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Differences and similarities of sex ratios between dioecious angiosperms and dioicous bryophytes

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ABSTRACT

Observation: For plant species with separate sexes, the average population sex ratio is female biased in mosses and liverworts, male biased in trees and shrubs, and unbiased in short-lived herbs.

Key assumption: When fertilization occurs, female investment in reproduction greatly outweighs male investment.

Questions: Can we explain the general pattern in population sex ratios from primary sex ratios in seeds or spores? What does genomic conflict theory predict for sex ratios of angiosperms and bryophytes? Are there other explanatory factors?

Data studied: Literature review of variation in sex ratio found in seeds and spores and of male and female performance.

Conclusion: Primary sex ratios were often variable, suggesting genomic conflict involving both cytoplasmic factors and sex-linked drive. Even though theory predicts some differences, primary sex ratios of angiosperms and bryophytes were remarkably similar. Males sometimes perform worse than females in the pre-reproductive phase and this may result in a female bias before reproduction occurs. In the reproductive stage, females invest more than males and may suffer greater mortality. The sex ratio of a cohort may then slowly change from female to male bias. This might explain the different population sex ratios of short-lived (herbs) and long-lived angiosperms (trees). In bryophytes, low fertilization rates, reducing female allocation to reproduction, could shift the balance towards consistent female bias in their populations.

Keywords: cytoplasmic DNA, dioecy, gene drive, genomic conflict, sex allocation theory.

INTRODUCTION

About 5% of all angiosperms are dioecious with separate male and female individuals (Renner, 2014). Much higher percentages of the moss (57–60%) and liverwort species (68%) have separate sexes (Glime and Bisang, 2017a) and bryologists refer to such taxa as dioicous. In dioecious angiosperms, females may be XX and males XY, with Y dominant over X

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(Westergaard, 1958; Ming *et al.*, 2011; Charlesworth, 2013; Heikrujam *et al.*, 2015; Harkess and Leebens-Mack, 2017). Y-chromosomes contain a non-recombining region that contains male sex-determining genes, plus some accompanying genes (Bachtrog *et al.*, 2011; Charlesworth, 2013). This region will initially be small and may increase over time (Ming *et al.*, 2011). Heteromorphic sex chromosomes (i.e. those with differences between X and Y visible under the microscope) are quite rare and were demonstrated for only 48 higher plant species (Ming *et al.*, 2011). In dioicous bryophytes, female and male sex chromosomes are nowadays denoted as U and V, respectively (Bachtrog *et al.*, 2011). As in higher plants, some bryophytes have heteromorphic sex chromosomes while visible differences are lacking in others (McDaniel and Perroud, 2012; Renner *et al.*, 2017).

In angiosperms, the plant is (functionally) diploid and gametes are haploid. The pollen carries the sperm that fertilizes the egg cell. In bryophytes, the haploid gametophyte is the ‘leafy moss’ that we observe in the field and in dioicous species this gametophyte is either female or male, U or V (Fig. 1). Antheridia release the V-carrying male sperm, which swims over rather short distances, in the order of centimetres, to fertilize an egg cell in a female archegonium. After fertilization, a diploid sporophyte develops on the female gametophyte. Meiosis occurs in the UV sporophyte, after which haploid spores are released that are either female or male, U or V (Fig. 1). The spores potentially disperse over large distances and give rise to new plants. Botany textbooks emphasize that the sporophyte is the dominant life form in higher plants, while the gametophyte is dominant in bryophytes. In species with separate sexes, a further difference is that in angiosperms two types of sporophytes exist, females (XX) and males (XY). In bryophytes there is only one sporophyte (UV) that develops on the female gametophyte (U) (Fig. 1). There is no UU in bryophytes. In angiosperms, competition between X- and Y-carrying gametophytes (pollen) may potentially change the sex ratio. Sperm competition may also occur in bryophytes but since all sperm cells carry V, this will not affect the sex ratio of the offspring.

In dioecious angiosperms, female allocation to flowers, fruits, and seeds greatly outweighs male allocation to flowers with pollen (Delph, 1999; Obeso, 2002; Sinclair *et al.*, 2012). Delph (1999) documented greater female allocation for 32 species, while in two species males allocated as much to reproduction as females but in no case did males allocate more to reproduction. For the species reviewed by Sinclair *et al.* (2012), females allocated between 1.69 and 31.5 times more (median value 9.1) to reproduction than males. In dioicous bryophytes, female reproduction involves making archegonia, supporting the sporophyte and making structures to initially protect these organs, such as a perianth in many liverworts. After fertilization, the diploid sporophyte with its stalk, capsule, and developing spores grows on the mother plant and largely depends on her for nutritional support. Sporophyte production reduces growth of females (see Stark *et al.*, 2009 and references therein) and is therefore costly to the mother plant. Females with sporophytes allocate more to reproduction than males (Glime and Bisang, 2017c). For instance, females of the liverwort *Lophozia silvicola* allocated 24% of their biomass to reproduction and males only 2.3% (Laaka-Lindberg, 2001).

It is to be expected that allocation to reproduction represents a ‘reproductive cost’ (Delph, 1999) in terms of reduced growth and reduced survival, so that when reproductive allocation differs between the sexes there may be a ‘differential reproductive cost’ (Ortiz *et al.*, 2002). Since fertilized females allocate more to reproduction than males, one would expect female growth and survival to be lower than that of males. Based on this trade-off between reproduction and survival, one would also expect that all plant populations gradually become male-biased. Rather surprisingly, this is not a general pattern.

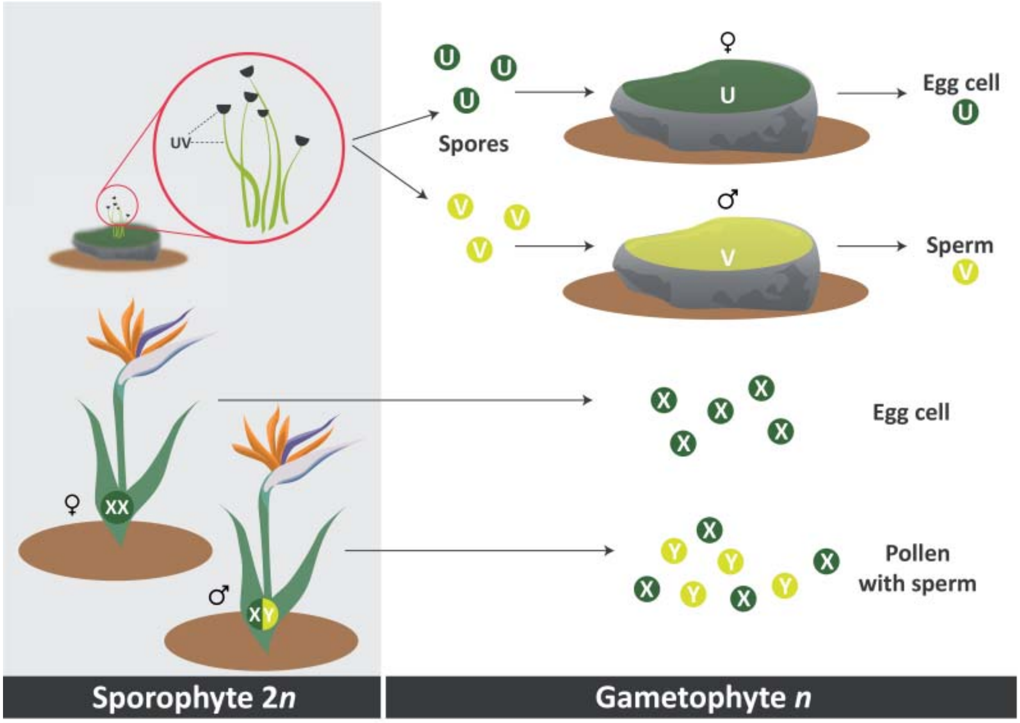

Fig. 1. Comparison of the life cycles of dioecious angiosperms and dioicous bryophytes. In angiosperms the sporophyte is typically the dominant phase, in bryophytes we mostly see the gametophyte (i.e. the 'leafy' moss) in nature. Another difference is that there are two types of sporophytes (XX females and XY males) in angiosperms but only one (UV) in bryophytes. Competition between X and Y sperm could change the primary sex ratio in angiosperms but not in bryophytes since all sperm carries V.

Sinclair *et al.* (2012) and Field *et al.* (2013) recently reviewed sex ratios in populations of dioecious angiosperms. Sex ratio is defined as the fraction of male individuals. In the review of Field *et al.* (2013) on 243 angiosperms, the population sex ratio of about half the species differed significantly from 0.5, while in the other half there were no significant differences. With respect to life form, Field *et al.* (2013) reported that the average sex ratio of various tree species was significantly male-biased (average 0.54), while the sex ratio of annual, herbaceous perennial, and shrub species was, on average, not significantly different from 0.5. If we dissect the data of Sinclair *et al.* (2012) into trees, shrubs, and herbs, a similar pattern emerges; male bias is most common in populations of shrubs and trees but male bias is as common as female bias in herbaceous perennials (Table 1). This pattern fits with the conclusions of Obeso (2002), who reported that male trees were typically larger, had higher relative growth rates, and survived better than female trees. Among herbaceous perennials, Obeso (2002) observed an opposite trend. Females were sometimes larger than males despite allocating more to reproduction (for more detail, see the discussion). Also, no consistent differences in survival existed between males and females in herbaceous perennials. The differential reproductive cost hypothesis fits the data for trees, but not for herbaceous plants. In 88% of the dioicous mosses and liverworts (excluding species with dwarf males), more

Table 1. Number of angiosperm species with male, female, or no bias reported in their populations

	Male bias	Female bias	No bias
Herbs	26	23	11
Shrubs	45	32	4
Trees	60	25	5

Note: Data obtained by pooling different categories in Sinclair *et al.* (2012).

female than male patches were counted in natural populations (Bisang and Hedenäs, 2005). In some bryophytes, males are extremely rare or have never been recorded (Bisang and Hedenäs, 2005). The different sex-ratio patterns between angiosperms and bryophytes require an explanation.

Sex-ratio evolution according to Fisher (1930)

For species with separate sexes and a simple sex-determination mechanism (such as XX/XY or U/V), meiosis dictates a fraction of 0.5 males in the 'just born' offspring and in the literature the primary sex ratio of these offspring is often considered to be 0.5. Primary sex ratios are also called seed sex ratios for higher plants and spore sex ratios or genetic sex ratios (McLetchie and García-Ramos, 2017) for bryophytes. Fisher (1930) suggested that a sex ratio of 0.5 is evolutionarily stable in a large, well-mixed population. For Fisher's prediction, it does not matter whether males and females differ in mortality later in their life (Charnov, 1982). If males have high mortality, then at reproduction more females are available per male, the surviving males are more successful, and in the basic models this compensates exactly for their increased mortality. In Fisher's model, both males and females disperse and mix fully in the population. This does not apply in full to plants in which genes are dispersed prior to mating through sperm only, then after mating are dispersed through seeds (higher plants) or spores (bryophytes). Making male offspring leads to better gene dispersal and less competition with relatives than making female offspring, and this mechanism potentially selects for a slight male bias in the primary sex ratio (de Jong *et al.*, 2002). We mention this refinement of Fisher's model for plants in passing and will not provide further detail here (but see Sinclair *et al.*, 2012). However, it should be noted that for bryophytes, distances of sperm movement are negligible compared with distances over which spores are dispersed. Bias in spore sex ratio due to this mechanism is not expected. In angiosperms, the sperm disperses with the pollen and, depending on the species, the distances of pollen movement could be considerable compared with distances of seed dispersal. With poor seed dispersal this could theoretically lead to a slightly male-biased sex ratio in the seeds (de Jong *et al.*, 2002).

More precisely, Fisher (1930) predicted that selection favours equal allocation of resources to sons and daughters. When each sex costs the same, this coincides with a sex ratio of 0.5. However, there might be cases where one of the sexes is less costly to produce than the other, for instance because weight at birth differs between sons and daughters. In those cases, a sex ratio of 0.5 is no longer evolutionarily stable and selection favours overproduction of the cheaper sex. In angiosperms, male and female seeds are generally of the same size (but see Stehlik and Barrett, 2005). The same holds for the spores of most bryophytes, although there are some examples of size differences (anisospory) with male spores smaller than female spores (Mogensen, 1981; Une, 1985; Glime and Bisang, 2017c). When male and female spores can be visually distinguished based on their size, anisosporous bryophytes offer excellent opportunities for testing Fisher's prediction of overproduction of the cheaper sex. This topic is, however, beyond the scope of the present paper. Fisher's (1930) theory is based on autosomal genes that affect sex ratio. Genomic conflict theory (Cosmides and Tooby, 1981; Burt and Trivers, 2006) is a recent extension that we outline in the next subsection.

Genomic conflict over sex ratio

Genomic conflict theory deals with genes that are (mostly) transmitted through one parent. First, genes linked to sex-determining factors are under selection to pass on as many copies of themselves as they can (Hamilton, 1967). Alleles that are overrepresented in the offspring, as compared to Mendelian inheritance, are said to drive. Second, when cytoplasmic genes are only maternally transmitted, these genes are under selection to produce a female bias. From the viewpoint of genomic conflict, primary sex ratios are the result of a tug-of-war between genes located at different places in the genome (Fig. 2) and are not necessarily 0.5. Even when the primary sex ratio is 0.5 there may exist, under the surface, a balance between genes that distort and restore the sex ratio. In many insect species, crosses between populations from different parts of the world resulted in biased sex ratios even though the sex ratio was 0.5 in each local population (Burt and Trivers, 2006).

Sex-linked drivers

In his seminal paper on gene drive and sex ratios of insects, Hamilton (1967) assumed that Y-carrying sperm competes with X-carrying sperm and either of the two could win. A driving allele on Y is under selection 100% of the time, whereas a similar driving allele on X is under selection only one-third of the time (only when it resides in a male and the X-carrying chromosome is involved in sperm competition). Therefore, in Hamilton's (1967) simulation models, Y-linked drivers increased three times as fast in the population as X-linked drivers.

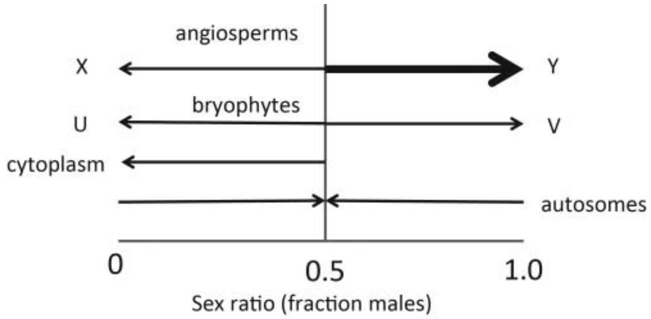

Fig. 2. Tug-of-war over sex ratio in dioecious angiosperms and dioicous bryophytes. Cytoplasmic genes select for a female-biased sex ratio. Under some assumptions (see text), Y-linked drive is stronger than X-linked drive in angiosperms (indicated by the bold arrow) while these forces are equally strong in bryophytes. In a well-mixed population, autosomes select for a sex ratio of 0.5.

Hamilton's concern was that Y-linked drive is most prominent, results in male bias and – eventually – in the extinction of populations.

The mechanism proposed by Hamilton (1967) may well apply to pollen competition in angiosperms. For *Silene latifolia*, for example, Taylor *et al.* (1999) showed different success of X and Y pollen in fertilization. In such cases, sex ratio of the offspring is a character that is inherited through the paternal parent in the cross and not through the maternal parent. In *Rumex nivalis*, the Y pollen was only successful with low stigmatic pollen loads (Stehlik and Barrett, 2006). With high stigmatic loads, inducing more competition, the Y pollen was outcompeted by X pollen, leading to seed sex ratios as low as 0.04. Note, however, that sperm competition is only one mechanism for gene drive. Other mechanisms for drive occur also in angiosperms (Lindholm *et al.*, 2016; see also Glawe and de Jong, 2007). U- and V-linked drive could also occur in bryophytes but the situation for these drivers is symmetrical. U- and V-linked drive could lead to variable sex ratios in spores around the average at 0.5.

Cytoplasmic factors

DNA in mitochondria and chloroplasts is usually inherited through the seeds in angiosperms and only rarely passes through the pollen (Mogensen, 1996). In bryophytes, the mitochondrial and chloroplast DNA is even more strictly maternally inherited (Natcheva and Cronberg, 2007; Jankowiak-Siuda *et al.*, 2008). Unlike animals that have compact mitochondria with little DNA (usually 11–28 kbp), angiosperms have large mitochondria (200–2000 kbp). The genome of bryophyte mitochondria is usually 100 kbp in size and is thus on the low side of the range for higher plants (Liu *et al.*, 2014). When the organelle genome becomes larger and more loosely organized, the probability of a mutation with a phenotypic effect increases.

Cytoplasmic male sterility has long been known in hermaphrodite plants (Lewis, 1941) and has been extensively studied up to the molecular level (Burt and Trivers, 2006). Mutations in mitochondrial DNA sometimes lead to degeneration of stamens or the production of inviable pollen and when male-sterile plants reallocate some of the resources, they will produce some extra seeds. Lewis (1941) was probably the first to realize that, when more seeds are produced, a cytoplasmic mutation for male sterility will increase in a population. The population is then gynodioecious, i.e. consists of hermaphrodites plus male-sterile (female) plants. When self-pollination provides the hermaphrodite with reproductive assurance, the population will arrive at some equilibrium at which females and hermaphrodites co-exist (Lewis, 1941). However, reproductive assurance does not exist in self-incompatible species that are 100% outcrossing. In outcrossing populations, a cytoplasmic mutation for male sterility will increase indefinitely in the population, to the point that the last female is not pollinated and the population goes extinct. Dioecious plants are 100% outcrossing, so the same principle applies. A cytoplasmic mutation that leads to more daughters will increase in the population until the last male dies and the population goes extinct. In these female-biased populations, autosomal genes that restore the sex ratio to 0.5 are strongly selected. For dioecious plants, only *Silene latifolia* has been studied from this angle (Taylor, 1994). By making reciprocal crosses, Taylor showed that the variable sex ratio in seeds is due to a balance between cytoplasmic factors and Y-linked nuclear restorers. Note that the terminology 'Y-linked restorers' might be misleading. In dioecious plants, genes on autosomes are selected to restore the sex ratio to 0.5, while genes on Y-chromosomes are selected to produce only males.

In bryophytes, cytoplasmic factors are also under selection to produce a female-biased spore sex ratio. When crossing a male plant of the moss *Ceratodon purpureus* from the

USA (New York) with a female from Ecuador, McDaniel *et al.* (2007) found that 45% of the autosomal loci showed distorted segregation and the allele from the mother was always overrepresented. The authors suggested that this bias was due to cytoplasmic factors.

Sexual dimorphisms

The evolution of separate sexes gives scope for males and females to gradually become different. This may be due to sexually antagonistic selection on flower characters (Eckhart, 1999), life-history and reproductive effort (Delph, 1999), or morphology (Dawson and Geber, 1999). In rare cases, males and females can already be distinguished in the vegetative stage. For instance, in *Leucadendron* species, males have much smaller leaves than females and this could be viewed as an adaptation of each sex to its later sexual function (Midgley, 2010). In dioicous bryophytes, males and females may differ in degree of branching, colour, erectness, shape (males slender, females wide), leaf size, leaf thickness, and presence/absence of hairs (Fuselier, 2004; Glime and Bisang, 2017c; Slate *et al.*, 2017). However, not all differences between males and females are necessarily the result of adaptation. Male and female structures produce signals that affect the plant. After separate sexes have evolved, one of the signals is lost and this could result in an immediate phenotypic difference between the sexes (Charlesworth, 2018).

Furthermore, it is well known for XX/XY sex-determination systems that genes close to the male-determining factor on Y do not recombine and accumulate mutations faster than similar genes on X (Charlesworth, 2013). This process is enhanced by the fact that a gene on Y has only one-third the effective population size of a gene on X (Bachtrog *et al.*, 2011). Hence genes linked to male sex determination on Y are prone to genetic drift and degeneration. This could result in males doing worse than females, not just in the reproductive phase but also before. None of this applies to bryophytes, since the non-recombining parts of U and V chromosomes are expected to accumulate mutations at the same rate (Bull, 1978; Bachtrog *et al.*, 2011).

Finally, differences between males and females could be due to different functioning of cytoplasmic genes. With strictly maternal inheritance of mitochondrial and chloroplast genes, there is no selection at all on the cytoplasmic genes to function properly in males (Cosmides and Tooby, 1981). Cytoplasmic genes are at a dead end in males – zero copies are transmitted to the next generation. Haig (2016) argued that the cytoplasmic gene spends all previous generations in a female plant and encounters each male as a new evolutionary environment. Cytoplasmic genomes only evolve to maximize reproductive success of female plants and not of males. The more males and females differ, the more problematic will be the functioning of the cytoplasmic gene in the male. This could lead, for instance, to higher photosynthetic rates in females (Dawson and Geber, 1999; Juvany and Munné-Bosch, 2015). This final explanation for sexual dimorphism has received the least attention. It applies to both angiosperms and bryophytes. As Cosmides and Tooby (1981) pointed out, the situation for males could be even worse than just suboptimal functioning of cytoplasmic genes. With strong sib competition, cytoplasmic genes are under selection to slow down or even kill their male bearer if this increases seed production of his sisters. His sisters contain the same cytoplasmic genes and are the only ones that pass them on. Sib competition for resources may occur in angiosperms when seeds fall next to the mother plant (see, for example, Holderegger and Stehlik, 1999). Sib competition may also occur in bryophytes when tetrads disperse as one unit (McLetchie, 1992) or when the majority of spores fall near the mother (Miles and Longton, 1992).

While adaptation and indirect effects of loss of sex organs would lead to differences between males and females mainly in the reproductive stage, the degeneration of Y and suboptimal functioning of cytoplasm would lead to males doing generally worse than females over their whole life cycle, including the non-reproductive stage.

Outline

Can we explain the general patterns in the population sex ratios of short- and long-lived angiosperms and bryophytes? Does the primary sex ratio correspond with the sex ratio in populations? Is the primary sex ratio typically close to 0.5 and constant, or biased and variable as expected under the hypothesis of genomic conflict? The occurrence of male bias in some families, batches of seeds or spores collected from the same mother plant, would suggest that Y- or V-linked drivers occur. Next we review additional factors that modify the sex ratio of populations, including the performance of males and females in the pre-reproductive phase. We discuss how these extra factors could help understand the different sex ratios in angiosperm and bryophyte populations that were the starting point of this paper.

METHODS AND RESULTS

Is primary sex ratio in angiosperms and bryophytes biased and variable?

We selected studies in which batches of seeds or spores taken from different mothers (families) were grown under controlled conditions. We also selected crossing studies, expecting that these studies generate different combinations of sex-ratio distorters and restorers and perhaps more extreme sex ratios. Studies on angiosperms in which results were pooled were excluded. An average sex ratio of 0.5 might mean that there is no variation, but the population could also consist of a mix of male- and female-biased families. A publication bias in documenting primary sex ratios is likely. Far fewer studies have been conducted for bryophytes than for angiosperms, so we included all studies on spore sex ratio. Glime and Bisang (2017b) have already reviewed most of these bryophyte studies in some detail.

Without unique markers for gender in the pre-reproductive state, estimating primary sex ratio involves growing plants from seeds or spores until maturity, preferably under conditions without mortality. This may present problems for long-lived organisms such as shrubs and trees; when mortality depends on gender, it will bias the estimate of the true sex ratio. Also for bryophytes it is not always easy to attain full spore germination, 100% survival of the early stage (protonema), and to create conditions under which all individuals express sex. Population sex ratio is the fraction of males among all flowering individuals counted in the field. Field data are usually collected in this way but the method has the disadvantage that individuals not expressing sex are left out of the count, which could lead to a biased estimate of the true sex ratio in the population when the probability to survive until first reproduction differs for males and females.

Population sex ratios of the selected species varied in angiosperms (2 male-biased, 5 female-biased, 1 unbiased) and were mostly female-biased in bryophytes (0 male-biased, 6 female-biased, 1 unbiased).

Six studies on dioecious angiosperms showed a significant female bias in the seed sex ratio, three studies showed no bias, and two studies showed a significant male bias. The female-biased sex ratios in the seeds of two *Salix* species and *Silene latifolia* matched the female bias in their natural populations. In three cases (*Hippophae rhamnoides*, *Rumex acetosa*, and *R. nivalis*), there was a partial match. The bias in the seed sex ratio was in the same direction as the population sex ratio but was less extreme, indicating that mortality later in life plays a role. The slight male bias in seed sex ratio of *Hippophae rhamnoides* may have been due to mortality during the period of growing plants until maturity (de Jong and van der Meijden, 2004). The strongly male-biased sex ratio (0.84) in *H. rhamnoides* populations in inward dunes is mainly caused by higher mortality of females than males. In *Rumex nivalis*, seed sex ratio was 0.41 while the population sex ratio was 0.13 (Stehlik and Barrett, 2006); in *R. acetosa*, seed sex ratio was 0.41 (Bizan *et al.*, 2014) and the population sex ratio ranged from 0.21 to 0.29 (Korpelainen, 1992a). A further complication of the *Rumex* studies is that this genus is wind-pollinated and seed sex ratio may depend on pollination intensity (Stehlik and Barrett, 2006). For two remaining studies in Table 2 (*Chamaelirium luteum* and *Urtica dioica*) there was no match between seed sex ratio and population sex ratio.

In eight out of ten cases, seed sex ratio varied significantly ($\alpha = 0.10$) between families. The frequency distribution of open pollinated seeds, collected in the field from various mother plants of *Urtica dioica*, is given in Fig. 3A. The sex ratio of different families ranged

Table 2. Sex ratios (fraction males) of individual seed crops in some dioecious angiosperms and how well sex ratio matches population sex ratio

Plant species	Sex ratio	Range	Method	Reference	Population sex ratio[2]	Match[3]
Actinidia deliciosa	0.54	0.44–0.66*	13 C[1]	Testolin *et al.* (1995)	?	?
Chamaelirium luteum	0.52	0.36–0.73	30 F	Meagher (1981)	M > F	No
Dioscorea floribunda	0.63[+]	0.35–0.87*	15 C	Martin (1966)	?	?
Hippophae rhamnoides	0.56[+]	0.42–0.63	5 F	de Jong and van der Meijden (2004)	M > F	Partly, see text
Rumex acetosa	0.32[+]	0.27–0.41*	3 C	Bizan *et al.* (2014)	F > M	Partly, see text
Rumex nivalis	0.41[+]	0.33–0.45*	18 F	Stehlik and Barrett (2005)	F > M	Partly, see text
Salix repens	0.29[+]	0.00–0.45*	7 F	de Jong and van der Meijden (2004)	F > M	Yes
Salix viminalis	0.41[+]	0.02–0.82*	13 C	Alström-Rapaport *et al.* (1997)	F > M	Yes
Silene latifolia	0.38[+]	0.15–0.60*	4 C	Taylor (1996)	F > M	Yes
Urtica dioica	0.42[+]	0.00–0.75*	33 F	de Jong *et al.* (2005b)	M = F	No
Urtica dioica	0.51	0.40–0.60[#]	7 C	Shannon and Holsinger (2007)	?	?

[+] Significantly different ($\alpha = 0.05$) from a sex ratio of 0.5.

* Significant variation in sex ratio at $\alpha = 0.05$.

[#] Significant variation in sex ratio at $\alpha = 0.10$.

[1] C = crosses, F = families; number of crosses or families indicated.

[2] Sex ratio in natural population is indicated when known: M > F male biased (more than 50% males); M = F unbiased (50% males); F > M female biased (less than 50% males).

[3] Match indicates qualitatively whether the bias in population sex ratio is consistent with bias in seed sex ratio.

from 0.05 to 0.75. The frequency distribution of family sex ratios was not symmetrical but instead skewed towards low values (female bias). Taylor (1999) drew a similar asymmetric frequency distribution for *Silene latifolia* (his figure 2A), in which the sex ratio of full-sib families ranged from 0 to 0.75 (average 0.415), also with a skew towards low values. Significantly male-biased families occurred in both *U. dioica* and *S. latifolia*. The frequency distribution of open pollinated seeds collected from 18 flowering plants of *Rumex nivalis* was symmetrical (Stehlik and Barrett, 2005, their figure 3). However, this frequency distribution appeared to be bimodal with high frequencies in the lower and upper tails.

For bryophytes, three cases showed a female bias, five showed no bias, and one case showed a significant male bias in spore sex ratio. In two cases, spore sex ratio matched the population sex ratio. In *Mnium hornum*, sex ratio in the spores was 0.5 and the population sex ratio was unbiased (Newton, 1972). For *Plagiomnium undulatum* (Newton, 1972), the female-biased spore sex ratio matched the female bias in populations of this species. In the hepatic *Sphaerocarpos texanus* (McLetchie, 1992), the match was partial, populations of this species showing a stronger female bias than the spores. In four cases, the spore sex ratio was 0.5 and populations were female-biased [*Bryum argenteum* (Stark *et al.*, 2010); *Drepanocladus*

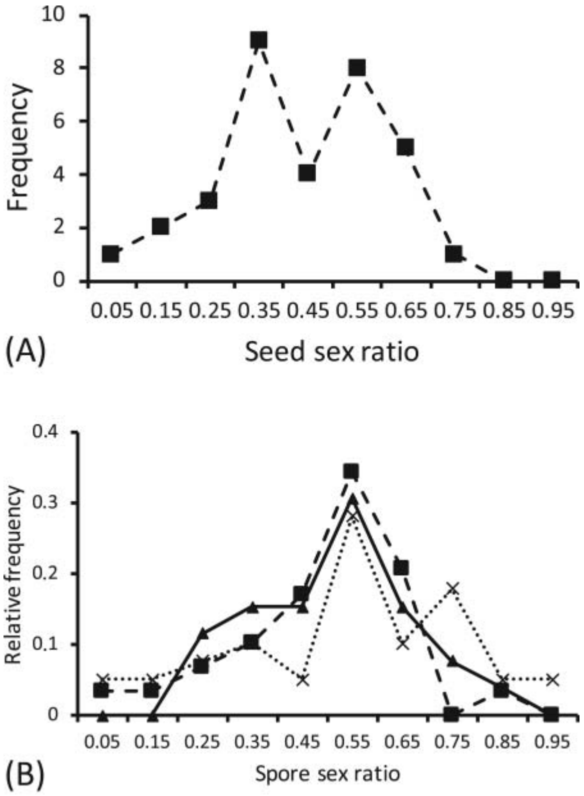

Fig. 3. (A) Absolute frequency of seed sex ratio in seeds of *Urtica dioica* sampled on 33 plants in the field. Modified from de Jong *et al.* (2005b). (B) Relative frequency of spore sex ratio of *Ceratodon purpureus.* Solid line (▲), data from Norrell *et al.* (2014); dotted line (×), Shaw and Beer (1999) Danby population; dashed line (■), Shaw and Beer (1999) Ithaca population.

lycopodioides (Bisang *et al.*, 2017); two populations of *Ceratodon purpureus* (Shaw and Beer, 1999)]. In these latter cases, the female bias in populations must be due to mortality later in life.

For only two moss species was variation in the sex ratio of batches of spores collected from different sporophytes studied. No significant variation existed between nine families of *Drepanocladus lycopodioides*, which had an average spore sex ratio of 0.5 (Bisang *et al.*, 2017). In the moss *Ceratodon purpureus*, several studies documented variation in spore sex ratio (Shaw and Beer, 1999; McDaniel *et al.*, 2007; Norrell *et al.*, 2014). McDaniel *et al.* (2007) found in a single cross a sex ratio of 0.59 ($n = 160$), i.e. a significant male bias in a binomial test (two-sided $P = 0.03$). However, for only 55% of the spores could sex be determined, so one cannot rule out that females were less likely to express sex. Norrell *et al.* (2014) isolated spores from 25 mother plants of *Ceratodon purpureus.* In 10 of these 25 families, the sex ratio differed significantly from 0.5 (Fig. 3B). In nine families there was a significant female bias, and in one family (Durham, NC) there was a male bias (0.72), significant at the $\alpha = 0.01$ level. Shaw and Beer (1999) compared sex ratio of spores from different mother plants from a population in Ithaca (CU) and one in Danby (DB), separated by 35 kilometres. Some of these families had strongly male-biased ratios in the spores, up to 0.85 (one sporophyte in CU) and 1.0 (two sporophytes in DB). The frequency distribution of sex ratios of the spores is remarkably symmetrical with an average around 0.5 (Fig. 3B).

We expected more male-biased primary sex ratios in higher plants than in bryophytes. This was not borne out by the data in Tables 2 and 3, which showed similar values for primary sex ratios of angiosperms and bryophytes. The variation in the spore sex ratio between families in the moss *Ceratodon purpureus* (Fig. 3B) was symmetric, while the frequency distributions of seed sex ratios of different families of *Urtica dioica* (Fig. 3A) and *Silene latifolia* (Taylor, 1999) were shifted to the left, i.e. towards female bias. From the viewpoint of genomic conflict, this may suggest a balance between X- and Y-linked drivers in the moss *C. purpureus* and a greater importance of the cytoplasmic factors for the two angiosperms.

Is sex ratio more biased in crosses between populations and varieties?

Crosses between populations yield novel combinations of drivers and restorers, which may result in more extreme sex ratios. Yet this was not the case for the angiosperms or bryophytes listed in Tables 2 and 3. Sex ratios in the half-sib families (denoted by F in Tables 2 and 3) were as variable as the crosses (denoted by C). Genotypes used in these crosses all came from widely different locations. Crosses between *Silene latifolia* and *S. dioica* gave similar variation in sex ratio as crosses within each of the two species (Taylor, 1993). However, while crosses within *S. latifolia* populations displayed no bias in seed sex ratio, crosses between different European populations did have a female bias (Hathaway *et al.*, 2008). Also in a Polish study on *Rumex acetosa*, reciprocal crosses between plants from two different populations produced more extreme seed sex ratios (0.27 and 0.28) than crosses within one population (0.41) (Bizan *et al.*, 2014). This result was based on only three crosses, so more detailed study is needed.

For the moss *Ceratodon purpureus*, the sex ratio of 0.59 in the interpopulation cross (McDaniel *et al.*, 2007) fell within the range of variation for sib families (Table 3). This does not indicate greater sex-ratio variation in crosses between populations than within populations.

Table 3. Average and variation in sex ratio (fraction males) of families – batches of spores taken from different sporophytes – in some dioicous bryophyte species

Bryophyte species	Sex ratio	Range	Method	Reference	Population sex ratio[3]	Match[4]
Bryum argenteum	0.50	pooled	2 S[+] 3 C	Stark *et al.* (2010)	F > M	No
Ceratodon purpureus	0.41[+]	0.17–0.62*	26 F[1]	Norrell *et al.* (2014)		?
C. purpureus	0.48	0.00–0.90*	29 F	Shaw and Beer (1999) CU[5]	F > M[2]	No
C. purpureus	0.53	0.00–1.00*	39 F	Shaw and Beer (1999) DB[5]	F > M[2]	No
C. purpureus	0.59[+]	one cross	C	McDaniel *et al.* (2007)		?
Drepanocladus lycopodioides	0.50	0.28–0.65	9 S	Bisang *et al.* (2017)	F > M	No
Mnium hornum	0.53	pooled	F	Newton (1972)	M = F	Yes
Plagiomnium undulatum	0.20[+]	pooled	F	Newton (1972)	F > M	Yes
Sphaerocarpos texanus	0.44[+]	pooled	F	McLetchie (1992)	F > M	Partly, see text

[+] Significantly different from a sex ratio of 0.5.

* Significant heterogeneity of sex ratio ($P < 0.05$) between sporophytes.

[1] F = the number of families, i.e. sporophytes from which spores were collected.

[2] A population sex ratio of 0.41 was reported for another (Michigan) population by Shaw and Gaughan (1993).

[3] Sex ratio in natural population is indicated when known: M > F male biased (more than 50% males); M = F unbiased (50% males); F > M female biased (less than 50% males).

[4] Match indicates qualitatively whether the bias in population sex ratio is consistent with bias in seed sex ratio.

[5] CU = population in Ithaca; DB = population in Danby.

Pre-reproductive performance of males and females

There are several ways to address this question.

Pre-reproductive growth

The most direct way is to allow plants to grow from germination until first reproduction and measure size before any reproduction occurs. In their review, Lloyd and Webb (1977) found no differences in growth between the sexes for spinach, while females grew better than males in hemp (at the start of reproduction females were 1.32–2.22 times larger than males), *Silene latifolia*, and *Mercurialis perennis*. Wade (1981) did not confirm the result for *M. perennis*, but found similar growth of males and females in monocultures. Females of *Mercurialis annua*, grown in a glasshouse from seeds collected in Spain, produced more vegetative biomass than males (Hesse and Pannell, 2011). A similar experiment with *M. annua* seeds collected in Belgium showed no difference between males and females in fresh weight, plant height, or root length (Orlofsky *et al.*, 2016). However, Orlofsky *et al.* (2016) noted that females made more reproductive nodes and survived longer than males, especially under salinity stress. Males of *Rumex acetosa* and *R. acetosella* were considerably smaller than females at first reproduction (Korpelainen, 1992a). During succession, males of *Rumex acetosa* disappeared from the vegetation, resulting in a strong female bias in the population sex ratio (Korpelainen, 1992b). Under greenhouse conditions, males of *Rumex nivalis* were slower in developing their first leaf, made shorter leaves and less basal shoots, suffered more from aphid damage, and experienced greater mortality than females (Stehlik and Barrett, 2005). Female seedlings of *Populus deltoides* grew better than male seedlings under salt stress (Li *et al.*, 2016). No sexual differences in growth or other characters were reported for *Populus balsamifera* and *P. trichocarpa* (McKown *et al.*, 2017). Eppley (2006) assessed the competitive ability of male and female seedlings (determined by molecular markers) of the grass *Distichlis spicata*. Seedlings were six times larger when grown with male versus female conspecific seedlings; males were therefore inferior competitors. In the pre-reproductive stage, male individuals of the shrubs *Sipanura grandifolia* (Nicotra, 1999) and *Ilex aquifolium* (Retuerto *et al.*, 2000) grew worse than females.

Before sex expression, no differences in growth rates of males and females existed in the bryophytes *Bryum argenteum* (Horsley *et al.*, 2011), *Pleurozium schreberi* (Longton and Greene, 1979), *Drepanocladus trifarius* (Bisang *et al.*, 2015), and *Syntrichia caninervis* (Stark *et al.*, 2004). Males grew less well than females in the moss *Ceratodon purpureus* (Shaw and Gaughan, 1993) – but note that the difference in dry mass for the same species was less in the study of Shaw and Beer (1999) – and the liverworts *Sphaerocarpos texanus* (McLetchie, 1992) and *Marchantia inflexa* (McLetchie and Puterbaugh, 2000).

Clones

Taking clones from males and females and propagating vegetative plants under the same conditions, Oñate and Munné-Bosch (2009) found no difference in growth for *Urtica dioica*. Male cuttings of *Acer negundo* showed slower growth in wet habitats, but grew as well as female cuttings in xeric habitats (Dawson and Geber, 1999). Male cuttings of willow grew worse than female cuttings under a range of conditions (Jiang *et al.*, 2016). Overall male cuttings had 16% higher aboveground biomass than female cuttings when averaged over various treatments in *Populus tremula* but this difference was not significant (Sobuj *et al.*, 2018).

Juvany and Munné-Bosch (2015) reviewed the literature on tolerance of dioecious angiosperms to abiotic and biotic stress, including many studies that used cuttings. They emphasized that the stress response was highly species-specific with males outperforming females in some species and females outperforming males in other species.

Fragmentation and regeneration of bryophytes occur frequently in nature and the method of cloning has often been used. Regeneration of leaves was not different between the sexes for the mosses *Plagiomnium undulatum* (Newton, 1972) and *Pleurozium schreberi* (Longton and Greene, 1979). Leaves taken from males regenerated worse than leaves from females in *Mnium hornum* (Newton, 1972) and *Syntrichia caninervis* (Stark *et al.*, 2004). When Stark *et al.* (2005) exposed *S. caninervis* to several cycles of dehydration, male survival was lower than female survival. Males were also less tolerant to dehydration in the tropical liverwort *Marchantia inflexa* (Marks *et al.*, 2016).

Reproducing individuals

When females allocate more to reproduction and still manage to outgrow reproducing males, this suggests that some additional factor slows males down. The alternative result, reproducing females growing less than reproducing males, can be interpreted in different ways and is inconclusive.

In reviewing the literature on *Silene latifolia*, Dawson and Geber wrote that it: '. . . remains a puzzle how females can achieve a larger size and sustain a larger reproductive investment than males' (1999, p. 202). If we limit the review of Obeso (2002) to (sub)dioecious species, male trees were bigger than female trees in 14 cases, sizes were equal in 10 cases, and males were smaller than females in only two cases. This contrasts sharply with the pattern in herbaceous perennials; males were bigger than females in six species, there was no difference in two species, and males were smaller than females in 10 species. These two trees and 10 herbaceous perennials in which reproducing males were smaller than females are inconsistent with the hypothesis of differential reproductive costs, suggesting that an additional factor may slow males down. This line of evidence is, however, indirect.

From the liverwort species reviewed by Fuselier (2004), in 49 cases the males were smaller, while females were smaller in only one case. Males were smaller in several moss genera and families (e.g. Ernst-Schwarzenbach, 1939). Dwarf males occur in some bryophytes. In 18 out of 72 species in which male dwarfism was documented, males were always dwarfed but more often (54 out of 72 species) this behaviour depended on the environment (Hedenäs and Bisang, 2011). Male spores can develop normally but when they germinate on top of a female of the same species they remain small, apparently under hormonal control of the female (Glime and Bisang, 2017c). In their review of bryophyte sex ratios, Bisang and Hedenäs (2005) omitted species with dwarf males. This topic is also beyond the scope of our paper.

Herbivory

Measuring herbivory on male and female plants is also informative for comparing performance. Cornelissen and Stiling (2005) performed a meta-analysis of sex-biased herbivory in angiosperms. Male plants hosted significantly higher numbers of herbivores than female plants. Males also had lower concentrations of secondary chemicals and invested less in other defences such as leaf toughness, amount of resin and trichomes. No differences in nutrient concentrations were observed among the sexes. To our knowledge, nothing is known about sex-differential herbivory in bryophytes.

Taken together, the results suggest that, for both angiosperms and bryophytes, there are either no differences between males and females in growth or survival before reproduction or that males perform worse. We could not find a single example in which the male significantly outperformed the female before reproduction.

DISCUSSION

Primary sex ratios

We discussed some theoretical reasons why primary sex ratios would be different in angiosperms and bryophytes. The drive of the male chromosome could be stronger in angiosperms, leading to male-biased sex ratios. In several cases, sex ratios in seeds (Table 2) and spores (Table 3) were biased and varied between families, consistent with the idea that sex ratio can be a tug-of-war between different genetic factors. Several families showed a male bias in their primary sex ratio, suggesting that Y- or V-linked drive occurs in nature. In several cases, primary sex ratio matched population sex ratio but in other cases it did not or the match was only partial (Tables 2 and 3). For the set of species we reviewed, the primary sex ratio was remarkably similar between angiosperms and bryophytes. This suggests that the general difference in sex ratio between angiosperm and bryophyte populations is not due to primary sex ratio and must originate later in life. This is still a very preliminary conclusion due to the selection of the species investigated. There were only a few species, all of which were chosen because the seeds and spores of these species could easily be grown to maturity.

An explanation for female-biased population sex ratios

Our starting point was the general difference in sex ratio in populations of some angiosperms and bryophytes. If primary sex ratios of these groups are similar (Tables 2 and 3), the difference must then emerge later in life. Plant mortality is typically high in the early life stages of seedling survival and establishment. When males are lagging behind in growth and have slightly lower survival, such differences could accumulate and lead to a female-biased sex ratio, even before any reproduction has occurred. In angiosperms, males typically reproduce earlier and/or at a smaller size than females (Delph, 1999). When early male reproduction leads to extra mortality, this accentuates the female bias in the true sex ratio of the cohort. From the moment that females begin reproducing, they invest much more in reproduction than males and it is likely that from this point onwards females pay a higher reproductive cost and grow and survive less well than males (Delph, 1999). Given enough time, this eventually leads to a male bias in the population. This scenario could explain the different patterns of sex-ratio bias in short-lived herbs and long-lived trees (Table 1) (Sinclair *et al.*, 2012; Field *et al.*, 2013). Short-lived plants can have male-, female-, or unbiased sex ratios depending on differences in survival between males and females in the pre-reproductive and reproductive stages. In long-lived plants like shrubs and trees, the reproductive period is longer and this may eventually lead to a female-biased sex ratio.

For bryophytes, the same scenario could work with an additional role for low levels of fertilization. The movement of sperm cells is limited to centimetres. Hence it can be expected that low levels of fertilization occur more frequently in dioicous bryophytes than in angiosperms. Frequency of developing a sporophyte from an archegonium was 36% in populations of the desert moss *Syntrichia caninervis* (Stark *et al.*, 2000). In the moss *Hylocomium splendens*, this probability rapidly decreased with distance to the nearest male in most years; the probability was ~25% at 5 cm to the nearest male and was less than 10% at 10 cm distance (Rydgren *et al.*, 2006). In dioecious angiosperms, seed set also typically declines with distance to the nearest male (reviewed in de Jong *et al.*, 2005a) but the effect is probably less drastic than for dioicous bryophytes. In general, low fertilization reduces female investment in reproduction, while male investment remains unchanged. Low fertilization levels may push populations towards female bias. Clonal reproduction is common in many dioicous mosses (During, 2007). For reproducing individuals of the moss *Hylocomium splendens*, Rydgren *et al.* (2010) showed that non-fertilized females grew better than males, which in turn grew better than sporophyte-producing females. With low fertilization this will result in a female-biased population. It would seem important to document the consistency of this rank order in more species. Male investment in antheridia exceeded female investment in archegonia in the mosses *Syntrichia caninervis* (Stark *et al.*, 2000; Stark, 2002) and *Bryum argenteum* (Horsley *et al.*, 2011) but not in *Drepanocladus trifarius* (= *Pseudocalliergon trifarium*) (Bisang *et al.*, 2006). To predict the change in sex ratio over time, it would be good to include the phase before reproduction also in these comparisons.

Mechanisms behind biased primary sex ratios

Selective abortion seems the only plausible mechanism for biased spore sex ratios in bryophytes (McDaniel and Perroud, 2012). Despite a low average spore viability of 50%, Norrell *et al.* (2014) found no correlation between viability and the sex ratio of different spore families of the moss *Ceratodon purpureus*. Information on selective seed abortion in angiosperms is scattered. For *Urtica dioica*, the sex ratio of the offspring depended entirely on characters from the mother (the sex ratio from the family from which she came) and not on that of the father (Glawe and de Jong, 2007). This could indicate abortion of some seeds depending on their gender.

Other mechanisms that result in bias in the primary sex ratio apply to angiosperms only. Competition for fertilization between pollen carrying an X- or Y-chromosome affected the seed sex ratio in *Silene latifolia* (Taylor *et al.*, 1999; Hathaway *et al.*, 2008) and *Rumex nivalis* (Stehlik and Barrett, 2006). Environmental effects on sex determination or more complex systems than XX/XY (Martin, 1966) could also result in biased primary sex ratios in angiosperms.

Males as the weaker sex: degeneration of Y or cytoplasmic factors?

The focus of much literature was upon the reproductive phase (e.g. Geber, 1999; Barrett and Hough, 2013) and differences between the performance of males and females in the pre-reproductive phase were rarely mentioned. Obeso (2002) acknowledged differences in the pre-reproductive stage in a single sentence, referring to the dioecious shrubs *Sipanura grandifolia* (Nicotra, 1999) and *Ilex aquifolium* (Retuerto *et al.*, 2000). Lloyd and Webb commented: ‘Not surprisingly then, there have been few observations of pre-reproductive growth of the sexes, although this subject is of considerable theoretical interest’ (1977, p. 181). In their review of stress tolerance, Juvany and Munné-Bosch remarked: ‘Interestingly . . . taken together these studies also show that sex-related differences can be observed in saplings obtained from cuttings and grown in the greenhouse when sex is not expressed’ (2015, p. 6084).

We think that it is incorrect to regard sexual differences in pre-reproductive performance as adaptations. Similarly to females, males are under selection to grow larger and survive better so as to produce as much pollen as possible in order to win the competition with other male plants. One expects the differences between males and females to increase through evolutionary time. The presence of weak males in *Silene latifolia* and *Rumex* species, all with heteromorphic sex chromosomes, and in the entirely dioecious Salicaceae family is in line with this idea. For bryophytes, there also appears to be an association between male weakness and ancient dioicy. Both *Sphaerocarpos texanus* (Allen, 1919) and *Mnium hornum* (Ono, 1967) have heteromorphic sex chromosomes with V being smaller than U. The sex chromosomes of *Ceratodon purpureus* are large with the sex-determining region covering 5% of the chromosome (McDaniel *et al.*, 2007). All species in the genus *Marchantia* are dioicous, the sex chromosomes are probably ancient (Renner *et al.*, 2017), and sexual dimorphisms are common (Fuselier, 2004). For the other bryophytes mentioned, including all species without weak males, descriptions of cytological differences between U and V are missing. This prevents a definite conclusion but the trends in angiosperms and bryophytes follow the pattern that weak males occur in species with ancient sex chromosomes.

The answer to which mechanism is most important for explaining differences in performance between males and females should come from detailed comparisons of gene expression, photosynthesis, respiration, and other physiological characters (see also Charlesworth, 2018). In bryophytes, degradation of U and V is similar, which suggests that when there are weak males, this is the result of suboptimal functioning of the cytoplasm. In angiosperms, degeneration of Y and suboptimal functioning of the cytoplasm both work in the same direction. Since they both compromise male performance, their joint effect in angiosperms may be stronger than in bryophytes. Whether this is indeed the case is not yet clear from the limited data set we analysed. To determine which mechanism is responsible for male weakness in angiosperms, one could look for situations where the two factors are decoupled.

(1) Sexual dimorphism in size appears to be variable between populations of *Rumex acetosa* and *R. acetosella*, with males in some populations reaching the same weight as females, but only about 45% of female weight in other populations (Korpelainen, 1992b). In such a case, it is straightforward to distinguish between the effects of the Y-chromosome (paternally transmitted) and cytoplasm (maternally transmitted) by making reciprocal crosses between plants from the different populations.

(2) Rarely in angiosperms, for instance in all characterized *Salix* species and in some *Populus* species (reviewed in Harkess and Leebens-Mack, 2017), females are the heterogametic sex (typically denoted as ZW) and males are homogametic (ZZ). In such species, degradation of W would lower the performance of the females, while suboptimal functioning of the cytoplasm would place a burden on the males. The net effect of the two factors is not obvious beforehand and we have no expectation that males do worse than females or the other way around. Studies on gymnosperms could also be useful in this context. In many gymnosperms, chloroplasts and mitochondria are only paternally inherited (Neale and Sederoff, 1987). In that case, degradation of Y would lower the performance of males, while suboptimal functioning of the cytoplasm would burden the females. Again the net effect is not obvious beforehand. Sixty-five percent of gymnosperm species are dioecious, with dioecy dominant in eight of the 12 families (Walas *et al.*, 2018). Yet data on sex determination (Ming *et al.*, 2011) and sex ratios of gymnosperms are scarce. The population sex ratio of *Juniperus communis* was unbiased at low elevations but became strongly male-biased at higher elevations (Ortiz *et al.*, 2002). Females invested, on average, 29.5% of their resources in reproduction, which was considerably more than the 5.8% that males invested. Despite sexual differences in reproductive investment, growth of males was similar to that of females. Therefore, Ortiz *et al.* (2002) concluded that their results did not fit the hypothesis of differential reproductive costs (Delph, 1999) and that, for some unknown physiological reason, males are better able to cope with stressful abiotic conditions. The complete opposite of our typical angiosperm with XY males and maternal inheritance of the cytoplasm would be a species with ZW females and paternal inheritance of the cytoplasm. Here, all suggested factors (degradation of W, suboptimal functioning of the cytoplasm in females, and higher female investment in reproduction) work in the same direction, bringing down the performance of the female. This should lead to male-biased populations in nature. Both assumptions could be correct for *Ginkgo biloba* (Ming *et al.*, 2011). However, a female-biased sex ratio of 0.4 was reported in natural populations of *G. biloba* (Tang *et al.*, 2012).

We have argued that, by itself, the hypothesis of differential reproductive costs does not provide a complete explanation for sex-ratio patterns in plant populations. One should also take fertilization rates into account, especially for bryophytes, since low fertilization rates reduce female allocation to reproduction but have no effect on male allocation. In this way, low fertilization rates could lead to female bias. Data on primary sex ratios and performance of males and females in the pre-reproductive stage, with a focus on male/female heterogamy and transmission of cytoplasmic factors, gives a more nuanced picture. Making reciprocal crosses or planned comparisons can help explain the variation in primary and secondary sex ratios that exist in the plant kingdom.

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