RESEARCH PAPER

Environmental stressors affect sex ratios in sexually dimorphic plant sexual systems

S. Varga* & C. D. Soulsbury*  
School of Life Sciences, University of Lincoln, Joseph Banks Laboratories, Lincoln, LN6 7TS, UK

Keywords  
sexual systems; dioecy; gynodioecy; sex ratio; subdioecy; trioecy.

Correspondence  
S. Varga, School of Life Sciences, University of Lincoln, Joseph Banks Laboratories, Lincoln LN6 7TS, UK.  
Email: svarga@lincoln.ac.uk or sandrivarga30@hotmail.com

*Both authors contributed equally to this work. Both authors jointly conceived the study; SV collected the data; CS analysed the data; both authors wrote the paper.

Editor  
M. Arista

Received: 6 January 2020; Accepted: 29 March 2020

doi:10.1111/plb.13125

INTRODUCTION

The evolution and maintenance of plant sexual systems has fascinated evolutionary ecologists for decades. Individual plant gender (sensu Lloyd & Bawa 1984; i.e. maleness or femaleness as a parent of the next generation at sexual maturity) is not always fixed, but instead sex expression (i.e. whether individuals contribute to the next generation via ovules, pollen or both) occurs on a continuum and can be variable through time (see Geber et al. 1999 for a review of the terminology used to describe gender in plants). For the sake of simplicity, here we use ‘gender’ to refer to whether individuals reproduce as males (i.e. only through pollen), as females (i.e. only through ovules) or as a hermaphrodite or monoecious individual (i.e. through the production of ovules and pollen in different proportions). Most plant species are hermaphroditic, with individuals having perfect or bisexual flowers possessing both the female (i.e. seed production) and male (i.e. pollen production) sexual functions within the same flower. During plant evolution, physical separation of the female and male sexual functions in different individuals (i.e. dioecy) has independently evolved repeatedly (Charlesworth 2002; Renner 2014). There are three major pathways leading to dioecy (Goldberg et al. 2017). In the dimorphic pathway, hermaphrodites coexist with single-sexed individuals (either female first and then male in the gynodioecious pathway, or male first and female after in the androdioecious pathway), and later on hermaphrodites are replaced with the opposite gender, although the three genders may coexist temporarily (i.e. subdioecy). In the monomorphic pathway, dioecy evolves with the spread of monoecious (i.e. plants bearing pistillate and staminate flowers) individuals first and then the evolution of unisexual individuals (see Käfer et al. 2017 for more details). And lastly, in the direct pathway, separate sexes appear via reciprocal reductions in male and female function of the style morphs. The macroevolutionary pathways in plant sexual system evolution are complex, with support for transitions both towards and away from sexual differentiation (Goldberg et al. 2017; Käfer et al. 2017). In addition, there are potentially multiple underlying genes and pathways underpinning sexual differentiation (Henry et al. 2018), highlighting the complex nature of plant sexual system evolution.

According to the different sexual phenotypes present, plant populations can be classified as: hermaphroditic (only hermaphrodite plants present, plants containing only bisexual flowers), monoecious (plants containing both pistillate and staminate flowers), dioecious (with female plants containing only pistillate flowers and male plants containing only staminate flowers), gynodioecious (with female plants containing only pistillate flowers and hermaphrodite plants containing bisexual flowers), androdioecious (with male plants containing staminate flowers and hermaphrodite plants containing bisexual flowers), and subdioecious or trioecious (with female plants containing only ovules and male plants containing only pollen).
containing only pistillate flowers, male plants containing staminate flowers and with hermaphrodite/monoecious plants containing both pistillate and staminate flowers on the same individual or bisexual flowers).

The sex ratios of plant populations are governed by several factors, including the relative fitness of each sexual morph and the inheritance of sex. Such modes of inheritance are complex (see e.g. Chase 2007, Sloan 2015 for discussions on this topic). Nevertheless, once separate sexes have arisen, population sex ratios (i.e. the proportion of females or male-sterile genets within a population) are predicted to vary not only due to the underlying genetic mechanisms of gender determination, but also due to ecological factors that can affect the relative seed and pollen production (see e.g. Dorken & Pannell 2008). Population sex ratios will ultimately be the result of negative frequency-dependent selection (Fisher 1930; Clarke et al. 1988). This is relatively well established for dioecious and gynodioecious species in connection with pollen limitation. In dioecious systems the rarer sex is the most fit and will be selected for, driving towards a 1:1 sex ratio, whereas in gynodioecious systems, sex ratios are predicted to vary more when male sterility is cytoplasmic rather than when it is nuclear (see Charlesworth 1981; Frank 1989; Gouyon et al. 1991; McCauley & Brock 1998; McCauley & Bailey 2009), even though variation will also depend on the degree of self-compatibility and inbreeding (see e.g. Purrigton and Schmitt 1998). McCauley and Bailey (2009) even though variation will also depend on the degree of self-compatibility and inbreeding depression (Yamauchi et al. 2019), but pollen limitation will limit female frequency when they become too abundant (e.g. Spigler & Ashman 2012). Therefore, understanding the ecological and environmental context of sex ratio variation is important for elucidating the selective forces that act on sexual polymorphism evolution and maintenance, irrespective of the mechanism.

Regardless of the sexual system, biased sex ratios can be the result of biased primary (i.e. seed) sex ratios (e.g. Stelllik et al. 2008), different germination requirements between the genders (e.g. Purrigton and Schmitt 1998), sex lability (e.g. Korpelainen 1998) or different gender-associated mortality associated with the costs of maintaining each sexual function (e.g. Obeso 2002). Indeed, when to start reproducing and how many resources should be allocated to reproduction are key factors determining plant fitness (Reekie & Bazzaz 2005). Unless large amounts of pollen are produced compared to amounts of seed (e.g. for wind-pollinated plants), the costs of producing seed are generally larger than the costs of producing pollen, even though this will depend on the currency used to measure reproductive costs (see Ashman 1994; Obeso 2002). Reproductive allocation is usually larger in females compared to males in dioecious systems, and similar or smaller in gynodioecious systems (e.g. Ashman 1994, Gibson & Diggle 1997, Van Etten et al. 2008), imposing important reproductive costs and trade-offs with other plant functions, such as growth and defence. Even though physiological and demographic compensation mechanisms exist to mitigate these reproductive costs, the availability of resources and the ecological context that plants encounter is expected to have profound effects on sex ratios. In dioecious plants, females appear more sensitive to the costs of reproduction as seed production is usually more costly than pollen production, whereas in gynodioecious species, hermaphrodites appear to pay a higher cost of reproduction than females because, in addition to seeds, hermaphrodites also produce pollen and usually have larger flower displays and rewards for pollinators (Ashman 1999; Geber et al. 1999; Obeso 2002; Shykoff et al. 2003).

The link between how environmental stressful conditions may impact sex ratios will ultimately be the result of the sexes experiencing the environmental pressures in a different way (Retuerto et al. 2018), which will determine differences in seed germination, generation-associated mortality or sex lability. The role of environmental conditions and their relationship to plant sex ratio has been observed since Darwin’s time. Whilst observing the gynodioecious Thymus serpyllum, Darwin wrote: “a very dry station apparently favours the presence of the female form” (Darwin 1877: p. 301). He further stated, “with some of the other above-named Labiatae the nature of the soil or climate likewise seems to determine the presence of one or both forms” (Darwin 1877: p. 301). This observation has been replicated many times; biased sex ratios have been reported in more than half of the dioecious species studied, with the majority of studies showing a sex ratio biased towards males, particularly in less favourable environments (Barrett et al. 2010), where female plants, possessing the relatively more costly gender, may experience higher mortality. In contrast, the reverse pattern tends to be reported in gynodioecious systems, with higher female frequencies linked to more stressful environments (e.g. Webb 1979, Ashman 1999, Asikainen & Mutikainen 2003), because reproductive allocation, and therefore costs, are usually similar or larger in hermaphrodite plants compared to female plants in this sexual system (e.g. Delph 1990, Ashman 1994). In subdioecious systems, females seem to be more common in more stressful habitats (Ashman 2006; Spigler & Ashman 2011). Thus, in all three systems, environmental stressors strongly impact female frequency, but seem to do so in opposite directions.

Environmental stressors known to affect plant sex ratios include water, light and nutrient availability, temperature, changes in CO₂ and O₂ or UV, and in most cases, environmental stresses induce maleness in plants (Sinclair et al. 2012; Field et al. 2013; Hultine et al. 2016; Retuerto et al. 2018). As yet, a broad understanding of how different types of environmental stressors influence intraspecific variation in female frequency is lacking. As discussed above, studies that focus on a single or a few species have made some predictions about how different plant sexual systems respond to environmental stressors, but as yet there has been no broad attempt to elucidate which environmental stressors affect sex ratio variation and in which direction, and no comparison between different sexual systems across broad environmental gradients exists. Here, we tested whether the same environmental stressors affect female frequency in dioecious, gynodioecious and subdioecious systems, and investigated the direction and magnitude of such effects. We further analysed whether the terrestrial biome where the population is located has a significant effect so as to more broadly test how female frequency may vary with climate conditions. We hypothesized that the relation between female frequency and stress level would differ among sexual systems, and that the importance of different stressors will differ among sexual systems, as the different sexes are differently affected by different abiotic stressors (Retuerto et al. 2018 and references therein).
MATERIAL AND METHODS

For our literature survey we ran a search in January 2017 using the ISI Web of Knowledge, with the search terms ‘(dioec* OR gynodioec* OR subdioec* OR tricioe*) AND (sex ratio OR frequency OR proportion) AND plant*’, which resulted in 991 articles. From these, we read each paper and secondary references. We included in our dataset only those studies reporting the exact location and the frequency of females (N = 263 papers, see Appendix S1: Data S1 for the full reference list).

The sexual system for each plant species was extracted as specified by the authors of each paper. Specifically, plants were classified as belonging to dioecious sexual systems when only female and male plants were observed within the population; gynodioecious when only female and hermaphrodite plants were observed within the population; and subdioecious when female, male and hermaphrodite plants were observed or when populations contained monococious plants. As noted earlier, sex expression, and therefore plant gender, may be variable among populations or over time. Discrepancy in the plant sexual system was noted for eight species. Old reports for Myrica gale and Thymelaea hirsuta noted the presence of some monoecious individuals between dioecious populations, but because recent, more comprehensive surveys report them as fully dioecious, we decided to classify them as dioecious. For Arisaema triphyllum, Atriplex canescens and Buchloë dactyloides, populations have been reported to be dioecious and subdioecious; and for Fuchsia microphylla, Ochradenus baccatus and Silene acaulis, populations have been reported as gynodioecious and subdioecious. In these cases, we classified them all as subdioecious. Because of the low number of cases, this classification did not affect the main findings.

Climate variables and biomes

Global climate data were downloaded from WorldClim at 30 arc-sec resolution (Hijmans et al. 2005, http://www.worldclim.org/) and all 19 bioclimatic variables were extracted for each location (O’Donnel & Ignizio 2012, http://www.worldclim.org/bioclim). We selected bioclimatic variables based on the following criteria: (1) we chose extreme or limiting environmental conditions that are known to impact plant growth and reproduction (temperature, precipitation); (2) we selected stressors that covered seasons (quarters), rather than maximum or minimum values; and (3) finally, we selected variables that are stressful, e.g. extremes of temperature (cold and warm), driest conditions versus wettest. This left us with three variables for temperature (Bio9: mean temperature of the driest quarter, Bio10: mean temperature of the warmest quarter, Bio11: mean temperature of the coldest quarter) and three equivalent for precipitation (Bio17: precipitation of the driest quarter, Bio18: precipitation of the warmest quarter, Bio19: precipitation of the coldest quarter) that were included in the models. These variables have strong links with plant growth and physiology as they represent heat/cold tolerance and drought/moisture tolerance and, together, tolerance to seasonal temperature and water variation, which can be directly related to stress. We checked for multicollinearity between variables using variance inflation factors (VIF) using a custom function. For the majority of these, VIF < 5.0 (range 2.0–4.6), but for dioecy two variables were > 5.0 (5.3 and 6.1). Although higher than we would like, they are still lower than thresholds where this may be an issue (i.e. > 10; Freckleton 2011). Finally, for each location, we include the terrestrial biome where the population was located (defined by Olson et al. 2001). This allowed us to more broadly test how female frequency may vary with climate conditions.

Phylogeny and final dataset

We constructed a time-calibrated plant phylogeny by grafting the families, genera and species included in our study onto a backbone phylogeny in the R package V.PhyloMaker (Jin & Qian 2019). The backbone of this supertree was the PhytoPhylo mega-phylogeny, an updated and expanded version of a previous species-level phylogeny (Zanne et al. 2014). Genera and species that were not found in the mega-phylogeny were handled by randomly adding species within their families where possible (see Fig. 1). The reported populations covered the five continents, even though some biomes were underrepresented (Olson et al. 2001; Appendix S2: Figure S1).

Data analysis

All statistical analyses were performed with R (R Core Team, 2018). We first tested whether female frequency was determined by different bioclimatic variables for each sexual system using phylogenetic mixed models (PMM) in the R package MCMCglmm (Hadfield 2010). All bioclimatic variables were introduced simultaneously, with the inclusion of a phylogenetic covariance matrix, with species retained as a second random effect within the models. We set parameter-expanded uninformative priors, a total of independent chains of 500,000 iterations, with sampling taking place every 500 iterations after a 10,000 burn-in. The phylogenetic heritability was estimated by dividing the variance explained by the phylogeny by the sum of all variance components. The phylogenetic heritability varies between 0 and 1, where 0 represents no evolutionary signal (no covariance in the residuals due to shared ancestry), and 1 indicates that the observed covariance in residuals follows that expected under a Brownian motion model of trait evolution (Freckleton et al. 2002).

We then tested the female frequency in relation to biome for each sexual system separately, using a linear mixed model (LMM), with species fitted as a random effect. In this case, we chose not to carry out a PMM, as global biotic units are characterized by similar vegetation characters, which are themselves phylogenetically determined. We considered that such circularity would mean that carrying out a PMM was of little value in this case. We concluded our analysis by carrying out a Pearson’s correlation between average female frequencies of each plant sexual system from each biome.

Among the bioclimatic variables, stressful conditions for plants are expected to occur at the highest or lowest values, depending on the type of environmental stressor. For example, the most stressful condition in the warmest quarter is at high temperatures, and during the coldest quarter it is at low temperatures. To counteract this non-intuitive scaling, we transformed all effect sizes so that they occurred on the same scale, i.e. a positive effect indicates an increase in female frequency with greater environmental stress, whereas a negative effect reflects a reduction in female frequency.
RESULTS
Differences in female frequency between sexual systems and across biomes

Female frequency ranged from 0 to 100% in all sexual systems (Figs 1, 2A). There was a significant relationship between female frequency and sexual system (Intercept (dioecious) = 44.9 (17.43/73.07); gynodioecious: posterior mean (95% CI) = 24.13 (19.06/29.33), pMCMC < 0.001; subdioecious: posterior mean (95% CI) = 4.98 (0.69/10.59), pMCMC = 0.223). Female frequency was close to 50% in dioecious (46.0 ± 0.6%) and subdioecious (48.4 ± 1.0%) systems, but gynodioecious species had significantly lower female frequency (25.0 ± 1.0%) than both dioecious and subdioecious sexual systems (Fig. 2A).

There was no difference in female frequency between biomes for gynodioecy ($F_{12,159.25} = 1.074$, $P = 0.385$), but we detected significant differences for subdioecy ($F_{11,141.04} = 7.34; P < 0.001$; Fig. 3) and dioecy ($F_{10,478.54} = 2.29; P = 0.012$; Fig. 3). For gynodioecious species, female frequencies were lowest in tundra, boreal forests and flooded grasslands and in savannas (biomes 11, 6, and 9) and highest in Mediterranean forest and...
tropical/subtropical grasslands (biomes 12 and 7; Fig. 3). For dioecious species, female frequencies were lowest in temperate broadleaved forest (biome 4) and highest in tundra and boreal forests (biome 11; Fig. 3). Finally, subdioecious species had lowest female frequency in tundra and Mediterranean forests (biomes 11 and 12) and highest in deserts/xeric environments, boreal forests and taiga and tundra (biomes 13, 11 and 6; Fig. 3). Across biomes, there was a significant negative correlation between average female frequency of gynodioecious and subdioecious plants from each biome.

Relationships between female frequency and climate variables

We found that different bioclimatic stressors were statistically affecting female frequency, depending on the sexual system. For gynodioecious plants, female frequency was explained by the mean temperatures of the coldest and driest quarters (Table 1). The mean temperature of the driest quarter was positively correlated with female frequency, whereas the mean temperature of the coldest quarter was negatively correlated with female frequency (Table 1; Fig. 4A). Similarly, in subdioecious systems, female frequency was also positively correlated to mean temperature of the warmest quarter and precipitation of the coldest quarter, and further negatively correlated with the mean temperature of the coldest quarter (Table 1; Fig. 4C). In contrast, none of the bioclimatic parameters were statistically significant in dioecious systems (Table 1; Fig. 4B).

Stressor direction and female frequency

Focusing on the direction of the stressor to female frequency, in gynodioecious sexual systems, two out of the six climatic stressors were significant in the PMM (Table 1); in both of these, higher female frequency was associated with greater temperature stress (Fig. 4A). In contrast, female frequency was unrelated to environmental stressors in dioecious plants (Fig. 4B). For subdioecious systems, four out of the six climatic stressors in the PMM were significant. Of these, female frequency was greater with higher temperature stress during the coldest and warmest quarters (Fig. 4C), but larger female frequencies at lower precipitation stress in the driest quarter and higher precipitation stress during the coldest quarter.

DISCUSSION

Across all three sexual systems, there were key differences in the type of environmental stressors that were impacting female frequency. Environmental variables were significantly impacting female frequency in subdioecious and correlated with mean temperature of the warmest quarter and precipitation of the coldest quarter, and further negatively correlated with the mean temperature of the coldest quarter (Table 1; Fig. 4C). In contrast, none of the bioclimatic parameters were statistically significant in dioecious systems (Table 1; Fig. 4B).

![Fig. 2.](image-url) (A) Beeswarm plot of female frequency (%) in relation to plant sexual system in dioecious (dark blue), subdioecious (dark green) and gynodioecious (yellow) sexual systems, and (B) scatterplot showing the significant correlation between average female frequency of gynodioecious and subdioecious plants from each biome.

![Fig. 3.](image-url) Mean female frequency ± SE (%) in each biome for dioecious (dark blue), subdioecious (dark green) and gynodioecious (yellow) plant breeding systems.
Dioecy differs from the other two sexual systems. Dioecy is functionally different from hermaphroditism and gynodioecy, and it is expected, based on the dual function of the sexes, that dioecy systems would be most responsive to environmental stressors. Therefore, female frequency in dioecious systems is likely to be more affected by environmental stressors than in gynodioecious sexual systems, but not in dioecious sexual systems, where none of the environmental variables explained female frequency. This may suggest that dioecy is a sexual system that is less responsive to environmental stressors impacting sex ratio variation than gynodioecy or subdioecy, or that environmental factors act in a more species-specific manner.

### Differences among sexual systems

We could corroborate our hypothesis that among sexual systems, different environmental stressors affect female frequency, joining previous studies showing different responses of the sexes to environmental stresses (Retuerto et al. 2018). In particular, female frequencies in gynodioecy and subdioecy increased with increased environmental stress, and moreover, female frequencies from the same biome in these two systems were negatively correlated to each other, emphasizing the idea that dioecy differs from the other two sexual systems.

We found that as environmental stressors became stronger, there was an increased female frequency in gynodioecious and subdioecious systems. This makes sense if we consider the costliness of the sexes; in gynodioecious systems, the costliest sex is hermaphrodites because of the dual allocation to pollen and seed production, including costs of maintaining male and female function simultaneously, inbreeding depression and pollinator type and limitation (Ashman 2006). In subdioecy, the situation is more complex because of the coexistence of three sex phenotypes; sex ratio in this system is also strongly

---

**Table 1.** Results from the phylogenetic analysis (pMCMC) showing the effect of environmental variables on female frequency, with phylogenetic covariance matrix fitted as a random effect. Statistically significant values (pMCMC ≤ 0.05) are indicated with boldface. We include Pagel’s λ (mean ± 95% CI) calculated from each model.

| Sexual system | Variable | Estimate | Lower 95%CI | Upper 95%CI | pMCMC |
|---------------|----------|----------|------------|-------------|-------|
| Gynodioecy    | Intercept| 12.217   | −38.478    | 61.812      | 0.600 |
| N = 63 species| Mean Temp Driest Q (Bio9)| 0.150 | 0.082 | 0.226 | 0.001 |
| n = 495 populations| Mean Temp Warmest Q (Bio10)| −0.001 | −0.099 | 0.092 | 1.000 |
| λ = 0.67 (0.48/0.84)| Mean Temp Coldest Q (Bio11)| −0.149 | −0.247 | −0.054 | 0.003 |
|                | Precipitation Driest Q (Bio17)| 0.017 | −0.040 | 0.073 | 0.551 |
|                | Precipitation Warmest Q (Bio18)| 0.014 | −0.017 | 0.045 | 0.377 |
|                | Precipitation Coldest Q (Bio19)| −0.027 | −0.063 | 0.007 | 0.122 |
| Dioecy         | Intercept| 34.282   | 9.673      | 60.381      | 0.008 |
| N = 242 species| Mean Temp Driest Q (Bio9)| 0.013 | −0.020 | 0.043 | 0.442 |
| n = 1128 populations| Mean Temp Warmest Q (Bio10)| 0.030 | −0.010 | 0.065 | 0.129 |
| λ = 0.70(0.59/0.80)| Mean Temp Coldest Q (Bio11)| −0.028 | −0.065 | 0.009 | 0.139 |
|                | Precipitation Driest Q (Bio17)| 0.009 | −0.015 | 0.031 | 0.462 |
|                | Precipitation Warmest Q (Bio18)| 0.000 | −0.012 | 0.011 | 0.918 |
|                | Precipitation Coldest Q (Bio19)| 0.007 | −0.001 | 0.017 | 0.127 |
| Subdioecy      | Intercept| 24.813   | −83.825    | 119.519     | 0.613 |
| N = 37 species | Mean Temp Driest Q (Bio9)| −0.009 | −0.051 | 0.028 | 0.664 |
| n = 388 populations| Mean Temp Warmest Q (Bio10)| 0.103 | 0.024 | 0.183 | 0.014 |
| λ = 0.97 (0.95/0.99)| Mean Temp Coldest Q (Bio11)| −0.014 | −0.018 | −0.051 | 0.002 |
|                | Precipitation Driest Q (Bio17)| 0.080 | 0.035 | 0.125 | 0.001 |
|                | Precipitation Warmest Q (Bio18)| 0.005 | −0.025 | 0.038 | 0.716 |
|                | Precipitation Coldest Q (Bio19)| −0.032 | −0.054 | −0.010 | 0.005 |

Q: quarter.

*Environmental stress and plant sex ratios* (2020) by Varga and Soulsby. Plant Biology published by John Wiley & Sons Ltd on behalf of German Society for Plant Sciences, Royal Botanical Society of the Netherlands.
linked to how fitness is obtained: via the male or the female function (Maurice & Fleming 1995; Wolf & Takebayashi 2004; Ehlers & Bataillon 2007), which probably reduces the flexibility of female frequency variation due to environmental stress. Nevertheless, the evidence for this is limited, and we still lack a broad analysis of sex-specific physiology, morphology and life history (Case and Ashman 2005).

The relative importance of different environmental stressors

The incidence and frequency of different sexual systems across environmental gradients is closely related to intrinsic plant life history characteristics, such as growth form, clonal habit and pollen and seed dispersal mechanisms (Thomson & Barrett 1981; Loveless & Hamrick 1984; Werren & Beukeboom 1998; Vamosi et al. 2003; Barrett et al. 2010; Field et al. 2012; Moeller et al. 2017). In addition to these, we found key differences in the types of stressors that were important for female frequency in each sexual system. After correcting for phylogenetic relationships, for gynodioecious sexual systems temperature appeared as the major factor in determining female frequency, while in subdioecious systems precipitation was an additional significant factor. Even though few studies have dissected the separate effect of temperature and precipitation, in gynodioecious systems it has been suggested that temperature is the driving force behind the female frequency–latitude relationship (Ruffatto et al. 2015). Our analysis corroborates this. More specifically, the mean temperature during the coldest and driest quarters were significantly affecting female frequency (Table 1). Temperature is likely to be most important, as gynodioecious systems generally have a more boreal–temperate–Mediterranean distribution (Caruso et al. 2016; Fig. S1), which makes them less likely to be water-limited during the growing season. Moreover, individual studies support the same pattern of greater female frequencies with higher temperature stress (e.g. Lobelia siphilitica: Caruso & Case 2007; Daphne laureola: Alonso & Hererra 2001). The specific mechanism causing higher female frequencies at increased temperature stress is not clear, but is probably the result of simultaneous effects at the flower, pollen and seed production levels (Hedhly et al. 2009); as for example, a reduced

**Fig. 4.** PMM effects and 95% CI for (A) gynodioecious, (B) dioecious and (C) subdioecious systems. A positive effect size indicates increasing female frequency, whereas a negative effect size indicates a decreasing female frequency. Variables that are significant from the PMM are indicated with black points.
male fitness caused by impaired pollen performance at higher or lower temperatures is well established (Delph et al. 1997; Zinn et al. 2010; Iossa 2019). Irrespective of the mechanism, this analysis across species emphasizes the importance of temperature in modifying female frequency and population sex ratios in gynodioecious plants.

As in gynodioecious systems, temperature-related stressors had similar positive effects on female frequency for subdioecious systems (Table 1). However, in this system there was also a significant effect of precipitation-related stressors on female frequency. Many subdioecious species typically have a more xeric distribution than gynodioecious and dioecious species, and most growth and reproduction in desert plants occurs during and immediately after the rainy season (e.g. Wolfe & Schmida 1997). Therefore, even though extreme as well as seasonal changes in precipitation may dramatically affect all plant sexual systems in general (Zeppel et al. 2014), it is perhaps more evident in subdioecious plants. From our study, it appears that greater precipitation stress in the coldest quarter, but also lower precipitation stress in the driest one, would favor greater female frequency. Several studies have reported that gender dimorphism is more prevalent in drier conditions, which is probably related to the costs of reproduction of the different genders, in addition to the effects on pollination and mating patterns of plants (e.g. Case & Barrett 2004, Vaughton & Ramsey 2004).

It is important to keep in mind that sexual plasticity (i.e. sexual lability or gender diphany) in response to the environment has been documented in members of all three sexual systems (Korpelainen 1998; Vega-Frutis et al. 2014). This fact highlights the need for long-term observational studies, where the same plant individuals are monitored for several years to ensure reliable estimates of population sex ratios.

CONCLUSIONS

In summary, we found intrinsic differences in drivers of female frequencies across sexual systems. Temperature stress positively affects female frequency in gynodioecious and subdioecious plants, whereas precipitation stress positively and negatively affects female frequency in subdioecious plants. Our results support the idea that environmental stressors act as important precursors to plant sexual system change (Ashman 2006), especially by increasing the frequency of females in the population in gynodioecious systems. In most instances, the exact mechanisms underpinning environment-controlled sex ratios may be still unknown, but our study shows the sex ratios of flowering plants is variable and can respond to environmental conditions. In light of the climate emergency, future studies examining plant sexual system evolution due to increased environmental stress are needed, alongside disentangling the role that water-related or temperature-related stressors and their synergistic effects have on plant reproduction and fitness.

ACKNOWLEDGEMENTS

The authors thank Stuart Humphries for computing facilities, all the authors that sent additional information when requested and two reviewers for providing constructive criticism on a previous draft. SV was supported by H2020 Marie Sklodowska-Curie IF (grant agreement 660104).

Data Availability Statement

Data are available on FigShare (https://doi.org/10.6084/m9.figshare.12280298).

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Full reference list of the papers included in our study.

Figure S1. Global distribution of the populations included in this study coloured to signal plant sexual system (dioecious: dark blue, subdioecious: dark green, gynodioecious: yellow).

REFERENCES

Alons C., Herrera C.M. (2001) Neither vegetative nor reproductive advantages account for high frequency of male-steriles in southern Spanish gynodioecious Daphne laureola (Thymelaeaceae). American Journal of Botany, 88, 1016–1024.

Ashman T.-L. (1994) Reproductive allocation in hermaphrodite and female plants of Sidalcea oregana ssp. psiatii (Malvaceae) using four currencies. American Journal of Botany, 81, 433–438.

Ashman T.-L. (1999) Determinants of sex allocation in a gynodioecious wild strawberry: implications for the evolution of dioecy and sexual dimorphism. Journal of Evolutionary Biology, 12, 648–661.

Ashman T.-L. (2006) The evolution of separate sexes: a focus on the ecological context. In: Harder L.D., Barrett S.C.H. (Eds), Ecology and evolution of flowers. Oxford University Press, New York, USA, pp 204–222.

Asikainen E., Mutikainen P. (2003) Female frequency and relative fitness of females and hermaphrodites in gynodioecious Geranium sylvaticum (Geraniaceae). American Journal of Botany, 90, 226–234.

Barrett S.C.H., Yakimowski S.B., Field D.L., Pickup D.L. (2010) Ecological genetics of sex ratios in plant populations. Philosophical Transactions of the Royal Society of London, series B: Biological Sciences, 365, 2549–2557.

Bierzychudek P., Eckhart V. (1988) Spatial segregation of the sexes of dioecious plants. The American Naturalist, 132, 34–43.

Caruso C.M., Case A.L. (2007) Sex ratio variation in gynodioecious Lobelia siphilitica: effects of population size and geographic location. Journal of Evolutionary Biology, 20, 1396–1405.

Caruso C.M., Eisen K., Case A.L. (2016) An angiosperm-wide analysis of the correlates of gynodioecy. International Journal of Plant Sciences, 177, 115–121.

Case A.L., Ashman T.-L. (2005) Sex-specific physiology and its implications for the cost of reproduction. In: Reeke E.G., Bazzaz F.A. (Eds), Reproductive allocation in plants. Elsevier/Academic Press, Cambridge, MA, USA, pp 129–157.

Case A.L., Barrett S.C.H. (2004) Environmental stress and the evolution of dioecy: Warmbea duva (Colchicaceae) in Western Australia. Evolutionary Ecology, 18, 145–164.

Charlesworth D. (1981) Allocation of resources to male and female functions in hermaphrodites. Biological Journal of the Linnean Society, 15, 57–74.

Charlesworth D. (2002) Plant sex determination and sex chromosomes. Heredity, 88, 94–101.

Chase D.L. (2007) Cytoplasmic male sterility: a window to the world of plant mitochondrial–nuclear interactions. Trends in Genetics, 23, 81–90.

Clarke B.C., Shelton P.R., Mani G.S. (1988) Frequency-dependent selection, metrical characters and molecular evolution. Philosophical Transactions of the Royal Society of London, series B Biological Sciences, 319, 631–40.

Core Team R. (2018) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria Available from: https://www.R-project.org.

Darwin C.R. (1877) The different forms of flowers on plants of the same species. John Murray, London, UK.

Delph L.F. (1990) Sex-differential resource allocation patterns in the subdioecious shrub Hebe subalpina. Ecology, 71, 1342–1351.

Delph L.F., Johannsson M.H., Stephenson A.G. (1997) How environmental factors affect pollen
Environmental stress and plant sex ratios

Sloan D.B. (2015) Using plants to elucidate the mechanisms of cytoplasmic co-evolution. New Phytologist, 205, 1040–1046.

Spigler R.B., Ashman T.L. (2011) Sex ratio and dioecy in Fragaria virginiana: the roles of plasticity and gene flow examined. New Phytologist, 190, 158–168.

Spigler R.B., Ashman T.L. (2012) Dioecy to dioecy: are we there yet? Annals of Botany, 109, 531–543.

Stehlik I., Friedman J., Barrett S.C.H. (2008) Environmental influence on primary sex ratio in a dioecious plant. Proceedings of the National Academy of Sciences, USA, 105, 10847–10852.

Thomson J.D., Barrett S.C.H. (1981) Selection for outcrossing, sexual selection, and the evolution of dioecy in plants. The American Naturalist, 118, 443–449.

Vamosi J.C., Otto S.P., Barrett S.C.H. (2005) Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. Journal of Evolutionary Biology, 18, 1006–1018.

Van Etten M.L., Prevost L.B., Degen A.C., Ortiz B.V., Donovan L.A., Chang S.M. (2008) Gender differences in reproductive and physiological traits in a gynodioecious species, Geranium maculatum (Geraniaceae). Evolutionary Ecology, 18, 323–341.

Vega-Frutos R., Macias-Ordonez B.Guerrera R. Fromhage L. (2014) Sex change in plants and animals: a unified perspective. Journal of Evolutionary Biology, 27, 667–675.

Webb C.J. (1979) Breeding systems and the evolution of dioecy in New Zealand aroid Umbelliferae. Evolution, 33, 662–672.

Werren J.H., Beukeboom L.W. (1998) Sex determination, sex ratios, and genetic conflict. Annual Review of Ecology and Systematics, 29, 233–261.

Weitz D.E., Takebayashi N. (2004) Pollen limitation and the evolution of androdioecy from dioecy. The American Naturalist, 163, 122–137.

Wolfe L.M., Shimida A. (1997) The ecology of sex expression in a gynodioecious Israeli desert shrub (Ochrardes baccatus). Ecology, 78, 101–110.

Yamauchi A., Yamagishi T., Booston R., Telschow A., Kudo G. (2019) Theory of covolution of cytoplasmic male-sterylity, nuclear restorer and selving. Journal of Theoretical Biology, 477, 96–107.

Yang J., Hu L., Wang Z., Zhu W., Meng L. (2014) Responses to drought stress among sex morphs of Oxyria sinensis (Polygonaceae), a subdioecious perennial herb native to the East Himalayas. Ecology and Evolution, 4, 4033–4040.

Zaren A.E., Tatak D.C., Connwell W.K., Eastman L.M., Smith S.A., FitzJohn R.G., … Beaulieu J.M. (2013) Three keys to the radiations of angiosperms into freezing environments. Nature, 506, 89–92.

Zeppel M.I.B., Wilks J.I., Lewis J.D. (2014) Impacts of extreme precipitation and seasonal changes in precipitation on plants. Biogeosciences, 11, 3083–3093.

Zinn K.E., Tun-Ondermier M., Harper J.F. (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. Journal of Experimental Botany, 61, 1959–1968.