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Sex-specific morphological and physiological differences in the moss *Ceratodon purpureus* (Dicranales)

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- **Background and Aims** Dioecy and sexual dimorphism occur in many terrestrial plant species but are especially widespread among the bryophytes. Despite the prevalence of dioecy in non-vascular plants, surprisingly little is known about how fine-scale sex-specific cell and leaf morphological traits are correlated with sex-specific physiology and population sex ratios. Such data are critical to understanding the inter-relationship between sex-specific morphological and physiological characters and how their relationship influences population structure. In this study, these data types were assessed to determine how they vary across three populations within one moss species and whether fine-scale morphological traits scale up to physiological and sex ratio characteristics.
- **Methods** Twenty cell-, leaf- and canopy-level traits and two photochemical measurements were compared between sexes and populations of the dioecious moss *Ceratodon purpureus*. Field population-expressed sex ratios were obtained for the same populations.
- **Key Results** Male and female plants differed in cell, leaf and photochemical measures. These sexual dimorphisms were female biased, with females having larger and thicker leaves and greater values for chlorophyll fluorescence-based, leaf photochemistry measurements than males. Female traits were also more variable than male traits. Interestingly, field population sex ratios were significantly male biased in two study populations and female biased in the third study population.
- **Conclusions** The results demonstrate that the larger morphology and the greater physiological output of female *C. purpureus* gametophytes compared with males occurs across populations and is likely to have significant effects on resource allocation and biotic interactions. However, this high level of dimorphism does not explain population sex ratio variation in the three study populations tested. This research lays the groundwork for future studies on how differential sex-specific variation in cell and leaf traits influences bryophyte plant fitness.

Key words: *Ceratodon purpureus*, dioecious, life history, morphology, moss, physiology, sexual dimorphism, expressed sex ratio, trait comparisons.

INTRODUCTION

Separate sexes occur in $>4\,\%$ of angiosperm (Renner and Ricklefs, 1995) and in $>50\,\%$ of bryophyte species (Wyatt and Anderson, 1984; Bisang and Hedenäs, 2005), evolving independently in angiosperms and bryophytes hundreds of times (Charlesworth, 2002; McDaniel *et al*., 2013). When male and female functions are separated onto distinct morphs, sexual specialization may evolve, and such sexual specialization is particularly likely when differential resource needs (e.g. phosphorus vs. nitrogen demands) occur between males and females (Case and Ashman, 2005). Differential selection on males and females, whether the result of resource needs or sexual selection, may result in sexual dimorphism. In vascular plants with separate sexes, sexual dimorphism has been well documented (for reviews, see Geber *et al*., 1999; Barrett and Hough, 2013), with males and females differing in vegetative and reproductive morphology (e.g. Ackerly and Jasienski, 1990; Delph *et al*., 1996; Harris and Pannell, 2008; Teitel *et al*., 2016), physiology (e.g. Dawson and Bliss, 1989; Dawson and Ehleringer, 1993; Zhang *et al*., 2014), life history (e.g. Cipollini and Stiles, 1991; Espirito-Santo *et al*., 2003; Yang *et al*., 2014) and biotic interactions (e.g. Cornelissen and Stiling, 2005; Eppley *et al*., 2009; Petry *et al*., 2013; Varga *et al*., 2013; Li *et al*., 2015).

If male and female plants differ in size or reproductive output, it is hypothesized that there will be a sex-specific differential in energy assimilation (Dawson and Geber, 1999). In angiosperms, males and females have been found to differ substantially in gas exchange, with females often having a greater capacity for photosynthetic assimilation than males, presumably due to the greater reproductive costs for females which are associated with bearing fruit (Dawson and Ehleringer, 1993; Dawson *et al*., 2004). However, exceptions to this pattern have been found, with males having higher rates of assimilation than females (Marshall *et al*., 1993; Gehring and Monson, 1994). As many dioecious species may be spatially segregated on the landscape, understanding how physiological response varies with genotype and sex is important to understanding how differing resource availability may contribute to altering population and community structure (Dawson *et al*., 2004). However, only a handful of studies have quantified sexual dimorphism from several populations in a common garden experiment or across environments to determine whether genetics or environmental variation controls variation in these traits (Delph *et al.*, 2002; Delph and Bell, 2008; Yu *et al.*, 2011), and the few studies that have examined whether sex-specific physiology was genetically controlled in plants have generally concentrated on a single population (Wang and Griffin, 2003; Dudley and Galen, 2007).

Here, we examine whether differential selection on males and females of the cosmopolitan moss *Ceratodon purpureus* has led to differences in cell, leaf, canopy and physiological traits between male and female plants across three populations. Sexual dimorphism has been reported for this species (Shaw and Gaughan, 1993) and is assumed to be relatively common across moss species, yet on such small organisms these subtleties can be difficult to distinguish (Shaw and Beer, 1999). Additionally, in mosses, differences between sexes can border on the extreme (Hedenäs and Bisang, 2011) or not be present at all (Horsley *et al.*, 2011), making mosses an excellent and globally widespread system for studying the evolutionary ecology of sexual dimorphism in plants. Sex-specific differences in life history traits have inspired investigations of bryophyte sex ratios, reproductive trade-offs and functional traits (Shaw and Gaughan, 1993; Shaw and Beer, 1999; Bowker *et al.*, 2000; McLetchie and Puterbaugh, 2000; Bisang and Ehrlén, 2002; Bisang and Hedenäs, 2005, 2013; Stark *et al.*, 2010; Alvarenga *et al.*, 2013). However, in bryophytes, we know little about the breadth of between-sex diversity in morphological traits (Shaw and Beer, 1999; McLetchie and Puterbaugh, 2000; Horsley *et al.*, 2011) or physiological traits (Stark *et al.*, 2005; McLetchie and Stark, 2006; Groen *et al.*, 2010), and comparatively little emphasis has been placed on understanding the underlying sex-specific physiological differences in dioecious mosses at a finer scale and their contributions to sexual dimorphisms (Stark *et al.*, 2009). Developing this sex-specific framework is also important for understanding how moss-dominated ecosystems (the bryosphere), which occur on every continent and dominate major portions of the earth's surface, shape the function and diversity of heterotrophic communities (Lindo and Gonzalez, 2010; Balkan, 2016) and consequently nutrient cycling (Cornelissen *et al.*, 2007; Turetsky *et al.*, 2012; Delgado-Baquerizo *et al.*, 2016) in ecosystems.

Mosses are a particularly ideal study system for such analyses because population sex ratios of expressing individuals are often highly skewed, generally with a female bias (Bisang and Hedenäs, 2005; but see McDaniel *et al.*, 2007). Additionally, while we expect selection to act rapidly on physiological traits that maximize reproductive success and minimize the differential costs of reproduction for the separate sexes, selection may in fact act differently on the gametophyte (pre-zygotic) or sporophyte (post-zygotic) generation to produce biased population sex ratios (Norrell *et al.*, 2014). For instance, early work by Shaw and Gaughan (1993) determined that expressed sex ratios of *C. purpureus* populations were more female biased than male biased. Male gametophytes in these populations were significantly smaller in overall biomass and had shorter leaves than females (Shaw and Gaughan, 1993). Since mosses often lack storage organs to ameliorate interactions with the environment, mosses are often physiologically limited by water status (Ehrlén *et al.*, 2000; Rydgren and Økland, 2003). In fact, most mosses have leaves that are only one cell thick, so altering cell or leaf size could benefit their overall water status and prolong hydration during desiccating events. Male gametophytes bear antheridia (sperm-producing reproductive structures) which along with their gametes are in fact noted for their ability to tolerate desiccation (Shortlidge *et al.*, 2012; Stark *et al.*, 2016). Since sexual reproduction in mosses is known to be sperm limited, this creates a scenario where sexual selection may exert pressure on morphological and physiological traits that maximize the reproductive potential of gametophytic males by maximizing their tolerance of desiccation (McLetchie, 1996; Bisang *et al.*, 2004). If fertilization is successful, however, female gametophytes would need to stay hydrated longer than males to support the growth of the sporophyte which is physiologically dependent on the maternal gametophyte for maturation. Female gametophytes and their attached sporophytes appear to vary in their tolerance of desiccation depending on when in the maturation process drying occurs (Stark *et al.*, 2007; Stark and Brinda, 2015). Building on these observations, here we predict that the sex having larger morphological trait values will also have been selected to have higher physiological trait values to offset reproductive costs, as has been found in angiosperms (Caruso *et al.*, 2003).

To examine sex-specific patterns in morphology and physiology in *C. purpureus*, we concentrated our measurements on a sub-set of 20 of the cell-, leaf- and canopy-level traits that Waite and Sack (2010) demonstrated were critical to explaining moss gas exchange physiology, and that have been used to demonstrate differences across moss species in gas exchange physiology (Waite and Sack, 2010; Wang *et al.*, 2016). We expect these traits may be important in sex-specific allocation differences within *C. purpureus*. However, mosses are not just scaled-down angiosperms, as area and volume scale down differentially. The efficiency of water diffusion for water transport scales with plant size, and plant–atmosphere interactions are a matter of scale, as small plants are trapped in the laminar boundary layer of the surface on which they grow, unlike larger plants (Proctor *et al.*, 2007). These unique characteristics of small-scale moss canopies mean that the limited data we have on sexual dimorphism and sex-specific physiology in angiosperms may not translate to this system. Using the *C. purpureus* model system, we addressed the following questions. (1) Are there sex-specific differences in cell-, leaf- and canopy-level traits and are these observations consistent among populations of the same species? (2) How are sexual dimorphisms in *C. purpureus* related to measures of leaf photochemistry and/or field expressed sex ratios in this study species? To address these questions, plants from three populations were grown in a common-garden environment to evaluate the amount of phenotypic trait variation among populations that can be attributed to genetic differences, and expressed sex ratios were assessed for each of the three field populations.

MATERIALS AND METHODS

Study species and greenhouse component

Ceratodon purpureus (Hedw.) Brid. (Dicranales) is a dioecious moss that is ubiquitous across all continents and varies dramatically in its ecological tolerances (Crum and Anderson, 1981; Jules and Shaw, 1994). Previous investigations have noted discrepancies from the expected 1:1 ratio of males to females in natural populations of this species (Shaw and Gaughan, 1993), and more recent work suggests that the meiotic sex ratio apparent at spore germination can vary greatly in *C. purpureus*, potentially expaining some of the population sex ratio variation in this species (Norrell *et al*., 2014). We collected plants from three locations. Two are urban locales within Portland, OR: these will henceforth be referred to as Neuberger Hall (NH) (45°30′N, 122°41′W), on the downtown campus of Portland State University (PSU), and North East 35th (NE 35) (45°32′N, 122°37′W), in a neighbourhood 8 km north-east of downtown. A population in a third location, UCUT (45°36′N, 123°02′W), 32 km west of the Portland, OR metropolitan area, was also utilized. All three populations are assumed to be genetically distinct due to the distance between these sites. Regional climate conditions were similar between all sites, but overstorey canopy cover was noticeably different between the NE 35 and NH sites and the UCUT site. Therefore, canopy openness was measured at each site with a densiometer (NE 35, 84 % cover; NH, 98 % cover; UCUT, 0 % cover).

In order to distinguish between genetic and environmentally controlled life history traits, we grew plants from all three populations through the protonemal stage in an environmentally controlled glasshouse on the PSU campus (Shaw, 1986). Plants were collected from the field as gametophytes in clumps from disjointed areas (to decrease the likelihood of collecting ramets of the same genet) within each of the three study sites and allowed to air-dry. One shoot per clump (assumed to be an individual) was finely ground, and plant fragments were sprinkled on the soil surface of a single 10 cm^2 plastic pot filled with 2:1 sand/coir mixture (Down to Earth, Eugene, OR, USA). Each individual was grown to sexual maturity for identification and then allowed to continue growth until the pot was full of ramets of that single individual (approx. 12 months). All plants received the same water regime (misted six times daily), light levels (no artificial lighting) and temperatures (ranged from 10 to 25° C) so that any field effects from the separate populations were removed. We started with about 30 individuals per population. Of those that survived and thrived, four randomly selected individuals of each sex per population provided the plant materials from which the comprehensive cell, leaf and canopy trait measurements were derived. All individuals were of reproductive age, thus allowing verification of the sex by observance of distinct sexual structures, antheridia (male) and archegonia (female), prior to analyses. In bryophytes, it is common to have gametophytes that do not produce reproductive structures even though their sex may be genetically determined. For the following trait measurements, only sexually expressing shoots of individuals are used. Determining the sex of non-sex-expressing gametophytes was beyond the scope of this study.

Leaf and cell measurements

In order to differentiate leaf and cell morphology between the sexes, two ramets per individual were selected from the same location within each pot as designated by a wire grid. Plant material was mounted fresh on a slide, then hydrated and photographed (Leica compound and dissecting microscope, Germany; Leica Application Suite 3.5.0, Germany) at × 4 and ×40 magnification to obtain images of the cells, leaves and leaf cross-sections. Lower leaves rather than parachietal leaves were measured to assess pre-reproductive sexual dimorphism (Shaw and Gaughan, 1993). Methods follow those of Waite and Sack (2010). Images were later analysed using Image-Pro Express (Media Cybernetics, MD, USA). Each leaf or cell trait was measured from two whole leaf or two leaf cross-sectional images (one leaf or cross-section per ramet) and include by category: cell traits: CLA, cell lumen area ($\mu\mathrm{m}^2$); CLL, cell lumen length (μm); CLW, cell lumen width (μm); SWT, adaxial cell wall thickness (μm); IWT, abaxial cell wall thickness (μm); CWLG, interior longitudinal cell wall thickness (μm); CWLT, interior latitudinal cell wall thickness (μm); and leaf traits: COD, costa depth (mm), COW, costa width (mm); LA, leaf area (mm^2); LL, leaf length (mm); LT, lamina thickness (μm); LW, leaf width (mm); L/W, length/width (Supplementary Data Additional methods).

Canopy measurements

We quantified six canopy traits, selected for their potential association with leaf and cell traits, by dividing glasshouse-grown pots of *C. purpureus* into grids. From three randomly selected grid squares (repeated for each individual), three cores (4·37 cm^2) of fresh plant material were collected for analysis. After a canopy projected area image was acquired with a dissecting scope, we measured the canopy height (CH) of three averaged sized gametophyte shoots of each core. One shoot, of average height, was photographed to determine the shoot projected area. Shoots were then defoliated, and leaves and stems photographed separately to allow for measurements of leaf projected area and stem projected area. All plant material was then oven-dried at 80 °C, and dried plant weights were used to quantify canopy mass, leaf mass, stem mass and shoot mass. Canopy metrics determined from shoot and canopy images include: canopy traits: LMA, leaf mass area ($\mathrm{g\ m}^{-2}$); CMA, canopy mass area ($\mathrm{g\ m}^{-2}$); CH, canopy height (cm); CD, canopy density ($\mathrm{g\ cm}^{-3}$); LMF, leaf mass fraction; LAR, leaf area ratio (mm^2/g). Equations and exact methods follow those of Waite and Sack (2010) (Supplementary Data Additional methods).

Leaf photochemistry measurements

Leaf photochemistry measurements [in this study chlorophyll fluorescence-based F_v/F_m and electron transport rate (ETR)] allow a non-destructive glimpse of a plant's photosynthetic functioning under natural conditions. All photochemical measurements were made using the Junior-PAM chlorophyll fluorometer (Walz, Effeltrich, Germany), fitted with a small diameter fibre optic probe. All plants were dark adapted overnight prior to analyses. Fluorescence measurements were made in a dark room, and care was taken to ensure that plants did not desiccate.

We used F_v/F_m to measure the maximum photochemical efficiency of dark-adapted photosystem II (PSII). Four F_v/F_m measurements were taken for each individual and a mean was calculated for each. Initial chlorophyll fluorescence measurements corresponded with cell, leaf and canopy imaging dates. Subsequent measurements were repeated four times at 2 week intervals.

Photosynthetic photon flux density (PPFD) response curves followed the methods of Marschall and Proctor (2004) and were used to attain a relative measure of photosynthetic ability. The Junior-PAM chlorophyll fluorometer emits consecutive increasing intensities of light and records the instantaneous relative ETR of PSII, with 6 min at each level for equilibration. Two replicate ETR measurements were attained for each dark-acclimated individual and a mean was calculated for each.

Field population expressed sex ratio

To understand how these morphological and physiological trait investigations relate to population expressed sex ratios, we collected plants from the field in May of 2012 from the three populations mentioned above. Every 30 cm, along 152 cm transects, three 4·37 cm^2 moss cores were sampled with a soil corer, placed in numbered envelopes to reduce observer bias, and stored at room temperature until analysis. Two transects per population were sampled, yielding 36 moss cores per population. In the laboratory using a dissecting microscope (Leica, Germany), we determined counts of sexually expressing male and female ramets per core, and we recorded the total number of ramets per core. We pooled the counts from all cores within each population to obtain the sex ratio per population of expressing ramets. Population sex ratio values (SR_{pop}) were determined by dividing the number of expressing male ramets by the total number of sexually expressing ramets (male and female). Determining the sex of non-sex-expressing ramets was beyond the scope of this study.

Statistical analyses

Analyses of variance (ANOVAs) were performed to determine the effect of sex, population and the interaction between sex and population on measured leaf, cell and canopy traits, using JMP Version 9.0 (SAS Institute, 2010). Data fit the assumptions of the ANOVA models. Because we used multiple analyses from data on the same samples, we used a Benjamini and Hochberg procedure to control for a false discovery rate (Benjamini and Hochberg, 1995). We used Tukey's tests to determine significance among factors. We used repeated measures ANOVA to determine the effect of sex, population and the interaction between sex and population on F_v/F_m and ETR across time and light levels, respectively, using JMP Version 9.0. *t*-tests were performed at each time point (for F_v/F_m) and light level (for ETR) to compare the performance of males and females across populations. We used Pearson's and Spearman's correlation coefficients on log-transformed data to meet normality assumptions and created a correlation matrix of the 20 measured cell, leaf and canopy traits. Principal components analysis (PCA) was performed on the correlation matrix using the R package factoextra (Kassambara and Mundt, 2016; R Core Team, 2016). Three traits (LW, LL and LA) were excluded from the PCA because of their strong correlation (Waite and Sack, 2010). Using JMP Version 12.0.1 (SAS Institute, 2015), likelihood χ^2 tests were used to determine whether population sex ratios varied from 1:1.

RESULTS

Cell, leaf and canopy traits

One cell trait (longitudinal measure of the cell wall; CWLG) differed significantly between the sexes, with female plants having thicker cell walls than males (Fig. 1A; Table 1). No cell traits varied among populations or in the interaction between population and sex. However, six of seven leaf traits varied significantly between expressing males and females, with greater values for females than males in each trait (Fig. 1B–F; Table 1). For four leaf traits (leaf length; LL, leaf area; LA, costa depth; COD, and costa width; COW; Table 1), population was a significant factor. For all four traits, post-hoc analyses via Tukey's tests showed that plants from the NE 35th and NH populations had significantly larger traits than plants from the UCUT population (Supplementary Data Table S1). For two leaf traits (LL and LA), the interaction between population and sex was a significant factor indicating that the degree of sexual dimorphism of this trait differed among populations. The post-hoc analysis suggests that for leaf length the NE 35th and NH populations showed significant sexual dimorphism while the UCUT population did not (Fig. 1B). The post-hoc analysis for leaf area suggests that all three populations exhibit sexual dimorphism for this trait, but that the degree of dimorphism is significantly greater in the NE 35th population than in the other two populations (Fig. 1C). Canopy traits did not differ between the sexes or among populations, and no interactions were significant for canopy traits (Table 1).

Photochemical measures

Sex and population were both significant factors in explaining dark-adapted PSII efficiency (F_v/F_m) in this moss species ($F_{1,18} = 7·32$; $P = 0·02$ and $F_{2,18} = 3·47$; $P = 0·05$, respectively). Females had significantly higher F_v/F_m values than did males (Fig. 2), and the NE 35 and NH populations had higher values than the UCUT population (data not shown). The interaction between sex and population was not significant ($F_{2,18} = 1·67$; $P = 0·22$). Time was also a significant factor in the model ($F_{5,14} = 91·98$; $P < 0·0001$), and interactions between time and sex ($F_{5,14} = 3·99$; $P = 0·02$) and time, sex and population were significant ($F_{10,28} = 2·16$; $P = 0·05$), indicating that F_v/F_m decreased overall during our sampling time period, that the overall differential in F_v/F_m between males and females increased during our sampling time period (Fig. 2), and that this increase varied among populations. The interaction between time and population was not significant ($F_{10,28} = 0·41$; $P = 0·93$), indicating that the difference in F_v/F_m among populations did not vary during the sampling time period but remained more or less the same.

Sex and population overall did not have a significant effect on ETR ($F_{1,18} = 2·17$; $P = 0·12$ and $F_{2,18} = 1·88$; $P = 0·18$, respectively), and the interaction was only marginally significant ($F_{2,18} = 3·22$; $P = 0·06$). However, when we analysed our data on light levels we found that light level ($F_{7,12} = 214·33$; $P < 0·0001$)

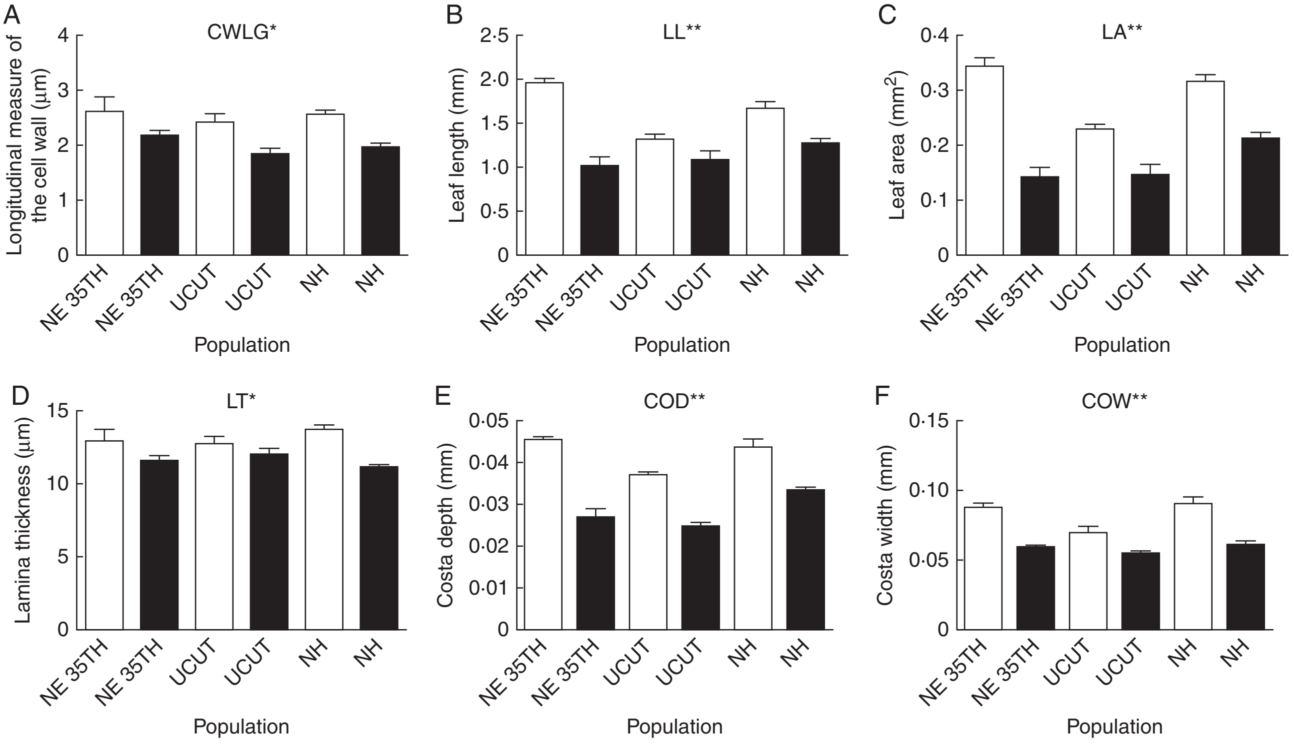

FIG. 1. Sex-specific comparison of six secondary reproductive traits in *Ceratodon purpureus* for three populations: (A) longitudinal measure of the cell wall (CWLG), (B) leaf length (LL), (C) leaf area (LA), (D) lamina thickness (LT), (E) costa depth (COD) and (F) costa width (COW). In all panels, females are represented by white bars and males are represented by black bars. Error bars indicate standard error. Asterisks indicate that the effect of sex was significant for ANOVA for each trait (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; full results in Table 1).

was significant, as ETR increased with light level, and interactions with light levels and sex ($F_{7,12} = 3·74$; $P = 0·02$) and light level, sex and population ($F_{14,24} = 3·48$; $P = 0·004$) were significant, as the greatest differences in ETR in this moss species occurred at higher light levels (Fig. 3). We did not reach saturation of electron flow in the light levels examined in this study, as the relative ETR continued to increase linearly with PPFD, and this increase corresponded with a greater divergence in values between the sexes (Proctor, 2003; Marschall and Proctor, 2004).

Correlation structure among cell, leaf and canopy traits

The first principal component (PC1) separated the sexes in the PCA, explained 32·9 % of the overall trait variation and pointed to a tight grouping of highly correlated cell and leaf traits which arose largely due to the allocation of females to greater leaf, costa, cell and cell wall size (Fig. 4) (Supplementary Data Fig. S1; Table S2 and S3). The second PCA axis (PC2; 15·6 % of the overall variation) primarily reflected variation in cell and canopy traits (Fig. 4). Distance between the sexes within a population on the PCA is indicative of a greater level of sexual dimorphism in life history traits. Within sex, females were more variable than males within and across populations. Populations of *C. purpureus* overlapped greatly in space (Supplementary Data Fig. S2). Along PC1, variation was highest in the NE 35th population. The UCUT population was the most variable along PC2 while the NE 35th population had the least variance along this axis.

Field population expressed sex ratio

The NE 35 (SR_{pop}, 0·63; 111 males, 66 females, 26 % of shoots were fertile) and UCUT (SR_{pop}, 0·59; 558 males, 381 females, 57 % of shoots were fertile) populations exhibited significantly male-biased expressed sex ratios ($\chi^2 = 11·57$; $P = 0·0007$ and $\chi^2 = 33·57$; $P < 0·0001$, respectively). However, the NH population (SR_{pop}, 0·08; 16 males, 182 females, 26 % of shoots were fertile) was extremely female biased in sex expression ($\chi^2 = 163·31$; $P < 0·0001$).

DISCUSSION

Our results present sexual dimorphism in cell, leaf and photochemical measures in the ubiquitous dioecious moss *Ceratodon purpureus*, with females expressing significantly larger trait values and exhibiting enhanced photochemical measures compared with males. Females had significantly larger measures for seven of 20 cell and leaf traits (including larger leaves in most measures) than males, but no canopy traits differed between sexes. Besides these sex-specific differences, our study reveals variation between the three common-garden populations in four leaf traits and in photochemical measures. In contrast to our prediction, we demonstrate that the sex with the higher trait

TABLE 1. *Mean values and statistical analyses of leaf, cell and canopy traits from* Ceratodon purpureus *plants*

Trait	Symbol	Units	Mean (±s.d.)	Sex	Population	Sex × population
Cell traits						
Cell lumen area	CLA	μm^2	160·6 (50·7) 136·1 (42·3)	1·42	0·34	0·17
Cell lumen length	CLL	μm	21·09 (5·03) 18·60 (4·80)	1·43	0·60	0·57
Cell lumen width	CLW	μm	7·872 (1·11) 7·584 (1·21)	0·45	1·90	2·35
Adaxial laminar cell wall thickness	SWT	μm	2·168 (0·52) 1·777 (0·27)	4·64	0·59	0·03
Abaxial laminar cell wall thickness	IWT	μm	2·238 (0·46) 1·862 (0·33)	4·76	0·72	0·15
Longitudinal measure of the cell wall	CWLG	μm	2·537 (0·33) 1·986 (0·23)	**22·06***	1·70	0·19
Latitudinal measure of the cell wall	CWLT	μm	2·184 (0·32) 1·906 (0·22)	5·76	0·48	0·78
Leaf traits						
Leaf length	LL	mm	1·651 (0·30) 1·122 (0·20)	**69·30****	**8·50***	**11·56****
Leaf width	LW	mm	0·311 (0·04) 0·251 (0·04)	**16·55***	5·49	0·94
Leaf area	LA	mm^2	0·297 (0·06) 0·167 (0·04)	**123·51****	**15·07****	**9·82****
Lamina thickness	LT	μm	13·13 (1·17) 11·57 (0·73)	**15·51***	0·07	1·93
Costa depth	COD	mm	0·042 (0·00) 0·028 (0·00)	**162·76****	**17·24****	5·29
Costa width	COW	mm	0·083 (0·01) 0·058 (0·01)	**81·14****	**9·90***	2·96
Length/width	L/W	–	5·558(1·47) 4·494(0·71)	6·50	1·26	3·80
Canopy traits						
Leaf mass area	LMA	$g\ m^{-2}$	9·813 (2·41) 14·27 (13·7)	1·27	1·72	0·58
Canopy mass area	CMA	$g\ m^{-2}$	5·075 (0·22) 3·948 (1·07)	4·14	0·66	0·20
Canopy height	CH	cm	0·718 (0·22) 0·556 (0·15)	4·77	0·40	2·24
Canopy density	CD	$g\ cm^{-3}$	0·008 (0·00) 0·008 (0·00)	0·27	0·51	0·89
Leaf mass fraction	LMF	–	0·560 (0·06) 0·573 (0·10)	0·15	0·66	2·15
Leaf area ratio	LAR	$mm^2\ g^{-1}$	9042 (4568) 7680 (4977)	0·53	2·65	0·18

The means for each trait are for expressing females (upper number) and males (lower number) with the s.d. in parentheses across study populations. The statistical analyses are ANOVA for the effects of sex (male vs. female), population (UCUT, NE 35 and NH) and the interaction between sex and population on each trait, and the *F* statistics are provided. Significant results are in bold; $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$, and are based on the Benjamini and Hochberg procedure to control for false discovery rate with multiple comparisons (Benjamini and Hochberg, 1995); $n = 24$.

values (in our case females) is sometimes less common in natural populations, as sexually expressing males are more common in two of our study populations than sexually expressing females. Our third study population, however, exhibits the expected female-biased sex ratio. Below, we discuss the evolutionary and ecological implications of our results with respect to sex-specific water relations, reproduction and biotic interactions.

Sex-specific differences

Female plants, in our study, have leaves that are on average 13·5 % thicker (LT; Fig. 1D) than those of male plants, a potential desiccation tolerance mechanism (Dilks and Proctor, 1979). As found in earlier studies on this species, female plants also have larger and longer leaves than males (LA, LL; Fig. 1B, C) which may prolong hydration via greater water storage in cells or by increasing the boundary layer, as conduction of water is external in most mosses, rather than internal as in angiosperms (Table 1; Shaw and Gaughan, 1993; Shaw and Beer, 1999; Proctor, 2000). Female plants in this study have wider and deeper costae than male plants (COD, COW; Fig. 1E, F), another trait in mosses often associated with water storage. The costa of *C. purpureus* has thick-walled cells, stereids, that are presumed to prolong leaf hydration (Frahm, 1985) and help the plant avoid desiccation. From studies on other mosses, costa

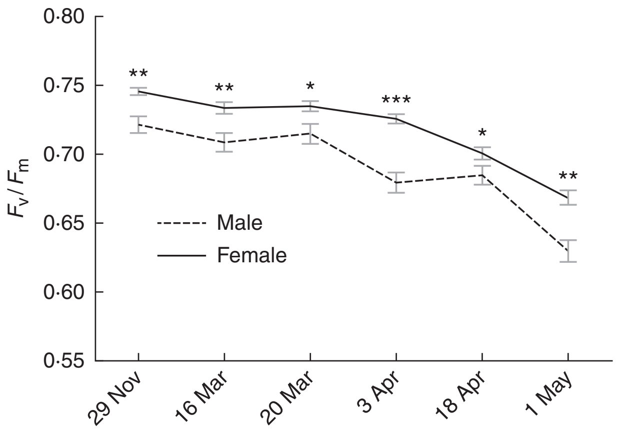

FIG. 2. F_v/F_m values for males and females over time. Each point represents a mean of 12 *Ceratodon purpureus* individuals (four from each population) in three common-garden populations. Error bars indicate the s.e. A repeated measure ANOVA showed a significant effect of sex ($P = 0.02$) and a significant interaction between time and sex ($P = 0.02$); asterisks indicate significant differences between males and females for *t*-tests at each date ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

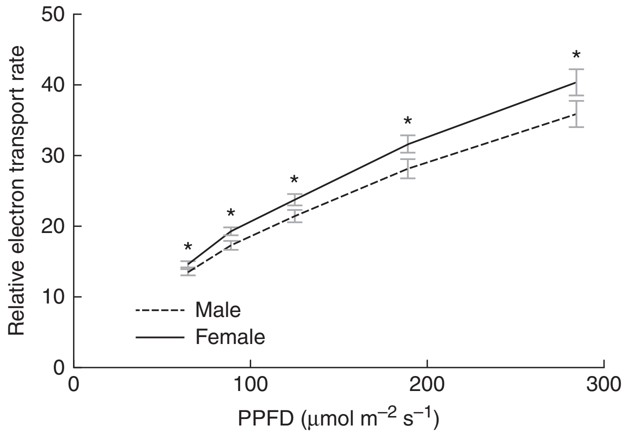

FIG. 3. Photosynthetic photon flux density (PPFD) response curves comparing the relative electron transport rates (ETR) for males and females. Each point represents a mean of 12 *Ceratodon purpureus* individuals (four from each of three populations). Error bars indicate the s.e. A repeated measure ANOVA showed a significant interaction between light levels and sex ($P = 0.02$); asterisks indicate significant differences between males and females for *t*-tests at each light level ($*P < 0.05$).

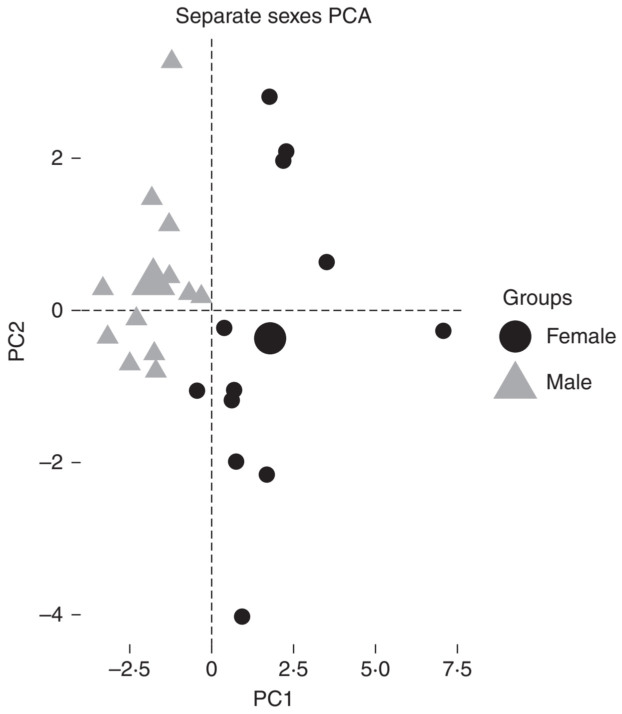

FIG. 4. Principle components analysis (PCA) of 17 cell, leaf and canopy traits. PC1 = 32.9 % of the variance; PC2 = 15.6 % of the variance (see Supplementary Data Table S2). Each point is an individual *Ceratodon purpureus* grown in a common garden.

size and structure has been found to vary within a species depending on the moisture conditions of a particular habitat, with larger costae being found in drier locations (Zastrow, 1934; Frahm, 1985). The increased size of these traits is likely to benefit *C. purpureus* females by prolonging the duration of hydration-limited physiological activity thus allowing females to assimilate carbon for longer. In contrast, cell and canopy traits were, for the most part, not sexually dimorphic in *C. purpureus* (with the exception of CWLG; Fig. 1A). *Ceratodon purpureus* cell and leaf traits did share many strong positive correlations, yet interestingly there were few correlations between cell or leaf traits and canopy traits, a pattern which deserves further investigation (Supplementary Data Table S3). Cellular desiccation poses a grave threat to the structural stability of cells, a limitation that may explain the lack of variation in cell traits between sexes or among populations in this study. Conversely, the fact that there were few correlations among cell or leaf and canopy traits could provide an opportunity for greater plasticity in canopy traits or may alternatively suggest that canopy traits are under a different selective pressure (Sultan 2000; Delph *et al.*, 2002). Our photochemical measures suggest that females may assimilate carbon at a greater rate than males (Figs 2 and 3). Studies are currently under way to analyse more comprehensively sex-specific differences in CO_2/H_2O exchange and photochemistry in *C. purpureus*. When life histories vary between the sexes, morphological differences between the males and females are common (Geber *et al.*, 1999). In mosses, females must remain hydrated for much longer than males to facilitate water-mediated fertilization and embryo development (Stark *et al.*, 2007; Stark and Brinda, 2015). It seems likely that these dimorphic morphologies, particularly larger leaves, should prolong hydration and benefit females and specifically the sporophyte generation (Moore *et al.*, 2016). Additionally, besides maximizing the amount of time spent hydrated, it follows intuitively that plasticity in these same or additional water-related traits might also ensure reproductive success in the face of environmental heterogeneity. This need for plasticity could help explain the increased trait variation measured in females vs. that of males within and across our study populations (Fig. 4).

In addition to impacts on water relations and reproduction, differences in leaf thickness between the sexes could also influence how the sexes interact with the biotic community. In vascular plants, male and female plants have been found to interact differentially with insects and fungi (Cornelissen and Stiling, 2005; Varga and Kytöviita, 2008; Varga, 2010), and sex-specific differences in plants have been shown to lead to sex-specific heterotrophic communities (Petry *et al*., 2013). Thus, larger leaf traits in females compared with males are likely to lead to differences in how each sex interacts with local heterotrophs. In mosses, thicker and larger leaves may allow females greater protection against fungal pathogens, which occur at high densities in moss communities (Davey and Currah, 2006; Davey *et al*., 2013). Indeed, males from these same study populations, with thinner leaves, were recently found to have higher fungal biomass and richness than females (Balkan, 2016). In fact, if the higher photochemical values in females allow for greater overall net assimilation, this could provide the resources needed for fungal chemical defences and/or volatile compounds that have been hypothesized as contributing to an early ‘plant pollinator’-like syndrome in *C. purpureus* (Cronberg *et al*., 2006; Cronberg, 2012; Rosenstiel *et al*., 2012).

Population-level differences

While trait variation between males and females occurs more often (seven out of 20 traits) than trait variation among populations in this study (four out of 20 traits), the population-level differences help differentiate those traits that are genetically constrained from those that are more plastic. None of the cell or canopy traits measured here demonstrate population differences, suggesting that those traits and the sexual dimorphisms found for one cell trait (CWLG) may be more constrained in this species. Waite and Sack (2010) found that these same cell traits varied drastically across bryophyte species, thus perhaps cell traits are generally conserved at the species level in mosses. Leaf traits, conversely, are dimorphic between sexes and variable across these study populations. For all four leaf traits that vary by population (LL, LA, COD and COW), these traits are greatest for females in the NE 35 and NH populations where canopy cover is densest (84 and 98 % cover, respectively) and least in the UCUT population (0 % cover). F_v/F_m values follow this same trend, with the NH population having the highest values and the UCUT population the lowest values. Consideration of those traits that are consistent vs. traits that vary across populations sheds light on how natural and sexual selection interact in *C. purpureus* to create separate sexes with trait dimorphisms.

Although our work only considers plants from three populations with limited environmental variance, it suggests that *C. purpureus* may be more genetically constrained between the sexes and across populations when considering cell and canopy traits but sexually dimorphic and more plastic in its ability to modify leaf traits differentially across environments. Such remarkable fine-tuned adaptability probably contributes to the ability of this species of moss in particular to grow on every continent along almost any environmental gradient. These findings suggest that across-environment stability in *C. purpureus* is partially achieved by integrating its sexually selected water-related needs with environmentally mediated leaf morphologies. In addition, the fact that females express greater variability than males in this study may in part be explained by the sensitivity of the growing sporophyte to desiccation (Fig. 4; Stark *et al*., 2007; Stark and Brinda, 2015). Support for the effects of plasticity in leaf morphology on stabilizing fitness across environments has been found in vascular plants (Schlichting, 1986; Sultan, 2000; Pigliucci, 2001), though additional studies are needed to understand how constraints and modifications of suites of traits are influenced by environmental heterogeneity.

Leaf photochemistry and expressed sex ratios

We predicted that the sex with the larger morphology would have greater physiological output to offset potential costs. Our measures of leaf photochemistry support this prediction as females have higher F_v/F_m values across a 5 month time frame and higher relative ETRs across different light levels (Figs 2 and 3). Consistent with previous studies (Shaw and Gaughan, 1993; Shaw and Beer, 1999), *C. purpureus* displays biased sex ratios in our study populations; however, two of the three populations in our study were significantly male biased compared with a female bias noted in previous studies. While we cannot discount the possibility that sexual determination of non-sex-expressing shoots might alter these findings, our result is contrary to our sex ratio prediction which presumed that the sex associated with larger sized traits and greater physiological advantage would also have a fitness advantage. One explanation could be that females bear an added cost by allocating more energy to growth and that this cost is not offset sufficiently by the sex-specific difference in physiology, and thus males outcompete females. This species occurs in disturbed habitats that vary considerably in abiotic conditions. While the majority of *C. purpureus* populations may be female biased, males may dominate in some populations, such as our NE 35 and UCUT populations, where abiotic conditions may alter the physiological balance between the sexes to favour males, as has been found to occur in dioecious angiosperms such as *Acer negundo* (Ward *et al*., 2002). More site-specific variables would need to be examined for such an assessment.

CONCLUSION

Overall we find that sexual dimorphism occurs consistently for leaf traits and that this dimorphism is somewhat population specific. Females usually have larger and thicker leaves than males, and this correlates with greater photochemical output for females than males. Additionally, higher levels of variation among females within and among populations contrasts with the consistency of male traits, which vary little within or among populations. Traits that do vary among populations (LL, LA, COD and COW) should be considered with regards to how habitat heterogeneity might influence resource allocations and their corresponding dimorphic patterns in *C. purpureus*. Long-term studies are needed to determine how small-scale cell and leaf traits influence survival and mating differences among *C. purpureus* genotypes.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Additional methods. Figure S1: biplot of PCA trait scores. Figure S2: PCA of separate populations. Table S1: means (±s.d.), shown separately for each population, for traits in which population was significant in the full ANOVA model. Table S2: factor loadings of 17 traits in the first two principal components. Table S3: correlation matrix of cell leaf, and canopy traits of *Ceratodon purpureus*.

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