Indeed, sampling populations over a broad geographical range, encompassing wide environmental and demographic contexts, should enable future studies to disentangle the contribution of ecology and demography to among-population sex-ratio variation.

Influence of life history and ecological factors on sex-ratio variation

If greater reproductive costs in females results in delayed or less frequent flowering, then male bias may be expected in young populations, or those containing a greater proportion of non-reproductive individuals (Lloyd, 1973; Allen and Antos, 1993). Although not universal, in several species we found that the proportion of non-reproductive individuals in a population was indeed correlated with the degree of male bias. This pattern has also been demonstrated in systems where populations can be aged. For example, in fire-adapted *Leucadendron gandogeri* male-biased sex ratios occur in younger populations containing many non-reproductive individuals, whereas older populations, in which all plants were reproductively mature, exhibited equal sex ratios (Barrett et al., 2010). Similar patterns have been found in populations of different age in *Oemleria cerasiformis*, in which both young and older populations were male biased (Allen and Antos, 1993). In the latter case the temporal variation in sex-ratio bias was

interpreted as the result of both the delayed onset of female flowering and greater female mortality after successive years of reproduction. Future studies could usefully employ sex-specific genetic markers for determining the sex of non-reproductive individuals and for identifying at which stage during the life cycle biases become established, particularly in long-lived perennial species (e.g. Stehlik and Barrett, 2005; Shelton, 2010a).

Information on sex-specific differences in life history, such as survival and flowering, can also provide important insights into the mechanisms underlying sex-ratio bias (Meagher, 1981; Shelton, 2010b). For example, Meagher (1981) used data from long-term monitoring of *Chamaelirium luteum* populations to demonstrate that expected sex ratios, based on life-history characteristics and demographic projection models, closely matched those that were observed. This approach has rarely been used in studies of dioecious plant populations due, in part, to the lack of detailed life-history and sex-ratio data over multiple years, a challenging task for studies of multiple populations occurring in differing ecological contexts. Because sex-specific markers are only available for a few dioecious species, projection models may be useful for detecting departures from equilibrium, and for determining some of the demographic influences on among-population sex-ratio variation.

As a consequence of differences in reproductive investment, males and females may differ in susceptibility to stress and in their response to resource gradients (Delph, 1999). Among-population variation in sex ratios may therefore align with gradients in moisture (e.g. Freeman et al., 1976; Waser, 1984), altitude (e.g. Pickering and Hill, 2002) or latitude (e.g. *Sagittaria latifolia*, S. B. Yakimowski and S. C. H. Barrett, unpubl. res.). Our results varied among species, but for decreasing soil moisture and increasing altitude and latitude they generally followed patterns predicted by higher female reproductive expenditure. Greater male bias was found in populations at higher altitude (Fig. 1A) and latitude, and in those occurring in more xeric conditions (Fig. 1B), likely reflecting reduced growth and/or survival of females in more stressful environments. These results indicate the importance of environmental heterogeneity in affecting patterns of sex-ratio variation and highlight the value of sampling populations across gradients of stress.

Influence of non-equilibrium conditions and mode of reproduction on sex-ratio variation

Many populations of dioecious plants may not be at equilibrium when sampled, but the role of stochastic forces in affecting sex-ratio variation is rarely mentioned in the literature. Our simulations, which tracked variation in sex ratios among demes in a metapopulation, demonstrated that life history and founder effects are likely to play an important role in generating heterogeneity in sex ratios, particularly in populations of clonal species (Fig. 2A–D). An important assumption in our model is that population size is positively correlated with population age (i.e. time since colonization). Although this assumption may be violated in some natural populations, the similarity between our simulation results and empirical data suggests that metapopulation processes and mode of

reproduction (clonal vs. non-clonal) are important features contributing to sex-ratio heterogeneity.

The greater heterogeneity in sex ratios among small compared with large populations in our simulations indicates the role of chance events and metapopulation dynamics in producing skewed ratios. Moreover, the deeper funnel-like distribution of sex ratios produced from simulations of clonal populations (Fig. 2B, D) closely matched the empirical data on sex ratios from natural populations (Fig. 2F). These results indicate that clonal populations with limited sexual recruitment can preserve non-equilibrium sex ratios for long periods of time. We found that this effect was further accentuated in simulations with fewer founding individuals (panel B compared with panel D in Fig. 2). Founder effects and clonal propagation are important in causing departures from equilibrium morph ratios in gynodioecious (Stevens and Van Damme, 1988) and heterostylous (Eckert and Barrett, 1995) populations, but they have been overlooked as a cause of sex-ratio variation in dioecious species, in part, due to limited population sampling. Genetic estimates of population structure and migration (Obbard *et al.*, 2006), combined with measures of patch occupancy and abundance (Eppley and Pannell, 2007), are needed to confirm the potential role of metapopulation processes and non-equilibrium conditions in generating sex-ratio variation in dioecious species, especially those that are long-lived and possess dual reproductive modes.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Table S1: mean sex ratio, heterogeneity and reproductive mode (clonal, non-clonal) of flowering plant species used in the among-population within-species analyses of sex-ratio variation, together with a full list of references for the studies included.

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