DR. SANDRA VARGA (Orcid ID : 0000-0001-9799-714X)

Running Head: Environmental stress and plant sex ratios.

Environmental stressors affect sex ratios in sexually dimorphic plant sexual systems

Sandra Varga[1][*] and Carl D. Soulsbury[*]

School of Life Sciences, University of Lincoln, Joseph Banks Laboratories, Lincoln LN6 7TS, UK.

[1]Author for correspondence: svarga@lincoln.ac.uk or sandravarga30@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.

Key words: sexual systems, dioecy, gynodioecy, sex ratio, subdioecy, trioecy.

Abstract

- Revealing the environmental pressures determining the frequency of females amongst populations of sexually dimorphic plants is a key research question. Analyses of sex-ratio variation have been mainly done in dioecious plants, which misses key plant sexual systems that might represent intermediate stages in the evolution of dioecy from hermaphroditism.

- We investigated female frequency across populations of sexually dimorphic plant species in relation to environmental stressors (temperature, precipitation), totaling 342 species, 2011 populations, representing 40 orders and 3 different sexual systems (dioecy, gynodioecy, and subdioecy). We also included the biome where the population was located to test how female frequency may vary more broadly with climatic conditions.

- After correcting for phylogeny, our results for gynodioecious systems showed a positive relationship between female frequency and increased environmental stress, with the main effects being temperature-related. Subdioecious systems showed also strong positive relationships with temperature and positive and negative relationships related to precipitation, whilst no significant effects on sex ratio in dioecious plants were detected.

- Combined, we show that female frequencies in intermediate sexual system on the pathway from hermaphroditism to dioecy respond strongly to environmental stressors and have different selective agents driving female frequency.

Introduction

The evolution and maintenance of plant sexual systems has been fascinating evolutionary ecologists for decades. Individual plant gender (*sensu* Lloyd and 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 as 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 males in the gynodioecious pathway; or male first and females 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 gene 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 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 the 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 the 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 and 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 conection with pollen limitation. In dioecious systems the rarer sex is the most fit and will be selected for, driving to a 1:1 sex ratio, whereas in gynodioecious systems, sex ratios are predicted to vary more when male sterility is cytoplasmic than when it is nuclear (see Charlesworth 1981, Frank 1989, Gouyon et al. 1991; McCauley and Brock 1998; McCauley an 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 and 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. Stehlik 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 and Bazzaz 2005). Unless large amounts of pollen are produced compared to seeds (for example for wind pollinated plants), the costs of producing seeds are generally larger than the costs of producing pollen, even though this will depend on the currency used to measure reproductive costs (see Obeso 2002, Ashman 1994).

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 and Diggle 1997, Van Etten et al. 2008), imposing important reproductive costs and trade-offs with other plant functions such as growth and defense. 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 display larger flower display 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, gender-associated mortality, or sex lability. The role of environmental conditions and their relationship to plant sex ratio has been observed since Darwin's time (Darwin 1877). 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 carried on "*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 greater 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 and Mutikainen 2003), because reproductive allocation and therefore costs, are usually similar or larger in hermaphrodite plants compared to female ones 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 and 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 CO_2 and O_2, 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, 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 was located has a significant effect to more broadly test how female frequency may vary with climatic conditions. We hypothesised 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).

Materials and Methods

For our literature survey we ran a search in January 2017 using ISI Web of Knowledge with the search terms '(dioec* OR gynodioec* OR subdioec* OR trioec*) 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 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 subdioecy when female, male, and hermaphrodite plants were observed or when populations contained monoecious plants. As noted earlier, sex expression and therefore plant gender may be variable among populations or time. Discrepancy in the plant sexual system was noted for 8 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 *Buchloe dactyloides*, populations have been reported to be dioecious and subdioecious; and for *Fuchsia microphyla*, *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.

Climatic variables and biomes

Global climatic data were downloaded from WorldClim at 30 arc-second resolution (Hijmans et al. 2005, http://www.worldclim.org/) and all 19 bioclimatic variables were extracted for each location (O'Donnel and Ignizio 2012, http://www.worldclim.org/bioclim). We selected bioclimatic variables 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 vs. 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) were included in the models. These variables have strong links with plant growth and physiology as they represent heat/cold tolerance and drought/moist 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 them, VIF < 5 (range 2 - 4.6), but for dioecy two variables were >5 (5.3 & 6.1). Though 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 climatic 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 'S. PhyloMaker' (Jin and 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 (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) using the R package MCMCglmm (Hadfield 2010). All bioclimate 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 felt, such circularity meant 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.

Amongst 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 conditions in the warmest quarter is at high temperatures and during the coldest quarter at low temperatures values respectively. 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 (Fig. 1 and 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 (-29.33/-19.06), pMCMC < 0.001; subdioecious: posterior mean (95% CI) = -4.98 (-10.59/0.69), pMCMC = 0.223). Female frequency was close to 50% in dioecious ($46.0 \pm 0.6\%$) and subdioecious systems ($48.4 \pm 1.0\%$), but gynodioecious species had significantly lower female frequency ($25.0 \pm 1.0\%$) 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 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 (biomess 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 plants and average female frequency of subdioecious plants ($r_p = -0.719$, $P = 0.008$; Fig. 2B), but not between dioecious and gynodioecious ($r_p = 0.221$, $P = 0.513$) or subdioecious and dioecious plants ($r_p = -0.448$, $P = 0.167$).

Relationships between female frequency and climatic 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 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. 3A). Similarly, in subdioecious systems, female frequency was also positively correlated to mean temperature of the driest quarter in addition to being positively correlated to mean temperature of the coldest quarter (Table 1). In this system, female frequency was positively 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. 3C). In contrast, none of the bioclimatic parameters were statistically significant in dioecious systems (Table 1; Fig. 3B).

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, higher female frequency was associated with greater temperature stress (Fig. 3A). In contrast, female frequency was unrelated to environmental stressors in dioecious plants (Fig. 3B). For subdioecious systems, four out of the six climatic stressors in the PMM were significant (Fig. 3C). Of these, female frequency was greater with higher temperature stress during the coldest and warmest quarters (Fig. 3C), but greater 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 on subdioecious and gynodioecious sexual systems, but not on 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 greater 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 differ 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 seeds in addition to larger floral display (Shykoff et al. 2003). Hence, female frequency should be higher where stressors are greater. In contrast, subdioecious systems contain predominantly male and female individuals with a few hermaphrodites. Here the costly sexes are hermaphrodite and females over males and so, as stressors increase, so the male function should be favoured over females and therefore, female frequency should decrease. Single-species evidence is mixed. Some studies report that female function declines with reduced precipitation (Dudley 2006), whereas other found no difference between sexes (Yang et al. 2014). Though not analysed in this study, we might expect that hermaphrodite frequency declines in these systems too.

In dioecious systems, sex ratios are expected to approach 1:1 due to negative frequency-dependent selection (Fisher 1930), although departures from this have been often reported (Barrett et al. 2010, Field et al. 2012, Munné-Bosch 2015). The ecological and evolutionary mechanisms producing biased sex ratio variation are relatively well established in dioecious plants and have been explained by a biased primary sex ratio (de Jong and van de Meijden 2004), different germination requirements (Purrington and Schmitt 1998), different mortality associated with the two sexes (Obeso 2002), spatial segregation of the sexes (Bierzychudek and Eckhart 1988) and sex lability (Korpelainen 1998). Theoretically, females are more susceptible to stress levels than males because of the higher costs of reproduction associated with seed production (Obeso 2002). The overall lack of effect between stress levels and female frequency in dioecious plants might be explained by the existence of compensatory growth and physiological mechanisms (Obeso 2002, Case and Ashman 2005). Even though relatively understudied, the same factors apply to gynodioecious and subdioecious systems (e.g. Gomez and Shaw 2006, Varga and Kytöviita 2016). In these two sexual systems, female frequency is also explained by the underlying genetics as both genders achieve their fitness via 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 linked to how fitness is obtained: via the male or the female function (Maurice and Fleming 1995, Wolf and Takebayashi 2004, Ehlers and 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 and Barrett 1981, Loveless and Hamrick 1984, Werren and 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 type 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, whilst 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 and Case 2007; *Daphne laureola*: Alonso and 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 male fitness caused by impaired pollen performance at higher or low 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.

Like 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 occur during and immediately after the rainy season (e.g. Wolfe and 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 favour 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 and Barrett 2004, Vaughton and Ramsey 2004).

It is important to keep in mind that sexual plasticity (i.e. sexual lability or gender diphasy) 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 indidivual 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 environmental controlled sex ratios may be still unknown, but our study show 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 its synergetic effects have on plant reproduction and fitness.

References

Alonso, 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. *spicata* (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 L. D. Harder and S. C. H. Barrett (Eds.), Ecology and evolution of flowers (pp. 204–222). Oxford University Press, USA.

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 B: Biological Sciences 365:2549–2557.

Bierzychudek, P., Eckhart V. (1988) Spatial segregation of the sexes of dioecious plants. 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., Barrett S.C.H. (2004) Environmental stress and the evolution of dioecy: *Wurmbea dioica* (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.

Case, A.L., Ashman T.-L. (2009). Sex-specific physiology and its implications for the cost of reproduction. In: E. G. Reekie and F. A. Bazzaz (Eds.), Reproductive allocation in plants (pp. 129-157). Elsevier Academic Press.

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 B Biological Sciences 319:631–40.

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

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 performance: ecological and evolutionary perspectives. Ecology 78:1632–1639.

Dorken, M.E., Pannell J.R. (2008) Density-dependent regulation of the sex ratio in an annual plant. The American Naturalist 171:824–830.

Dudley, L.S. (2006) Ecological correlates of secondary sexual dimorphism in *Salix glauca* (Salicaceae). American Journal of Botany 93:1775–1783.

Ehlers, B.K., Bataillon, T. (2007) "Inconstant males" and the maintenance of labile sex expression in subdioecious plants. New Phytologist 174:194–211.

Field, D.L., Pickup, M., Barrett, S.C.H. (2012) Comparative analyses of sex-ratio variation in dioecious flowering plants. Evolution 67:661–672.

Field, D.L., Pickup, M., Barrett, S.C.H. (2013) Ecological context and metapopulation dynamics affect sex-ratio variation among dioecious plant populations. Annals of Btany 111:917–923.

Fisher, R.A. (1930) The genetical theory of natural selection. Clarendon Press, Oxford.

Frank, S.A. (1989) The evolutionary dynamics of cytoplasmic male sterility. The American Naturalist 133:345–376.

Freckleton, R.P., Harvey, P.H., Pagel, M. (2002) Phylogenetic analysis and comparative data: a test and review of evidence. The American Naturalist 160:712–726.

Freckleton, R.P. (2011). Dealing with collinearity in behavioural and ecological data: model averaging and the problems of measurement error. Behavioral Ecology and Sociobiology, 65:91–101.

Geber, M.A., Dawson, T.E., Delph, L.F. (1999) Gender and sexual dimorphism in flowering plants. Springer-Verlag, Berlin.

Gibson, J., Diggle, P. (1997) Structural analysis of female and hermaphroditic flowers of a gynodioecious tree, *Ocotea tenera* (Lauraceae). American Journal of Botany 84:298–298.

Goldberg, E.E., Otto, S.P., Vamosi, J.C., Mayrose, I., Sabath, N., Ming, R., Ashman, T.-L. (2017) Macroevolutionary synthesis of flowering plant sexual systems. Evolution 71: 898–912.

Gomez, N.N., Shaw, R.G. (2006) Inbreeding effect on male and female fertility and inheritance of male sterility in *Nemophila menziesii* (Hydrophyllaceae). American Journal of Botany 93:739–746.

Gouyon, P.H., Vichot, F., Van Damme, J.M.M. (1991) Nuclear-cytoplasmic male sterility: single point equilibria versus limit cycles. The American Naturalist 137:498–514.

Hadfield, J.D. (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R Package. Journal of Statistical Software 33:1–22.

Hedly, A., Hormaza, J.I., Herrero, M. (2009) Global warming and sexual plant reproduction. Trends in Plant Science 14: 30–36.

Henry, I.M., Akagi, T., Tao, R., Comai, I. (2018) One hundred ways to invent the sexes: theoretical and observed paths to dioecy in plants. Annual Review in Plant Biology 69:553–575.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G., Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25:1965–1978.

Hultine, K.R., Grady, K.C., Wood, T.E., Shuster, S.M., Stella, J.C. (2016) Climat change perils for dioecious plant species. Nature Plants, article number 16109. DOI: 10.1038/NPLANTS.2016.109

Iossa, G. (2019) Sex-specific differences in thermal fertility limits. Trends in Ecology and Evolution 34: 490–492.

Jin, Y., Qian, H. (2019) V.PhyloMaker: an R package that can generate very large phylogenis for vascular plants. Ecography 42: 1353–1359.

de Jong, T.J., van de Meijden, E. (2004) Sex ratio of some long-lived dioecious plants in a sand dune area. Plant Biology 6:616–620.

Käfer, J., Marais, G.A.B., Pannell, J.R. (2017) On the rarity of dioecy in flowering plants. Molecular Ecology 26:1225–1241.

Korpelainen, H. (1998) Labile sex expression in plants. Biological Reviews, 73:157–180.

Lloyd, D.G., Bawa, K.S. (1984) Modification of the gender of seed plants in varying conditions. Evolutionary Biology 17: 255–338.

Loveless, M.D., Hamrick, J.L. (1984) Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15:65–95.

Maurice, S., Fleming, T. (1995) The effect of pollen limitation on plant reproductive systems and the maintenance of sexual polymorphisms. Oikos 74:55–60.

McCauley, D.E., Brock, M.T. (1998) Frequency-dependnt fitness in *Silene vulgaris*, a gynodioecious plant. Evolution 52:30–36.

McCauley, D.E., Bailey, M.F. (2009) Recent advance in the study of gynodioecy: the interface of theory and empiricism. Annals of Botany 104: 611–620.

Moeller, D.A., Briscoe Runquist, R.D., Moe, A.M., Geber, M.A., Goodwillie, C., Cheptou, P.-O. … Winn, A.A. (2017) Global biogeography of mating system variation in seed plants. Ecology Letters 20:375–384.

Munné-Bosch, S. (2015) Sex ratios in dioecious plants in the framework of global change. Environmental and Experimental Botany 109:99–102.

O'Donnel, M.S., Ignizio, D.A. (2012) Bioclimatic predictors for supporting ecological applications in the conterminous United States. U.S. Geological Survey Data Series 691.

Obeso, J.-R. (2002) The costs of reproduction in plants. New Phytologist 155:321–348.

Olson, D.M., Dinerstein, E., Wikramanayake, E.D. (2001) Terrestrial ecoregions of the world: A new map of life on Earth: A new global map of terrestrial ecoregions provides an innovative tool for conserving biodiversity. BioScience 51:933–938.

Purrington, C.B., Schmitt, J. (1998) Consequences of sexually dimorphic timing of emergence and flowering in *Silene latifolia*. Journal of Ecology 86:397–404.

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

Reekie, E.G., Bazzaz, F.A. (2005) Reproductive allocation in plants. Elsevier Academic Press.

Renner, S.S. (2014) The relative and absolute frequencies of angiosperm sexual systems: Dioecy, monoecy, gynodioecy, and an updated online database. American Journal of Botany 101:1588–1596.

Retuerto, R., Sánchez-Vilas, J., Varga, S. (2018) Sexual dimorphism in response to stress. Environmental and Experimental Botany 146:1–4.

Ruffatto, D.M., Zaya, D.N., Molano-Flores, B. (2015) Reproductive success of the gynodioecious *Lobelia spicata* Lam. (Campanulaceae): Female frequency, population demographics, and latitudinal patterns. International Journal of Plant Sciences 176:120–130.

Shykoff, J.A., Kolokotronis, S.-O., Collin, C.L., López-Villavicencio, M. (2003) Effects of male sterility in reproductive traits in gynodioecious plants: a meta-analysis. Oecologia 135: 1–9.

Sinclair, J.P., emlen, J., Freeman, D.C. (2012) Biased sex ratios in plants: theory and trends. Botanical Reviews 78: 63–86.

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

Stehlik, I., Friedman, J., Barrett, S.C.H. (2008) Environmental influence on primary sex ratio in a dioecious plant. PNAS 105:10847–10852.

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

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

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. (2003) Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. Journal of Evolutionary Biology 16:1006–1018.

Van Etten, M.L., Prevost, L.B., Deen, 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). International Journal of Plant Sciences 169:271–279.

Vaughton, G., Ramsey, M. (2004) Dry environments promote the establishmnt of females in monomorphic populations of *Wurmbea biglandulosa* (Colchicaceae). Evolutionary Ecology 18:323–341.

Varga, S., Kytöviita, M.-M. (2016) Light availability affects sex lability in a gynodioecious plant. American Journal of Botany 103:1928–1936.

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

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

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

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

Yamauchi, A., Yamagishi, T., Booton, R., Telschow, A., Kudo, G. (2019) Theory of coevolution of cytoplasmic male-sterility, nuclear restorer and selfing. 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.

Zanne A.E., Tank, D.C., Cornwell, W.K., Eastman, L.M.., Smith, S.A., FitzJohn, R.G., … Beaulieu, J.M. (2014) Three keys to the radiation of angiosperms into freezing environments. Nature 506:89–92.

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

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

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 Statistical 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 N = 63 species n = 495 populations λ=0.67 (0.48/0.84)	Intercept	12.217	-38.478	61.812	0.600
	Mean Temp Driest Q (Bio9)	0.150	0.082	0.226	**0.001**
	Mean Temp Warmest Q (Bio10)	-0.001	-0.099	0.092	1.000
	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 N = 242 species n = 1128 populations λ=0.70(0.59/0.80)	Intercept	34.282	9.673	60.381	0.008
	Mean Temp Driest Q (Bio9)	0.013	-0.020	0.043	0.442
	Mean Temp Warmest Q (Bio10)	0.030	-0.010	0.065	0.129
	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)	-0.114	-0.180	-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.

Figure legends

Fig. 1. Phylogenetic barplot showing mean + SE female frequency (%) for each species. Bars are coloured to signal plant sexual system (dioecious: dark blue, subdioecious: dark green, gynodioecious: yellow).
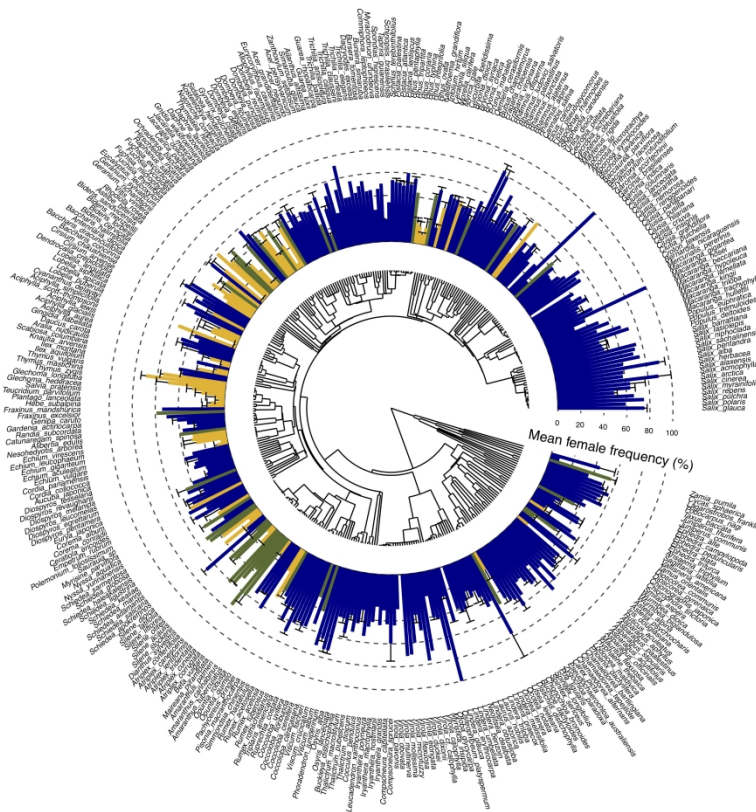
Fig. 2. (A) Beeswarm plot of female frequency (%) in relation to plant sexual system in dioecious (dark blue), subdioecious (dark green), and gynodioecious (yellow) sexual systems and (B) scatterplot showing the significant correlation between average female frequency of gynodioecious and subdioecious plants from each biome.

Fig. 3. Mean female frequency + SE (%) in each biome for dioecious (dark blue), subdioecious (dark green) and gynodioecious (yellow) plant breeding systems.

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 in black points.

1   **Fig. 1**. Phylogenetic barplot showing mean + SE female frequency (%) for each species. Bars

2   are coloured to signal plant sexual system (dioecious: dark blue, subdioecious: dark green,

3   gynodioecious: yellow).

4

5

plb_13125_f1.jpg

1   **Fig. 2**. (A) Beeswarm plot of female frequency (%) in relation to plant sexual system in

2   dioecious (dark blue), subdioecious (dark green), and gynodioecious (yellow) sexual systems

3   and (B) scatterplot showing the significant correlation between average female frequency of

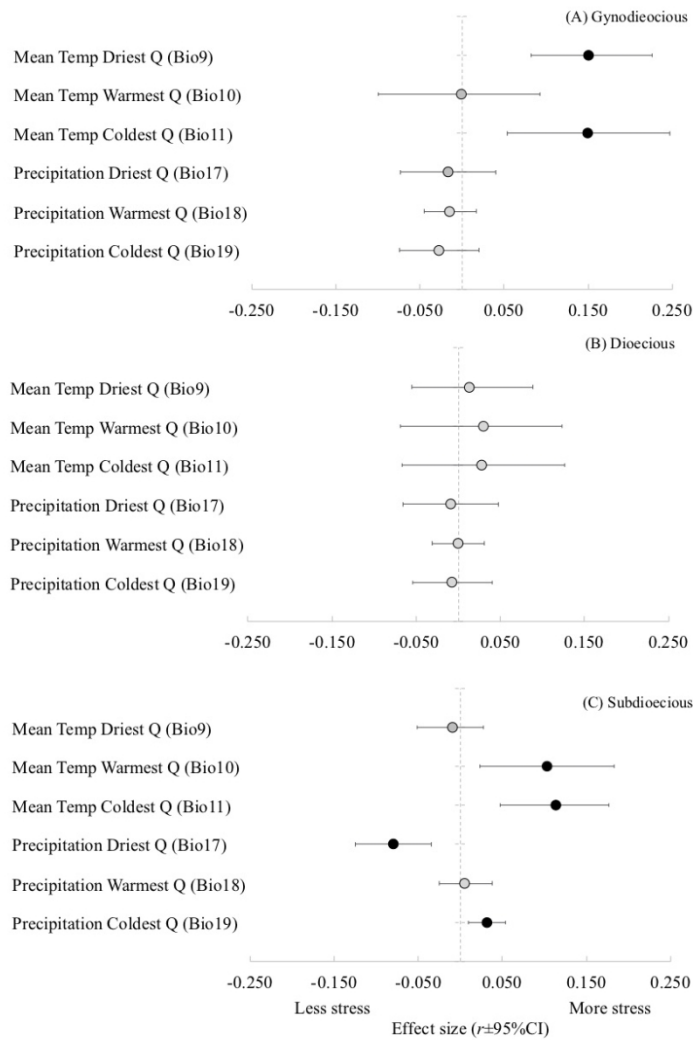4   gynodioecious and subdioecious plants from each biome.

5   **A)**   **B)**

6
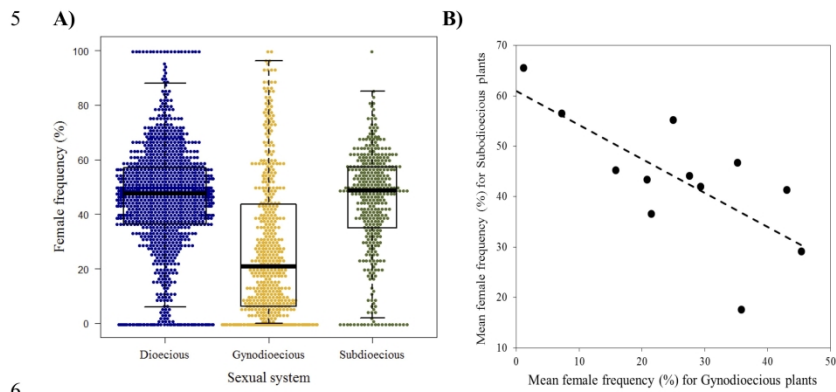
plb_13125_f2.jpg

Fig. 3. Mean female frequencies + SE (%) in each biome for dioecious (dark blue), subdioecious (dark green) and gynodioecious (yellow) plant breeding systems.
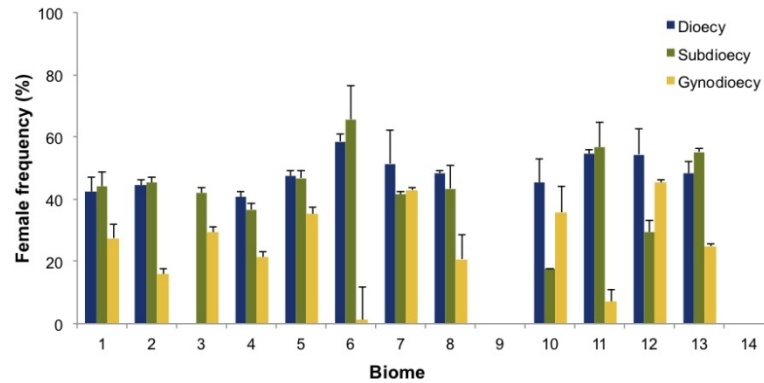
Note: Biome 1: Tropical and subtropical moist broadleaf forests (tropical and subtropical, humid); Biome 2: Tropical and subtropical dry broadleaf forests (tropical and subtropical, semihumid); Biome 3: Tropical and subtropical coniferous forests (tropical and subtropical, semihumid); Biome 4: Temperate broadleaf and mixed forests (temperate, humid); Biome 5: Temperate coniferous forests (temperate, humid to semihumid): Biome 6: Boreal forests/taiga (subarctic, humid); Biome 7: Tropical and subtropical grasslands, savannas, and shrublands (tropical and subtropical, semiarid); Biome 8: Temperate grasslands, savannas, and shrublands (temperate, semiarid); Biome 9: Flooded grasslands and savannas (temperate to tropical, fresh or brackish water inundated); Biome 10: Montane grasslands and shrublands (alpine or montane climate); Biome 11: Tundra (Arctic); Biome 12: Mediterranean forests, scrub or sclerophyll forests (temperate warm, semihumid to semiarid with winter rainfall); Biome 13: Deserts and xeric shrublands (temperate to tropical, arid); Biome 14: Mangrove (subtropical and tropical, salt water inundated).

plb_13125_f3.jpg

1   **Fig. 4**. PMM effects and 95% CI for (A) gynodioecious, (B) dioecious, and (C) subdioecious

2   systems. A positive effect size indicates increasing female frequency, whereas a negative

3   effect size indicates a decreasing female frequency. Variables that are significant from the

4   PMM are indicated in black points.

5

plb_13125_f4.jpg